European Commission

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BAS 750F
(Mefentrifluconazole)

Volume 3 – B.5 (AS)

Rapporteur Member State: United Kingdom
Co-Rapporteur Member State: France & Austria

Version History

When	What
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Table of contents

B.5. METHODS OF ANALYSIS

B.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA

B.5.1.1. Methods for the analysis of the active substance as manufactured

B.5.1.1.1. APL0669/01: Determination of Reg.No. 5834378 in BAS 750 F TGAI by HPLC

Report:	KCA 4.1.1/1 Bentz A., 2013 a Analytical method APL0669/01 - Determination of the active ingredient Reg.No.5834378 in Reg.No. 5834378 TGAI 2013/1140545
Guidelines:	none
GLP:	no
Report:	KCA 4.1.1/2 Bentz A.,Harsch M., 2013 a Validation of the analytical method APL0669/01: Determination of the active ingredient Reg.No. 5834378 in Reg.No. 5834378 TGAI 2013/1140546\
Guidelines:	OECD Principles of Good Laboratory Practice, GLP Principles of the German Chemikaliengesetz (Chemicals Act), 2004/10/EC, EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, CIPAC Guidelines on method validation, SANCO/3029/99 rev. 4 (11 July 2000), US EPA OPPTS Harmonized Test Guideline 830.1000, US EPA OPPTS Harmonized Test Guideline 830.1800
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Studies supported:	*KCA 1.11/1* *Harsch M., 2015 a* *Chemical analysis of five batches BAS 750 F - Technical Grade Active Ingredient (TGAI)* *2015/1111987* *KCA 1.11/3* *Fries J, 2015 a* *BAS 750 F TGAI - Analytical profiles of three batches of BAS 750 F TGAI* *2015/1164015* *Harsch M, 2016a* *Chemical Analysis of Five Batches BAS 750 F Technical Grade Active Ingredient (TGAI)* *2016/1138208*

Principle of the method

The analysis of the active substance was performed by HPLC applying UV detection at 230 nm and external calibration. The column used was an Aquity BEH C18 (50 mm x 2.1 mm, 1.7 µm) at 40 °C. A gradient elution was used (mobile phase A: 1000 mL water + 0.5 mL formic acid, mobile phase B: 1000 mL acetonitrile + 0.5 mL formic acid). The retention time as well as the spectra were found to be identical. No CIPAC methods are available for the determination of the active substance in the TGAI.

Approximately 50 mg of the test item was added to a 50 mL volumetric flask and made to volume with acetonitrile. 10 mL of this solution was added to a 50 mL volumetric flask and made to volume with acetonitrile.

Validation summary

HPLC-UV DAD is a highly specific method hence no further confirmatory techniques were required. The identity of the analyte was additionally confirmed by comparison of the UV, IR and mass spectra of the reference and test items. Chromatograms of a reference item, test item and solvent were presented showing no interferences >3% of test item peak at the retention time of interest. To assess method precision, 5 replicate sample determinations were made at a level of 99.387 % and the RSDs were within the Modified Horwitz RSDr acceptable limit of 1.34%. The accuracy of the method was assessed to be acceptable due to the lack of interference and an acceptable level of precision. The linear range encompassed the nominal concentration of the analyte ± at least 20%. The method is satisfactorily validated in accordance with SANCO/3030/99 rev.4.

Validation data

Table 5.1-1: Validation data

Analyte	**LOQ (% w/w)**	**Recovery fortification level (% w/w)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
BAS 750F Reg.No. 5834378	As there is no interference and an acceptable level of precision, the accuracy of the method is assessed to be acceptable.			0.364 @ 99.387 % w/w (5) Modified Horwitz % RSDr = 1.34	85.94 – 311.26 mg/L (43 – 156 % of the nominal concentration) 5 standards, r = 1.0000 y = 8855533x + 397542	Acceptable chromatograms presented for reference item, test item and solvent. No interference >3% of test item peak Identity confirmed by comparison of retention time (1.3 min), UV-spectra, IR-spectra and MS-spectra (*m/z* 398.2, 400.2) of the analyte in the reference item and the test item

B.5.1.1.2. Determination of the enantiomeric ratio of BAS 750 F in TGAI and formulations

Report:	CA 1.11/2 Harsch M., 2015b Determination of the enantiomeric ratio of BAS 750 F in TGAI and formulations 2015/1180118
Guidelines:	OECD Principles of Good Laboratory Practice, GLP Principles of the German Chemikaliengesetz (Chemicals Act)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

[redacted]

Validation summary

[redacted]

Validation data

Table 5.1-2: Validation data

B.5.1.1.3. Determination of impurity Reg.No. [redacted] in BAS 750 F

Report:	CA 4.1.1/9 Harsch M., 2014 b Analytical method APL0685/01 - Determination of [redacted] in Reg.No. 5834378 TGAI (Technical Grade Active Ingredient) by GC 2014/1010801
Guidelines:	none
GLP:	no

Report:	CA 4.1.1/10 Harsch M., 2014 c Validation of the analytical method APL0685/01: Determination of [redacted] in Reg.No. 5834378 TGAI (Technical Grade Active Ingredient) by GC 2014/1010802
Guidelines:	EPA 830.1800, EPA 830.1000, SANCO/3030/99, CIPAC Guidelines on method validation, EC 1107/2009 of the European Parliament, 2004/10/EC, GLP Principles of the German Chemikaliengesetz (Chemicals Act), OECD Principles of Good Laboratory Practice
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

[redacted]

Validation summary

[redacted]

Validation data

Table 5.1-3: Validation data for the analytical method APL0685/01

B.5.1.2. Methods for risk assessment

B.5.1.2.1. Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

Report:	KCA 4.1.2/1 Studenroth S.,Luer D., 2015 a Validation of analytical method L0214/01 for the determination of BAS No. 750 F (Reg.No. 5834378) and metabolites of Reg.No. 5924326 and 1,2,4-Triazole (Reg.No. 87084) in soil by LC-MS/MS 2015/1039006
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Report:	KCA 4.1.2/2 Lueer D., 2016 a Report Amendment No. 1: Validation of analytical method L0214/01 for the determination of BAS No. 750 F (Reg.No. 5834378) and its metabolites Reg.No. 5924326 and 1,2,4-Triazole (Reg.No. 87084) in soil by LC-MS/MS 2016/1030227
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Report:	KCA 4.1.2/3 Obermann M., 2016 a Report Amendment No. 2: Validation of analytical method L0214/01 for the determination of BAS No. 750 F (Reg.No. 5834378) and its metabolites Reg.No. 5924326 and 1,2,4-Triazole (Reg.No. 87084) in soil by LC-MS/MS 2016/1215646
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Studies supported:	*KCA 7.1.2.2.1/5* *Staudenmaier H., Dalkmann P., 2015 b* *Investigation of the extractability of BAS 750 F in samples from 14C soil degradation studies* *2015/1182724*

Principle of the method

A 5 g soil sample was extracted with 40 mL of a mixture of acetonitrile/water (70/30, v/v). After centrifugation an aliquot of 10 mL was taken (extract 1). The same extraction procedure was repeated and after centrifugation a second aliquot of 10 mL (extract 2) was combined with extract 1 and thoroughly mixed. BAS 750 F and Reg. No. 5924326 (4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenol) were directly analysed at this stage. For analysis of 1,2,4-Triazole, 5 mL of the combined extracts 1 and 2 were transferred into a tared glass tube and the volume was reduced in a nitrogen evaporator to a volume less than 1 mL. The concentrated extract was filled up to a volume of 1 mL with ultra-pure water.

For BAS 750 F and Reg.No. 5924326, analysis was performed by LC-MS/MS on an Aquasil C-18 column (150 x 3 mm, 3 µm) at 25 °C with external standardisation and ESI^+ detection monitoring the following selected mass transitions:

Reg. No. 5834378 (BAS 750 F)	398 → 182*
	398 → 133
Reg. No. 5924326	288 → 159*
	288 → 103

*Proposed as quantification transition for further studies but during method validation both mass transitions were used for quantification to confirm identity.

A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

For Reg. No. 87084 analysis was performed on both a Hypercarb column (100 x 4.6 mm, 5 µm) at 30 °C and a Synergi Hydro RP column (150 x 4.6 mm, 4 µm) at 40 °C. Both techniques used ESI^+ detection monitoring a single selected mass transition (*m/z* 70 → 43), used external calibration and employed a gradient elution (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

Soil characteristics

Presented in Table 5.1-4 are the characteristics of the soil samples used in the analytical method.

Table 5.1-4: Soil characteristics

	LUFA 2.2	**LUFA 2.3**
Soil class (USDA)	Loamy fine sand	Sandy loam
Soil class (DIN)	Loamy sand (SI2)	Silty sand (Su3)
Total nitrogen (%)	0.13	0.06
TOC (Total Organic Carbon)	1.72	0.67
TC (total carbon)	1.72	0.67
pH ($CaCl_2$)	5.8	5.4
pH (H_2O)	6.4	6.1

Matrix effects

The influence of matrix effects on Reg. No. 5834378, Reg. No. 5924326 and Reg. No. 87084 was determined by comparison of the mean response factor in solvent standards with the response factor in soil matrix-matched standards. The mean response factor was determined over all concentrations (for Reg. No. 5834378 and Reg. No. 5924326: 0.025 – 3.0 ng/mL; for Reg. No. 87084: 0.125 – 15.0 ng/mL)

Table 5.1-5: Matrix effects

Matrix	**Analyte**	**Mass transition**	**Response factor in matrix matched standards compared to solvent standards (%)***
LUFA 2.2 soil	Reg.No. 5834378 (BAS 750 F)	398 → 182	82, 88**
		398 → 133	81, 89**
	metabolite Reg.No. 5924326	288 → 159	97
		288 → 103	100
	Reg.No. 87084 (1,2,4-Triazole)	70 → 43 (Hypercarb)	105
		70 → 43 (Synergi)	102
LUFA 2.3 soil	Reg.No. 5834378 (BAS 750 F)	398 → 182	79, 85, 85***
		398 → 133	83, 85, 89***
	metabolite Reg.No. 5924326	288 → 159	93, 96, 101***
		288 → 103	93, 94, 92***
	Reg.No. 87084 (1,2,4-Triazole)	70 → 43 (Hypercarb)	106
		70 → 43 (Synergi)	104

*These are mean values calculated from all concentrations tested
**Two values are quoted here as the experiment was repeated to confirm the observed influence of the matrix on the detection of the analyte
***The first and second values result from matrix matched standards produced by concentrating the extract prior to dilution with solvent and the third value from matrix matched standards produced by direct dilution of the extract with solvent.

No significant matrix effects were observed for Reg. No. 5924326 or Reg. No. 87084 however, the matrix in the matrix matched standards of Reg. No. 5834378 had an influence on the detection of Reg. No. 5834378 which could not be neglected. Therefore, validation of Reg. No. 5834378 was conducted using matrix-matched standards.

Stability of standards

The stability of Reg. No. 5834378, Reg. No. 5924326 and Reg. No. 87084 in standard solutions was tested by determination of recovery at a fortification level of 0.002 mg/kg before and after storage at 4 ± 2 °C in the dark. Both before and after storage, the concentrations were measured against freshly prepared standards.

Table 5.1-6: Stability of standards

Matrix	Analyte	Mass transition	Days of storage	Mean % recovery pre-storage	Mean % recovery post-storage
Water	Reg.No. 5834378 (BAS 750 F)	398 → 182	43	105	96
		398 → 133	43	98	99
	metabolite Reg.No. 5924326	288 → 159	43	97	96
		288 → 103	43	98	96
	Reg.No. 87084 (1,2,4-Triazole)	70 → 43 (Hypercarb)	31	98	102
		70 → 43 (Synergi)	31	97	104

Standard solutions of Reg. No. 5834378 and Reg. No. 5924326 were stable (≤10 % decline) for 43 days when stored at 4 °C. Standard solutions of Reg. No. 87084 were stable (≤10 % decline) for 31 days when stored at 4 °C.

Stability of extracts

The stability of Reg. No. 5834378, Reg. No. 5924326 and Reg. No. 87084 in soil extracts was tested by determination of recovery at a fortification level of 0.002 mg/kg before and after storage at 4 ± 2 °C in the dark. Both before and after storage, the concentrations were measured against freshly prepared standards.

Table 5.1-7: Stability of extracts

Matrix	Analyte	Mass transition	Days of storage	Mean % recovery pre-storage	Mean % recovery post-storage
LUFA 2.2 soil	Reg.No. 5834378 (BAS 750 F)	398 → 182	8	-	101
		398 → 133	8	-	104
	metabolite Reg.No. 5924326	288 → 159	10	103	100
		288 → 103	10	100	99
	Reg.No. 87084 (1,2,4-Triazole)	70 → 43 (Hypercarb)	10	93	103
		70 → 43 (Synergi)	10	100	90
LUFA 2.3 soil	Reg.No. 5834378 (BAS 750 F)	398 → 182	8	-	94
		398 → 133	8	-	94
	metabolite Reg.No. 5924326	288 → 159	10	93	100
		288 → 103	10	94	101
	Reg.No. 87084 (1,2,4-Triazole)	70 → 43 (Hypercarb)	10	96	100
		70 → 43 (Synergi)	10	98	95

Reg. No. 5834378, Reg. No. 5924326 and Reg. No. 87084 were stable in the extracts of the two soil types over a period of at least 7 days when stored at 4 ± 2 °C in the dark.

Validation summary

HPLC-MS/MS is a highly specific technique and two mass transitions (3 ions) are monitored for BAS 750 F and Reg.No. 5924326 whereas for Reg.No. 87084, a single mass transition is monitored with confirmation achieved by a different column. Chromatograms of standard solutions, control samples and fortified samples have been presented showing no interferences >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels for each analyte of interest corresponding to LOQ and 10xLOQ. In all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and the RSDs were within the acceptable limit of 20%. The overall RSDs were between 1.4 - 13.3%. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched standards for BAS 750F and using solvent-based standards for Reg.No. 5924326 and Reg.No. 87084. The LOQ of the method is 0.002 mg/kg. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1. SANCO/825/00 rev.8.1 has been considered here as the method has also been submitted as a method of analysis in soil for post-approval control and monitoring purposes (see Section B.5.2.2).

Report:	KCA 4.1.2/4 Geschke S., 2014 a Validation of an analytical method for determination of BAS 555 F (Metconazole) and its metabolite 1,2,4-(1H)-Triazole in soil 2013/1377001
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)
Study supported:	*CA 7.1.2.2.1/9* *Geschke S., 2015 a* *Determination of storage stability of BAS 555 F (Metconazole) and its metabolite 1,2,4-Triazole in soil* *2015/1204922*

Principle of the method

A 5 g soil sample was extracted twice with 40 mL of a mixture of acetonitrile/water (70/30, v/v), shaken and centrifuged. A 10 mL aliquot of the extract was taken. The process was repeated and the extracts combined.

Analysis was performed by LC-MS/MS using a Thermo Aquasil C18 column (150 x 3 mm, 3 µm) with a 4 mm guard column from Phenomenex at 25 °C with external standardisation and Turbo Spray (ESI) monitoring the following three mass transitions of 1,2,4-(1H)-triazole: *m/z* 70 → 28 (quantification), 70 → 43 (confirmation), 70 → 70 (confirmation). A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in methanol)

Soil characteristics

Presented in Table 5.1-8 are the characteristics of the soil samples used in the analytical method.

Table 5.1-8: Soil characteristics

Trial from field study S12-04438, 407650	L120313	L120317
pH (calcium chloride)	5.01	7.33
pH (Water suspension)	5.59	7.82
TC (coulometric titration) (%)	0.74	0.59
TOC (coulometric titration) (%)	0.72	0.57
Organic matter (Calculated TOC x 1.724) (%)	1.24	0.98
Soil type (DIN 4220)	SI2 poor loamy sand	St2 poor clay sand
Soil type (USDA)	Loamy sand	Sand

Matrix effects

Residues of 1,2,4-triazole were detected in blank specimens therefore extracts from the soil blank samples were spiked with 1ng/mL 1,2,4-triazole. The matrix-matched standards were then quantified against standards in acetonitrile/water (70:30, v/v). The matrix effect was calculated as a ratio of the mean response factor in matrix matched standards to the mean response factor in solvent standards expressed as a percentage.

Table 5.1-9: Matrix effects

Matrix	Analyte	Mass transition	Response factor in matrix matched standards compared to solvent standards (%)*
L120313	1,2,4-triazole	70 → 28	91
		70 → 43	106
		70 → 70	96
L120317	1,2,4-triazole	70 → 28	93
		70 → 43	110
		70 → 70	101

*These are mean values calculated from all concentrations tested

No significant matrix effects were observed for 1,2,4-triazole. Therefore, calibration could be performed with standards in acetonitrile/water (70:30, v/v).

Stability of stock solutions

The stability of 1,2,4-triazole in stock solutions (1000 µg/mL) was tested by determination of recovery at a fortification level of 1 ng/mL.

Table 5.1-10: Stability of stock solutions

Matrix	Analyte	Mass transition	Days of storage	Recovery (%)
Demineralised water	1,2,4-Triazole	70 → 28	0	114
			15	112
			47	116
			75	104
		70 → 43	0	102
			15	100
			47	100
			75	98
		70 → 70	0	102
			15	104
			47	103
			75	100

Stock solutions of 1,2,4-triazole were stable (≤10 % decline) when stored refrigerated for 75 days.

Stability of extracts

The stability of 1,2,4-triazole in soil extracts was tested by determination of recovery at a fortification level of 0.02 mg/kg after storage for 7 days.

Table 5.1-11: Stability of extracts

Matrix	Analyte	Mass transition	Recoveries % range (mean, n) after 7 days storage
Soil L120313	1,2,4-Triazole	70 → 28	94 – 95 (95, 5)
		70 → 43	83 – 93 (87, 5)
		70 → 70	90 – 95 (93, 5)
Soil L120317	1,2,4-Triazole	70 → 28	84 – 90 (91, 5)
		70 → 43	95 – 101 (98, 5)
		70 → 70	84 – 89 (86, 5)

1,2,4-triazole was stable in the extracts of the two soil types over a period of 7 days.

Validation summary

HPLC-MS/MS is a highly specific technique and 3 mass transitions (4 ions) were monitored as outlined in the guidance document. Chromatograms of control samples, standards solutions and fortified samples have been

presented showing interferences >30% of LOQ at the retention time of interest therefore interferences in the control samples were determined and blank correction in the recovery data was needed. Accuracy was assessed at 2 fortification levels corresponding to the LOQ and 10xLOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20%. The overall RSDs were between 8.7 – 13.9 %. The linear range is appropriate for the nominal test concentrations and was determined using solvent-based standards as no matrix effects were observed. The LOQ of the method is 0.002 mg/kg. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 7.1.2.2.1/1 Schaeufele M., 2015 d Field soil dissipation study of Reg.No. 5834378 in the formulation EXP 5834378 F-AV on bare soil at six sites in Europe, 2013 2015/1046920
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes
Report:	KCA 7.1.2.2.1/2 Schaeufele M., 2015 e Final Report: amendment No. 1: Field soil dissipation study of Reg.No. 5834378 in the formulation EXP 5834378 F-AV on bare soil at six sites in Europe, 2013 2015/1242234
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes
Studies supported:	*KCA 7.1.2.2.1/1* *Schaeufele M., 2015 d* *Field soil dissipation study of Reg.No. 5834378 in the formulation EXP 5834378 F-AV on bare soil at six sites in Europe, 2013* *2015/1046920* *KCA 7.1.2.2.1/2* *Schaeufele M., 2015 e* *Final Report: amendment No. 1: Field soil dissipation study of Reg.No. 5834378 in the formulation EXP 5834378 F-AV on bare soil at six sites in Europe, 2013* *2015/1242234*

BASF method L0214/01 was used. The analytical method L0214/01 has been previously validated in KCA 4.1.2/1. In deviation from this method, an Agilent Porosil 120 EC C18 column (2.1 x 150 mm, 2.7 µm) was used without a column oven with ionspray positive ionisation monitoring the following mass transitions:

- Reg. No. 5834378 (BAS 750 F): *m/z* 398 → 70
- Reg. No. 5924326 (M750F003) (4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenol): *m/z* 288 → 70
- Reg. No. 87084 (1,2,4-triazole): *m/z* 70→43

An additional deviation is the gradient elution was used (mobile phase A: water: methanol (90:10 v:v) + 0.01M ammonium formate + 0.1% formic acid; mobile phase B: methanol:formic acid (100:0.1 v:v)).
Due to the differences in the LC-MS/MS conditions, a test was performed to confirm that no significant matrix effects were observed in the analysis of the soil samples; the results of which are presented in

Table 5.1-13.

Soil characteristics

Presented in Table 5.1-8 are the characteristics of the soil samples used in the analytical method.

Table 5.1-12: Soil characteristics

Trial	L130556		L130557	
Location	Bogense, Denmark		Lentzke, Germany (East)	
Soil properties	0 – 30 cm	30 – 50 cm	0 – 35 cm	35 – 50 cm
Soil class (DIN)	Sandy Loam (SI3)	Sandy Loam (SI3)	Strong loamy sand (SI2)	Strong loamy sand (Su3)
Soil class (USDA)	Sandy Loam	Sandy Loam	Loamy sand	Loamy sand
Total organic C [%]	1.1	0.5	0.7	0.2
Organic matter [%]	1.8	0.9	1.2	0.4
pH ($CaCl_2$)	6.4	7.4	5.4	4.5
pH (H_2O)	6.9	7.9	5.9	5.4
Soil taxonomy	Haplic Luvisol		Podzoluvisols - Luvisol	

Trial	L130558		L130559	
Location	Goch-Nierswalde, Germany (West)		Stotzheim, France (North)	
Soil properties	0 – 30 cm	30 – 50 cm	0 – 30 cm	30 – 50 cm
Soil class (DIN)	Loamy Silt (Ut2)	Loamy Silt (Uls)	Silt Loam (Ut4)	Silt Loam (Tu4)
Soil class (USDA)	Silt loam	Loam	Silty Clay Loam	Silty Clay Loam
Total organic C [%]	1.6	0.3	0.8	0.7
Organic matter [%]	2.8	0.5	1.4	1.2
pH ($CaCl_2$)	6.5	6.0	7.4	7.6
pH (H_2O)	7.1	6.7	8.0	8.3
Soil taxonomy	Gleyic cambisol and Stagnic Luvisol		Haplic Calcisol	

Trial	L130560		L130561		
Location	Poggio Renatico, Italy		Utrera, Spain		
Soil properties	0 – 30 cm	30 – 50 cm	0 – 20 cm	20 – 40 cm	40 – 50 cm
Soil class (DIN)	Silt Loam (Ut4)	Silt Loam (Ut4)	Weak Loamy Sand (St2)	Strong Loamy Sand (Sl3)	Sandy Clay (Ts4)
Soil class (USDA)	Silty Clay Loam	Silty Clay Loam	Loamy Sand	Loamy Sand	Sandy Clay
Total organic C [%]	1.1	1.1	0.4	0.2	0.4
Organic matter [%]	1.8	1.9	0.7	0.4	0.6
pH ($CaCl_2$)	7.6	7.6	7.4	7.0	6.7
pH (H_2O)	8.3	8.2	7.9	7.6	7.1
Soil taxonomy	Calcari Endostagnic Fluviosols		Eutric Planosols, Glegic luviosols and Plinthic luviosols		

Matrix effects

Due to differences in the HPLC-MS/MS conditions compared to method L0214/01, the effects of the matrix on the analysis were determined. The response of solvent based calibration solutions was compared to control soil samples fortified with the analytes (1 ng/mL for BAS 750F and Reg.No. 592436; 5 ng/mL for 1,2,4-triazole).

Table 5.1-13: Matrix effects

Matrix	Mean matrix effect based on 2 replicates (%)		
	BAS 750 F	**Reg.No. 5924326**	**1,2,4-triazole**
L130556	101	97	94
L130557	101	99	99
L130558	103	97	99
L130559	99	95	104
L130560	99	97	94
L130561	101	97	98

No significant matrix effects (>20%) were observed therefore calibration was performed using solvent-based standards.

Validation summary

HPLC-MS/MS is a highly specific technique and a single mass transition monitored. Chromatograms of calibration solutions, solvent blank, extracts from control soils and extracts from treated soils have been presented showing no interferences >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels for each analyte of interest corresponding to LOQ and 10xLOQ. In all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, between 7 and 20 determinations were made at each fortification level and the RSDs were within the acceptable limit of 20% (except for 1,2,4-triazole in field soil sample L130566 (Denmark) fortified at 0.02 mg/kg which has an RSD of 20.2, however this is considered close enough to 20 for the exceedance to be regarded as negligible. The overall RSDs were between 3.2 – 16.9%. The linear range is appropriate for the nominal test concentrations and was determined using solvent-based standards. The LOQ of the method is 0.002 mg/kg. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 7.1.2.2.1/6 Brewin S., 2015 a Storage stability of residues of BAS 750 F- Reg.No. 5834378 and its metabolite Reg.No. 5924326 in soil when stored at approximately -20°C for 540 days - Interim Report 2015/1050221
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes

Report:	KCA 7.1.2.2.1/7 Brewin S., 2015 b Interim report Amendment No. 1: Storage stability of residues of BAS 750 F- Reg.No. 5834378 and its metabolite Reg.No. 5924326 in soil when stored at approximately -20°C for 540 days - Interim Report 2015/1249072
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes

Report:	KCA 7.1.2.2.1/8 Brewin S., 2016 a Storage stability of residues of BAS 750 – Reg.No. 5834378 and its metabolite Reg.No.5924326 in soil when stored at approximately -20°C for 650 days 2015/1106725
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes

Studies supported:	*KCA 7.1.2.2.1/6* *Brewin S., 2015 a* *Storage stability of residues of BAS 750 F- Reg.No. 5834378 and its metabolite Reg.No. 5924326 in soil when stored at approximately -20°C for 540 days - Interim Report* *2015/1050221* *KCA 7.1.2.2.1/7* *Brewin S., 2015 b* *Interim report Amendment No. 1: Storage stability of residues of BAS 750 F- Reg.No. 5834378 and its metabolite Reg.No. 5924326 in soil when stored at approximately -20°C for 540 days - Interim Report* *2015/1249072* *KCA 7.1.2.2.1/8* *Brewin S., 2016 a* *Storage stability of residues of BAS 750 – Reg.No. 5834378 and its metabolite Reg.No.5924326 in soil when stored at approximately -20°C for 650 days* *2015/1106725*

The analytical method L0214/01 has been previously validated in KCA 4.1.2/1. A number of changes were made to the method. They include the following:

1. The preparation of analytical standards uses different volumes but the final concentrations are achieved
2. Different flasks, tubes and vials were used as available
3. Different shakers were used with slightly different speed settings
4. Different LC-MS/MS conditions were used. An Aglient Porosil 120 EC C18 column (2.1 mm x 150 mm, 2.7 µm) was used without an oven. A gradient elution was used (mobile phase A: water: methanol (90:10 v:v) + 0.01 M ammonium formate + 0.1 % formic acid; mobile phase B: methanol:formic acid (100:0.1 v:v)) with ESI^+ detection monitoring the mass transitions: *m/z* 398 → 70 and 288 → 70.

Due to differences in the LC-MS/MS conditions, a test was performed as part of the Field Dissipation Study to confirm that no significant matrix effects were observed during analysis of the soil samples which originate from the same trial locations for both studies. These matrix effects are presented in

Table 5.1-13. Therefore, the changes to the analytical method L0214/01 are considered sufficiently minor that the validation data previously provided for method L0214/01 are applicable. Additional validation data has been provided in KCA 7.1.2.2.1/1 for the same sample sites and using the modified LC-MS/MS conditions stated in this study.

This analytical method was previously validated in KCA 7.1.2.2.1/1 in accordance with SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1. Additional linearity and specificity data have been provided for this study. Chromatograms of calibration solutions, solvent blank, extracts from control soil and fortified extracts have been presented showing no interferences >30% of LOQ at the retention time of interest. The linear range is appropriate for the nominal test concentrations. The LOQ of the method is 0.002 mg/kg. The additional validation data support the method being satisfactorily validated in accordance with SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1.

Report: KCA 7.1.2.2.1/3
Jacobson B. et al., 2016 a
Terrestrial field dissipation of the fungicide BAS 750 F following broadcast applications of BAS 750 01 F (EC) or BAS 750 UA F (SC)
2015/7006396
Guidelines: SANCO/3029/99 rev. 4 (11 July 2000)
GLP: yes

Study supported: *KCA 7.1.2.2.1/3*
Jacobson B. et al., 2016 a
Terrestrial field dissipation of the fungicide BAS 750 F following broadcast applications of BAS 750 01 F (EC) or BAS 750 UA F (SC)
2015/7006396

Principle of the method
BASF method number L0214/01 has been used with the following deviations: For BAS 750 F and Reg.No. 5924326, a column temperature of 30 °C was used in place of 25 °C, for 1,2,4-triazole, one set was run with a column temperature wrongly set to 40 °C instead of 30 °C; these are not expected to have any effect on the results. The analytical method L0214/01 has been previously validated in KCA 4.1.2/1.

Soil characteristics
Presented in the following tables are the characteristics of the soil samples used in the analytical method.

Table 5.1-14: Characteristics of soil from New York site NY (R140591)

Depth (inches)	0-6	6-12	12-18	18-24	24-30	30-36	36-42	42-48
Textural classification	Silt Loam	Silt Loam	Silt Loam	Silt Loam	Silt Loam	Silt Loam	Silt Loam	Silt Loam
pH (saturated paste)	5	4.9	5	5.1	5.1	4.9	5	5.7
Organic matter (%)	4.3	2.7	1.09	0.31	0.31	0.22	0.17	0.22
Organic carbon (%)	2.5	1.6	0.63	0.18	0.18	0.13	0.1	0.13

Table 5.1-15: Characteristics of soil from North Dakota site ND (R140592)

Depth (inches)	0-6	6-12	12-18	18-24	24-30	30-36	36-42	42-48
Textural classification	Clay	Clay	Clay	Clay	Sandy Clay Loam	Clay	Clay	Clay
pH (saturated paste)	7.5	7.6	7.7	7.8	7.8	8	7.9	8
Organic matter (%)	3.2	1.9	1.6	1.4	1.4	1.2	0.95	0.95
Organic carbon (%)	1.9	1.1	0.9	0.83	0.8	0.68	0.55	0.55

Table 5.1-16: Characteristics of soil from Washington site NY (R140591)

Depth (inches)	0-6	6-12	12-18	18-24	24-30	30-36	36-42	42-48
Textural classification	Loamy Sand	Sand	Sand	Loamy Sand	Loamy Sand	Sandy Loam	Sandy Loam	Sandy Loam
pH (saturated paste)	5	4.9	5	5.1	5.1	4.9	5	5.7
Organic matter (%)	4.3	2.7	1.09	0.31	0.31	0.22	0.17	0.22
Organic carbon (%)	2.5	1.6	0.63	0.18	0.18	0.13	0.1	0.13

Table 5.1-17: Characteristics of soil from California site CA (R140594)

Depth (inches)	0-6	6-12	12-18	18-24	24-30	30-36	36-42	42-48
Textural classification	Loamy Sand	Loamy Sand	Loamy Sand	Loamy Sand	Loamy Sand	Loamy Sand	Loamy Sand	Loamy Sand
pH (saturated paste)	7.6	8	8.3	8.3	8.3	8.3	8.5	8.4
Organic matter (%)	0.7	0.48	0.31	0.13	0.13	0.09	0.26	0.31
Organic carbon (%)	0.41	0.28	0.18	0.08	0.08	0.05	0.15	0.18

Table 5.1-18: Characteristics of soil from Oklahoma site OK (R140595)

Depth (inches)	0-6	6-12	12-18	18-24	24-30	30-36	36-42	42-48
Textural classification	Sandy Loam	Sandy Loam	Sandy Loam	Loam	Loam	Loam	Loam	Sandy Clay Loam
pH (saturated paste)	7.1	5.8	5.8	6.3	6.4	6.7	6.8	7
Organic matter (%)	0.67	0.97	0.76	0.71	0.63	0.63	0.5	0.42
Organic carbon (%)	0.39	0.56	0.44	0.42	0.37	0.37	0.29	0.24

Table 5.1-19: Characteristics of soil from Illinois site IL (R140596)

Depth (inches)	0-6	6-12	12-18	18-24	24-30	30-36	36-42	42-48
Textural classification	Silty Clay Loam	Silty Clay Loam	Silty Clay Loam	Clay Loam	Silty Clay Loam	Silty Clay Loam	Silty Clay Loam	Clay Loam
pH (saturated paste)	6	6	6.3	6.6	6.8	7	7.2	7.4
Organic matter (%)	4.3	3.3	1.8	0.82	0.56	0.56	0.47	0.34
Organic carbon (%)	2.5	1.9	1	0.47	0.32	0.32	0.27	0.2

Validation summary

HPLC-MS/MS is a highly specific technique and a single mass transition monitored. Chromatograms of standards, untreated (control) soil, fortified soil and treated soil have been presented showing no interferences >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels for each analyte of interest corresponding to LOQ and 10xLOQ. In all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, between 19 and 30 determinations were made at each fortification level and the RSDs were within the acceptable limit of 20%. The overall RSDs were between 8.2 - 14%. The linear range is appropriate for the nominal test concentrations and was determined using solvent-based standards. The LOQ of the method is 1 µg/kg. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 7.1.4/1 Sandt H.J. van de 2015 a Determination of foliar DT50 of Triazole (BAS 750 F) after application of BAS 750 01 F to wheat surfaces 2015/1130156
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes
Studies supported:	*KCA 7.1.4/1* *Sandt H.J. van de 2015 a* *Determination of foliar DT50 of Triazole (BAS 750 F) after application of BAS 750 01 F to wheat surfaces* *2015/1130156*

Principle of the method

The dislodgeable foliar residues (DFR) samples were shaken and an aliquot of 0.5 mL was diluted with methanol to a final volume of 1 mL. Further dilution with methanol/water 1/1 was sometimes necessary to bring the concentration to within the calibration range.

Analysis was performed by HPLC-MS/MS using a Thermo Betasil C18 column (100 x 2.1 mm, 5 µm) at 25 °C with Turbo Ion Spray (ESI) detection monitoring the following mass transitions: *m/z* 398 → 182 (quantification) and 398 → 133 (confirmation) and external standardisation. A gradient elution was used (mobile phase A: water/formic acid 1000/1, v/v; mobile phase B: methanol/formic acid 1000/1, v/v).

Validation summary

HPLC-MS/MS is a highly specific technique and two mass transitions (3 ions) monitored in accordance with the guidance. Chromatograms of calibration standard, control solution and fortified solution have been presented showing no interferences >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels for each analyte of interest corresponding to LOQ and 10xLOQ. In all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 3 determinations were made at each fortification level (less than the 5 replicates in accordance with the guidance) and the RSDs were within the acceptable limit of 20%. The overall RSD was 9.9%. The linear range is appropriate for the nominal test concentrations and was determined using solvent-based standards. The LOQ of the method is 0.05 µg/mL. The method is fit for purpose but not fully validated in accordance with SANCO/3029/99 rev.4 as only 3 replicates were used to determine recoveries and repeatability at each fortification level.

Report:	KCA 7.1.2.2.1/9 Geschke S., 2015 a Determination of storage stability of BAS 555 F (Metconazole) and its metabolite 1,2,4-Triazole in soil 2015/1204922
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes
Studies supported:	*KCA 7.1.2.2.1/9* *Geschke S., 2015 a* *Determination of storage stability of BAS 555 F (Metconazole) and its metabolite 1,2,4-Triazole in soil* *2015/1204922*

Principle of the method

The analytical method used in this study was validated during a parallel study in KCA 4.1.2/4 earlier in this section.

Soil characteristics

Presented in Table 5.1-20 are the characteristics of the soil samples used in the analytical method. For the characteristic of soil from trials L120313 and L120317, see earlier in this section.

Table 5.1-20: Soil characteristics

Trial from field study S12-04438, 407650	L120312	L120314	L120315	L120316
pH (calcium chloride)	6.29	6.55	7.63	7.75
pH (Water suspension)	6.80	7.04	8.12	8.19
TC (coulometric titration) (%)	0.98	1.70	2.41	3.18
TOC (coulometric titration) (%)	0.96	1.67	2.03	1.78
Organic matter (Calculated TOC x 1.724) (%)	1.66	2.88	3.50	3.07
Soil type (DIN 4220)	Su3 Medium silty sand	Us Sandy silt	Uu Pure silt	Ut4 High clay silt
Soil type (USDA)	Sandy loam	Silt loam	Silt	Silt loam

Matrix effects

In addition to the original method validation, as further matrices were investigated, then the effect of these matrices was determined.

Due to the residues of the 1,2,4-(1H)-triazole in blank specimens, standard solutions in the range of 10 ng/mL to 0.5 ng/mL were used. Matrix effects were calculated as the proportion of mean response factor of standards in matrix versus standards in acetonitrile/water (70:30, v/v) expressed as a percentage.

Table 5.1-21: Matrix effects

Matrix	Analyte	Mass transition	Matrix effect* (%)
L120312	1,2,4-triazole	70 → 70	115, 100, 120, 87
L120313	1,2,4-triazole	70 → 70	100
L120314	1,2,4-triazole	70 → 70	94
L120315	1,2,4-triazole	70 → 70	89
L120316	1,2,4-triazole	70 → 70	101, 117, 106, 90
L120317	1,2,4-triazole	70 → 70	101

*Number of results reflects number of tests conducted

No significant matrix effects (< 20 %) were observed for soil from trials L120312, L120313, L120314, L120315, L120316 and L120317. Therefore, calibration was performed with standards in acetonitrile/water (70:30, v/v).

Stability in final extracts

As samples were analysed within 24 hours of extraction, the storage stability of the extracts was not required to be tested.

Stability of fortified samples

Storage stability samples were prepared and stored deep frozen until analysis. Residues were determined immediately and after storage for up to 720 days. Two samples were analysed before and after storage and the results are reported in Table 5.1-22. Significant interferences (> 30 % of LOQ) were observed in some samples at the retention time and mass transitions considered for 1,2,4-(1H)-triazole. Therefore, interferences in some control samples were determined and blank correction in the stored sample data were needed.

Table 5.1-22: Stability of fortified samples

Matrix	Analyte	Fortification level (mg/kg)	Storage timing (days)	Mean recovery pre-storage (%)	Mean procedural recovery (%)
Soil from trial L120312	1,2,4-triazole	0.02	0	97	94
			32	80	82
			60	80	85
			120	76	74
			242	72	98
			361	71	75
			540	73	94
			720	72	72
Soil from trial L120313	1,2,4-triazole	0.02	0	99	96
			32	93	95
			60	78	90
			120	77	92
			242	79	90
			361	75	86
			540	75	82
			720	76	86
Soil from trial L120314	1,2,4-triazole	0.02	0	88	98
			32	94	100
			60	83	90
			120	84	85
			242	80	102
			361	71	80
			540	94	86
			720	77	88
Soil from trial L120315	1,2,4-triazole	0.02	0	99	101
			32	82	86
			60	81	83
			120	75	80
			242	76	89
			361	75	79
			540	84	91
			720	81	82
Soil from trial L120316	1,2,4-triazole	0.02	0	92	100
			32	95	100
			60	88	86
			120	79	83
			242	76	86
			361	71	72
			540	83	91
			720	90	97
Soil from trial L120317	1,2,4-triazole	0.02	0	94	97
			32	105	108
			60	95	102
			120	87	94
			242	78	97
			361	77	79
			540	89	103
			720	92	94

1,2,4-(1H)-triazole is stable under deep-frozen conditions (≤ -18 °C) for at least 720 days of storage.

Validation summary

In Section KCA 4.1.2/4, this analytical method was shown to be satisfactorily validated in accordance with SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1. Additional validation data has been provided in this section for additional soil types. One mass transition (two ions) was monitored. Chromatograms of standard solution, untreated soil sample, treated soil sample, and fortified sample have been presented showing interferences >30% of LOQ at the retention time of interest hence blank correction in the recovery data was needed. Accuracy was assessed at a single fortification level corresponding to 10xLOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20%. The linear range is appropriate for the nominal test concentrations and was determined using solvent-based standards as no matrix effects were observed. The LOQ of the method is 0.002 mg/kg. The method is fit for purpose but not fully validated in accordance with SANCO/3029/99 rev.4 as, in the additional validation data, recovery has not been performed at the LOQ level even though there are significant interferences observed in control samples

Report:	KCA 4.1.2/7 Penning H. et al., 2013 a Validation of analytical method L0199/01 for the determination of 1,2,4-Triazole (Reg.No. 87084) in water by LC-MS/MS 2012/1297158
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

2mL of a water sample was introduced onto an SPE column. The column was washed with water and the filtrate was evaporated to dryness. The residue was dissolved in 0.5 mL water.

Analysis was performed by HPLC-MS/MS using a Thermo Aquasil C18 column (3 µm, 150 x 3 mm) and, for confirmatory purposes, a Thermo Hypercarb column (3 µm, 50 x 4.6 mm) at 20-25 °C with internal calibration and detection by ESI MS each monitoring the following ion transition: *m/z* 70→43. A gradient elution was used (mobile phase A: 1% formic acid in water, mobile phase B: 1% formic acid in methanol).

Water characteristics

Table 5.1-23: Water characteristics

	Surface water	Ground water
Sampling site	Böhler Wald, Kastenbergheide, Bohl-Iggelheim, Rheinland-Pfalz, Germany	Wasserwerk Schifferstadt (WW), RW, T8 18-3A EDV.nr. 2391961984, Rheinland-Pfalz, Germany
TOC (total organic carbon) (mg/L)	16.7	3.5
TC (total carbon) (mg/L)	37.6	43.1
TIC (total inorganic carbon) (mg/L)	20.8	39.5
DOC (dissolved organic carbon) (mg/L)	14.6	1.7
DC (dissolved carbon) (mg/L)	35.3	41.3
DIC (dissolved inorganic carbon (mg/L)	20.7	39.6
pH	6.26	6.85
Conductivity (µS/cm)	291.0	346
Dissolved oxygen (mg/L)	8.66	3.5
Carbonate hardness (mmol/L)	0.88	1.50
Carbonate hardness (°dH)	4.9	8.4
Total hardness (mmol/L)	1.22	1.39
Total hardness (°dH)	6.8	7.8
Magnesium (mg/L)	6.04	7.4
Calcium (mg/L)	38.8	43.7
Non filterable substances (mg/L)	5	18

Matrix effects

The method used an internal standard (stable isotope labelled 1,2,4-triazole) for quantification. Any influence from the matrix in the samples affect the analyte 1,2,4-triazole in the same way as the internal standard. Therefore there was no influence of the matrix on the results of the validation. Therefore, validation of 1,2,4-triazole was conducted using solvent-based standards.

Stability of standards

The stability of 1,2,4-triazole in the standard solution (1 ng/mL) was tested by storage at 4 °C for 7, 16 and 30 days and measurement of recovery of 1,2,4-triazole against freshly prepared standards at each time point. Quantification was performed at one mass transition using an internal standard and a Hypercarb HPLC column.

Table 5.1-24: Stability of standards

Matrix	Analyte	Days of storage	Recovery % of 1 ng/mL (n)
Solvent	1,2,4-triazole	0	98.4 (6)
		7	99.2 (6)
		16	97.9 (6)
		30	98.4 (6)

The standard solution of 1,2,4-triazole was stable (≤10 % decline) for at least 30 days when stored at 4 °C.

Stability of extracts

The stability of 1,2,4-triazole in the filtrates obtained from fortified ground water samples was determined at a fortification level of 0.00025 mg/kg. Data was obtained before and after storage for 7 days at 4 °C through measurement of the recovery of 1,2,4-triazole measured against freshly prepared standards. Quantification was performed at the mass transition *m/z* 70→43 using an internal standard and a Hypercarb HPLC column.

Table 5.1-25: Stability of extracts

Matrix	Analyte	Days of storage	Mean % recovery (n)
Ground water SPE-filtrates	1,2,4-triazole	0	99.0 (4)
		7	98.5 (4)

1,2,4-triazole was stable in the SPE-filtrates of ground water over a period of at least 7 days.

Validation summary

HPLC-MS/MS is a highly specific technique and a single mass transition was monitored with identity confirmed using a second chromatographic column as outlined in the guidance document. Chromatograms of control samples, fortified samples and standard solutions have been presented showing no interferences >30% of LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels for the analyte in each matrix of interest corresponding to LOQ and 10xLOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSDs were between 2.0 % and 10.6 %. The linear range is appropriate for the nominal test concentrations, and was determined using solvent-based standards as no matrix effects were observed. The LOQ of the method is 0.05 µg/kg. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 4.1.2/8 Obermann M.,Studenroth S., 2015 a Validation of analytical method L0327/01, for the determination of BAS 750 F in air by LC-MS/MS 2015/1111330
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4, EPA 850.6100, OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

The test item (BAS 750 F) was spiked onto the front filter of an adsorbent tube (ORBO™). 540 L air with a relative humidity of 80 % and a temperature of about 35 °C was passed over the filter. The content of the ORBO™ adsorber tube was extracted with 10 mL of acetonitrile before ultrasonification and centrifugation. The extract was decanted and evaporated. Extraction was repeated by adding 10 mL of acetonitrile to the tube containing the adsorber material, vortexing and shaking before ultrasonification and centrifugation. The two extracts were combined and evaporated to dryness. The residue was reconstituted with 2 mL of acetonitrile/water (70/30, v/v) before being diluted 1:10 with acetonitrile/water (70/30, v/v). For samples containing higher residues (100xLOQ), dilution 1:100 with acetonitrile/water (70/30, v/v) was performed. Further dilutions were made as appropriate.

Analysis was performed by LC-MS/MS on an Aquasil C18 column (150 x 3 mm, 3 µm) at 25 °C with external calibration and ESI^+ detection at two mass transitions: *m/z* 398 → 182 (quantification) and 398 → 133 (confirmation). A gradient elution was used (mobile phase A: water/formic acid, 1000/1 v/v; mobile phase B: acetonitrile/formic acid, 1000/1, v/v).

Matrix effects

The response of BAS 750 F in the presence of matrix compared to standard prepared in acetonitrile/water (70/30, v/v) was determined. The mean response factors were calculated for each concentration from 0.05 ng/mL to 5 ng/mL for the solvent-based standards and matrix-matched standards and compared by setting solvent-based standards to 100%.

Table 5.1-26: Matrix effects

Matrix	**Analyte**	**Mass transition**	**% response factor in matrix matched standards compared to solvent standards* (n)**
Air/Orbo™ adsorber material	BAS 750 F	398 → 182	91 (7)
		398 → 133	92 (7)

*These are mean values calculated from all concentrations tested

The mean response factors obtained from matrix-matched standards were between 91% and 92% demonstrating that no significant matrix effects were identified. Therefore matrix-matched standards were not used for the validation of the method.

Stability of standards

The stability of BAS 750 F was determined after 30 days at 4 °C in Stock (1000 mg/L) and fortification (2 mg/L) solutions prepared in acetonitrile, and calibration (0.25, 1, 2.5 ng/mL) solutions prepared in acetonitrile/water (70/30, v/v). After storage, the concentration of BAS 750 F was measured against freshly prepared standards.

Table 5.1-27: Stability of standards

Matrix	Analyte	Mass transition	Days of storage	Recovery fortification level (mg/L)	Mean % recovery (n)
Stock solution in acetonitrile	BAS 750 F	398 → 182	30	97	97 (3)
Fortification solution in acetonitrile	BAS 750 F	398 → 182	30	105	105 (3)
Calibration solution in acetonitrile/water (70/30, v/v)	BAS 750 F	398 → 182	30	0.00025 0.001 0.0025	99 (3) 100 (3) 93 (3)

Stock, fortification and calibration solutions of BAS 750 F were stable (≤10% decline) for at least 30 days when stored at 4 °C.

Stability of extracts

The stability of BAS 750 F on adsorber material was tested at a fortification level of 10 x LOQ after 7 days storage at 4 °C. The recovery of BAS 750 F was measured after storage against freshly prepared standards.

Table 5.1-28: Stability of extracts

Matrix	Analyte	Mass transition	Days of storage	Recovery fortification level (ng/L air)	Mean % recovery (n)
Adsorber material	BAS 750 F	398 → 182	7	0.1	86 (3)

BAS 750 F was stable on the adsorbed material after 7 days storage at 4 °C.

Retention capacity of sorbent material/breakthough

To check for breakthrough in the back up bed in the Part B of the adsorber tube, 500 ng (100 x LOQ) were spiked into the adsorber material (corresponding to 540 L air) resulting in a fortified concentration of 1 ng/L air. A volume of 540 L air with 80 % humidity and a temperature of about 35 °C was drawn through the adsorber tube for 6 hours. The mean recoveries for BAS 750 F in the front and back-up beds was determined. Quantification was performed for both mass transitions.

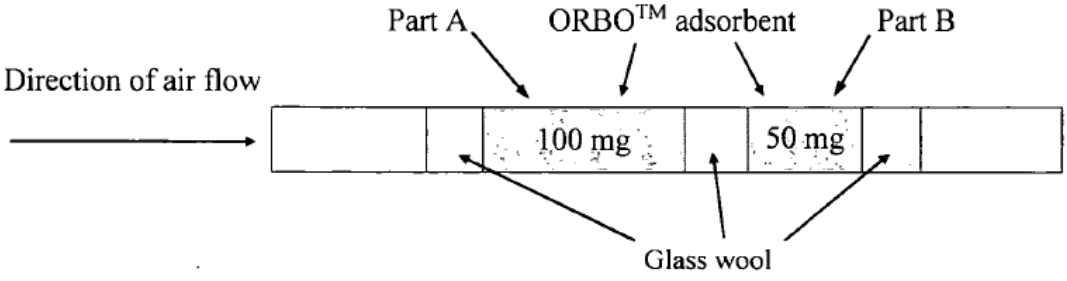

Table 5.1-29: Retention capacity of sorbent material/breakthrough

Position	Analyte	Mass transition	Recovery fortification level (ng/L air)	Mean % recovery (n)
Front	BAS 750 F	398 → 182	1	101
		398 → 133	1	98
Back	BAS 750 F	398 → 182	-	<LOD (0.002 ng/L)
		398 → 133	-	<LOD (0.002 ng/L)

The mean recoveries for BAS 750 F measured in the front bed declined less than 10 %. In the back-up bed, the recoveries of BAS 750 F were below the limit of detection. Therefore, the retention capacity of the front bed was adequate to collect BAS 750 F in concentrations up to 1 ng/L air.

Sorbent characteristics

As the ORBO™ adsorbent is a polymer based sorbent, no additional proof of ability to adsorb particle associated residues is required.

Validation summary

HPLC-MS/MS is a highly specific technique and two mass transitions (3 ions) were monitored as outlined in the guidance document. Chromatograms of standard solutions, control samples, fortified samples and reagent blanks were presented showing no interferences >30% of LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels corresponding to LOQ and 10xLOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSDs were 2.5% for both mass transitions. The linear range is appropriate for the nominal test concentrations and was determined using solvent-based standards as no matrix effects were observed. The LOQ of the method is 0.01 ng/L which complies with the concentration C, calculated from the $AOEL_{systematic}$ (33 µg/m^3 = 33 ng/L). The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1. SANCO/825/00 rev.8.1 has been considered here as the method has also been submitted as a method of analysis in air for post-approval control and monitoring purposes (see Section B.5.2.4)..

Compilation of validation data for methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

Table 5.1-30: Validation data in support of environmental fate studies

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
KCA 4.1.2/1 Studenroth S., Luer D., 2015 a	Soil LUFA 2.2	Reg.No. 5834378 (BAS 750 F)	0.02	398 → 182	0.002 0.02	100 – 104 (102, 5) 102 – 105 (102, 5)	1.6 (5) 1.4 (5) Overall: 1.4 (10)	0.025 – 3.0 ng/mL [Approx. 0.0004 – 0.048 mg/kg] r = 0.9998, y = 2.74×10^{5}x + 1.13×10^{3}, 7 standards	Acceptable chromatograms presented for standard solutions, control samples and fortified samples. No interference >30% of LOQ Identity confirmed by additional mass transition
				398 → 133	0.002 0.02	96 – 101 (98, 5) 102 – 104 (102, 5)	2.1 (5) 1.0 (5) Overall: 2.8 (10)	0.025 – 3.0 ng/mL [Approx. 0.0004 – 0.048 mg/kg] r = 0.9997, y = 1.21×10^{5}x + 534, 7	

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
								standards	
		metabolite Reg.No. 5924326	0.02	288 → 159	0.002 0.02	98 – 103 (101, 5) 100 – 104 (102, 5)	2.3 (5) 1.6 (5) Overall: 2.0(10)	0.025 – 3.0 ng/mL [Approx. 0.0004 – 0.048 mg/kg] r = 0.9972, y = 3.64×10^4x – 402, 7 standards	Acceptable chromatograms presented for standard solutions, control samples and fortified samples. No interference >30% of LOQ Identity confirmed by additional mass transition
				288 → 103	0.002 0.02	94 – 102 (98, 5) 99 – 106 (103, 5)	3.7 (5) 2.7 (5) Overall: 4.0 (10)	0.025 – 3.0 ng/mL [Approx. 0.0004 – 0.048 mg/kg] r = 0.9985, y = 1.74×10^4x – 24.5, 7 standards	

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
		Reg.No. 87084 (1,2,4-Triazole)	0.02	70 → 43 (Hypercarb)	0.002 0.02	98 – 106 (102, 5) 82 – 99 (88, 5)	3.1 (5) 7.5 (5) Overall: 9.3 (10)	0.125 – 15 ng/mL [Approx. 0.0004 – 0.048 mg/kg] r = 0.9972, y = 5.99×10^{4}x + 3.84×10^{3}, 8 standards	Acceptable chromatograms presented for standard solutions, control samples and fortified samples. No interference >30% of LOQ Identity confirmed by a second column
				70 → 43 (Synergi)	0.002 0.02	93 – 94 (93, 5) 93 – 99 (97, 5)	0.5 (5) 2.5 (5) Overall: 2.5 (10)	0.125 – 15 ng/mL [Approx. 0.0004 – 0.048 mg/kg] r = 1.000, y = 5.21×10^{4}x - 627, 8 standards	
	Soil LUFA 2.3	Reg.No. 5834378 (BAS 750 F)	0.02	398 → 182	0.002	105 – 112 (109, 5)	2.4 (5)	0.025 – 3.0 ng/mL	Acceptable chromatograms

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
					0.02	82 – 108 (96, 5)	12.1 (5) Overall: 10.2 (10)	[Approx. 0.0004 – 0.048 mg/kg] r = 0.9993, y = 1.37×10^{5}x + 228, 7 standards	presented for standard solutions, control samples and fortified samples. No interference >30% of LOQ Identity confirmed by additional mass transition
				398 → 133	0.002 0.02	102 – 110 (107, 5) 81 – 110 (96, 5)	3.2 (5) 15.0 (5) Overall: 11.2 (10)	0.025 – 3.0 ng/mL [Approx. 0.0004 – 0.048 mg/kg] r = 0.9994, y = 5.88×10^{4}x – 31.5, 7 standards	
		metabolite Reg.No. 5924326	0.02	288 → 159	0.002 0.02	80 – 106 (99, 5) 79 – 84 (82, 5)	11.2 (5) 2.7 (5) Overall:	0.025 – 3.0 ng/mL [Approx. 0.0004 –	Acceptable chromatograms presented for standard solutions, control samples and fortified samples.

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
							13.3 (10)	0.048 mg/kg] r = 0.9972, y = 3.64×10^4x – 402, 7 standards	No interference >30% of LOQ Identity confirmed by additional mass transition
				288 → 103	0.002 0.02	88 – 106 (99, 5) 78 – 86 (82, 5)	7.3 (5) 4.1 (5) Overall: 11.6 (10)	0.025 – 3.0 ng/mL [Approx. 0.0004 – 0.048 mg/kg] r = 0.9985, y = 1.74×10^4x – 24.5, 7 standards	
		Reg.No. 87084 (1,2,4-Triazole)	0.02	70 → 43 (Hypercarb)	0.002 0.02	76 – 92 (88, 5) 80 – 88 (85, 5)	8.4 (5) 3.7 (5) Overall: 6.7 (10)	0.125 – 15 ng/mL [Approx. 0.0004 – 0.048 mg/kg]	

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
								r = 0.9972, y = 5.99×10^{4}x + 3.84×10^{3}, 8 standards	
				70 → 43 (Synergi)	0.002 0.02	86 – 99 (95, 5) 87 – 100 (96, 5)	5.6 (5) 5.6 (5) Overall: 5.3 (10)	0.125 – 15 ng/mL [Approx. 0.0004 – 0.048 mg/kg] r = 1.000, y = 5.21×10^{4}x - 627, 8 standards	
KCA 4.1.2/4 Geschke S., 2014 a	L120313 soil	1,2,4-(1H)-Triazole	0.002	70 → 28	0.002 0.02	62 – 94 (79, 5) 89 – 104 (97, 5)	17.6 (5) 6.4 (5) Overall: 15.8 (10)	0.03 – 10 ng/mL [0.00048 – 0.16 mg/kg] r = 0.9994, y = 1.15×104x + 376, 9	Chromatograms presented for control samples, standard solutions and fortified samples. Significant interferences (>30% of LOQ) were observed.

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
								standards	Therefore, interferences in the control samples were determined and blank correction in the recovery data was needed. Identity was confirmed by monitoring three mass transitions
				70 → 43	0.002 0.02	67 – 112 (87, 5) 82 – 98 (92, 5)	19.2 (5) 6.5 (5) Overall: 13.6 (10)	0.03 – 10 ng/mL [0.00048 – 0.16 mg/kg] r = 0.9996, y = 1.08×10^5 - 608, 9 standards	
				70 → 70	0.002 0.02	76 – 114 (92, 5) 87 – 106 (98, 5)	15.2 (5) 7.4 (5) Overall: 11.6 (10)	0.03 – 10 ng/mL [0.00048 – 0.16 mg/kg] r = 0.9996, y = 5.3×10^5 - 3.62×10^3, 9 standards	
	L120317 soil	1,2,4-(1H)-Triazole	0.002	70 → 28	0.002 0.02	97 – 106 (95, 5) 79 – 93 (87, 5)	7.7 (5) 8.3 (5)	0.03 – 10 ng/mL [0.00048 – 0.16	Chromatograms presented for control samples, standard solutions and fortified

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
							Overall: 8.7 (10)	mg/kg] r = 0.9994, y = 1.15×104x + 376, 9 standards	samples. Significant interferences (>30% of LOQ) were observed. Therefore, interferences in the control samples were determined and blank correction in the recovery data was needed. Identity was confirmed by monitoring three mass transitions
				70 → 43	0.002 0.02	80 – 124 (105, 5) 91 – 95 (94, 5)	16.5 (5) 3.3 (5) Overall: 13.1 (10)	0.03 – 10 ng/mL [0.00048 – 0.16 mg/kg] r = 0.9996, y = 1.08×10^5 - 608, 9 standards	
				70 → 70	0.002 0.02	73 – 110 (84, 5) 90 – 101 (95, 5)	18.8 (5) 4.7 (5) Overall: 13.9 (10)	0.03 – 10 ng/mL [0.00048 – 0.16 mg/kg] r = 0.9996, y = 5.3×10^5 - 3.62×10^3,	

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
								9 standards	
KCA 7.1.2.2.1/6 Brewin S., 2015 a KCA 7.1.2.2.1/7 Brewin S., 2015 b KCA 7.1.2.2.1/8 Brewin S., 2016 a	Soil	BAS 750 F (Reg. No. 5834378)	0.002	Validation data previously provided for method L0214/01.				0.025 – 2 ng/mL [Approx. 0.0004 – 0.032 mg/kg] 7 standards, r = 0.9996, y = 39368.1x +1206.33	Acceptable chromatograms presented for calibration solutions, solvent blank, extracts from control soil and fortified extracts. No interference >30% LOQ
		Reg. No. 5924326 (M750F003) (4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenol)	0.002					0.025 – 2 ng/mL [Approx. 0.0004 – 0.032 mg/kg] 7 standards, r = 0.999, y = 17638.8x + 560.961	Acceptable chromatograms presented for calibration solutions, solvent blank, extracts from control soil and fortified extracts. No interference >30% LOQ

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
KCA 7.1.2.2.1/1 Schaeufele M., 2015 d KCA 7.1.2.2.1/2 Schaeufele M., 2015 e	L130566 soil (Denmark)	BAS 750 F	0.002	398 → 70	0.002 0.02	83 – 108 (93, 15) 85-111 (100, 20)	8.2 (15) 6.0 (20) Overall: 7.5 (35)	0.025 – 2 ng/mL [Approx. 0.0004 – 0.032 mg/kg] 7 standards, y = 63613 x + 1283.08, r = 0.9988	Acceptable chromatograms presented for calibration solutions, solvent blank, extracts from control soils and extracts from treated soils. No interference >30% of LOQ
	L130557 soil (Germany - East)				0.002 0.02	79 – 104 (91, 8) 93 – 105 (99, 13)	10.3 (8) 3.8 (13) Overall: 7.9 (21)		
	L130558 soil (Germany - West)				0.002 0.02	72 – 108 (85, 11) 83 – 107 (93, 16)	13.8 (11) 7.0 (16) Overall: 10.9 (27)		
	L130559 soil (France-North)				0.002 0.02	73 – 109 (89, 10) 78 – 107 (96, 15)	12.0 (10) 7.5 (15) Overall: 10.0 (25)		
	L130560 soil (Italy)				0.002	70 – 108 (90, 13)	12.7 (13)		

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
					0.02	91 – 119 (102, 20)	7.2 (20) Overall: 11.0 (33)		
	L140561 soil (Spain)				0.002 0.02	76 – 103 (88, 7) 92 – 112 (97, 11)	10.3 (7) 5.7 (11) Overall: 8.6 (18)		
	L130566 soil (Denmark)	Reg. No. 5924326 (M750F003) (4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenol)	0.002	288 → 70	0.002 0.02	87-105 (97, 15) 92-107 (100, 18)	5.8 (15) 3.7 (18) Overall: 4.9 (33)	0.025 – 2 ng/mL Approx. 0.4 – 32 µg/kg 7 standards, y = 3433.2x + 329.437, r = 0.9993	Acceptable chromatograms presented for calibration solutions, solvent blank, extracts from control soils and extracts from treated soils. No interference >30% of LOQ
	L130557 soil (Germany - East)				0.002 0.02	87-105 (97, 15) 92-107 (100, 18)	8.9 (8) 2.5 (13) Overall: 6.3 (21)		

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	L130558 soil (Germany - West)				0.002 0.02	81-107 (93, 10) 87-108 (99, 15)	8.3 (10) 5.6 (15) Overall: 7.1 (25)		
	L130559 soil (France-North)				0.002 0.02	81-99 (91, 10) 95-107 (100, 15)	7.6 (10) 4.1 (15) Overall: 7.2 (25)		
	L130560 soil (Italy)				0.002 0.02	89-107 (100, 10) 93-109 (100, 16)	5.8 (10) 4.5 (16) Overall: 4.9 (26)		
	L140561 soil (Spain)				0.002 0.02	91-99 (96, 7) 92-102 (99, 11)	3.5 (7) 2.6 (11) Overall: 3.2 (18)		

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	L130566 soil (Denmark)	1,2,4-triazole	0.002	70→43	0.002 0.02	71-97 (83, 15) 61-119 (89, 18)	9 (15) 20.2 (18) Overall: 16.7 (33)	0.025 – 2 ng/mL Approx. 0.4 – 32 µg/kg 7 standards, y = 63613 x + 1283.08, r = 0.9988	Acceptable chromatograms presented for calibration solutions, solvent blank, extracts from control soils and extracts from treated soils. No interference >30% of LOQ
	L130557 soil (Germany - East)				0.002 0.02	76-100 (85, 11) 71-119 (93, 19)	8.9 (11) 19 (19) Overall: 16.9 (30)		
	L130558 soil (Germany - West)				0.002 0.02	72-106 (84, 10) 70-116 (90, 15)	13.3 (10) 17.4 (15) Overall: 16.3 (25)		
	L130559 soil (France-North)				0.002 0.02	71-91 (80, 13) 71-110 (86, 17)	7.6 (13) 12.7 (17) Overall: 11.4 (30)		

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	L130560 soil (Italy)				0.002 0.02	73-104 (83, 11) 75-112 (87, 17)	11 (11) 13.8 (17) Overall: 12.8 (28)		
	L140561 soil (Spain)				0.002 0.02	72-85 (77, 7) 65-106 (82, 11)	6.4 (7) 15.7 (11) Overall: 13.4 (18)		
KCA 7.1.2.2.1/3 Jacobson B. et al., 2016 a	NY soil (R140591)	BAS 750 F	0.001	398 → 182	0.001 0.1	75.6-128 (93.8, 20) 73.6-113 (90.5, 20)	13 (20) 13 (20) Overall: 13 (40)	0.025 - 20 ng/mL [Approx. 0.0004 – 0.32 mg/kg] 8 standards, $y = 1.44\times10^{6}x + 5.43\times10^{4}$, r = 0.9992	Acceptable chromatograms presented for standards, untreated (control) soil, fortified soil and treated soil. No interference >30% LOQ
	ND soil (R140592)				0.001 0.1	73.3-118 (97, 19) 68.4-124 (95.2, 20)	12 (19) 12 (20) Overall: 13 (39)		
	WA soil (R140593)				0.001 0.1	80-127 (102, 28) 81.3-116	11 (28) 10 (27)		

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
						(97.4, 27)	Overall: 11 (56)		
	CA soil (R140594)				0.001 0.1	64.7-123 (96.9, 26) 74.4-119 (96.7, 26)	16 (26) 12 (26) Overall: 14 (52)		
	OK soil (R140595)				0.001 0.1	79.8-122 (99.2, 31) 74.6-113 (93.4, 27)	9 (31) 8.9 (27) Overall: 9.1 (62)		
	IL soil (R140596)				0.001 0.1	75-106 (86.9, 24) 71.2-103 (86.2, 23)	10 (24) 10 (23) Overall: 10 (49)		
	NY soil (R140591)	Reg. No. 5924326		288 → 159	0.001 0.1	70.2-124 (98.4, 20) 82.3-106 (94, 20)	14 (20) 7.8 (20) Overall: 12 (40)	0.025 - 20 ng/mL [Approx. 0.0004 – 0.32 mg/kg]	Acceptable chromatograms presented for standards, untreated (control) soil, fortified soil and treated soil.
	ND soil				0.001	66-117	14 (20)		

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	(R140592)				0.1	(97.1, 20) 69.4-103 (93.5, 20)	9.4 (20) Overall: 12 (40)	8 standards, y = 3.36×10^{5}x - 611, r = 0.9998	No interference >30% LOQ
	WA soil (R140593)				0.001 0.1	68.6-157 (96.9, 28) 75.2-102 (93.1, 27)	12 (28) 8 (27) Overall: 10 (56)		
	CA soil (R140594)				0.001 0.1	81.7-118 (99.2, 26) 67.9-108 (89.4, 26)	11 (26) 12 (26) Overall: 13 (52)		
	OK soil (R140595)				0.001 0.1	79.8-118 (97.3, 31) 78.3-108 (92.6, 26)	9.2 (31) 7 (26) Overall: 8.2 (61)		
	IL soil (R140596)				0.001 0.1	89.7-116 (102, 23) 74.6-107 (90.2, 23)	6.9 (23) 7.9 (23)		

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
							Overall: 9.5 (46)		
	NY soil (R140591)	1,2,4-triazole		70 → 43	0.001 0.1	70.8-110 (91.2, 20) 72.8-109 (91.9, 20)	11 (20) 9.1 (20) Overall: 10 (40)	0.025 - 20 ng/mL [Approx. 0.0004 – 0.32 mg/kg]	Acceptable chromatograms presented for standards, untreated (control) soil, fortified soil and treated soil.
	ND soil (R140592)				0.001 0.1	63.7-108 (88.4, 23) 83.4-107 (95.2, 22)	14 (23) 7 (22) Overall: 11 (45)	7 standards, y = 3.05×10^4x – 170, r = 0.9998	No interference >30% LOQ
	WA soil (R140593)				1 100	72.1-120 (95.7, 28) 75.6-114 (93.4, 28)	14 (28) 10 (28) Overall: 12 (56)		
	CA soil (R140594)				0.001 0.1	64.6-123 (88.3, 26) 64.7-109 (89, 26)	14 (26) 13 (26) Overall: 13 (52)		

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	OK soil (R140595)				0.001 0.1	71.1-126 (94, 30) 75.5-111 (94.7, 30)	13 (30) 9.2 (30) Overall: 11 (60)		
	IL soil (R140596)				0.001 0.1	74.1-112 (90.9, 24) 82.1-109 (94.8, 24)	11 (24) 6.3 (24) Overall: 9.2 (48)		
KCA 7.1.4/1 Sandt H.J. van de 2015 a	DFR dislodging solution	BAS 750 F	0.05 µg/mL	398 → 182	0.05 µg/mL 5 µg/mL	95.7 – 101.3 (98.4, 3) 84.6 – 113.4 (96.8, 3)	2.84 (3) 15.4 (3) Overall: 9.9 (6)	0.001- 0.25 µg/mL 5 standards, y = 4.19×10^{3}x +22.2, r = 0.9995	Acceptable chromatograms presented for calibration standard, control solution and fortified solution. No interference >30% LOQ Identity confirmed by a second mass transition

KCA 7.1.2.2.1/9 Geschke S., 2015 a	Soil from trial L 120312	1,2,4-(1H)-triazole	0.002	70 → 70	0.02	72 – 98 (84, 8)	10 (8)	0.03 – 10 ng/mL [0.00048 – 0.16 mg/kg] r = 0.9997, y = 5.16×10^5 – 5.78×10^4, 9 standards	Acceptable chromatograms presented for standard solution, untreated soil sample, treated soil sample, and fortified sample. Significant interferences (>30% of LOQ) were observed. Therefore, interferences in the control samples were determined and blank correction in the recovery data was needed. Identity was confirmed by comparison of retention times and monitoring two fragment ions
	Soil from trial L 120313				0.02	82 – 96 (89, 8)	5.4 (8)		
	Soil from trial L 120314				0.02	80 – 102 (90, 8)	8.4 (8)		
	Soil from trial L 120315				0.02	79 – 101 (86, 8)	7.3 (8)		
	Soil from trial L 120316				0.02	72 – 100 (89, 8)	9.6 (8)		
	Soil from trial L 120317				0.02	79 – 108 (96, 8)	9.5 (8)		
KCA 4.1.2/7 Penning H. et al., 2013 a	Surface water	1,2,4-triazole	0.05 µg/kg	Hypercarb	0.05 µg/kg 0.50 µg/kg	84.8 – 119.3 (97.2, 5) 86.1 – 93.1 (90.7, 5)	14.0 (5) 3.2 (5) Overall: 10.6 (10)	0.05 – 5 ng/mL [Approx. 0.0125 – 1.25 µg/kg] 7 standards, r^2 =	Acceptable chromatograms presented for control samples, fortified samples and standard solutions. No interference >30% of LOQ

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
								0.9999, y = 0.7708 x – 0.001	Identity confirmed by monitoring a single mass transition (*m/z* 70→43) using two different chromatographic columns
			0.05 µg/kg	Aquasil C18	0.05 µg/kg 0.50 µg/kg	91.8 – 98.8 (94.1, 5) 94.0 – 97.0 (95.5, 5)	3.0 (5) 1.1 (5) Overall: 9.3 (10)	0.05 – 5 ng/mL [Approx. 0.0125 – 1.25 µg/kg] 7 standards, r^2 = 1.000, y = 0.7507 x – 0.0094	
	Ground water	1,2,4-triazole	0.05 µg/kg	Hypercarb	0.05 µg/kg 0.50 µg/kg	76.0 – 105.0 (87.2, 5) 97.0 – 99.6 (95.0, 5)	13.0 (5) 4.0 (5) Overall: 9.3 (10)	0.05 – 5 ng/mL [Approx. 0.0125 – 1.25 µg/kg] 7 standards, r^2 = 1.000, y = 0.8204 x – 0.0003	Acceptable chromatograms presented for control samples, fortified samples and standard solutions. No interference >30% of LOQ Identity confirmed by monitoring a single mass transition (*m/z*
			0.05 µg/kg	Aquasil C18	0.05 µg/kg	94.5 – 98.5 (97.2, 5)	1.7 (5)	0.05 – 5 ng/mL	

Reference	**Matrix**	**Analyte**	**LOQ (mg/kg unless otherwise stated)**	**Mass transition (*m/z*)**	**Recovery fortification level (mg/kg unless otherwise stated)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
					0.50 µg/kg	95.0 – 101.0 (97.2, 5)	2.6 (5) Overall: 2.0 (10)	[Approx. 0.0125 – 1.25 µg/kg] 7 standards, r^2 = 0.9993, y = 0.7858 x – 0.0034	70→43) using two different chromatographic columns
KCA 4.1.2/8 Obermann M.,Studenroth S., 2015 a	Air	BAS 750 F	0.01 ng/L air	398 → 182	0.01 ng/L air 0.1 ng/L air	87 – 92 (89, 5) 86 – 92 (89, 5)	2.5 (5) 2.8 (5) Overall: 2.5 (10)	0.05 – 5 ng/mL [Approx 0.002 – 0.2 ng/L air] r = 0.9999, 7 standards, y = 1.34×10^5x + 546	Acceptable chromatograms presented for standard solutions, control samples, fortified samples and reagent blanks. No interference >30% of LOQ Identity confirmed by MS/MS with two mass transitions: *m/z* 398 → 182 and 398 → 133
				398 → 133	0.01 ng/L air 0.1 ng/L air	87 – 90 (88, 5) 88 – 91 (90, 5)	2.1 (5) 2.2 (5)	0.05 – 5 ng/mL [Approx 0.002 – 0.2	

Reference	Matrix	Analyte	LOQ (mg/kg unless otherwise stated)	Mass transition (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
							Overall: 2.5 (10)	ng/L air] r = 0.9996, 7 standards, y = 5.11×10^4 + 233	

B.5.1.2.2. Methods in soil, water and any additional matrices used in support of efficacy studies

No stand-alone validation of analytical methods was required in support of efficacy studies.

B.5.1.2.3. Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

Report:	KCA 4.1.2/9 Baltussen E., 2013 a Development and validation of an analytical method for the analysis of BAS 750 F in diet 2015/1189151
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5)
GLP:	yes (certified by Ministry of Health, Welfare and Sport, The Hague, The Netherlands)
Report:	KCA 4.1.2/10 Baltussen E., 2016 a Report Amendment Number 1: Development and validation of an analytical method for the analysis of BAS 750 F in diet 2016/1041496
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5)
GLP:	yes (certified by Ministry of Health, Welfare and Sport, The Hague, The Netherlands)
Studies supported:	*KCA 5.3.2/2* [redacted] *2015 a* *90-day oral dietary toxicity study with BAS 750 F in C57BL/6JRj mice* *2014/1046542*

Principle of the method

10g blank powder diet was extracted 3 times by shaking with 30 mL 1% formic acid in acetonitrile before filtering and combining in a 100 mL volumetric flask and making to volume with 1% formic acid in acetonitrile. The solution was filtered and diluted in a 1:1 (v/v) ratio with water.

BAS 750 F was analysed by HPLC-UV on an Acquity UPLC BEH Shield RP-18 (100 x 2.1 mm, 1.7 µm) at 40 °C with UV detection at 210 nm and external calibration. The mobile phase employed a gradient elution (mobile phase A: 0.1% trifluoroacetic acid in acetonitrile; mobile phase B – 0.1% trifluoroacetic acid in water).

Validation summary

HPLC-UV is not a highly specific method however validation has shown that it is specific to the analyte of interest. Chromatograms of the test substance and a blank accuracy sample were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, between 5 and 10 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 7.2%. The linear range is appropriate for the nominal test concentrations and was determined using solvent-based standards, however, matrix effects were not considered. The LOQ of the method is 10 mg/kg. The method is fit for purpose, although not fully validated in accordance with SANCO/3029/99 rev.4 as the suitability of matrix-matched standards has not been addressed.

Report:	KCA 4.1.2/11 Becker M.,Kamp H., 2015 i BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in diet using HPLC-UV 2015/1174512
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Study supported:	*KCA 5.5/1* [redacted] *2016 b* *BAS 750 F - Combined chronic toxicity/carcinogenicity study in Wistar rats - Administration via the diet up to 24 months* *2015/1000531* *KCA 5.5/3* [redacted] *2015 b* *18-month carcinogenicity study with BAS 750 F in male and female C57BL/6JRJ mice* *2015/1000532*

Principle of the method

10 g of the Ground Kliba maintenance diet mouse/rat "GLP" meal were extracted 3 times with 30 mL extraction solution (1% formic acid in acetonitrile). After centrifugation, the supernatants were collected and combined in a 100 mL volumetric flask and made to volume with extraction solution before filtering. If the amount of test substance in the sample solution was outside the calibration range, an adequate dilution step with matrix solution had to be performed to match the described concentration range.

Analysis was performed by HPLC-UV DAD using an Ascentis Express C18 (150 x 4.6 mm, 2.7 µm) at ambient temperature with UV detection at 210 nm and external calibration. A gradient elution was used (mobile phase A: 0.1% trifluoroacetic acid in acetonitrile; mobile phase B: 0.1% trifluoroacetic acid in water)

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms for the matrix solution and calibration solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 3.5%. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched solutions. The LOQ of the method is 20 mg/kg. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 4.1.2/12 Becker M.,Kamp H., 2015 j BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in diet using HPLC-UV 2015/1175541
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

10 g (for the concentration range of 607 – 6249 mg/kg and 7591 –37955 mg/kg) and 5 g (for the concentration range of 6250 – 7590 mg/kg) of the Ground Kliba maintenance diet quail/duck "GLP" meal were extracted 3 times by shaking with 30 mL extraction solution (1% formic acid in acetonitrile). After centrifugation the supernatants were combined in a 100 mL volumetric flask and made to volume with extraction solution before being filtered. If the amount of test substance in the sample solution was outside the calibration range, an adequate dilution step with matrix solution had to be performed to match the described concentration range.

Analysis was performed by HPLC-UV DAD using an Ascentis Express C18 column (150 x 4.6 mm, 2.7 µm) at ambient temperature with UV detection at 210 nm and external calibration. A gradient elution was used (mobile phase A: 0.1% trifluoroacetic acid in acetonitrile; mobile phase B: 0.1% trifluoroacetic acid in water).

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the matrix solution and calibration solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 4.0%. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched solutions. The LOQ of the method is 607 mg/kg. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 4.1.2/13 Becker M.,Kamp H., 2015 a BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in diet using LC-MS/MS 2015/1175542
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Study supported:	*KCA 5.8.2/5* [redacted] *2015 d* *BAS 750 F- S-Phase response study in Wistar rats - Administration via the diet for 3, 7, 14 and 28 days* *2014/1170772*

Principle of the method

10 g of the Ground Kliba maintenance diet quail/duck “GLP” meal were extracted 3 times by shaking with 30 mL extraction solution (1% formic acid in acetonitrile). After centrifugation the supernatants were combined in a 100 mL volumetric flask and made to volume with extraction solution before being filtered. If the amount of test substance in the sample solution was outside the calibration range, an adequate dilution step with matrix solution had to be performed to match the described concentration range.

Analysis was performed by HPLC-MS using an Ascentis Express C18 column (100 x 2.1 mm, 2.7 µm) at ambient temperature with electrospray ionisation (positive mode) MRM (monitoring parent ion (*m/z*): 398.0; product ion (*m/z*): 181.9) and external calibration. An isocratic elution was used (60% mobile phase A: acetonitrile; 40% mobile phase B: 0.01% formic acid in water).

Validation summary

HPLC-MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the matrix solution and calibration solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 2.7%. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched solutions. The LOQ of the method is 10 mg/kg. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 4.1.2/14 Becker M.,Kamp H., 2015b BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in aqueous Carboxymethylcellulose (CMC) using HPLC-UV 2015/1177605
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Study supported:	*KCA 5.3.3/1* [redacted] *2015 b* *BAS 750 F - Repeated dose 28-day dermal toxicity study in Wistar rats* *2014/1170751* *KCA 5.7.1/1* [redacted] *2015 c* *BAS 750 F - Acute oral neurotoxicity study in Wistar rats - Administration by gavage* *2014/1170759*

Principle of the method

The sample (aqueous carboxymethyl cellulose (CMC) formulation (1 % CMC, w/v)) was transferred to a volumetric flask using 10 mL mixture of acetonitrile/water (1:1 v/v) before making to volume with acetonitrile before filtering. If the amount of test substance in the sample solution was outside the calibration range, an adequate dilution step with matrix solution had to be performed to match the described concentration range.

Analysis was performed by HPLC-UV DAD using a Chromolith Performance Rp-18e column (100 x 4.6 mm) at ambient temperature with UV detection at 230 nm and external calibration. An isocratic elution was used (50% mobile phase A: 0.1% formic acid in acetonitrile; 50% mobile phase B: 0.1% formic acid in water).

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the test substance, a solvent blank and a blank accuracy sample were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 4 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 4.1%. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched solutions. The LOQ of the method is 0.10 mg/mL. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 4.1.2/15 Becker M.,Kamp H., 2015 c BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in a mixture of Dimethyl Sulfoxide and corn oil using HPLC-UV 2015/1185311
Guidelines:	EEC 91/414, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Study supported:	*KCA 5.4.2/1* [redacted] *2014 a* *BAS 750 F - Micronucleus test in bone marrow cells of the mouse* *2014/1043159*

Principle of the method

The samples (mixtures of dimethyl sulfoxide and corn oil (2+3, v/v) were diluted with acetone using appropriate volumetric flasks to obtain sample solution with concentrations that match the calibration range. If the amount of test substance in the sample solution was greater than the calibration range, dilution was required with matrix solution to bring the concentration within the linear range.

Analysis was performed by HPLC-UV using a Kinetex C18 column (100 x 4.6 mm, 5 µm) at ambient temperature with UV detection at 230 nm and external calibration. A gradient elution was used (mobile phase A: 0.1% formic acid in acetonitrile; mobile phase B: 0.1% formic acid in water).

Validation summary

HPLC-UV is not a highly specific method however validation has shown that it is specific to the analyte of interest. Chromatograms of the test substance, a solvent blank and a blank accuracy sample were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 0.7%. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched solutions. The LOQ of the method is 0.20 mg/mL. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 4.1.2/16 Becker M.,Kamp H., 2015 d BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in corn oil using HPLC-UV 2015/1184812
Guidelines:	EEC 91/414, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Study supported:	*KCA 5.2.1/2* *Becker M., Kamp H., 2013 a* *BAS 750 F - Homogeneity and concentration control analyses in corn oil* *2013/1395622* *KCA 5.2.2/2* *Becker M., Kamp K., 2013 a* *Analytical report - BAS 750 F - Homogeneity and concentration control analyses in corn oil* *2013/1395620*

Principle of the method

The samples (corn oil (PH. EUR.)) were diluted with acetone using appropriate volumetric flasks to obtain sample solution with concentrations that match the calibration range. If the amount of test substance in the sample solution was greater than the calibration range, dilution was required with matrix solution to bring the concentration within the linear range.

Analysis was performed by HPLC-UV DAD using a Kinetex C18 column (100 x 4.6 mm, 5 µm) at ambient temperature with UV detection at 230 nm and external calibration. A gradient elution was used (mobile phase A: 0.1% formic acid in acetonitrile; mobile phase B: 0.1% formic acid in water).

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the test substance, a solvent blank and a blank accuracy sample were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 0.7%. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched solutions. The LOQ of the method is 0.1 mg/mL. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 4.1.2/17 Becker M.,Kamp H., 2015 e BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in Dimethyl sulfoxide using HPLC-UV 2015/1184813
Guidelines:	EEC 91/414, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Study supported:	*KCA 5.4.1/2* *Becker M.,Kamp H., 2013 b* *BAS 750 F - Stability analysis in Dimethyl sulfoxide* *2015/1040886*

Principle of the method

The dimethyl sulfoxide samples were diluted with acetonitrile using appropriate volumetric flasks to obtain sample solution with concentrations that match the calibration range. If the amount of test substance in the sample solution was greater than the calibration range, dilution was required with matrix solution to bring the concentration within the linear range

Analysis was performed by HPLC-UV DAD using a Chromolith Performance 18e column (100 x 4.6 mm) at ambient temperature with UV detection at 230 nm and external calibration. An isocratic elution was used (50% mobile phase A: 0.1% formic acid in acetonitrile; 50% mobile phase B: 0.1% formic acid in water).

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the test substance, a solvent blank and a blank accuracy sample were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 1.3 %. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched solutions. The LOQ of the method is 0.5 mg/mL. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 4.1.2/18 Becker M., Kamp H., 2015 f BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in plasma using LC-MS/MS 2015/1186912
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Study supported:	*KCA 5.5/5* *Becker M., Kamp H., 2015 a* *BAS 750 F - Plasma analysis for external studies* *2015/1186254* *KCA 5.3.2/5* [redacted] *2015 a* *BAS 750 F - Repeated-dose 90-day oral toxicity study in beagle dogs - Oral administration (capsule)* *2015/1000530*

Principle of the method

30 µL of plasma were mixed with 270 µL acetonitrile in a centrifuge tube (1.5 mL). After vortex mixing and protein precipitation, the samples were centrifuged and the clear supernatant used directly in analysis. If the amount of test substance in the sample solution was greater than the calibration range, dilution was required with matrix solution to bring the concentration within the linear range.

Analysis was performed by HPLC-MS/MS using an Ascentis Express C18 column (100 x 2.1 mm, 2.7 µm) at 30 °C with electrospray ionisation (positive mode) MRM (monitoring parent ion (*m/z*): 398.0; product ion (*m/z*): 181.9) and external calibration. An isocratic elution was used (60% mobile phase A: acetonitrile; 40% mobile phase B: 0.01% formic acid in water).

Validation summary

HPLC-MS/MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the test substance, a solvent blank and a blank accuracy sample were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 3.3%. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched solutions. The LOQ of the method is 0.05 mg/L. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 4.1.2/19 Becker M.,Kamp H., 2015 g BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in Paraffinum subliquidum using HPLC-UV 2015/1186913
Guidelines:	EEC 91/414, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Studies supported:	*KCA 5.2.6/2* *Grauert E., Kamp H., 2014 a* *Analytical report - BAS 750 F - Homogeneity and concentration control analyses in paraffinum subliquidum* *2014/1116448*

Principle of the method

The Paraffinum subliquidum (PH. EUR.) samples were diluted completely with tetrahydrofuran using appropriate volumetric flasks to obtain sample solution with test substance concentrations that match the calibration range. If the amount of test substance in the sample solution was greater than the calibration range, dilution was required with matrix solution to bring the concentration within the linear range.

Analysis was performed by HPLC-UV DAD using a Chromolith Performance RP-18e column (100 x 4.6 mm) at ambient temperature with UV detection at 230 nm and external calibration. A gradient elution was used (mobile phase A: 0.1% formic acid in acetonitrile; mobile phase B: 0.1% formic acid in water).

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the test substance, a solvent blank and a blank accuracy sample were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 0.8 %. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched solutions. The LOQ of the method is 100 g/kg. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 4.1.2/20 Becker M.,Kamp H., 2015 k Reg.No. 6011210 - Validation of an analytical method for the analysis of Reg.No. 6011210 in diet using HPLC-UV 2015/1188594
Guidelines:	EEC 91/414, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Study supported:	*KCA 5.8.1/8* [redacted] *2016 a* *Reg.No. 6011210: Repeated-dose 28-day toxicity study in C57BL/6 J Rj mice - Administration via the diet* *2016/1000646*

Principle of the method

10 g of the sample (Ground Kliba maintenance diet mouse/rat "GLP" meal) were weighed into a 50 mL centrifuge tube and extracted 3 times by shaking with 30 mL 5% formic acid in acetonitrile. After centrifugation, the supernatants were collected in a 100 mL volumetric flask and made to volume with acetonitrile before filtering. If the amount of test substance in the sample solution was greater than the calibration range, dilution was required with matrix solution to bring the concentration within the linear range.

Analysis was performed by HPLC-UV DAD with an Ascentis Express C18 column (150 x 4.6 mm, 2.7 µm) at 40 °C with UV detection at 234 nm and external calibration. A gradient elution was used (mobile phase A: 5% water and 0.1% trifluoroacetic acid in acetonitrile; mobile phase B: 5% acetonitrile and 0.1% in trifluoroacetic acid in water).

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the test substance, a solvent blank and a blank accuracy sample were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 2.9%. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched solutions. The LOQ of the method is 50 mg/kg. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 4.1.2/21 Hedrich R., 2015 a Validation of an analytical method for the analysis of Reg.No. 6011210 in corn oil using HPLC 2015/1189154
Guidelines:	OECD-DOC ENV/MC/CHEM(98)17 Paris 1998, EEC 91/414, SANCO/3029/99 rev. 4
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Study supported:	*KCA 5.8.1/2* *Schmitt D., 2015 a* *Concentration control analysis and homogeneity control analysis of Reg.No. 6011210 in vehicle corn oil* *2015/1186900*

Principle of the method

A 1.0 mL corn oil sample was added to a 100 mL volumetric flask and made to volume with THF. 75 µL of this solution was added to a 10 mL volumetric flask and made to volume with acetonitrile.

Analysis was performed by reversed phase UHPLC-UV DAD on a Phenomenex Kinetex 1.3u C18 100 column (50 x 2.1 mm, 1.3 µm) at 40 °C with UV detection at 250 nm and external calibration. A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the lowest calibration level, matrix blank and solvent blank were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 2.0 %. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched solutions. The LOQ of the method is 200 g/L. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 4.1.2/22 Becker M.,Kamp H., 2015 l Reg.No. 6011210 - Validation of an analytical method for the analysis of Reg.No. 6011210 in Dimethyl sulfoxide using HPLC-UV 2015/1188599
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Study supported:	*KCA 5.8.1/5* *Becker M., Kamp M., 2015 a* *Reg.No. 6011210 - Stability analysis in dimethyl sulfoxide* *2015/1186975*

Principle of the method

The dimethyl sulfoxide samples were diluted with acetonitrile using appropriate volumetric flasks to obtain sample solution with concentrations that match the calibration range. If the amount of test substance in the sample solution was greater than the calibration range, dilution was required with matrix solution to bring the concentration within the linear range.

Analysis was performed by HPLC-UV DAD using a Kinetex C18 column (100 x 4.6 mm, 5 µm) at ambient temperature with UV detection at 250 nm and external calibration. An isocratic elution was used (50% mobile phase A: 5% water and 0.1% trifluoroacetic acid in acetonitrile; mobile phase B: 5% acetonitrile and 0.1% in trifluoroacetic acid in water).

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the lowest calibration level, matrix blank and solvent blank were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 0.7 %. The linear range is appropriate for the nominal test concentrations and was determined using matrix matched solutions. The LOQ of the method is 0.05 mg/mL. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 4.1.2/23 Mallat E., 2015 a The validation of the determination of Reg.No. 6011210 in mouse EDTA plasma samples using LC-MS/MS 2015/1186930
Guidelines:	SANCO/3029/99 rev.4
GLP:	yes (certified by Ministry of Health, Welfare and Sport, The Hague, The Netherlands)

Principle of the method

M750F022(Reg.No. 6011210) (2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]propane-1,2-diol) was extracted from mouse K2-EDTA plasma by liquid-liquid extraction. The extract was then evaporated under a stream of nitrogen.

After reconstitution, Analysis was performed by LC-MS/MS using an XBridge phenyl column (4.6 x 100 mm, 3.5 µm) at 40 °C with MRM monitoring *m/z* 364 → 309 and internal calibration. A gradient elution was used (mobile phase A: 5% methanol in pure water; mobile phase B: 500 mM ammonium formate in pure water).

Matrix effects

The influence of matrix effects was determined by spiking mouse EDTA plasma with 30 ng/mL and 1600 ng/mL Reg.No. 6011210 and internal standard and comparing the peak areas with solvent-based samples.

Table 5.1-31: Matrix effects

Matrix	Analyte	Concentration (ng/mL)	Matrix effect* (%)
Mouse EDTA plasma	Reg.No.6011210	30	94
		1600	101

*These are mean values calculated from the five samples tested normalised against the internal standard

No significant matrix effects were observed therefore the validation samples were analysed using solvent-based standards.

Validation summary

HPLC-MS/MS is a highly specific method and additional confirmation was not necessary. Chromatograms of test substance, a solvent blank and a blank accuracy sample were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 4 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 6 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 5.5 %. The linear range is appropriate for the nominal test concentrations and was determined using solvent-based solutions as no significant matrix effects were observed. The LOQ of the method is 10 ng/mL. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Compilation of validation data for methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

Table 5.1-32: Validation data for methods used in support of toxicological studies

Reference	Analyte	LOQ (mg/kg unless otherwise stated)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
KCA 4.1.2/9 Baltussen E., 2013 a KCA 4.1.2/10 Baltussen E., 2016 a	BAS 750 F	10	10 100 750	87 – 98 (94, 6) 87 – 106 (98, 5) 80 – 93 (89, 10)	7.3 (6) 7.8 (5) 5.7 (10) Overall: 7.2 (21)	0.100 – 49.8 mg/L [Corresponds to 2 – 996 mg/kg] r = 0.9992, 5 standards, y = 70400x + 962	Acceptable chromatograms presented for test substance and blank accuracy sample. No interference >30% of LOQ
KCA 4.1.2/11 Becker M.,Kamp H., 2015 i	BAS 750 F	30	30 250 6000	93.8 – 97.9 (95.4, 5) 97.7 – 103.1 (100.5, 5) 100.5 – 103.8 (102.2, 5)	2.2 (5) 2.5 (5) 1.3 (5) Overall: 3.5 (15)	0.20 – 2.0 mg/100 mL r^2 = 0.99997, 7 standards, y = 5.797x + 0.1245	Acceptable chromatograms presented for matrix solution and calibration solution. No interference >30% of LOQ
KCA 4.1.2/12 Becker M.,Kamp H., 2015 j	BAS 750 F	607	607 1250 9996	96.1 – 101.7 (99.4, 5) 102.9 – 108.4 (106.8, 5) 95.7 – 105.5	2.2 (5) 2.2 (5) 3.8 (5)	0.31 – 7.6 mg/100 mL r^2 = 0.9998, 5 standards, y = 5.466x + 0.1916	Acceptable chromatograms presented for matrix solution and calibration solution. No interference >30% of LOQ

Reference	Analyte	LOQ (mg/kg unless otherwise stated)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
				(102.0, 5)	Overall: 4.0 (15)		
KCA 4.1.2/13 Becker M.,Kamp H., 2015 a	BAS 750 F	10	10 20 30	97.6 – 106.4 (102.2, 5) 94.8 – 102.5 (99.9, 5) 99.3 – 103.3 (100.6, 5)	3.3 (5) 3.0 (5) 1.6 (5) Overall: 2.7 (15)	499 – 2994 ng/mL r^2 = 0.99989, 7 standards, y = 4143x + 16219	Acceptable chromatograms presented for matrix solution and calibration solution. No interference >30% of LOQ
KCA 4.1.2/14 Becker M.,Kamp H., 2015b	BAS 750 F	0.10 mg/mL	0.10 mg/mL 2.50 mg/mL 100 mg/mL 250 mg/mL	98.9 – 102.2 (100.6, 5) 87.4 – 99.1 (95.8, 5) 104.8 – 105.5 (105.2, 5) 99.4 – 100.0 (99.7, 5)	1.5 (5) 5.0 (5) 0.3 (5) 0.2 (5) Overall: 4.1 (20)	0.0050 – 0.050 mg/mL r^2 = 0.99990, 7 standards, y = 0.9937x – 0.0018	Acceptable chromatograms presented for test substance, a solvent blank and a blank accuracy sample. No interference >30% of LOQ

Reference	Analyte	LOQ (mg/kg unless otherwise stated)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
KCA 4.1.2/15 Becker M.,Kamp H., 2015 c	BAS 750 F	0.20 mg/mL	0.20 mg/mL 1 mg/mL 2 mg/mL	98.5 – 99.6 (99,1, 5) 98.2 – 98.5 (98.3, 5) 97.8 – 100.2 (99.0, 5)	0.5 (5) 0.2 (5) 0.9 (5) Overall: 0.7 (15)	0.01 – 0.1 mg/mL r^2 = 0.99969, 7 standards, y = 0.9355x – 0.0338	Acceptable chromatograms presented for test substance, a solvent blank and a blank accuracy sample. No interference >30% of LOQ
KCA 4.1.2/16 Becker M.,Kamp H., 2015 d	BAS 750 F	0.1 mg/mL	0.1 mg/mL 0.35 mg/mL 0.7 mg/mL	96.9 – 98.6 (97.7, 5) 97.6 – 99.1 (98.4, 5) 97.0 – 98.3 (97.9, 5)	0.8 (5) 0.6 (5) 0.6 (5) Overall: 0.7 (15)	0.0052 – 0.1 mg/mL r^2 = 0.99964, 7 standards, y = 1.004x – 0.0454	Acceptable chromatograms presented for test substance, a solvent blank and a blank accuracy sample. No interference >30% of LOQ
KCA 4.1.2/17 Becker M.,Kamp H., 2015 e	BAS 750 F	0.5 mg/mL	0.5 mg/mL 1 mg/mL 1.5 mg/mL	100.8 – 102.7 (101.4, 5) 98.7 – 99.2 (99.0, 5) 99.1 – 102.5 (100.5, 5)	0.7 (5) 0.4 (5) 1.3 (5)	0.00488 – 0.0488 mg/mL r^2 = 0.99993, 7 standards, y = 0.9822x + 0.0065	Acceptable chromatograms presented for test substance, a solvent blank and a blank accuracy sample. No interference >30% of LOQ

Reference	Analyte	LOQ (mg/kg unless otherwise stated)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
					Overall: 1.3 (15)		
KCA 4.1.2/18 Becker M.,Kamp H., 2015 f	BAS 750 F	50 ng/mL	50.1 ng/mL 1002 ng/mL 10020 ng/mL	102.9 – 110.8 (108.4, 5) 108.3 – 110.5 (108.8, 5) 102.6 – 105.2 (103.8, 5)	4.5 (5) 0.9 (5) 1.0 (5) Overall: 3.3 (15)	Low range: 4.0 – 75 ng/mL r^2 = 0.99974, 6 standards, y = 4030.7x - 1188 High range: 100 - 1202 ng/mL r^2 = 0.99975, 6 standards, y = 3855.1x + 13696	Acceptable chromatograms presented for test substance, a solvent blank and a blank accuracy sample. No interference >30% of LOQ
KCA 4.1.2/19 Becker M.,Kamp H., 2015 g	BAS 750 F	100 mg/g	100 mg/g 500 mg/g 600 mg/g	98.9 – 100.1 (99.4, 5) 98.0 – 99.6 (98.5, 5) 97.6 – 99.1 (98.3, 5)	0.4 (5) 0.9 (5) 0.6 (5) Overall: 0.8 (15)	2.0 – 10 mg/100 mL r^2 = 0.99995, 7 standards, y = 0.5009x + 0.0025	Acceptable chromatograms presented for test substance, a solvent blank and a blank accuracy sample. No interference >30% of LOQ

Reference	Analyte	LOQ (mg/kg unless otherwise stated)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
KCA 4.1.2/20 Becker M.,Kamp H., 2015 k	Reg.No. 6011210 (Metabolite M750F022) (2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]propane-1,2-diol)	50	50 500 5000	102.4 – 107.5 (100.6, 5) 98.4 – 103.1 (100.7, 5) 100.2 – 101.7 (101.2, 5)	2.0 (5) 2.0 (5) 0.9 (5) Overall: 2.9 (15)	0.31 – 5.13 mg/100 mL r^2 = 0.99994, 7 standards, y = 3.625x + 0.0758	Acceptable chromatograms presented for test substance, a solvent blank and a blank accuracy sample. No interference >30% of LOQ
KCA 4.1.2/21 Hedrich R., 2015 a	Reg.No. 6011210 (Metabolite M750F022) (2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]propane-1,2-diol)	200 g/L	200 g/L 400 g/L 800 g/L	96.6 – 101.6 (99.2, 5) 98.6 – 100.8 (99.4, 5) 99.6 – 104.1 (101.9, 5)	2.0 (5) 1.0 (5) 1.9 (5) Overall: 2.0 (15)	4 – 70 mg/L [Approx. 53 – 933 g/L] r = 1.000, 7 standards, y = 0.258x + 0.043	Acceptable chromatograms presented for the lowest calibration level, matrix blank and solvent blank. No interference >30% of LOQ
KCA 4.1.2/22 Becker M., Kamp H., 2015 l	Reg.No. 6011210 (Metabolite M750F022) (2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]propane-1,2-diol)	0.05 mg/mL	0.0505 mg/mL 0.101 mg/mL 0.202 mg/mL	99.6 – 101.9 (100.8, 5) 99.2 – 101.2 (100.5, 5) 99.7 – 101.2 (100.6, 5)	0.9 (5) 0.8 (5) 0.6 (5) Overall: 0.7 (15)	0.005 – 0.051 mg/mL r = 0.99998, 7 standards, y = 0.5731x – 0.0034	Acceptable chromatograms presented for test substance, a solvent blank and a blank accuracy sample. No interference >30% of LOQ

Reference	Analyte	LOQ (mg/kg unless otherwise stated)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
KCA 4.1.2/23 Mallat E., 2015 a	Reg.No. 6011210 (Metabolite M750F022) (2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]propane-1,2-diol)	10 ng/mL	10 ng/mL 30.0 ng/mL 200 ng/mL 1600 ng/mL	96.4 – 114 (103, 6) 80.1 – 102.1 (90.8, 6) 79.6 – 90.0 (84.4, 6) 85.6 – 108.6 (90.7, 6)	6.9 (6) 6.1 (6) 5.3 (6) 6.5 (6) Overall: 5.5 (24)	10.0 – 2000 ng/mL y = 0.000493x + 0.000489, r = 0.9987, 9 standards	Acceptable chromatograms presented for test substance, a solvent blank and a blank accuracy sample. No interference >30% of LOQ

B.5.1.2.4. Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies

No stand-alone validated analytical methods for the determination of BAS 750 F were required for exposure studies.

B.5.1.2.5. Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

Plant matrices

BAS 750 F

Report: CA 4.1.2/25
Paula Jose W.F. de, 2015 a
Validation of BASF Method Number L0076/09 for the determination of BAS 750 F in citrus (whole fruit), coffee (grain), dry beans (seed), soybeans (grain), tomato (whole fruit), wheat (grain) and wheat (straw) using LC-MS/MS
2015/3001681

Guidelines: Resolucao RDC No. 4 - ANVISA (18/01/2012)

GLP: yes
(certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Studies supported:

CA 6.1/1
Guedez-Orozco A.-A.,Eilers B., 2015 a
Storage stability of BAS 750 F in plant matrices
2015/1106709

CA 6.3.1/1
Erdmann H.-P., 2015 a
Study on the residue behaviour of Reg.No. 5834378 (BAS 750 F) in wheat after application of EXP 5834378 F-AV (BAS 750 00 F) under field condition in Germany, The Netherlands, United Kingdom, Southern France, Greece, Italy and Spain, 2013
2014/1010809

CA 6.3.1/2
Ale E., 2015 a
Residue study (Decline) with BAS 750 01 F, BAS 750 00 F and BAS 750 BU F applied to wheat in Northern and Southern Europe in 2014
2015/1099704

CA 6.3.2/1
Erdmann H.-P., 2015 b
Study on the residue behaviour of Reg.No. 5834378 (BAS 750 F) in barley after application of EXP 5834378 F-AV (BAS 750 00 F) under field condition in Germany, The Netherlands, United Kingdom, Southern France, Greece, Italy and Spain, 2013
2014/1010808

CA 6.3.2/2
Ale E., 2015 b
Residue study (Decline) with BAS 750 01 F, BAS 750 00 F and BAS 750 BU F applied to barley in Northern and Southern Europe in 2014
2015/1099703

CA 6.5.3/1
Plier S.,Elze M., 2015 a
Determination of residues of BAS 750 F (Reg.No. 5834378) in wheat and its processed products after two applications of BAS 750 01 F in Germany, 2014
2014/1315283

CA 6.5.3/2
Plier S.,Elze M., 2015 b
Determination of residues of BAS 750 F (Reg.No. 5834378) in barley and its processed products after two applications of BAS 750 01 F in Germany, 2014

2014/1315282

CA 6.6.2/1
Martin T., 2015 a
Study on the residue behavior of BAS 750 F on the rotational crops: wheat, carrots or radish, broccoli or cauliflower and spinach or lettuce after one application of BAS 750 01 F to bare soil under field conditions, 2014-2015
2015/1106682

Principle of the method L0076/09

Citrus, coffee, dry beans, soybean, tomato and wheat grains: Residues of BAS 750 F were extracted using 100 mL extraction solution (methanol/Milli-Q water/2 mol/L HCl solution (70/25/5, v/v/v)) before being centrifuged. A 1 mL aliquot was transferred to a centrifuge tube containing 1mL 0.2 mol/L NaOH solution and partitioned against 5 mL cyclohexane and centrifuged. A 4 mL aliquot was taken from the organic phase and evaporated to dryness before being dissolved in 2 mL methanol/Milli-Q water (50/50, v/v) and filtered. For samples with analyte concentrations outside the calibration range, further dilution was performed as required.

Wheat straw: Residues of BAS 750 F were extracted using 100 mL extraction solution (methanol/Milli-Q water/2 mol/L HCl solution (70/25/5, v/v/v)) before being centrifuged, filtered and collected in a 200 mL volumetric flask. The produce was repeated with another 100 mL extraction solution before being homogenised, filtered, added to the first extract and making to volume with extraction solution. An aliquot was centrifuged and a 1 mL aliquot was transferred to a centrifuge tube containing 1mL 0.2 mol/L NaOH solution and partitioned against 5 mL cyclohexane and centrifuged. A 4 mL aliquot was taken from the organic phase and evaporated to dryness before being dissolved in 1 mL methanol/Milli-Q water (50/50, v/v) and filtered. For samples with analyte concentrations outside the calibration range, further dilution was performed as required.

Analysis was performed by HPLC-MS/MS and UPLC-MS/MS with MRM monitoring the following ion transitions: *m/z* 398→133 (quantification) and *m/z* 398→133 (confirmation) with external standardisation.

For HPLC-MS/MS a Thermo Scientific Betasil C18 column (100 mm x 2.1 mm, 5 µm) was used at 20 °C with a gradient elution (mobile phase A: 0.1% formic acid in Milli-Q Water; mobile phase B: 0.1% formic acid in methanol).

For UPLC-MS/MS a Waters Acquity UPLC BEH C18 column (50 mm x 2.1 mm, 1.7 µm) was used at 40 °C with a gradient elution (mobile phase A: 0.1% formic acid in Milli-Q Water; mobile phase B: 0.1% formic acid in acetonitrile).

Matrix effects

To test the influence of the matrix on the analysis, the response of the analyte in the matrix was compared to the response of the solvent-based standards at 3 concentrations with 3 injections of each at both mass transitions.

Table 5.1-33: Matrix effects

Chromatographic technique	**Matrix**	**Mass transition (*m/z*)**	**Mean matrix effect across 3 concentrations (%)**
HPLC	Citrus	398→182	103.1
		398→133	101.4
	Coffee	398→182	100.7
		398→133	104
	Dry beans	398→182	103.7
		398→133	106.4
	Soybeans	398→182	102.6
		398→133	106.7
	Tomato	398→182	103.4
		398→133	104.4
	Wheat grain	398→182	103.5
		398→133	105.7
	Wheat straw	398→182	105.3
		398→133	105
UPLC	Citrus	398→182	104.5
		398→133	104.9
	Coffee	398→182	101.4
		398→133	105.6
	Dry beans	398→182	103.6
		398→133	103.1
	Soybeans	398→182	106.5
		398→133	109.9
	Tomato	398→182	104.7
		398→133	103.8
	Wheat grain	398→182	101.9
		398→133	102.3
	Wheat straw	398→182	106.2
		398→133	106

No significant matrix effects (>20%) were observed therefore matrix-matched standard solutions were not used for quantification.

Stability of stock, fortification and calibration standard solutions

The stability of BAS 750 F in stock, fortification and calibration solutions was investigated by storing at 5±3°C for 14 and 30 days. At each time point the concentration of BAS 750 F was measured against freshly prepared standards. Quantification was performed for both mass transitions.

Table 5.1-34: Stability of stock, fortification and calibration standard solutions

Matrix	**Analyte**	**Nominal concentration (ng/mL)**	**Mass transition (*m/z*)**	**Storage interval (days)**	**Mean recovery of stored solution against fresh solution (%)**
Stock solution	BAS 750 F	0.500	398→182	14	104.6
				30	101.9
			398→133	14	116
				30	107.5
Fortification solution	BAS 750 F	0.500	398→182	14	97.4
				30	104.8
			398→133	14	98.9
				30	105.8
Calibration solution BAS 750 F	BAS 750 F	0.200	398→182	14	95.7
				30	90.6
			398→133	14	96.7
				30	81
		0.500	398→182	14	92.0
				30	97.2
			398→133	14	94.4
				30	96.7
		1.00	398→182	14	98.2
			398→133	14	99.4

The analyte was shown to be stable in stock, fortification and calibration standard solutions for up to 30 days at 5±3 °C except for calibration solutions at 1.00 ng/mL for which data is only available for storage after 14 days.

Stability of extract and final volume solutions

The stability of extract and final volume solutions of 7 matrices was investigated by storing under refrigerator conditions for 7 or 8 days. At each time point the concentration of BAS 750 F was measured for the primary mass transition (*m/z* 398→182).

Table 5.1-35: Stability of extract and final volume solutions

Solution type for stability analysis	Matrix	Storage interval (days)	Mean recovery (%)
Extract	Citrus	0	79.1
		7	69.1
	Coffee	0	85.4
		7	83.7
	Dry beans	0	85.1
		7	86.5
	Soybeans	0	91.5
		7	76.2
	Tomato	0	83.6
		7	81.8
	Wheat grain	0	83.9
		7	76.9
	Wheat straw	0	75.9
		8	76
Final volume	Citrus	0	79.1
		7	76.2
	Coffee	0	85.4
		7	90.1
	Dry beans	0	85.1
		7	86.9
	Soybeans	0	91.5
		7	79.3
	Tomato	0	83.6
		7	83.3
	Wheat grain	0	83.9
		7	79.6
	Wheat straw	0	75.9
		8	76.3

The stability of BAS 750 F in extract and final volume solutions was shown for up to 7 days for all matrices except for wheat straw which was stable for up to 8 days.

Validation summary

HPLC-MS/MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the calibration standards, reagent blank, control sample and fortified samples were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels corresponding to LOQ, 10xLOQ and 100xLOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, either 5 or 6 determinations were made at each fortification level and RSDs were within the acceptable limit of 15 %. The overall RSDs were within the range 4.0 – 12 %. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched solutions. The LOQ of the method is 0.01 mg/kg. The analytical method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report: CA 4.1.2/26
Class T., 2011 a
Modification M004 of BCS residue analytical method 01062 for the determination of 1,2,4-Triazole, Triazolylalanine, Triazole acetic acid and Triazole lactic acid by LC/DMS/MS/MS in plant materials
2012/1294644

Guidelines: Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4), OECD-ENV/JM/MONO/(2007)17 (OECD No. 39)

GLP: yes
(certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Studies supported:

CA 6.3.1/1
Erdmann H.-P., 2015 a
Study on the residue behaviour of Reg.No. 5834378 (BAS 750 F) in wheat after application of EXP 5834378 F-AV (BAS 750 00 F) under field condition in Germany, The Netherlands, United Kingdom, Southern France, Greece, Italy and Spain, 2013
2014/1010809

CA 6.3.1/2
Ale E., 2015 a
Residue study (Decline) with BAS 750 01 F, BAS 750 00 F and BAS 750 BU F applied to wheat in Northern and Southern Europe in 2014
2015/1099704

CA 6.3.2/1
Erdmann H.-P., 2015 b
Study on the residue behaviour of Reg.No. 5834378 (BAS 750 F) in barley after application of EXP 5834378 F-AV (BAS 750 00 F) under field condition in Germany, The Netherlands, United Kingdom, Southern France, Greece, Italy and Spain, 2013
2014/1010808

CA 6.3.2/2
Ale E., 2015 b
Residue study (Decline) with BAS 750 01 F, BAS 750 00 F and BAS 750 BU F applied to barley in Northern and Southern Europe in 2014
2015/1099703

CA 6.5.3/1
Plier S.,Elze M., 2015 a
Determination of residues of BAS 750 F (Reg.No. 5834378) in wheat and its processed products after two applications of BAS 750 01 F in Germany, 2014
2014/1315283

CA 6.5.3/2
Plier S.,Elze M., 2015 b
Determination of residues of BAS 750 F (Reg.No. 5834378) in barley and its processed products after two applications of BAS 750 01 F in Germany, 2014
2014/1315282

CA 6.6.2/1
Martin T., 2015 a
Study on the residue behavior of BAS 750 F on the rotational crops: wheat, carrots or radish, broccoli or cauliflower and spinach or lettuce after one application of BAS 750 01 F to bare soil under field conditions, 2014-2015
2015/1106682

Principle of the method 01062/M004

5.0g of the plant material was blended with 60 mL extraction solution (methanol/water (4/1, v/v)), filtered and made to 100 mL with extraction solution. A 10 mL aliquot was filtered, 0.20 mL internal standard added, concentrated and volume adjusted to about 1 mL with water before being vortexed with Bakerbond C18 SPE material and filtered.

For the determination of triazole (T); triazole acetic acid (TAA) except for in dry bean seed; triazole lactic acid (TLA); and triazole-alanine (triazolylalanine) (TA) in tomato, cucumber, lettuce and carrot leaf; analysis was performed by LC-DMS/MS/MS using a Thermo Aquasil C18 column (150 x 3 mm, 3 µm).

For the determination of triazole-alanine (triazolylalanine) (TA) in all matrices except the ones listed above; and triazole lactic acid (TLA) in dry bean seed; and as a confirmatory column for the other analytes; analysis was performed by LC-DMS/MS/MS using a Thermo Hypercarb column (100 x 4.6 mm, 5 µm).

For both columns, a gradient elution was used (mobile phase A: 0.5% formic acid in water; mobile phase B: 0.5% formic acid in methanol) with internal standardisation and detection was by Turbo IonSpray (ESI) with MRM monitoring the following transitions:

- 1,2,4-triazole *m/z* 70→43 (quantification)
- Triazolylalanine *m/z* 157→70 (quantification)
  m/z 157→88 (confirmation)
- Triazole acetic acid *m/z* 128→70 (quantification)
- Triazole lactic acid *m/z* 158→70 (quantification)

Matrix effects

Stable isotopically labelled internal standards of 1,2,4-Triazole, Triazolylalanine, Triazole acetic acid and Triazole lactic acid were used which compensates for matrix effects.

Stability in solution and extracts

The stability of analytes and internal standards in solution and extracts was not tested. Acceptable recoveries obtained with calibration solutions sufficiently demonstrate stability.

Validation summary

HPLC-MS/MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the calibration standards, and control and fortified samples for analytes in tomato, grain, orange, bean seed and oilseed rape/sunflower seed were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels corresponding to LOQ and 100xLOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 % except for triazole in carrot leaf (112 %), cereal grain (115 %, 118 %), cereal green plant (116 %), triazolylalanine in tomato (111 %), cucumber (111 %), lettuce (116 %), carrot leaf (118 %), and triazole lactic acid in carrot leaf (114 %). To assess method precision, 5 determinations were made at each fortification level, except for residues of all analytes in cucumber, lettuce, sweet pepper, carrot leaf, carrot root, cereal straw, cereal green plant, melon peel, melon fruit and melon pulp where 3 determinations were made. RSDs were within the acceptable limit of 20 % except for triazolylalanine residues in sweet pepper and sunflower seed. The overall RSDs were within the range 3 - 25%. The linear range is appropriate for the nominal test concentrations and was determined using solvent-based solutions. The LOQ of the method is 0.01 mg/kg. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4 except for residues of all analytes in cucumber, lettuce, sweet pepper, carrot leaf, carrot root, cereal straw, cereal green plant, melon peel, melon fruit and melon pulp and residues of triazole in cereal grain, and triazolylalanine in tomato where it is fit for purpose.

Animal matrices

Report:	CA 4.1.2/27 Devine C., 2015 a Validation of the BASF analytical method L0272/01 for BAS 750 F in animal matrices 2015/1106707
Guidelines:	EPA 860.1340 (1996), SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010), OECD-ENV/JM/MONO/(2007)17
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
Studies supported:	*CA 6.1/3* *Heger N.,Guedez-Orozco A.-A., 2015 b* *Storage stability of BAS 750 F in animal matrices* *2015/1106711* *CA 6.2.2/2* *Thiaener J.,Glaessgen W.E., 2015 b* *Investigation of the extractability of BAS 750 F and M750F022 in samples from 14C animal metabolism studies* *2015/1161960* *CA 6.4.1/1* [redacted] *2015 a* *Magnitude of residues in tissues and eggs of laying hens following multiple oral administrations of BAS 750 F* *2015/1106667* *CA 6.4.2/1* [redacted] *2015 a* *Magnitude of residues in milk and tissues of dairy cows following multiple oral administration of BAS 750 F* *2015/1107649*

Principle of the method L0272/01

For matrices containing proteins (muscle, kidney, liver and egg): 5 g of the matrix was extracted with a 100 mL mixture of methanol, water and 2N hydrochloric acid (70+25+5). A 10 mL of this aliquot of this was centrifuged. 1 mL of the supernatant was partitioned twice with 5mL cyclohexane in alkaline conditions and the supernatants combined in a 10 mL culture tube. A 4 mL aliquot of the organic layer was evaporated to dryness and dissolved in 0.5 mL methanol and 0.5 mL water.

For matrices containing fat (milk, cream and fat): 5 g of the matrix was extracted with 100 mL acetonitrile and 40 mL iso-hexane. A 10 mL aliquot was centrifuged. 4 mL of the supernatant was twice partitioned with 4 mL iso-hexane and 1 mL acetonitrile evaporated to dryness and dissolved in 0.5 mL methanol and 0.5 mL water.

Analysis was performed by LC-MS/MS using a Betasil C18 column (100 x 2.1 mm, 5 µm) at 30 °C with Turbospray Ionisation monitoring the following ion transitions: *m/z* 398 → 182 (quantification) and 298 → 133 (confirmation), and external standardisation. A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

Matrix effects

The response of the analyte in matrix-matched standards compared to solvent standards was compared. Concentrations ranging from 0.044 – 10 ng/mL were prepared. The matrix effect is the peak area of the analyte in the matrix-matched standard expressed as a percentage of the peak area of the analyte in non-matrix matched standard.

Table 5.1-36: Matrix effects

Matrix	Analyte	Mass transition (*m/z*)	**Peak area in matrix matched standards compared to solvent standards (%)***
Bovine meat	BAS 750 F	398 → 182	112
		298 → 133	113
Bovine milk	BAS 750 F	398 → 182	101.1
		298 → 133	103.9
Bovine cream	BAS 750 F	398 → 182	102.4
		298 → 133	101.9
Bovine fat	BAS 750 F	398 → 182	101.7
		298 → 133	102.4
Bovine liver	BAS 750 F	398 → 182	103.5
		298 → 133	105.2
Bovine kidney	BAS 750 F	398 → 182	94.9
		298 → 133	98.2
Hen eggs	BAS 750 F	398 → 182	116
		298 → 133	118

*These are mean values calculated from all concentrations tested

Matrix effects were less than 20 % and therefore the effects were not significant and either matrix-matched standards or non-matrix standards can be used for their analysis. However, for eggs the matrix effect was close to 20 % hence it was appropriate that matrix-matched standards are used for analysis in eggs. In the validation of the method, experiments were performed with matrix-matched standards for bovine meat, fat, liver and hen eggs, and solvent based standards for bovine milk and cream.

Stability of standards

An assessment of the stability of working (fortification/calibration) solutions in methanol/water (50/50, v/v) was made by comparing the peak area of a mid-range stored calibration solution prepared at least 7 days previously with a calibration solution freshly prepared from new stock solutions at the same nominal concentration.

An assessment of the stability of the stock solutions (1000 µg/mL) was made. This was achieved by injecting a dilution of a stored stock solution of the reference item against a dilution of a stock standard solution prepared on the day of analysis and comparing the results.

Table 5.1-37: Stability of standards

Matrix	Analyte	Standard concentration	Storage period	Mass transition (*m/z*)	% difference in peak area
Methanol/water (50/50, v/v) working solution	BAS 750 F	1.0 ng/mL	7	398→182	7.8
				398→133	9.4
Methanol stock solution	BAS 750 F	1000 µg/mL	98	398→182	12
				398→133	12

The working solutions proved to be stable (<20% difference) for at least 7 days.
The stock solutions proved to be stable (<20% difference) for at least 98 days.

Stability of extracts

An assessment of the stability of the final extracts in vials for all matrices was made after storing the final samples refrigerated for 7 days and re-analysing the set of samples that have been fortified at the LOQ (0.01mg/kg), using the primary transition only.

Table 5.1-38: Stability of extracts

Matrix	Analyte	Mass transition (*m/z*)	Days of storage	Mean % recovery pre-storage (n)	Mean % recovery post-storage (n)
Bovine meat	BAS 750 F	398 → 182	7	85.0 (5)	103 (5)
Bovine milk	BAS 750 F	398 → 182	8	82.0 (5)	85.4 (5)
Bovine cream	BAS 750 F	398 → 182	7	72.6 (5)	80.5 (5)
Bovine fat	BAS 750 F	398 → 182	7	80.2 (5)	80.7 (5)
Bovine liver	BAS 750 F	398 → 182	7	87.5 (5)	93.8 (5)
Bovine kidney	BAS 750 F	398 → 182	7	96.3 (5)	84.0 (5)
Hen eggs	BAS 750 F	398 → 182	7	97.6 (5)	85.9 (5)

The extracts were all stable for at least 7 days in all matrices as all recoveries in the fortified samples were within the range 70 – 120%. The change in recovery in bovine meat matrix was greater than 20%, however, the recovery was still within the range 70 – 120 %.

Validation summary

HPLC-MS/MS is a highly specific technique and two mass transitions (3 ions) were monitored as outlined in the guidance document. Chromatograms of calibration standards, blank matrix and fortified sample have been presented showing no interferences >30% of LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels for the analyte in each matrix of interest corresponding to LOQ and 10xLOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level (with the exception of two fortification levels where only 4 determinations were made) and RSDs were within the acceptable limit of 20 %. The overall RSDs were between 4.4 % and 14 %. The linear range is appropriate for the nominal test concentrations, and was determined using matrix-matched standards for bovine meat, fat, liver and hen eggs, and solvent based standards for bovine milk and cream. The LOQ of the method is 0.01 mg/kg. The analytical method is satisfactorily validated in accordance SANCO/825/00 rev.8.1 and SANCO/3029/99 rev.4. SANCO/825/00 rev.8.1 has been considered here as the method has also been submitted as a method of analysis in animal matrices for post-approval control and monitoring purposes (see Section B.5.2.1.2).

Report:	CA 4.1.2/28 Heger N.,Taraschewski I., 2016 b Validation of the BASF analytical method L0309/01: For the determination of M750F022 (Reg.No. 6011210) in animal matrices 2015/1106706
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), SANCO/825/00 rev. 8.1 (16 November 2010), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Report:	CA 4.1.2/32 Heger N., 2017 a Revalidation of the BASF analytical method L0309/01: For the determination of M750F022 (Reg.No. 6011210) in cow fat and milk 2017/1002385
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), SANCO/825/00 rev. 8.1 (16 November 2010), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07
GLP:	yes

Studies supported:

CA 6.1/2
Heger N., Taraschewski I., 2015 a
Storage stability of Reg.No. 6011210 in animal matrices
2015/1106710

CA 6.2.2/2
Thiaener J.,Glaessgen W.E., 2015 b
Investigation of the extractability of BAS 750 F and M750F022 in samples from 14C animal metabolism studies
2015/1161960

CA 6.4.1/1
[redacted] *2015 a*
Magnitude of residues in tissues and eggs of laying hens following multiple oral administrations of BAS 750 F
2015/1106667

CA 6.4.2/2
Guedez Orozco A.A.,Heger N., 2016 a
Determination of the fatty conjugates metabolites of M750F022 (Reg. No. 6011210) in animal matrices
2016/1001326

CA 6.4.2/1
[redacted] *2015 a*
Magnitude of residues in milk and tissues of dairy cows following multiple oral administration of BAS 750 F
2015/1107649

Principle of the method L0309/01

For fat-containing matrices (milk and fat): 5g matrix was extracted by shaking or macerating with 50 mL acetonitrile and 20 ml iso-hexane. 20 mL of the acetonitrile extract was shaken with 20 mL iso-hexane then centrifuged and then repeated. 15mL of the acetonitrile phase was dried, dissolved in 2mL methanol/water

(50/50) and diluted with 3 mL water. An SPE clean up step was carried out at the end of which the dried residue was dissolved with acetonitrile into the calibration range. Prior to analysis an Analyte Protectant mix (AP-mix) was added.

For protein-containing matrices (egg, muscle, liver and kidney): 5g matrix was extracted by macerating with 50 mL methanol/water/2N HCl (70/25/5) and centrifuging. 20 mL of this extract was shaken with 20 mL 0.2N NaOH and 100 mL cyclohexane (muscle and liver) or dichloromethane (egg and kidney) and centrifuged. The cyclohexane or dichloromethane phase was dried, dissolved in 2 mL methanol/water (50/50) and diluted with 3 mL water. An SPE clean up step was carried out and the dried residue dissolved with acetonitrile into the calibration range. Prior to analysis an Analyte Protectant mix (AP-mix) was added.

Analysis was performed by GC-MS using a Restek GmbH, RTX-5 Amine column (30 m x 0.25 mm, 0.25 µm) at an oven temperature rising from 100°C to 310 °C with EI detection monitoring 3 fragment ions (molecular weight 346.7): *m/z* 295 (quantification), 297 (confirmation) and 317 (confirmation), and external standardisation. The carrier gas was helium.

Matrix effects

The response of each analyte in matrix-matched standards compared to solvent based standards was compared. The influence of the matrix on the response of the analyte is the ratio of the slopes from solvent-based calibration curves to the slope obtained from matrix-matched standards expressed as a percentage (with the slope for the solvent standards set to 100 %).

Table 5.1-39: Matrix effects

Matrix	**Analyte**	**Fragment ion (*m/z*)**	**Slope in matrix matched standards compared to solvent* (%)**
Cow liver	BAS 750 F	295	157
		297	162
		317	162
Cow kidney	BAS 750 F	295	141
		297	142
		317	136
Cow muscle	BAS 750 F	295	113
		297	123
		317	124
Cow fat	BAS 750 F	295	167
		297	172
		317	178
Cow milk	BAS 750 F	295	131
		297	130
		317	134
Hen egg	BAS 750 F	295	103
		297	105
		317	99.8

*These are mean values calculated from all concentrations tested

In all matrices, except hen egg (for all fragment ions) and cow muscle (for quantitative fragment ion *m/z* 295 only), the influence of the matrix is greater than 20 %. Validation data were generated using matrix-matched standards for liver, kidney, muscle, milk and fat matrices. Solvent-based standards in acetonitrile were used for egg matrices.

Stability of stock, calibration and AP-mix solutions

The storage stability of M750F022 in stock, calibration and AP-mix solutions was investigated by measuring the concentration of the analyte before and after storage. All solutions were stored at 4 °C in the dark: stock solutions for 91 days, calibration solutions for 29 days and AP-mix solutions for 30 days. Quantification was performed for three fragment ions. The results are presented in Table 5.1-40.

Table 5.1-40: Stability of stock, calibration and AP-mix solutions

Matrix	Analyte	Days of storage	Fragment ion (*m/z*)	Mean % recovery before storage	Mean % recovery after storage
Stock solution	M750F022	91	295	98.9	99.3
			297	99.5	102
			317	96.9	102
Calibration solution (Acetonitrile)	M750F022	29	295	96.8	99.0
			297	95.2	95.6
			317	94.4	97.9
AP-mix solution (acetonitrile + AP-mix)	M750F022	30	295	103	97.3
			297	101	97.4
			317	94.1	97.6

Less than 10 % difference in recovery was observed after storage in all solutions tested demonstrating that M750F022 was stable in stock, calibration and AP-mix solutions for at least 91, 29 or 30 days, respectively when stored at 4 °C in the dark.

Stability of extracts and final volumes

The storage stability of M750F022 in animal extracts and final volumes was tested at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10xLOQ) by measuring the concentrations of the analyte against freshly prepared standards before and after storage. The extracts and final volumes were stored 7 days at 4 °C in the dark. Quantification was performed for three fragment ions. The results are presented in Table 5.1-41. Extract stability in kidney was only demonstrated for 4 days.

Table 5.1-41: Stability of extracts and final volumes

Matrix	Analyte	Days of storage	Fragment ion (*m/z*)	Mean % recovery before storage	Mean % recovery after storage
Cow liver extract	M750F022	7	295	77.5	62.1
			297	76.8	63.5
			317	76.7	64.7
Cow muscle extract	M750F022	7	295	81.0	83.8
			297	79.8	82.5
			317	79.4	81.4
Cow fat extract	M750F022	7	295	119	85.4
			297	117	96.3
			317	111	96.3
Cow milk extract	M750F022	7	295	78.2	80.0
			297	78.4	80.6
			317	78.3	80.6
Hen egg extract	M750F022	7	295	93.9	83.5
			297	92.9	82.6
			317	92.4	82.9
Cow liver final volume	M750F022	7	295	77.5	69.0
			297	76.8	70.4
			317	76.7	71.5
Cow kidney final volume	M750F022	4	295	82.4	85.4
			297	79.2	84.6
			317	81.9	85.5
Cow muscle final volume	M750F022	7	295	81.0	77.1
			297	79.8	74.3
			317	79.4	78.7
Cow fat final volume	M750F022	8	295	119	106.8
			297	117	105.0
			317	111	108.3
Cow milk final	M750F022	5	295	78.2	68.3

volume			297	78.4	67.8
			317	78.3	68.6
Hen egg final volume	M750F022	8	295	93.9	84.4
			297	92.9	83.0
			317	92.4	84.5

M750F022 was stable in extracts and final volumes over the tested time period of at least 7 days except for cow kidney which was only stable for 4 days in the final volume and cow milk which was only stable for 5 days in the final volume.

Validation summary

GC-MS is a highly specific technique using 3 fragment ions therefore a confirmatory technique was not required. Chromatograms of standard solutions, controls and fortified samples have been presented showing no interferences >30% of LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels for the analyte in each matrix of interest corresponding to LOQ and 10xLOQ and in all cases the mean recovery was within the acceptable range of 70 – 120 %. To assess method precision, 5 determinations were made at each fortification level (with the exception of two fortification levels for cow kidney where only 4 determinations were made) and RSDs were within the acceptable limit of 20 %. The overall RSDs were between 3.82 % and 15 %. The linear range is appropriate for the nominal test concentrations, and was determined using matrix-matched standards except for egg where solvent-based standards were used. The LOQ of the method is 0.01 mg/kg. The analytical method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	CA 4.1.2/29 Guedez Orozco A.A.,Heger N., 2016 a Determination of the fatty conjugates metabolites of M750F022 (Reg. No. 6011210) in animal matrices 2016/1001326
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Studies supported:	*CA 6.4.2/2* *Guedez Orozco A.A.,Heger N., 2016 a* *Determination of the fatty conjugates metabolites of M750F022 (Reg. No. 6011210) in animal matrices* *2016/1001326*

Principle of the method L0309/02

The fatty acid conjugates of M750F022 are cleaved to M750F022 using NaOH (10 M). To verify the functionality of the method, fortifications were done using the conjugate M750F025 as typically representative of this class of compounds (fatty acid conjugates) which are measured as M750F022. The conditions for the alkaline cleavage were tested and reported in the metabolism study (Study number 433801, DocID 2015/1001001) (derivatisation factor 1.6876)

For fat containing matrices (fat, skin), 5 g sample was extracted by macerating with 50 mL acetonitrile and 20 mL isohexane. The isohexane extract was shaken again with 20 mL acetonitrile, twice. The whole acetontrile extract was evaporated until dryness and then dissolved in 5 mL THF. 20 mL of NaOH (10M) was added and shaken. The extract was shaken with 20 mL THF and centrifuged. An aliquot of 10 mL or 15 mL of the THF phase is dried and dissolved in MeOH/H_2O (50/50). Then a SPE clean-up step was carried out.

For protein containing matrices (muscle, liver and egg) 5 g sample was extracted by macerating with 50 mL MeOH. 20 mL of the extract was evaporated until dryness and dissolved in 5 mL THF. 20 mL of NaOH (10 M) was added and the solution shaken. The extract was shaken with 20 mL THF and centrifuged. An aliquot of 20 mL of the THF phase is dried and dissolved in MeOH/H_20 (50/50). Then a SPE clean-up step was carried out.

Analysis was performed by GC/MS using a Restek GmbH RTX-5 Amine column (30 m x 0.25 mm, 0.25 µm) with helium as the carrier gas at 300 °C with EI detection of fragments *m/z* 295 (quantification), *m/z* 297 (confirmation) and *m/z* 317 (confirmation), although any of the fragments could be used for quantitation.

Matrix effects

In the study it was outlined that matrix-matched standards were not used because quality control samples were measured together with the samples and the recoveries ranged between 70-110%.

Validation summary

GC/MS is a highly specific method monitoring 3 mass fragments and additional confirmation was not necessary. Chromatograms of the calibration standards, fortified samples and control samples were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels corresponding to LOQ and 10xLOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 % except for residues in hen fat. To assess method precision, 5 determinations were made at each fortification level. RSDs were within the acceptable limit of 20 %. The overall RSDs were within the range 5.1 – 16 %. The linear range is appropriate for the nominal test concentrations and was determined using solvent-based solutions. The LOQ of the method is 0.01 mg/kg. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4 except for residues in hen fat where it is fit for purpose due to deficiencies in accuracy.

Report:	CA 4.1.2/30 Billian P.,Druskus M., 2009 a Residue analytical method 01132 for the determination of 1,2,4-Triazole, Triazole Alanine, Triazole Acetic Acid and Triazole Lactic Acid in/on milk, egg, muscle, fat, liver and kidney by HPLC-MS/MS (including amendment No. 1) 2010/1230632
Guidelines:	EEC 91/414, EEC 96/68, EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), SANCO/3029/99, SANCO/825/00 rev. 7 (17 March 2004), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07
GLP:	yes (certified by Ministerium fuer Arbeit, Gesundheit und Soziales des Landes Nordrhein-Westfalen Duesseldorf)
Studies supported:	*CA 6.4.1/1* [redacted] *2015 a* *Magnitude of residues in tissues and eggs of laying hens following multiple oral administrations of BAS 750 F* *2015/1106667* *CA 6.4.2/1* [redacted] *2015 a* *Magnitude of residues in milk and tissues of dairy cows following multiple oral administration of BAS 750 F* *2015/1107649*

Principle of the method 01132

For whole milk, cream, skimmed milk, muscle, egg yolk: 5.0 g sample were added to 40 mL extraction solution (methanol/water 4/1 (v/v)) and blended before being washed with 5 mL extraction solution. The sample was then filtered and any remaining residues washed with 30 mL extraction solution then made to volume with extraction solution to 100 mL. 100 µL acetic acid was added to a 5 mL aliquot of sample solution and filtered into a test tube, eluting with 5 mL methanol/water (7/3, v/v) before evaporating to dryness. The remaining residue was mixed with 2.0 mL internal standard solution and filtered.

For kidney: 5.0 g sample were added to 40 mL extraction solution (methanol/water 4/1 (v/v)) and blended before being washed with 5 mL extraction solution. The sample was then filtered and any remaining residues washed with 30 mL extraction solution then made to volume with extraction solution to 100 mL. 100 µL acetic acid was added to a 5 mL aliquot of sample solution and filtered into a test tube, eluting with 2 mL water before evaporating to dryness. The remaining residue was mixed with 2.0 mL internal standard solution and filtered.

For liver: 5.0 g sample were added to 40 mL extraction solution (methanol/water 4/1 (v/v)) and blended before being washed with 5 mL extraction solution. The sample was centrifuged, filtered and any remaining residues washed with 30 mL extraction solution then made to volume with extraction solution to 100 mL. 100 µL acetic acid was added to a 5 mL aliquot of sample solution and filtered into a test tube, eluting with 2 mL water before evaporating to dryness. The remaining residue was mixed with 2.0 mL internal standard solution and filtered.

For fat, whole egg and egg white: 5.0 g sample were added to 40 mL extraction solution (methanol/water 4/1 (v/v)) and blended before being washed with 5 mL extraction solution. The sample was centrifuged, filtered and any remaining residues washed with 30 mL extraction solution then made to volume with extraction solution to 100 mL. 100 µL acetic acid was added to a 5 mL aliquot of sample solution and filtered into a test tube, eluting with 5 mL methanol/water (7/3, v/v) before evaporating to dryness. The remaining residue was mixed with 2.0 mL internal standard solution and filtered.

Derivatisation for determination of 1,2,4-triazole (for all matrices): A 0.2 mL aliquot of the filtered sample was added to 0.5 mL of internal standard solution, 2 mL 0.1 M sodium bicarbonate in water and 2 mL 2 mM dansyl chloride in acetone and mixed. 100 µL 25% NH_4OH was added and mixed. 4 mL ethyl acetate was added

and the sample shaken, dried and evaporated to dryness. The remaining residue was dissolved in 1.25 mL acetone/water (1/1, v/v) and filtered.

Analysis was performed by LC-MS/MS using different conditions depending on the combination of analyte/matrix as shown in Table 5.1-42.

Table 5.1-42: Chromatographic conditions

LC-system	**Description**	**Analytes**
SCX	A Luna SCX column (150 x 2 mm, 5 µm) at 40°C with a guard column. A gradient elution was used (mobile phase A: 15 mmol/L ammonium formate; mobile phase B: 5 % acetic acid)	TAA (triazolylacetic acid) TLA (triazolylalanine) TA (triazolyllactic acid)
SCX neg	A Luna SCX column (150 x 2 mm, 5 µm) at 40°C with a guard column. A gradient elution was used (mobile phase A: 15 mmol/L ammonium formate; mobile phase B: 5 % acetic acid).	TAA TLA TA
Hypercarb	A Hypercarb column (100 x 3.0 mm, 5 µm) at 60 °C. A gradient elution was used (mobile phase A: water + 5 mL/L formic acid; mobile phase B: methanol + 5 mL/L formic acid)	TAA TLA TA
T	A Synergi Fusion-RP column (150 x 2 mm, 4 µm) at 60 °C with a guard column. A gradient elution was used (mobile phase A: water/methanol (9/1, v/v) + 10 mmol/L ammonium formate + 120 µL/L formic acid; mobile phase B: water/methanol (1/9, v/v) + 10 mmol/L ammonium formate + 120 µL/L formic acid).	TD (dansyl 1,2,4-triazole)
T 100	A Synergi Fusion-RP column (150 x 2 mm, 4 µm) at 60 °C with a guard column. A gradient elution was used (mobile phase A: water/methanol (9/1, v/v) + 10 mmol/L ammonium formate + 120 µL/L formic acid; mobile phase B: water/methanol (1/9, v/v) + 10 mmol/L ammonium formate + 120 µL/L formic acid).	TD

In all cases the collision gas was nitrogen and electrospray ionisation with MRM monitored the mass transitions shown in

Table 5.1-43.

Table 5.1-43: Mass transitions monitored

Matrix	LC-system	TD	TA	TAA	TLA
Whole milk	SCX		157→70 (quantification) 157→88 (confirmation)	128→70 (quantification)	158→70 (quantification)
	SCX-neg			128→70 (confirmation)	156→68 (confirmation)
	T	303→170 (quantification) 303→324 (confirmation)			
Skimmed milk	SCX		157→70 (quantification) 157→88 (confirmation)	128→70 (quantification)	158→70 (quantification)
	SCX-neg			128→70 (confirmation)	156→68 (confirmation)
	T	303→170 (quantification) 303→324 (confirmation)			
Cream	SCX		157→70 (quantification) 157→88 (confirmation)	128→70 (quantification)	158→70 (quantification)
	SCX-neg			128→70 (confirmation)	156→68 (confirmation)
	T	303→170 (quantification) 303→324 (confirmation)			
Bovine muscle	Hypercarb		157→88 (confirmation)	128→70 (quantification)	158→70 (quantification)
	SCX-neg		155→68 (quantification)	128→70 (confirmation)	156→68 (confirmation)
	T	303→170 (quantification) 303→324 (confirmation)			
Bovine kidney	Hypercarb		157→70 (quantification) 157→88 (confirmation)	128→70 (quantification)	158→70 (quantification)
	SCX-neg			128→70 (confirmation)	156→68 (confirmation)
	T 100	303→170 (quantification) 303→324 (confirmation)			
Bovine liver	Hypercarb		157→70 (quantification) 157→88 (confirmation)	128→70 (quantification)	158→70 (quantification)
	SCX-neg			128→70 (confirmation)	156→68 (confirmation)
	T	303→170			

		(quantification) 303→324 (confirmation)			
Fat	Hypercarb		157→70 (quantification) 157→88 (confirmation)	128→70 (quantification)	158→70 (quantification)
	SCX-neg			128→70 (confirmation)	156→68 (confirmation)
	T	303→170 (quantification) 303→324 (confirmation)			
Whole egg	Hypercarb		157→70 (quantification) 157→88 (confirmation)	128→70 (quantification)	158→70 (quantification)
	SCX-neg			128→70 (confirmation)	156→68 (confirmation)
	T	303→170 (quantification) 303→324 (confirmation)			
Egg white	Hypercarb		157→70 (quantification) 157→88 (confirmation)	128→70 (quantification)	158→70 (quantification)
	SCX-neg			128→70 (confirmation)	156→68 (confirmation)
	T	303→170 (quantification) 303→324 (confirmation)			
Egg yolk	Hypercarb		157→70 (quantification) 157→88 (confirmation)	128→70 (quantification)	158→70 (quantification)
	SCX-neg			128→70 (confirmation)	156→68 (confirmation)
	T	303→170 (quantification) 303→324 (confirmation)			

Matrix effects

For quantitation, stable isotopically labelled internal standards of 1,2,4-Triazole, Triazole Alanine, Triazole Acetic Acid and Triazole Lactic Acid were used which compensates for any matrix effects.

Stability in final extracts

The stability in final extracts was checked by comparing pre- and post-storage recoveries of samples stored at 4±3 °C in the dark.

Matrix	Fortification level (mg/kg)	Analyte	Storage interval	Recovery (%)
Whole milk	0.10	TD	Initial	100.8
			After 4 weeks	99.6
		TA	Initial	88
			After 4 weeks	88.8
		TAA	Initial	88.2
			After 4 weeks	89.6
		TLA	Initial	83.6
			After 4 weeks	84.2
Bovine muscle	0.10	TD	Initial	104.4
			After 5 weeks	101
		TA	Initial	96.6
			After 5 weeks	86.2
		TAA	Initial	98.2
			After 5 weeks	97.6
		TLA	Initial	95
			After 5 weeks	101
Whole egg	0.10	TD	Initial	98
			After 5 weeks	100.6
		TA	Initial	95.4
			After 5 weeks	99.2
		TAA	Initial	95.8
			After 5 weeks	81.8
		TLA	Initial	93.6
			After 5 weeks	117.2

Final extracts are stable for at least 4 weeks under refrigerated conditions. It is noted that TLA in whole egg shows an increase from 93.6 % to 117.2 %.

Validation summary

HPLC-MS/MS is a highly specific method using two ion transitions, chromatographic conditions or ionisation techniques and additional confirmation was not necessary. Chromatograms of the standard, control sample and recovery sample for whole milk, bovine muscle, bovine liver, fat, bovine kidney, whole egg and whole milk were provided showing interference >30% LOQ at the retention time of interest hence blank values were subtracted in the calculation of recoveries. Accuracy was assessed at 2 fortification levels corresponding to LOQ and 10xLOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 % except for the following:

- The second ion transition for 1,2,4-triazole in whole milk where the mean recovery at the lower fortification level was 114%.
- The second ion transitions for 1,2,4-triazole in cream where the mean recovery at the upper fortification level was 113%
- The first and second ion transitions for 1,2,4-triazole in bovine muscle where the mean recoveries at the lower fortification levels were 113% and 115% respectively
- The first and second ion transitions for triazole alanine in fat (bovine and pig) where the mean recoveries at the upper fortification levels were 119% and 122% respectively
- The first ion transition for triazole acetic acid in egg yolk where the mean recovery at the lower fortification level was 111%.
- The first ion transitions for triazole acetic acid in egg white where the mean recovery at the upper fortification level was 123%
- The second ion transition for triazole lactic acid in skimmed milk where the mean recovery at the lower fortification level was 112%.

- The first ion transition for triazole lactic acid in bovine kidney where the mean recovery at the lower fortification level was 111%.
- The first and second ion transitions for triazole lactic acid in egg white where the mean recoveries at the upper fortification levels were 121% and 115% respectively

To assess method precision, 5 determinations were made at each fortification level except for residues in skimmed milk, cream, egg yolk and egg white where 3 determinations were made per level. RSDs were within the acceptable limit of 20 %. The overall RSDs were within the range 1.9 – 15 %. The linear range is appropriate for the nominal test concentrations and was determined using solvent-based solutions. The LOQ of the method is 0.01 mg/kg. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4 except for residues of all analytes in skimmed milk, cream, egg yolk and egg white where it is fit for purpose due to deficiencies in repeatability and residues of 1,2,4-triazole in whole milk and bovine muscle, triazole alanine in fat (bovine and pig), and triazole lactic acid in bovine kidney due to deficiencies in accuracy.

Compilation of validation data for methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

Table 5.1-44: Validation data in support of residues studies

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
CA 4.1.2/25 Paula Jose W.F. de, 2015 a	BAS 750 F	HPLC	Citrus	0.01	398→182	0.01 0.10 1.0	71.7 – 79.2 (74.9, 6) 71.0 – 83.8 (79.5, 6) 74.3 – 86.7 (80.4, 5)	4.0 (6) 6.3 (6) 6.3 (5) Overall: 6.2 (17)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 34671x + 209.57, r^2 = 0.9996	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ
				0.01	398→133	0.01 0.10 1.0	72.1 – 81.7 (77.5, 6) 76.4 – 86.4 (83.0, 6) 72.2 – 84.9 (80.1, 5)	4.8 (6) 5.1 (6) 6.5 (5) Overall: 5.9 (17)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 13546x + 90.306, r^2 = 0.9996	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
										samples. No interference >30% of LOQ
			Coffee	0.01	398→182	0.01 0.10 1.0	93.3 – 105 (97.8, 6) 84.5 – 92.3 (89.0, 6) 88.2 – 93.9 (90.0, 6)	4.0 (6) 3.2 (6) 2.2 (6) Overall: 5.4 (6)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 27747x + 224.1, r^2 = 0.9995	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ
				0.01	398→133	0.01 0.10 1.0	90.0 – 107 (99.7, 6) 86.0 – 96.6 (93.7, 6) 87.7 – 95.0 (91.2, 6)	6.1 (6) 4.6 (6) 3.4 (6) Overall:	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 11853x +93.698, r^2 = 0.9985	Acceptable chromatograms presented for calibration standards, reagent blank, control

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
								6.5 (18)		sample and fortified samples. No interference >30% of LOQ
			Dry beans	0.01	398→182	0.01 0.10 1.0	82.4 – 89.4 (85.3, 6) 77.0 – 84.7 (81.7, 6) 74.5 – 83.4 (78.3, 6)	2.8 (6) 3.4 (6) 4.6 (6) Overall: 4.9 (18)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 82771x + 289.42, r^2 = 0.9996	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ
				0.01	398→133	0.01 0.10 1.0	77.1 – 89.7 (82.6, 6) 70.0 – 92.6 (82.9, 6) 75.8 – 84.6	6.5 (6) 9.2 (6) 4.1 (6)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 12740x + 70.108, r^2	Acceptable chromatograms presented for calibration standards, reagent

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
							(80.6, 6)	Overall: 6.7 (6)	= 0.9997	blank, control sample and fortified samples. No interference >30% of LOQ
			Soybeans	0.01	398→182	0.01 0.10 1.0	78.4 – 91.7 (82.9, 5) 84.6 - 92.3 (86.6, 6) 85.3 – 92.9 (87.9, 5)	6.2 (5) 3.3 (6) 3.3 (5) Overall: 5.0 (16)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 28723x +223.39, r^2 = 0.9995	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
				0.01	398→133	0.01 0.10 1.0	80.4 – 89.9 (84.3, 5) 83.4 – 96.4 (89.5, 6) 85.7 – 97.2 (90.8, 5)	4.5 (5) 6.2 (6) 4.7 (5) Overall: 5.9 (16)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 12007x + 131.34, r^2 = 0.9993	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ
			Tomato	0.01	398→182	0.01 0.10 1.0	77.8 – 96.2 (86.9, 5) 73.4 – 84.7 (78.8, 6) 70.1 – 78.9 (73.2, 5)	8.4 (5) 6.2 (6) 4.8 (5) Overall: 9.5 (16)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 187828x – 1266.1, r^2 = 0.9996	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
										>30% of LOQ
				0.01	398→133	0.01 0.10 1.0	81.1 – 91.2 (86.3, 5) 73.2 – 83.4 (79.1, 6) 70.5 – 80.3 (74.9, 5)	5.1 (5) 4.8 (6) 4.8 (5) Overall: 7.5 (16)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 49832x – 168.63, r^2 = 0.9994	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ
			Wheat grain	0.01	398→182	0.01 0.10 1.0	77.6 – 96.7 (83.8, 5) 82.5 – 85.0 (85.4, 6) 79.0 – 105 (87.2, 6)	9.2 (5) 2.8 (6) 10 (6) Overall:	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 34592x + 240.68, r^2 = 0.9998	Acceptable chromatograms presented for calibration standards, reagent blank, control

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
								7.8 (17)		sample and fortified samples. No interference >30% of LOQ
				0.01	398→133	0.01 0.10 1.0	73.1 – 97.1 (81.3, 5) 79.7 – 84.2 (82.5, 6) 72.7 – 102 (83.7, 6)	11 (5) 2.5 (6) 12 (6) Overall: 9.2 (17)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 13252x + 142.19, r^2 = 0.9996	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ
			Wheat straw	0.01	398→182	0.01 0.10 1.0	72.8 – 91.8 (85.9, 6) 74.9 – 86.5 (80.4, 6) 74.4 – 86.3	8.6 (6) 6.3 (6) 6.5 (6)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 117410x + 442.57, r^2	Acceptable chromatograms presented for calibration standards, reagent

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
							(80.3, 6)	Overall: 7.6 (18)	= 0.9989	blank, control sample and fortified samples. No interference >30% of LOQ
				0.01	398→133	0.01 0.10 1.0	71.8 – 96.5 (87.0, 6) 75.0 – 85.9 (81.0, 6) 77.2 – 86.3 (82.9, 6)	10.1 (6) 4.5 (6) 4.0 (6)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 28613 x + 1.5024, r^2 = 0.9981	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
		UPLC	Citrus	0.01	398→182	0.01 0.10 1.0	70.5 – 81.0 (78.4, 6) 75.0 – 87.0 (80.6, 6) 70.5 – 91.5 (79.0, 5)	5.1 (6) 5.4 (6) 12 (5) Overall: 7.5 (17)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 5441.3x – 4.034, r^2 = 0.9991	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ
				0.01	398→133	0.01 0.10 1.0	75.1 – 84.9 (81.2, 6) 74.4 – 92.8 (85.7, 6) 70.2 – 86.9 (77.5, 5)	4.3 (6) 9.6 (6) 9.3 (5) Overall: 8.6 (17)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 2168.8x + 21.357, r^2 = 0.9956	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
										>30% of LOQ
			Coffee	0.01	398→182	0.01 0.10 1.0	78.1 – 88.5 (82.7, 6) 83.1 – 90.3 (85.9, 6) 84.1 – 90.3 (87.6, 6)	4.4 (6) 3.3 (6) 3.3 (6) Overall: 4.0 (18)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 8296.8x + 0.2895, r^2 = 0.9978	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ
				0.01	398→133	0.01 0.10 1.0	74.8 – 88.4 (81.2, 6) 78.7 – 94.3 (86.9, 6) 78.5 – 89.1 (84.6, 6)	6.6 (6) 6.2 (6) 5.0 (6) Overall:	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 8778.6x + 14.394, r^2 = 0.9984	Acceptable chromatograms presented for calibration standards, reagent blank, control

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
								6.3 (18)		sample and fortified samples. No interference >30% of LOQ
			Dry beans	0.01	398→182	0.01 0.10 1.0	83.5 – 96.6 (91.0, 6) 80.2 – 98.7 (86.8, 6) 79.9 – 89.0 (83.5, 6)	5.7 (6) 7.9 (6) 4.8 (6) Overall: 6.9 (18)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y =24606x – 193.52, r^2 = 0.9994	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ
				0.01	398→133	0.01 0.10 1.0	81.5 – 103 (90.7, 6) 74.7 – 94.4 (84.0, 6) 75.0 – 91.9	10 (6) 9.2 (6) 8.2 (6)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 6682x – 66.158, r^2 =	Acceptable chromatograms presented for calibration standards, reagent

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
							(82.6, 6)	Overall: 9.7 (18)	0.9978	blank, control sample and fortified samples. No interference >30% of LOQ
			Soybeans	0.01	398→182	0.01 0.10 1.0	87.5 – 104 (94.1, 5) 84.3 – 97.0 (86.1, 6) 83.8 – 95.1 (88.6, 5)	7.0 (5) 1.1 (6) 4.7 (5) Overall: 6.0 (16)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 49017x – 93.864, r^2 = 0.9965	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
				0.01	398→133	0.01 0.10 1.0	71.8 – 79.9 (77.9, 5) 70.7 – 90.4 (82.0, 6) 79.6 – 88.6 (82.4, 5)	4.4 (5) 6.6 (6) 4.3 (5) Overall: 6.6 (16)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 9903.8x – 45.525, r^2 = 0.9991	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ
			Tomato	0.01	398→182	0.01 0.10 1.0	82.1 – 97.3 (88.7, 5) 73.4 – 92.2 (81.5, 6) 70.0 – 82.2 (76.5, 5)	6.9 (5) 8.1 (6) 7.4 (5) Overall: 9.3 (16)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 24599x – 153.69, r^2 = 0.9968	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
										>30% of LOQ
				0.01	398→133	0.01 0.10 1.0	71.8 – 85.2 (77.9, 5) 72.3 – 87.8 (78.8, 6) 72.3 – 87.3 (79.4, 5)	6.3 (5) 9.2 (6) 8.0 (5) Overall: 7.5 (16)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 6623.3x – 102.82, r^2 = 0.9992	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ
			Wheat grain	0.01	398→182	0.01 0.10 1.0	72.0 – 84.3 (77.5, 6) 70.0 – 93.4 (81.7, 6) 85.9 – 111 (92.2, 5)	6.8 (6) 9.4 (6) 11 (5) Overall:	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 5869.5x – 26.601, r^2 = 0.9978	Acceptable chromatograms presented for calibration standards, reagent blank, control

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
								12 (17)		sample and fortified samples. No interference >30% of LOQ
				0.01	398→133	0.01 0.10 1.0	73.9 – 106 (89.5, 6) 85.6 – 104 (95.1, 6) 81.0 – 109 (89.9, 5)	14 (6) 8.0 (6) 12 (5) Overall: 11 (17)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 1397x + 2.892, r^2 = 0.9967	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ
			Wheat straw	0.01	398→182	0.01 0.10 1.0	70.1 – 77.1 (73.4, 5) 71.1 – 82.6 (77.7, 6) 70.5 – 96.1	4.1 (5) 5.8 (6) 11 (6)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 7987.3x + 37.468, r^2	Acceptable chromatograms presented for calibration standards, reagent

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
							(84.5, 6)	Overall: 9.4 (17)	= 0.9968	blank, control sample and fortified samples. No interference >30% of LOQ
				0.01	398→133	0.01 0.10 1.0	70.4 – 84.7 (82.4, 5) 76.6 – 88.3 (84.1, 6) 76.7 – 90.4 (82.5, 6)	9.6 (5) 6.2 (6) 7.0 (6) Overall: 7.1 (17)	0.0400 – 2.00 ng/mL [Approx. 0.002 – 0.1 mg/kg] 6 standards, y = 5565.2x + 18.017, r^2 = 0.9991	Acceptable chromatograms presented for calibration standards, reagent blank, control sample and fortified samples. No interference >30% of LOQ

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
CA 4.1.2/26 Class T., 2011 a	Triazole	HPLC	Tomato	0.01	*m/z* 70→43	0.01 1.0	95 – 122 (105, 5) 92 – 103 (98, 5)	10 (5) 5 (5) Overall: 8 (10)	1 – 600 ng/mL [Approx. 0.002 – 1.20 mg/kg] C18 column: 8 standards, y = 0.837x + 0.00158, r = 0.9993	Acceptable chromatograms presented for calibration standards, and control and fortified samples for tomato, grain, orange, bean seed and oilseed rape No interference >30% of LOQ
			Cucumber	0.01	*m/z* 70→43	0.01 1.0	77 – 103 (90, 3) 97 – 102 (100, 3)	14 (3) 3 (3) Overall: 11 (6)		
			Lettuce	0.01	*m/z* 70→43	0.01 1.0	81 – 95 (88, 3) 101 – 103 (102, 3)	8 (3) 1 (3) Overall: 9 (6)		
			Sweet pepper	0.01	*m/z* 70→43	0.01 1.0	81 – 99 (87, 3) 96 – 113 (107, 3)	11 (3) 9 (3) Overall: 14 (6)		

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
			Carrot leaf	0.01	*m/z* 70→43	0.01 1.0	108 – 120 (112, 3) 90 – 102 (97, 3)	6 (3) 6 (3) Overall: 10 (6)		
			Carrot root	0.01	*m/z* 70→43	0.01 1.0	85 – 94 (90, 3) 95 – 104 (98, 3)	5 (3) 6 (3) Overall: 7 (6)		
			Cereal grain	0.01	*m/z* 70→43	0.01 1.0	108 – 121 (115, 5) 107 – 122 (118, 5)	4 (5) 5 (5) Overall: 5 (10)		
			Cereal straw	0.01	*m/z* 70→43	0.01 1.0	93 – 129 (109, 3) 83 – 121 (102, 3)	17 (3) 19 (3) Overall: 16 (6)		

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
			Cereal green plant	0.01	*m/z* 70→43	0.01 1.0	101 – 115 (109, 3) 112 – 121 (116, 3)	6 (3) 4 (3) Overall: 6 (6)		
			Dry bean seed	0.01	*m/z* 70→43	0.01 1.0	95 – 111 (104, 5) 89 – 106 (96, 5)	8 (5) 8 (5) Overall: 9 (10)		
			Whole orange	0.01	*m/z* 70→43	0.01 1.0	86 – 113 (100, 5) 96 – 102 (100, 5)	10 (5) 2 (5) Overall: 7 (10)		
			Oilseed rape seed	0.01	*m/z* 70→43	0.01 1.0	96 – 114 (102, 5) 83 – 98 (93, 5)	7 (5) 6 (5) Overall: 8 (10)		

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
			Melon peel	0.01	*m/z* 70→43	0.01 1.0	81 – 101 (94, 3) 100 – 114 (108, 3)	12 (3) 7 (3) Overall: 11 (6)		
			Melon fruit	0.01	*m/z* 70→43	0.01 1.0	96 – 100 (98, 3) 93 – 110 (100, 3)	2 (3) 9 (3) Overall: 6 (6)		
			Melon pulp	0.01	*m/z* 70→43	0.01 1.0	93 – 103 (97, 3) 108 – 113 (110, 3)	5 (3) 2 (3) Overall: 8 (6)		
	Triazolyl acetic acid		Tomato	0.01	*m/z* 70→43	0.01 1.0	85 – 96 (90, 5) 97 – 109 (101, 5)	5 (5) 5 (5) Overall: 7 (10)	1 – 600 ng/mL [Approx. 0.002 – 1.20 mg/kg] C18 column: 8 standards, y =	Acceptable chromatograms presented for calibration standards, and control

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
			Cucumber	0.01	*m/z* 70→43	0.01 1.0	90 – 109 (100, 3) 101 – 109 (105, 3)	10 (3) 4 (3) Overall: 7 (6)	1.01x + 0.0018, r = 0.9991	and fortified samples for tomato, grain, orange, bean seed and oilseed rape No interference >30% of LOQ
			Lettuce	0.01	*m/z* 70→43	0.01 1.0	100 – 110 (105, 3) 99 – 108 (104, 3)	5 (3) 5 (3) Overall: 4 (6)		
			Sweet pepper	0.01	*m/z* 70→43	0.01 1.0	104 – 107 (106, 3) 110 – 111 (110, 3)	1 (3) 1 (3) Overall: 3 (6)		
			Carrot leaf	0.01	*m/z* 70→43	0.01 1.0	97 – 120 (106, 3) 104 – 111 (108, 3)	11 (3) 3 (3) Overall: 8 (6)		

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
			Carrot root	0.01	*m/z* 70→43	0.01 1.0	96 – 114 (104, 3) 100 – 108 (105, 3)	9 (3) 4 (3) 6 (6)		
			Cereal grain	0.01	*m/z* 70→43	0.01 1.0	84 – 105 (97, 5) 74 – 83 (80, 5)	9 (5) 5 (5) Overall: 13 (10)		
			Cereal straw	0.01	*m/z* 70→43	0.01 1.0	88 – 121 (109, 3) 82 – 98 (90, 3)	17 (3) 9 (3) Overall: 16 (6)		
			Cereal green plant	0.01	*m/z* 70→43	0.01 1.0	95 – 108 (103, 3) 98 – 107 (102, 3)	7 (3) 5 (3) Overall: 5 (6)		

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
			Dry bean seed	0.01	*m/z* 70→43	0.01 1.0	92 – 118 (103, 5) 67 – 80 (72, 5)	11 (5) 7 (5) Overall: 21 (10)		
			Whole orange	0.01	*m/z* 70→43	0.01 1.0	89 – 95 (92, 5) 89 – 96 (92, 5)	3 (5) 3 (5) Overall: 3 (10)		
			Oilseed rape seed	0.01	*m/z* 70→43	0.01 1.0	82 – 115 (99, 5) 91 – 100 (95, 5)	13 (5) 4 (5) Overall: 9 (10)		
			Melon peel	0.01	*m/z* 70→43	0.01 1.0	87 – 89 (92, 3) 94 – 98 (96, 3)	7 (3) 2 (3) Overall: 5 (6)		

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
			Melon fruit	0.01	*m/z* 70→43	0.01 1.0	92 – 102 (97, 3) 109 – 112 (110, 3)	5 (3) 2 (3) Overall: 8 (6)		
			Melon pulp	0.01	*m/z* 70→43	0.01 1.0	97 – 103 (99, 3) 97 – 110 (105, 3)	3 (3) 7 (3) Overall: 6 (6)		
	Triazolylalanine		Tomato	0.01	*m/z* 70→43	0.01 1.0	102 – 139 (111, 5) 92 – 127 (110, 5)	14 (5) 12 (5) Overall: 12 (10)	1 – 600 ng/mL [Approx. 0.002 – 1.20 mg/kg] C18 column: 8 standards, y = 1.95x – 0.000528, r = 0.9987 Hypercarb column: 8 standards, y = 1.61x + 0.00488, r = 0.9999	Acceptable chromatograms presented for calibration standards, and control and fortified samples for tomato, grain, orange, bean seed and sunflower
			Cucumber	0.01	*m/z* 70→43	0.01 1.0	94 – 122 (111, 3) 101 – 116 (109, 3)	13 (3) 7 (3) Overall: 10 (6)		

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
			Lettuce	0.01	*m/z* 70→43	0.01 1.0	110 – 124 (116, 3) 96 – 116 (106, 3)	6 (3) 9 (3) Overall: 9 (6)		seed. No interference >30% of LOQ
			Sweet pepper	0.01	*m/z* 70→43	0.01 1.0	79 – 118 (104, 3) 93 – 112 (103, 3)	21 (3) 9 (3) Overall: 15 (6)		
			Carrot leaf	0.01	*m/z* 70→43	0.01 1.0	107 – 130 (118, 3) 96 – 126 (110, 3)	10 (3) 14 (3) Overall: 11 (6)		
			Carrot root	0.01	*m/z* 70→43	0.01 1.0	89 – 106 (98, 3) 104 – 106 (105, 3)	9 (3) 1 (3) Overall: 6 (6)		

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
			Cereal grain	0.01	*m/z* 70→43	0.01 1.0	85 – 111 (91, 5) 78 – 91 (84, 5)	12 (5) 6 (5) Overall: 11 (10)		
			Cereal straw	0.01	*m/z* 70→43	0.01 1.0	67 – 96 (79, 3) 75 – 76 (76, 3)	19 (3) 1 (3) Overall: 12 (6)		
			Cereal green plant	0.01	*m/z* 70→43	0.01 1.0	98 – 116 (108, 3) 95 – 103 (100, 3)	8 (3) 4 (3) Overall: 7 (6)		
			Dry bean seed	0.01	*m/z* 70→43	0.01 1.0	78 – 103 (88, 5) 74 – 92 (81, 5)	12 (5) 9 (5) Overall: 11 (10)		

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
			Whole orange	0.01	*m/z* 70→43	0.01 1.0	82 – 96 (90, 5) 95 – 102 (100, 5)	6 (5) 3 (5) Overall: 7 (10)		
			Melon peel	0.01	*m/z* 70→43	0.01 1.0	68 – 119 (97, 3) 90 – 102 (96, 3)	27 (3) 7 (3) Overall: 18 (6)		
			Melon fruit	0.01	*m/z* 70→43	0.01 1.0	91 – 109 (101, 3) 101 – 114 (107, 3)	9 (3) 6 (3) Overall: 7 (6)		
			Melon pulp	0.01	*m/z* 70→43	0.01 1.0	55 – 100 (77, 3) 102 – 116 (110, 3)	29 (3) 7 (3) Overall: 25 (6)		

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
			Sunflower seed	0.01	*m/z* 70→43	0.01 1.0	72 – 126 (101, 5) 85 – 94 (92, 5)	25 (5) 5 (5) Overall: 19 (10)		
	Triazole lactic acid		Tomato	0.01	*m/z* 70→43	0.01 1.0	78 – 107 (92, 5) 108 – 122 (114, 5)	13 (5) 5 (5) Overall: 14 (10)	1 – 600 ng/mL [Approx. 0.002 – 1.20 mg/kg] C18 column: 8 standards, y = 1.07x – 0.00333, r = 0.9997 Hypercarb column: 8 standards, y = 0.801x + 0.0073, r = 0.9972	Acceptable chromatograms presented for calibration standards, and control and fortified samples for tomato, grain, orange, bean seed and oilseed rape No interference >30% of LOQ
			Cucumber	0.01	*m/z* 70→43	0.01 1.0	94 – 106 (100, 3) 107 – 111 (108, 3)	6 (3) 2 (3) Overall: 6 (6)		
			Lettuce	0.01	*m/z* 70→43	0.01 1.0	102 – 114 (108, 3) 99 – 112 (104, 3)	6 (3) 7 (3) Overall: 6 (6)		

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
			Sweet pepper	0.01	*m/z* 70→43	0.01 1.0	96 – 114 (107, 3) 109 – 110 (110, 3)	9 (3) 1 (3) Overall: 6 (6)		
			Carrot leaf	0.01	*m/z* 70→43	0.01 1.0	114 – 126 (118, 3) 99 – 106 (102, 3)	6 (3) 4 (3) Overall: 9 (6)		
			Carrot root	0.01	*m/z* 70→43	0.01 1.0	99 – 110 (105, 3) 101 – 109 (106, 3)	5 (3) 4 (3) Overall: 4 (6)		
			Cereal grain	0.01	*m/z* 70→43	0.01 1.0	78 – 83 (80, 5) 74 – 84 (79, 5)	3 (5) 5 (5) Overall: 4 (10)		

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
			Cereal straw	0.01	*m/z* 70→43	0.01 1.0	93 – 104 (100, 3) 76 – 95 (85, 3)	6 (3) 12 (3) Overall: 12 (6)		
			Cereal green plant	0.01	*m/z* 70→43	0.01 1.0	82 – 92 (89, 3) 94 – 103 (98, 3)	7 (3) 5 (3) Overall: 8 (6)		
			Dry bean seed	0.01	*m/z* 70→43	0.01 1.0	84 – 99 (91, 5) 88 – 99 (94, 5)	6 (5) 5 (5) Overall: 5 (10)		
			Whole orange	0.01	*m/z* 70→43	0.01 1.0	89 – 104 (95, 5) 81 – 97 (92, 5)	6 (5) 7 (5) Overall: 6 (10)		

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
			Oilseed rape seed	0.01	*m/z* 70→43	0.01 1.0	71 – 90 (82, 5) 94 – 101 (98, 5)	10 (5) 2 (5) Overall: 12 (10)		
			Melon peel	0.01	*m/z* 70→43	0.01 1.0	102 – 110 (105, 3) 89 – 97 (93, 3)	4 (3) 4 (3) Overall: 7 (6)		
			Melon fruit	0.01	*m/z* 70→43	0.01 1.0	94 – 113 (106, 3) 108 – 111 (109, 3)	10 (3) 2 (3) Overall: 6 (6)		
			Melon pulp	0.01	*m/z* 70→43	0.01 1.0	95 – 114 (103, 3) 103 – 112 (108, 3)	10 (3) 4 (3) Overall: 7 (6)		
CA 4.1.2/27 Devine C., 2015 a	BAS 750 F	LC-MS/MS	Bovine meat	398 → 182	0.01	0.01 0.10	80.4 – 89.4 (85.0, 5) 109 – 110	4.4 (5) 0.4 (5)	0.04 – 10 ng/mL [Approx. 0.002 – 0.5 mg/kg]	Acceptable chromatograms presented for

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
							(110, 5)	Overall: 14 (10)	8 standards, r = 1.0000, y = 91929x + 1603	calibration standards, blank matrix and fortified sample. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions (*m/z* 398 → 182, 398 → 133)
				298 → 133	0.01	0.01 0.10	85.3 – 98.3 (93.0, 5) 106 – 111 (108, 5)	5.8 (5) 1.8 (5) Overall: 8.8 (10)	0.04 – 10 ng/mL [Approx. 0.002 – 0.5 mg/kg] 8 standards, r = 1.0000, y = 22198x - 169	
	BAS 750 F		Bovine milk	398 → 182	0.01	0.01 0.10	71.9 – 89.1 (82.0, 5) 84.5 – 87.1 (85.8, 5)	8.8 (5) 1.4 (5) Overall: 6.3 (10)	0.04 – 10 ng/mL [Approx. 0.0008 – 0.2 mg/kg] 8 standards, r = 0.9996, y = 59437x + 828	Acceptable chromatograms presented for calibration standards, blank matrix and fortified sample. No
				298 → 133	0.01	0.01	68.3 – 82.7 (76.5, 5)	7.0 (5)	0.04 – 10 ng/mL [Approx. 0.0008 –	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
						0.10	83.4 – 89.4 (86.3, 5)	2.9 (5) Overall: 8.0 (10)	0.2 mg/kg] 8 standards, r = 0.9999, y = 13813x + 187	interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions (*m/z* 398 → 182, 398 → 133)
	BAS 750 F		Bovine cream	398 → 182	0.01	0.01 0.10	69.1 – 75.3 (72.6, 5) 79.2 – 92.8 (86.4, 5)	3.2 (5) 5.6 (5) Overall: 10 (10)	0.04 – 10 ng/mL [Approx. 0.0008 – 0.2 mg/kg] 8 standards, r = 0.9996, y = 59437x + 828	Acceptable chromatograms presented for calibration standards, blank matrix and fortified sample. No interference >30% of LOQ Identity confirmed by LC-
				298 → 133	0.01	0.01 0.10	69.0 – 75.2 (72.1, 5) 81.8 – 92.0 (86.9, 5)	3.8 (5) 4.4 (5) Overall: 11 (10)	0.04 – 10 ng/mL [Approx. 0.0008 – 0.2 mg/kg] 8 standards, r = 0.9999, y = 13813x + 187	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
										MS/MS monitoring two mass transitions (*m/z* 398 → 182, 398 → 133)
	BAS 750 F		Bovine fat	398 → 182	0.01	0.01 0.10	70.1 – 94.1 (80.2, 5) 99.0 – 111 (104, 5)	11 (5) 4.9 (5) Overall: 16 (10)	0.04 – 10 ng/mL [Approx. 0.0008 – 0.2 mg/kg] 8 standards, r = 0.9998, y = 66690x – 601	Acceptable chromatograms presented for calibration standards, blank matrix and fortified sample. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions (*m/z* 398 → 182, 398 → 133)
				298 → 133	0.01	0.01 0.10	71.4 – 89.0 (80.8, 5) 98.8 – 109 (104, 5)	8.6 (5) 4.7 (5) Overall: 15 (10)	0.04 – 10 ng/mL [Approx. 0.0008 – 0.2 mg/kg] 8 standards, r = 0.9997, y = 15869x - 305	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	BAS 750 F		Bovine liver	398 → 182	0.01	0.01 0.10	83.2 – 94.0 (87.5, 5) 92.5 – 101 (96.4, 5)	5.2 (5) 3.8 (5) Overall: 6.6 (10)	0.04 – 10 ng/mL [Approx. 0.002 – 0.5 mg/kg] 8 standards, r = 1.0000, y = 107278x - 573	Acceptable chromatograms presented for calibration standards, blank matrix and fortified sample. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions (*m/z* 398 → 182, 398 → 133)
				298 → 133	0.01	0.01 0.10	84.6 – 92.0 (88.3, 5) 91.4 – 99.6 (95.3, 5)	3.9 (5) 3.2 (5) Overall: 5.3 (10)	0.04 – 10 ng/mL [Approx. 0.002 – 0.5 mg/kg] 8 standards, r = 1.0000, y = 25104x - 281	
	BAS 750 F		Bovine kidney	398 → 182	0.01	0.01 0.10	94.5 – 100 (96.2, 5) 94.2 – 106 (101, 5)	2.3 (5) 4.6 (5) Overall: 4.4 (10)	0.04 – 10 ng/mL [Approx. 0.002 – 0.5 mg/kg] 8 standards, r = 0.9999, y = 73768x -	Acceptable chromatograms presented for calibration standards, blank matrix

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
									2677	and fortified sample. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions (*m/z* 398 → 182, 398 → 133)
				298 → 133	0.01	0.01 0.10	94.3 – 99.3 (95.9, 5) 89.1 – 104 (100, 5)	2.1 (5) 6.7 (5) Overall: 5.2 (10)	0.04 – 10 ng/mL [Approx. 0.002 – 0.5 mg/kg] 8 standards, r = 0.9998, y = 17670x - 604	
	BAS 750 F		Hen eggs	398 → 182	0.01	0.01 0.10	90.9 – 97.2 (93.3, 5) 102 – 107 (105, 4*) *Dixon outlier excluded	3.1 (5) 2.0 (4) Overall: 6.6 (9)	0.04 – 10 ng/mL [Approx. 0.002 – 0.5 mg/kg] 8 standards, r = 0.9999, y = 113828x + 3980	Acceptable chromatograms presented for calibration standards, blank matrix and fortified sample. No interference >30% of LOQ
				298 → 133	0.01	0.01 0.10	83.7 – 99.7 (92.9, 5) 110 – 111 (110, 4*)	6.4 (5) 0.5 (4)	0.04 – 10 ng/mL [Approx. 0.002 – 0.5 mg/kg]	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
							*Dixon outlier excluded	Overall: 10 (9)	8 standards, r = 1.0000, y = 26641x + 785	Identity confirmed by LC-MS/MS monitoring two mass transitions (*m/z* 398 → 182, 398 → 133)
CA 4.1.2/28 Heger N.,Taraschewski I., 2016 b CA 4.1.2/32 Heger N., 2017 a	M750F022	GC	Cow liver	295	0.01	0.01 0.1	78.1 – 89.0 (83.9, 5) 62.0 – 77.1 (71.0, 5)	5.8 (5) 8.6 (5) Overall: 11 (10)	2.5 – 100 ng/mL [Approx. 0.004 – 0.13 mg/kg] 6 standards, r = 0.9987, y = 1.76×10^{3}x + 592	Acceptable chromatograms presented for standard solutions, controls and fortified samples. No interference >30% of LOQ Identity confirmed by GC-MS monitoring 3 ion fragments (*m/z* 295,
				297	0.01	0.01 0.1	79.6 – 87.8 (82.8, 5) 64.3 – 77.1 (70.8, 5)	3.9 (5) 8.1 (5) Overall: 10 (10)	2.5 – 100 ng/mL [Approx. 0.004 – 0.13 mg/kg] 6 standards, r = 0.9978, y = 578x – 214	
				317	0.01	0.01 0.1	78.7 – 85.5 (82.1, 5) 65.4 – 76.6	3.4 (5) 7.0 (5)	2.5 – 100 ng/mL [Approx. 0.004 – 0.13 mg/kg]	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
							(71.2, 5)	Overall: 9.0 (10)	6 standards, r = 0.9983, y = 184x – 38.8	297, 317)
	M750F022		Cow kidney	295	0.01	0.01 0.1	81.6 – 93.4 (89.2, 5) 70.8 – 80.0 (75.6, 5)	5.6 (5) 4.6 (5) Overall: 10 (10)	2.5 – 100 ng/mL [Approx. 0.004 – 0.13 mg/kg] 6 standards, r = 0.9996, y = 1.39×10^3x – 412	Acceptable chromatograms presented for standard solutions, controls and fortified samples. No interference >30% of LOQ Identity confirmed by GC-MS monitoring 3 ion fragments (*m/z* 295, 297, 317)
				297	0.01	0.01 0.1	79.6 – 86.8 (84.1, 4*) 70.3 – 75.9 (73.4, 4*) *Only 4 replicates were used in the calculation of recoveries and RSDs for cow kidney at the m/z 297 transition as one sample	3.7 (4) 3.5 (4) Overall: 8.0 (8)	2.5 – 100 ng/mL [Approx. 0.004 – 0.13 mg/kg] 6 standards, r = 0.9993, y = 462x - 535	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
							had a large interfering signal.			
				317	0.01	0.01 0.1	81.6 – 91.5 (86.5, 5) 71.9 – 82.3 (77.3, 5)	4.3 (5) 5.0 (5) Overall: 7.3 (10)	2.5 – 100 ng/mL [Approx. 0.004 – 0.13 mg/kg] 6 standards, r = 0.9992, y = 135x – 93.6	
	M750F022		Cow muscle	295	0.01	0.01 0.1	75.1 – 89.7 (82.4, 5) 73.8 – 89.9 (79.5, 5)	7.7 (5) 7.9 (5) Overall: 7.6 (10)	2.5 – 100 ng/mL [Approx. 0.003 – 0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, r = 0.9993, y = 1.82×10^3x – 1.29×10^3	Acceptable chromatograms presented for standard solutions, controls and fortified samples. No interference >30% of LOQ Identity confirmed by GC-MS monitoring 3 ion fragments (*m/z* 295,
				297	0.01	0.01 0.1	74.6 – 84.4 (79.2, 5.5) 73.8 – 93.8 (80.4, 5)	5.5 (5) 9.9 (5) Overall: 7.6 (10)	2.5 – 100 ng/mL [Approx. 0.003 – 0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, r =	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
									0.9993, y = 608x - 374	297, 317)
				317	0.01	0.01 0.1	72.5 – 87.4 (79.9, 5) 73.1 – 91.9 (79.0, 5)	6.9 (5) 9.4 (5) Overall: 7.8 (10)	2.5 – 100 ng/mL [Approx. 0.003 – 0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, r = 0.9986, y = 180x + 67.6	
	M750F022		Cow fat	295	0.01	0.01 0.1	83.8 – 93.1 (87.9, 5) 84.4 – 99.2 (88.6, 5)	5.23 (5) 7.02 (5) Overall: 5.86 (10)	2.5 – 100 ng/mL [Approx. 0.003 – 0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, r = 0.9964, y = 1.18×10^3x + 1.62×10^3	Acceptable chromatograms presented for standard solutions, controls and fortified samples. No interference >30% of LOQ Identity confirmed by GC-MS monitoring 3 ion
				297	0.01	0.01 0.1	84.9 - 94 (87.7, 5) 83.0 – 99.2 (88.1, 5)	4.31 (5) 7.59 (5) Overall: 5.83 (10)	2.5 – 100 ng/mL [Approx. 0.003 – 0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range]	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
									6 standards, r = 0.9963, y = 397x + 383	fragments (*m/z* 295, 297, 317)
				317	0.01	0.01 0.1	83.1 – 93.8 (90.2, 5) 82.3 – 97.8 (89.3, 5)	8.66 (5) 6.04 (5) Overall: 5.35(10)	2.5 – 100 ng/mL [Approx. 0.003 – 0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, r = 0.9974, y = 122x + 50.2	
	M750F022		Cow milk	295	0.01	0.01 0.1	82.7 – 92.0 (86.1, 5) 85.5 – 91.9 (88.1, 5)	4.57 (5) 2.95 (5) Overall: 3.82 (10)	2.5 – 100 ng/mL [Approx. 0.003 – 0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, r = 0.9986, y = 1.39×10^{3}x – 939	Acceptable chromatograms presented for standard solutions, controls and fortified samples. No interference >30% of LOQ Identity confirmed by GC-MS
				297	0.01	0.01 0.1	74.0 – 92.4 (79.8, 5) 87.0 – 93.1 (89.5, 5)	9.19 (5) 2.72 (5) Overall:	2.5 – 100 ng/mL [Approx. 0.003 – 0.1 mg/kg assuming redissolved in 2 mL to bring within	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
								8.54 (10)	calibration range] 6 standards, r = 0.9985, y = 460x – 365	monitoring 3 ion fragments (*m/z* 295, 297, 317)
				317	0.01	0.01 0.1	74.0 - 82.9 (78.8, 5) 91.7 - 100 (96.2, 5)	5.52 (5) 3.32 (5) Overall: 11.3 (10)	2.5 – 100 ng/mL [Approx. 0.003 – 0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, r = 0.9989, y = 128x – 85.3	
	M750F022		Hen egg	295	0.01	0.01 0.1	77.0 – 95.4 (85.6, 5) 94.8 – 117 (102, 5)		2.5 – 100 ng/mL [Approx. 0.003 – 0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, r = 0.9992, y = 487 x – 366	Acceptable chromatograms presented for standard solutions, controls and fortified samples. No interference >30% of LOQ Identity confirmed
				297	0.01	0.01 0.1	76.5 – 91.3 (84.1, 5) 96.1 – 115 (102, 5)	7.1 (5) 7.5 (5)	2.5 – 100 ng/mL [Approx. 0.003 – 0.1 mg/kg assuming redissolved in 2 mL	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
								Overall: 12 (10)	to bring within calibration range] 6 standards, r = 0.9994, y = 163x – 142	by GC-MS monitoring 3 ion fragments (*m/z* 295, 297, 317)
				317	0.01	0.01 0.1	74.4 – 85.3 (81.1, 5) 95.1 – 119 (104, 5)	6.3 (5) 9.6 (5) Overall: 15 (10)	2.5 – 100 ng/mL [Approx. 0.003 – 0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, r = 0.9987, y = 50.3x – 2.51	
CA 4.1.2/29 Guedez Orozco A.A.,Heger N., 2016 a	M750F025 (Reg.No. 6056452) measured as M750F022	HPLC	Hen egg	0.01	*m/z* 295	0.01 0.1	81.2 – 93.3 (86.7, 5) 79.6 – 89.8 (84.4, 5)	6.6 (5) 5.3 (5) Overall: 5.8 (10)	Solvent based standards used: 2.5 – 100 ng/mL [Approx. 0.003-0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, y = 1.52×10^3 – 1.15×10^3, r = 0.9985	Acceptable chromatograms presented for calibration standards, fortified samples and control samples. No interference >30% of

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
				0.01	*m/z* 297	0.01 0.1	79.3 - 88.3 (83.4, 5) 79.3 – 90.1 (84.2, 5)	5 (5) 5.8 (5) Overall: 5.1 (10)	Solvent based standards used: 2.5 – 100 ng/mL [Approx. 0.003-0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, y = 509x – 609, r = 0.9975	LOQ
				0.01	*m/z* 317	0.01 0.1	77.0 – 90.8 (82.3, 5) 79.1 – 90.6 (84.1, 5)	7.1 (5) 5.4 (5) Overall: 6.1 (10)	Solvent based standards used: 2.5 – 100 ng/mL [Approx. 0.003-0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, y = 151x – 167, r = 0.9969	
			Hen liver	0.01	*m/z* 295	0.01 0.1	63.3 – 89.0 (75.4, 5) 85.0 – 93.3 (90, 5)	16 (5) 3.4 (5) Overall:	Solvent based standards used: 2.5 – 100 ng/mL [Approx. 0.003-0.1	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
								13 (10)	mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, y = 1.52×10^3 – 1.15×10^3, r = 0.9985	
				0.01	*m/z* 297	0.01 0.1	66.8 – 88.7 (75.3, 5) 86.0 – 92.3 (89.6, 5)	12 (5) 2.7 (5) Overall: 12 (10)	Solvent based standards used: 2.5 – 100 ng/mL [Approx. 0.003-0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, y = 509x – 609, r = 0.9975	
				0.01	*m/z* 317	0.01 0.1	70.7 – 78.1 (70.5, 5) 56.6 – 68.4 (88.1, 5)	15 (5) 8.1 (5) Overall: 16 (10)	Solvent based standards used: 2.5 – 100 ng/mL [Approx. 0.003-0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range]	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
									6 standards, y = 151x – 167, r = 0.9969	
			Hen muscle	0.01	*m/z* 295	0.01 0.1	89.5 – 107 (96.9, 5) 83.0 – 96.3 (90.7, 5)	7.3 (5) 6.6 (5) Overall: 7.4 (10)	Solvent based standards used: 2.5 – 100 ng/mL [Approx. 0.003-0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, y = 1.52×10^3 – 1.15×10^3, r = 0.9985	
				0.01	*m/z* 297	0.01 0.1	85.1 – 105 (94.5, 5) 81.8 – 95.5 (89.9, 5)	8.4 (5) 6.7 (5) Overall: 7.7 (10)	Solvent based standards used: 2.5 – 100 ng/mL [Approx. 0.003-0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, y = 509x	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
									– 609, r = 0.9975	
				0.01	*m/z* 317	0.01 0.1	78.0 – 103.0 (86.8, 5) 74.5 – 82.8 (77.5, 5)	13 (5) 4.8 (5) Overall: 11 (10)	Solvent based standards used: 2.5 – 100 ng/mL [Approx. 0.003-0.1 mg/kg assuming redissolved in 2 mL to bring within calibration range] 6 standards, y = 151x – 167, r = 0.9969	
			Hen fat	0.01	*m/z* 295	0.01 0.1	66.6 – 73.6 (70.8, 5) 58.8 – 71.1 (65.3, 5)	4.1 (5) 7.7 (5) Overall: 7.1 (10)	Solvent based standards used: 2.5 – 100 ng/mL [Approx. 0.002 – 0.08 mg/kg assuming redissolved in 1.5 mL to bring within calibration range] 6 standards, y = 1.52×10^3 – 1.15×10^3,	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
									r = 0.9985	
				0.01	*m/z* 297	0.01 0.1	68.2 – 74.1 (70.7, 5) 58.4 – 70.3 (65, 5)	3.5 (5) 7.6 (5) Overall: 7 (10)	Solvent based standards used: 2.5 – 100 ng/mL [Approx. 0.002 – 0.08 mg/kg assuming redissolved in 1.5 mL to bring within calibration range] 6 standards, y = 509x – 609, r = 0.9975	
				0.01	*m/z* 317	0.01 0.1	70.8 – 78.2 (73.7, 5) 56.6 – 68.5 (62.3, 5)	4.3 (5) 7.1 (5) Overall: 10 (10)	Solvent based standards used: 2.5 – 100 ng/mL [Approx. 0.002 – 0.08 mg/kg assuming redissolved in 1.5 mL to bring within calibration range] 6 standards, y = 151x – 167, r = 0.9969	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
CA 4.1.2/30 Billian P.,Druskus M., 2009 a	TD (fortified as 1,2,4-triazole, determined as TD and calculated as 1,2,4-triazole)	T	Whole milk	0.01	*m/z* 302.9 → 170.0	0.01 (blank values subtracted) 0.10	97 – 109 (105, 5) 91 – 108 (101, 5)	4.7 (5) 7.3 (5) Overall: 6.1 (10)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.11x + 0.0025 (r = 0.9998)	Acceptable chromatograms presented for standard, control sample and recovery sample for whole milk, bovine muscle, bovine liver, fat, bovine kidney, and whole egg. Interference >30% of LOQ was observed hence the blank values were subtracted in the calculation of recoveries
				0.01	*m/z* 302.9 → 234.0	0.01 (blank values subtracted) 0.10	110 – 119 (114, 5) 91 – 107 (100, 5)	2.8 (5) 6.2 (5) Overall: 8.1 (10)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.03x – 0.00181 (r = 0.9989)	
	TA	SCX		0.01	*m/z* 156.7 → 70.1	0.01 0.10	74 – 92 (83, 5) 86 – 92 (88, 5)	7.7 (5) 3.0 (5) Overall: 6.0 (10)	0.125 - 25 µg/L [Approx. 0.001 – 0.2 mg/kg] 6 standards, y = 0.836x + 0.0025 (r = 0.9995)	
				0.01	*m/z* 156.7 → 88	0.01 0.10	71 – 87 (77, 5) 84 – 94 (90, 5)	8.1 (5) 4.5 (5) Overall: 10.5 (5)	0.125 - 25 µg/L [Approx. 0.001 – 0.2 mg/kg] 6 standards, y = 0.506x + 0.00146 (r = 0.9995)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TAA	SCX		0.01	*m/z* 127.5 → 69.9	0.01 0.10	96 – 109 (103, 5) 80 – 94 (88, 5)	4.9 (5) 6.1 (5) Overall: 9.7 (10)	0.125 - 25 µg/L [Approx. 0.001 – 0.2 mg/kg] 6 standards, y = 0.789x + 0.00063 (r = 0.9987)	
		SCX neg.		0.01	*m/z* 126.0 → 81.9	0.01 0.10	93 – 120 (108, 5) 89 – 105 (97, 5)	10.4 (5) 6.1 (5) Overall: 9.9 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.17x – 0.0134 (r = 0.9981)	
	TLA	SCX		0.01	*m/z* 157.8 → 70.1	0.01 0.10	94 – 107 (99, 5) 78 – 88 (84, 5)	5.3 (5) 4.5 (5) Overall: 9.9 (10)	0.125 - 25 µg/L [Approx. 0.001 – 0.2 mg/kg] 6 standards, y = 1.07x + 0.0101 (r = 0.9936)	
		SCX neg.		0.01	*m/z* 155.9 → 68.0	0.01 0.10	87 – 95 (91, 5) 88 – 109 (100, 5)	3.3 (5) 8.1 (5) Overall: 7.6 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 7 standards, y = 0.84x – 0.00121 (r = 0.9995)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TD (fortified as 1,2,4-triazole, determined as TD and calculated as 1,2,4-triazole)	T	Skimmed milk	0.01	*m/z* 302.9 → 170.0	0.01 (blank values subtracted) 0.10	101 – 108 (105, 3) 103 – 108 (106, 3)	3.4 (3) 2.5 (3) Overall: 2.7 (6)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.11x + 0.0025 (r = 0.9998)	
				0.01	*m/z* 302.9 → 234.0	0.01 (blank values subtracted) 0.10	106 – 111 (108, 3) 107 – 112 (109, 3)	2.4 (3) 2.4 (3) Overall: 2.2 (6)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.03x – 0.00181 (r = 0.9989)	
	TA	SCX		0.01	*m/z* 156.6 → 70.1	0.01 0.10	87 – 92 (90, 3) 92 – 99 (96, 3)	3.2 (3) 3.9 (3) Overall: 4.8 (6)	0.125 - 25 µg/L [Approx. 0.001 – 0.2 mg/kg] 6 standards, y = 0.836x + 0.0025 (r = 0.9995)	
				0.01	*m/z* 156.6 → 88.0	0.01 0.10	96 – 112 (106, 3) 93 – 96 (95, 3)	8.2 (3) 1.8 (3) Overall: 8.2 (6)	0.125 - 25 µg/L [Approx. 0.001 – 0.2 mg/kg] 6 standards, y = 0.506x + 0.00146 (r = 0.9995)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TAA	SCX		0.01	*m/z* 127.5 → 69.9	0.01 0.10	83 – 97 (89, 3) 93 – 95 (94, 3)	7.9 (3) 1.1 (3) Overall: 5.7 (6)	0.125 - 25 µg/L [Approx. 0.001 – 0.2 mg/kg] 6 standards, y = 0.789x + 0.00063 (r = 0.9987)	
		SCX neg.		0.01	*m/z* 126.0 → 81.9	0.01 0.10	85 - 92 (89, 3) 102 - 106 (104, 3)	4 (3) 2 (3) Overall: 9.3 (6)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.17x – 0.0134 (r = 0.9981)	
	TLA	SCX		0.01	*m/z* 157.8 → 70.1	0.01 0.10	82 - 85 (84, 3) 85 – 87 (86, 3)	1.8 (3) 1.3 (3) Overall: 1.9 (6)	0.125 - 25 µg/L [Approx. 0.001 – 0.2 mg/kg] 7 standards, y = 1.07x + 0.0101 (r = 0.9936)	
		SCX neg.		0.01	*m/z* 155.9 → 68.0	0.01 0.10	107 – 118 (112, 3) 107 – 109 (108, 3)	4.9 (3) 1.1 (3) Overall: 4 (6)	0.125 - 125 µg/L 6 standards, y = 0.84x – 0.00121 (r = 0.9995)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TD (fortified as 1,2,4-triazole, determined as TD and calculated as 1,2,4-triazole)	T	Whole milk	0.01	*m/z* 302.9 → 170.0	0.01 (blank values subtracted) 0.10	103 – 117 (110, 3) 106 – 118 (110, 3)	6.4 (3) 6.3 (3) Overall: 5.7 (6)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.11x + 0.0025 (r = 0.9998)	
				0.01	*m/z* 302.9 → 234.0	0.01 (blank values subtracted) 0.10	107 – 110 (109, 3) 110 – 116 (113, 3)	1.4 (3) 2.7 (3) Overall: 2.9 (6)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.03x – 0.00181 (r = 0.9989)	
	TA	SCX		0.01	*m/z* 156.7 → 70.1	0.01 0.10	81 – 105 (94, 3) 87 – 92 (89, 3)	12.9 (3) 2.8 (3) Overall: 9.0 (6)	0.125 - 25 µg/L [Approx. 0.001 – 0.2 mg/kg] 6 standards, y = 0.836x + 0.0025 (r = 0.9995)	
				0.01	*m/z* 156.7 → 88	0.01 0.10	92 – 119 (103, 3) 85 – 90 (88, 3)	13.6 (3) 3.0 (3) Overall: 12.9 (6)	0.125 - 25 µg/L [Approx. 0.001 – 0.2 mg/kg] 6 standards, y = 0.506x + 0.00146 (r = 0.9995)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TAA	SCX		0.01	*m/z* 127.5 → 69.9	0.01 0.10	84 – 89 (86, 3) 93 – 96 (94, 3)	2.9 (3) 1.8 (3) Overall: 5.1 (6)	0.125 - 25 µg/L [Approx. 0.001 – 0.2 mg/kg] 6 standards, y = 0.789x + 0.00063 (r = 0.9987)	
		SCX neg.		0.01	*m/z* 126.0 → 81.9	0.01 0.10	89 – 97 (94, 3) 101 – 109 (104, 3)	4.9 (3) 4.0 (3) Overall: 6.8 (6)	0.125 - 125 µg/L [Approx. 0.001 – 0.2 mg/kg] 6 standards, y = 1.17x – 0.0134 (r = 0.9981)	
	TLA	SCX		0.01	*m/z* 157.8 → 70.1	0.01 0.10	75 – 90 (77, 3) 92 – 96 (94, 3)	3.4 (3) 2.2 (3) Overall: 11.0 (6)	0.125 - 25 µg/L [Approx. 0.001 – 0.2 mg/kg] 6 standards, y = 1.07x + 0.0101 (r = 0.9936)	
		SCX neg.		0.01	*m/z* 155.9 → 68.0	0.01 0.10	104 – 106 (105, 3) 99 – 108 (104, 3)	1.0 (3) 4.4 (3) Overall: 2.9 (6)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 7 standards, y = 0.84x – 0.00121 (r = 0.9995)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TD (fortified as 1,2,4-triazole, determined as TD and calculated as 1,2,4-triazole)	T	Bovine muscle (meat)	0.01	*m/z* 302.9 → 170.0	0.01 0.10	110 - 118 (113, 5) 99 – 107 (104, 5)	2.7 (3) 3.1 (5) Overall: 5.0 (10)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.11x + 0.0025 (r = 0.9998)	
				0.01	*m/z* 302.9 → 234.0	0.01 0.10	111 – 118 (115, 5) 99 – 110 (107, 3)	2.6 (5) 4.2 (5) Overall: 5.2 (10)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.03x – 0.00181 (r = 0.9989)	
	TA	Hypercarb		0.01	*m/z* 156.7 → 70.1	0.01 (blank values subtracted) 0.10	80 – 95 (87, 5) 91 – 101 (97, 5)	7.2 (5) 4.0 (5) Overall: 7.5 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.651x + 0.004 (r = 0.9964)	
		SCX neg.		0.01	*m/z* 154.9 → 68.0	0.01 (blank values subtracted) 0.10	75 – 85 (80, 5) 87 – 110 (100, 5)	5.4 (5) 8.4 (5) Overall: 13.8 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.668x + 0.00547 (r = 0.9969)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TAA	Hypercarb		0.01	*m/z* 127.5 → 69.9	0.01 0.10	96 – 102 (98, 5) 96 – 102 (98, 5)	2.4 (5) 1.3 (5) Overall: 1.8 (10)	0.125 – 62.5 µg/L [Approx. 0.001 – 0.5 mg/kg] 6 standards, y = 0.678x + 0.00635 (r = 0.9907)	
		SCX neg.		0.01	*m/z* 126.0 → 81.9	0.01 0.10	81 – 94 (87, 5) 86 – 90 (87, 5)	6.1 (5) 1.9 (5) Overall: 4.3 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.17x – 0.0134 (r = 0.9981)	
	TLA	Hypercarb		0.01	*m/z* 157.8 → 70.1	0.01 0.10	80 – 93 (90, 5) 93 – 97 (95, 5)	6.7 (5) 2.0 (5) Overall: 5.5 (10)	0.125 – 62.5 µg/L [Approx. 0.001 – 0.5 mg/kg] 7 standards, y = 1.12x + 0.0236 (r = 0.9909)	
		SCX neg.		0.01	*m/z* 155.9 → 68.0	0.01 0.10	103 – 115 (107, 5) 103 – 106 (104, 5)	4.4 (5) 1.3 (5) Overal: 3.4 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 7 standards, y = 0.84x – 0.00121 (r = 0.9995)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TD (fortified as 1,2,4-triazole, determined as TD and calculated as 1,2,4-triazole)	T	Bovine liver	0.01	*m/z* 302.9 → 170.0	0.01 0.10	92 – 101 (96, 5) 97 – 105 (100, 5)	3.5 (5) 3.3 (5) Overall: 3.8 (10)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.11x + 0.0025 (r = 0.9998)	
				0.01	*m/z* 302.9 → 234.0	0.01 0.10	90 – 97 (94, 5) 97 – 95 (91, 5)	2.9 (5) 3.5 (5) Overall: 3.6 (10)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.03x – 0.00181 (r = 0.9989)	
	TA	Hypercarb		0.01	*m/z* 156.7 → 70.1	0.01 (blank values subtracted) 0.10	66 – 97 (84, 5) 94 – 97 (95, 5)	14.0 (5) 1.6 (5) Overall: 10.9 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.651x + 0.004 (r = 0.9964)	
		Hypercarb		0.01	*m/z* 156.7 → 88.0	0.01 (blank values subtracted) 0.10	62 – 91 (77, 5) 94 – 98 (97, 5)	13.5 (5) 1.8 (5) Overall: 14.4 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.413x – 0.002 (r = 0.9985)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TAA	Hypercarb		0.01	*m/z* 127.5 → 69.9	0.01 0.10	76 – 82 (81, 5) 93 – 103 (97, 5)	3.5 (5) 4.0 (5) Overall: 10.2 (10)	0.125 – 62.5 µg/L [Approx. 0.001 – 0.5 mg/kg] 6 standards, y = 0.678x + 0.00635 (r = 0.9907)	
		SCX neg.		0.01	*m/z* 126.0 → 81.9	0.01 0.10	86 – 96 (91, 5) 92 – 105 (98, 5)	4.6 (5) 6.2 (5) Overall: 6.4 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.17x – 0.0134 (r = 0.9981)	
	TLA	Hypercarb		0.01	*m/z* 157.8 → 70.1	0.01 0.10	82 – 88 (84, 5) 94 – 97 (95, 5)	2.7 (5) 1.2 (5) Overall: 6.7 (10)	0.125 – 62.5 µg/L [Approx. 0.001 – 0.5 mg/kg] 7 standards, y = 1.12x + 0.0236 (r = 0.9909)	
		SCX neg.		0.01	*m/z* 155.9 → 68.0	0.01 0.10	97 – 100 (99, 5) 95 – 102 (99, 5)	1.2 (5) 3.1 (5) Overall: 2.2 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 7 standards, y = 0.84x – 0.00121 (r = 0.9995)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TD (fortified as 1,2,4-triazole, determined as TD and calculated as 1,2,4-triazole)	T	Fat (bovine and pig)	0.01	*m/z* 302.9 → 170.0	0.01 0.10	96 – 117 (105, 5) 96 – 108 (100, 5)	8.3 (5) 5.1 (5) Overall: 7.0 (10)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.11x + 0.0025 (r = 0.9998)	
				0.01	*m/z* 302.9 → 234.0	0.01 0.10	98 – 115 (105, 5) 100 – 107 (104, 5)	6.1 (5) 2.6 (5) Overall: 4.4 (10)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.03x – 0.00181 (r = 0.9989)	
	TA	Hypercarb		0.01	*m/z* 156.7 → 70.1	0.01 (blank values subtracted) 0.10	95 – 105 (99, 5) 117 – 121 (119, 5)	3.6 (5) 1.5 (5) Overall: 9.9 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.651x + 0.004 (r = 0.9964)	
				0.01	*m/z* 156.7 → 88.0	0.01 (blank values subtracted) 0.10	90 – 104 (97, 5) 118 – 125 (122, 5)	5.8 (5) 2.1 (5) Overall: 12.6 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.413x – 0.002 (r = 0.9985)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TAA	Hypercarb		0.01	*m/z* 127.5 → 69.9	0.01 0.10	93 – 100 (96, 5) 95 – 99 (97, 5)	2.8 (5) 1.6 (5) Overall: 2.2 (10)	0.125 – 62.5 µg/L [Approx. 0.001 – 0.5 mg/kg] 6 standards, y = 0.678x + 0.00635 (r = 0.9907)	
		SCX neg.		0.01	*m/z* 126.0 → 81.9	0.01 0.10	87 – 98 (91, 5) 88 – 99 (93, 5)	5.1 (5) 5.4 (5) Overall: 5.1 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.17x – 0.0134 (r = 0.9981)	
	TLA	Hypercarb		0.01	*m/z* 157.8 → 70.1	0.01 0.10	83 – 99 (88, 5) 100 – 104 (101, 5)	7.2 (5) 1.7 (5) Overall: 8.5 (10)	0.125 – 62.5 µg/L [Approx. 0.001 – 0.5 mg/kg] 7 standards, y = 1.12x + 0.0236 (r = 0.9909)	
		SCX neg.		0.01	*m/z* 155.9 → 68.0	0.01 0.10	95 – 97 (96, 5) 96 – 103 (98, 5)	1.0 (5) 3.0 (5) Overall: 2.4 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 7 standards, y = 0.84x – 0.00121 (r = 0.9995)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TD (fortified as 1,2,4-triazole, determined as TD and calculated as 1,2,4-triazole)	T 100	Bovine kidney	0.01	*m/z* 302.9 → 170.0	0.01 0.10	73 – 89 (82, 5) 96 – 98 (98, 5)	9.0 (5) 0.9 (5) Overall: 10.7 (10)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.123x + 0.0468 (r = 0.9984)	
				0.01	*m/z* 302.9 → 234.0	0.01 0.10	87 – 100 (93, 5) 88 – 95 (92, 5)	6.0 (5) 2.9 (5) Overall: 4.5 (10)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.113x + 0.0416 (r = 0.9982)	
	TA	Hypercarb		0.01	*m/z* 156.7 → 70.1	0.01 (blank values subtracted) 0.10	98 – 108 (103, 5) 106 – 111 (108, 5)	4.2 (5) 1.8 (5) Overall: 4.5 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.651x + 0.004 (r = 0.9964)	
				0.01	*m/z* 156.7 → 88.0	0.01 (blank values subtracted) 0.10	95 – 107 (100, 5) 99 – 110 (107, 5)	6.2 (5) 4.4 (5) Overall: 6.2 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.413x – 0.002 (r = 0.9985)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TAA	Hypercarb		0.01	*m/z* 127.5 → 69.9	0.01 0.10	93 – 110 (100, 5) 98 – 105 (102, 5)	7.5 (5) 2.7 (5) Overall: 5.4 (10)	0.125 – 62.5 µg/L [Approx. 0.001 – 0.5 mg/kg] 6 standards, y = 0.678x + 0.00635 (r = 0.9907)	
		SCX neg.		0.01	*m/z* 126.0 → 81.9	0.01 0.10	67 – 104 (92, 5) 101 – 110 (106, 5)	16.0 (5) 3.2 (5) Overall: 12.4 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.17x – 0.0134 (r = 0.9981)	
	TLA	Hypercarb		0.01	*m/z* 157.8 → 70.1	0.01 0.10	106 – 120 (111, 5) 89 – 96 (92, 5)	5.0 (5) 2.9 (5) Overall: 10.7 (10)	0.125 – 62.5 µg/L [Approx. 0.001 – 0.5 mg/kg] 7 standards, y = 1.12x + 0.0236 (r = 0.9909)	
		SCX neg.		0.01	*m/z* 155.9 → 68.0	0.01 0.10	88 – 102 (93, 5) 90 – 94 (93, 5)	6.0 (5) 1.8 (5) Overall: 4.2 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 7 standards, y = 0.84x – 0.00121 (r = 0.9995)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TD (fortified as 1,2,4-triazole, determined as TD and calculated as 1,2,4-triazole)	T	Whole egg	0.01	*m/z* 302.9 → 170.0	0.01 (blank values subtracted) 0.10	89 – 105 (95, 5) 92 – 102 (98, 5)	7.0 (5) 4.1 (5) Overall: 5.6 (10)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.11x + 0.0025 (r = 0.9998)	
				0.01	*m/z* 302.9 → 234.0	0.01 (blank values subtracted) 0.10	95 – 105 (100, 5) 91 – 96 (93, 5)	4.2 (5) 2.5 (5) Overall: 4.7 (10)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.03x – 0.00181 (r = 0.9989)	
	TA	Hypercarb		0.01	*m/z* 156.7 → 70.1	0.01 0.10	80 – 90 (86, 5) 92 – 98 (95, 5)	4.9 (5) 2.5 (5) Overall: 6.5 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.651x + 0.004 (r = 0.9964)	
				0.01	*m/z* 156.7 → 88.0	0.01 0.10	89 – 93 (86, 5) 97 – 103 (99, 5)	9.0 (5) 2.5 (5) Overall: 9.6 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.413x – 0.002 (r = 0.9985)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TAA	Hypercarb		0.01	*m/z* 127.5 → 69.9	0.01 0.10	90 – 99 (92, 5) 93 – 97 (96, 5)	4.1 (5) 1.7 (5) Overall: 3.5 (10)	0.125 – 62.5 µg/L [Approx. 0.001 – 0.5 mg/kg] 6 standards, y = 0.678x + 0.00635 (r = 0.9907)	
		SCX neg.		0.01	*m/z* 126.0 → 81.9	0.01 0.10	95 – 103 (98, 5) 91 – 100 (97, 5)	3.2 (5) 3.8 (5) Overall: 3.4 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.17x – 0.0134 (r = 0.9981)	
	TLA	Hypercarb		0.01	*m/z* 157.8 → 70.1	0.01 0.10	90 – 98 (94, 5) 91 – 98 (94, 5)	3.4 (5) 3.4 (5) Overall: 3.2 (10)	0.125 – 62.5 µg/L [Approx. 0.001 – 0.5 mg/kg] 7 standards, y = 1.12x + 0.0236 (r = 0.9909)	
		SCX neg.		0.01	*m/z* 155.9 → 68.0	0.01 0.10	82 – 88 (85, 5) 92 – 96 (94, 5)	2.6 (5) 1.6 (5) Overall: 5.9 (10)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 7 standards, y = 0.84x – 0.00121 (r = 0.9995)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TD (fortified as 1,2,4-triazole, determined as TD and calculated as 1,2,4-triazole)	T	Egg yolk	0.01	*m/z* 302.9 → 170.0	0.01 0.10	98 – 103 (100, 3) 83 – 90 (86, 3)	2.6 (3) 4.2 (3) Overall: 8.8 (6)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.11x + 0.0025 (r = 0.9998)	
				0.01	*m/z* 302.9 → 234.0	0.01 0.10	102 – 112 (107, 3) 83 – 878 (85, 3)	4.7 (3) 2.4 (3) Overall: 13.2 (6)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.03x – 0.00181 (r = 0.9989)	
	TA	Hypercarb		0.01	*m/z* 156.7 → 70.1	0.01 (blank values subtracted) 0.10	100 – 115 (108, 3) 78 – 91 (84, 3)	7.0 (3) 7.7 (3) Overall: 15.0 (6)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.651x + 0.004 (r = 0.9964)	
				0.01	*m/z* 156.7 → 88.0	0.01 (blank values subtracted) 0.10	85 – 110 (99, 3) 78 – 92 (84, 3)	12.9 (3) 8.6 (3) Overall: 13.5 (6)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.413x – 0.002 (r = 0.9985)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TAA	Hypercarb		0.01	*m/z* 127.5 → 69.9	0.01 0.10	109 – 113 (111, 3) 90 – 97 (94, 3)	1.9 (3) 3.8 (3) Overall: 9.6 (6)	0.125 – 62.5 µg/L [Approx. 0.001 – 0.5 mg/kg] 6 standards, y = 0.678x + 0.00635 (r = 0.9907)	
		SCX neg.		0.01	*m/z* 126.0 → 81.9	0.01 0.10	101 – 104 (102, 3) 89 – 95 (92, 3)	1.5 (3) 3.3 (3) Overall: 6.2 (6)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.17x – 0.0134 (r = 0.9981)	
	TLA	Hypercarb		0.01	*m/z* 157.8 → 70.1	0.01 0.10	85 – 90 (88, 3) 95 – 100 (97, 3)	3.0 (3) 2.7 (3) Overall: 3.9 (6)	0.125 – 62.5 µg/L [Approx. 0.001 – 0.5 mg/kg] 7 standards, y = 1.12x + 0.0236 (r = 0.9909)	
		SCX neg.		0.01	*m/z* 155.9 → 68.0	0.01 0.10	75 – 87 (81, 3) 80 – 82 (81, 3)	7.5 (3) 1.2 (3) Overall: 4.8 (6)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 7 standards, y = 0.84x – 0.00121 (r = 0.9995)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TD (fortified as 1,2,4-triazole, determined as TD and calculated as 1,2,4-triazole)	T	Egg white	0.01	*m/z* 302.9 → 170.0	0.01 0.10	86 – 98 (93, 3) 92 – 103 (98, 3)	6.7 (3) 5.8 (3) Overall: 6.4 (6)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.11x + 0.0025 (r = 0.9998)	
				0.01	*m/z* 302.9 → 234.0	0.01 0.10	85 – 104 (96, 3) 100 – 120 (109, 3)	10.3 (3) 9.2 (3) Overall: 11.2 (6)	0.02 – 20 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.03x – 0.00181 (r = 0.9989)	
	TA	Hypercarb		0.01	*m/z* 156.7 → 70.1	0.01 0.10	82 – 91 (86, 3) 82 – 103 (92, 3)	5.5 (3) 11.5 (3) Overall: 9.1 (6)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.651x + 0.004 (r = 0.9964)	
				0.01	*m/z* 156.7 → 88.0	0.01 0.10	75 – 80 (77, 3) 79 – 95 (87, 3)	3.3 (3) 9.2 (3) Overall: 9.1 (6)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 0.413x – 0.002 (r = 0.9985)	

Reference	Analyte	Chromatographic technique	Matrix	LOQ (mg/kg unless otherwise stated)	Mass transition/ fragment (*m/z*)	Recovery fortification level (mg/kg unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
	TAA	Hypercarb		0.01	*m/z* 127.5 → 69.9	0.01 0.10	94 – 103 (99, 3) 120 – 124 (123, 3)	4.6 (3) 1.9 (3) Overall: 12.1 (6)	0.125 – 62.5 µg/L [Approx. 0.001 – 0.5 mg/kg] 6 standards, y = 0.678x + 0.00635 (r = 0.9907)	
		SCX neg.		0.01	*m/z* 126.0 → 81.9	0.01 0.10	96 – 99 (97, 3) 106 – 113 (110, 3)	1.8 (3) 3.3 (3) Overall: 7.3 (6)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 6 standards, y = 1.17x – 0.0134 (r = 0.9981)	
	TLA	Hypercarb		0.01	*m/z* 157.8 → 70.1	0.01 0.10	96 – 101 (99, 3) 119 – 123 (121, 3)	2.7 (3) 1.7 (3) Overall: 11.3 (6)	0.125 – 62.5 µg/L [Approx. 0.001 – 0.5 mg/kg] 7 standards, y = 1.12x + 0.0236 (r = 0.9909)	
		SCX neg.		0.01	*m/z* 155.9 → 68.0	0.01 0.10	89 – 102 (95, 3) 111 – 119 (115, 3)	6.9 (3) 3.5 (3) Overall: 11.4 (6)	0.125 - 125 µg/L [Approx. 0.001 – 1 mg/kg] 7 standards, y = 0.84x – 0.00121 (r = 0.9995)	

B.5.1.2.6. Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

Report: CA 8.1.1.2/1
[redacted] 2014 c
BAS 750 F - Avian dietary toxicity test in chicks of the bobwhite quail (Colinus virginianus)
2014/1127963
Guidelines: SANCO/3029/99
GLP: Yes

Report: CA 8.1.1.2/2
[redacted] 2015 a
Amendment No. 1 - BAS 750 F - Avian dietary toxicity test in chicks of the bobwhite quail (Colinus virginianus)
2015/1223324
Guidelines: SANCO/3029/99
GLP: Yes

Report: CA 8.1.1.2/3
[redacted] 2014 d
BAS 750 F - Avian dietary toxicity test in ducklings of the mallard duck (Anas platyrhynchos)
2014/1117035
Guidelines: SANCO/3029/99
GLP: Yes

Studies supported: *CA 8.1.1.2/1*
[redacted] *2014 c*
BAS 750 F - Avian dietary toxicity test in chicks of the bobwhite quail (Colinus virginianus)
2014/1127963

CA 8.1.1.2/2
[redacted] *2015 a*
Amendment No. 1 - BAS 750 F - Avian dietary toxicity test in chicks of the bobwhite quail (Colinus virginianus)
2015/1223324

CA 8.1.1.2/3
[redacted] *2014 d*
BAS 750 F - Avian dietary toxicity test in ducklings of the mallard duck (Anas platyrhynchos)
2014/1117035

Principle of the method

The method used in these studies has been satisfactorily validated under KCA 4.1.2/12 in accordance with SANCO/3029/99 rev.4.

The sample solution was prepared by addition of 10g (for concentration range 607 – 6249 mg/kg and 7591 – 37955 mg/kg) and 5g (for concentration range 6250 – 7590 mg/kg) of the Ground Kliba maintenance diet quail/duck "GLP" meal into a 50 mL centrifuge tube and extracted 3 times by shaking with 30 mL extraction solution (1% formic acid in acetonitrile). After centrifugation the supernatants were collected in a 100 mL volumetric flask and made to volume with extraction solution. If the amount of test substance in the sample solution was greater than the calibration range, dilution was required with matrix solution to bring the concentration within the linear range.

Analysis was performed by HPLC-UV DAD using an Ascentis Express C18 column (150 x 4.6 mm, 2.7 µm) at ambient temperature with UV detection at 210 nm and external standardization. A gradient elution was used (mobile phase A: 0.1% trifluoroacetic acid in acetonitrile; mobile phase B: 0.1% trifluoroacetic acid in water).

Supplementary validation data

The validation data generated under KCA 4.1.2/12 are available to support the validation of this method; and these data are reported at the end of Section B.5.1.2.6. To supplement these data, specific recoveries have been provided for certain fortifications from QC data to verify the suitability of the method for CA 8.1.1.2/1 and 8.1.1.2/2, and CA 8.1.1.2/3:

Table 5.1-45: Additional fortification samples for CA 8.1.1.2/1 and CA 8.1.1.2/2

Matrix	**Analyte**	**Recovery fortification level (mg/kg)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**
Ground Kliba maintenance diet quail and duck 'GLP' meals	BAS 750 F	1498	98.6 – 100.8 (100.1, 3)	1.3 (3)
		7591	92.0 – 96.8 (94.5, 4)	2.2 (4)

Table 5.1-46: Additional fortification samples for CA 8.1.1.2/3

Matrix	**Analyte**	**Recovery fortification level (mg/kg)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**
Ground Kliba maintenance diet quail and duck 'GLP' meals	BAS 750 F	1498	99.8 – 104.2 (102.3, 6)	1.5 (6)
		7591	96.3 - 107.4 (102.0, 8)	3.2 (9)

Validation summary

As stated under KCA 4.1.2/12, HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the matrix solution and calibration solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 4.0%. The additional mean recoveries presented are within the range 70 – 110 % and RSDs are within the acceptable limit of 20%. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched solutions. The LOQ of the method is 607 mg/kg. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 8.1.1.3/1 [redacted] 2014 a BAS 750 F: A reproduction study with the Northern bobwhite 2013/1281276
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.1.1.3/1* *[redacted] 2014 a* *BAS 750 F: A reproduction study with the Northern bobwhite* *2013/1281276*

Principle of the method

3 g of avian feed was added to a 50 mL centrifuge tube. 30 mL acetonitrile was added and centrifuged. Samples with concentrations 0 to 150 mg/kg were diluted 2.00 mL to 4.00 mL with water. Samples with concentrations of 285 to 600 mg/kg a.i., were diluted 1.00 mL to 25.0 mL with 50:50 (v/v) acetonitrile: water.

Analysis was performed by HPLC-UV using a YMC-PACK ODS-AM analytical column (150 x 4.6 mm, 5µm) at 40 °C with UV detection at 233 nm and external calibration. A gradient elution was used (mobile phase A: 0.1% phosphoric acid in water; mobile phase B: acetonitrile).

Validation summary

HPLC-UV is not a highly specific method however validation has shown that it is specific to the analyte of interest. Chromatograms of the calibration standard, matrix blank, fortified matrix and sample were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 7 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 4.2 %. The linear range is appropriate for the nominal test concentrations. The LOQ of the method is 30 mg/kg.

The RMS highlighted to the applicant concerns that the suitability of matrix matched standards for calibration purposes had not been addressed. The applicant provided the following justification:

"BASF is the opinion that the evaluation of matrix matched standards are necessary, if a mass spectrometer is used as detector and not an UV or DAD (diode array detector). Matrix effects occur due to co-eluting matrix components, impurities or degradation products, which can affect the ionization process of the target analyte in the MS detector. The most common ionization methods used in MS are ESI (electrospray ionization) and APCI (atmospheric pressure chemical ionization), in these cases a signal enhancement or suppression can be observed. In the case of an UV/DAD detector, the transmission or the extinction coefficient are not changed and any interference is directly apparent and can be observed in the chromatogram of the matrix control sample. Therefore, an assessment of the matrix effects is not necessary in the case of an UV/DAD detector, because any interference would be seen in the matrix control sample and this was free of interferences."

The RMS considers the applicant's case to be acceptable and, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.1.1.3/2 [redacted]., 2015 a BAS 750 F: A reproduction study with the mallard 2015/7005819
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.1.1.3/2* [redacted] *2015 a* *BAS 750 F: A reproduction study with the mallard* *2015/7005819*

Principle of the method

3 g of avian feed was added to a 50 mL centrifuge tube. 30 mL acetonitrile was added and centrifuged. Samples with concentrations of 0 to 30.0 mg/kg were diluted 2.00 mL to 4.00 mL (dilute with water). Samples with concentrations of 150 mg/kg, were diluted 1.00 mL to 5.00 mL (with 50:50 (v/v) acetonitrile:water). Samples with concentrations of 300 mg/kg, were diluted 1.00 mL to 10.0 mL (with 50:50 (v/v) acetonitrile:water). Samples with concentrations of 600 mg/kg, were diluted 1.00 mL to 25.0 mL (with 50:50 (v/v) acetonitrile:water).

Analysis was performed by HPLC-UV using a YMC-PACK ODS-AM analytical column (150 x 4.6 mm, 5µm) at 40 °C with UV detection at 233 nm and external calibration. A gradient elution was used (mobile phase A: 0.1% phosphoric acid in water; mobile phase B: acetonitrile).

Validation summary

HPLC-UV is not a highly specific method however validation has shown that it is specific to the analyte of interest.. Chromatograms of the calibration standard, matrix blank, fortified matrix and sample were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 7 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 4.2 %. The linear range is appropriate for the nominal test concentrations. The LOQ of the method is 30 mg/kg. Although matrix matched standards have not been used for calibration purposes, recoveries upon fortification confirm the expected nominal concentration of the samples. This shows that the matrix does not have an effect on the quantification of the analyte. Additionally, in this method UV detection has been used and the effects of co-eluting matrix components on the detection are negligible compared to a MS detector. The matrix blank chromatogram has been provided which shows negligible interference. Therefore, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.1/1 [redacted] 2014 a BAS 750 F - Acute toxicity study in the rainbow trout (Oncorhynchus mykiss) 2014/1036951
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.2.1/1* *[redacted], 2014 a* *BAS 750 F - Acute toxicity study in the rainbow trout (Oncorhynchus mykiss)* *2014/1036951*

Principle of the method

The mixing-water samples were diluted to within the calibration range using an acetonitrile/water mixture and acidified with formic acid.

Analysis was performed by HPLC-MS using a YMC Pro C18 column (50 x 3 mm, 3µm) with a guard column (10 x 3 mm) of the same material at 40 °C with ESI^+ MS detection at *m/z* 398 and external calibration. A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

Validation summary

HPLC-MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the blank solution, standard solution and sample solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels including the LOQ and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 3 determinations injected in duplicate were made at each fortification level (less than the 5 replicates in accordance with the guidance) and RSDs were within the acceptable limit of 20 %. The overall RSD was 1.8 %. The linear range is appropriate for the nominal test concentrations. The LOQ of the method is 0.001 mg/L.

The RMS highlighted to the applicant concerns that only 3 replicates were used to determine recoveries and repeatability at each fortification level and that the suitability of matrix matched standards for calibration purposes had not been addressed. The applicant provided the following justification:

"It should be noted that the method was validated with enough replicates in other study (DocID 2014/1098028, KCA 8.2.5.1/1), where 23 fortifications at LOQ (0.001 mg/L) and 100 LOQ (0.1 mg/L) were done in M4-Medium. The mean recoveries for these two levels were 89% (RSD:5.5%) and 99% (2.1%) and the relative standard deviations (RSD) were < 5.5 %. This proves that the method is suitable to determine BAS 750 F. Due to matrix comparability a reduced validation data set (3LOQs, 3 10xLOQs) should be considered acceptable for the matrix used in this study (Frankenthaler Water). It should also be noted that the method was also used for the analysis of BAS 750 F in Frankenthaler Water in report 2014/1262160 (KCA 8.2.2.1/2). In this study, 6 additional fortifications were done. Therefore, sufficient fortifications were run with this method with this matrix and analyte.
Regarding the suitability of matrix matched standards: In the present study, solvent standards were used and the recoveries varied between 93% and 101%. This shows that the use of solvent standards is suitable for the determination of BAS 750 F in mixing water, because the source of the analyte was known. Therefore, the recoveries of the method confirmed the values expected.
It should be noted, that samples analyzed in this study were diluted between 1:10 and 1:10000, therefore, matrix effects are not expected due to the high dilution of the samples. A validation of analytical method APL0500/03 will be done in M4-Medium for BAS 750 F, M750F007 and M750F003 including two fortification levels . Furthermore, the asssement of matrix effects in Frankenthaler water, M4-Medium, OECD 201 Medium will be adressed too. Due to matrix comparability a reduced validation data set (3LOQs, 3 10xLOQs) should be considered acceptable for the matrix used in this study (Frankenthaler Water). It should also be noted, that the method was also use for the analysis of BAS 750 F in Frankenthaler Water in report 2014/1262160, in this study, two sets of three fortifications were done. Therefore, sufficient fortifications were run with this method."

The RMS considers the applicant's case to be acceptable and, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.1/5 [redacted] 2014 a BAS 750 F: Acute toxicity to the sheepshead minnow, Cyprinodon variegatus, determined under static-renewal test conditions 2014/7002810
Guidelines:	SANCO/3029/99
GLP:	Yes
Report:	KCA 8.2.2.1/1 [redacted] 2015 a BAS 750 F: Early life-stage toxicity test with the sheepshead minnow, Cyprinodon variegatus, under flow-through conditions 2015/7000619
Guidelines:	SANCO/3029/99
GLP:	Yes
Report:	KCA 8.2.4.2/1 VanHooser A., 2014 a BAS 750 F: Acute toxicity test with the saltwater mysid, Americamysis bahia, determined under flow-through test conditions 2014/7002845
Guidelines:	SANCO/3029/99
GLP:	Yes
Report:	KCA 8.2.4.2/2 VanHooser A., 2015 a BAS 750 F: Effect on new shell growth of the eastern oyster (Crassostrea virginica) 2015/7000021
Guidelines:	SANCO/3029/99
GLP:	Yes
Report:	KCA 8.2.5.2/4 Dinehart S., 2016 a BAS 750 F: Life-cycle toxicity test of the saltwater mysid, Americamysis bahia, conducted under flow-through conditions 2016/7001293
Guidelines:	SANCO/3029/99
GLP:	Yes
Studies supported:	*KCA 8.2.1/5* *[redacted] 2014 a* *BAS 750 F: Acute toxicity to the sheepshead minnow, Cyprinodon variegatus, determined under static-renewal test conditions* *2014/7002810* *KCA 8.2.2.1/1* *[redacted], 2015 a* *BAS 750 F: Early life-stage toxicity test with the sheepshead minnow, Cyprinodon variegatus, under flow-through conditions* *2015/7000619* *KCA 8.2.4.2/1* *VanHooser A., 2014 a* *BAS 750 F: Acute toxicity test with the saltwater mysid, Americamysis bahia, determined under flow-through test conditions* *2014/7002845* *KCA 8.2.5.2/4* *Dinehart S., 2016 a*

BAS 750 F: Life-cycle toxicity test of the saltwater mysid, Americamysis bahia, conducted under flow-through conditions
2016/7001293

Principle of the method

5 mL of the saltwater sample was added to 5 or 10 mL hexane and vortexed to mix. After separation, 1 mL hexane was removed, transferred to a culture tube and blown to dryness. The evaporated sample was reconstituted in 50:50 water:acetonitrile and further diluted, if necessary, to produce sample concentrations within the linear range.

Analysis was performed by LC-MS/MS using a Waters Acquity HSS T3 column (50 mm x 2.1 mm, 1.8 µm) at 40 °C with TurboIon Spray ionization MRM monitoring Q1 mass 398.0, Q3 mass 182.0 and external calibration. A gradient elution was used (Mobile Phase A: 0.1% formic acid in water; Mobile Phase B: 0.1% formic acid in Methanol).

Validation summary

HPLC-MS/MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the blank solution, standard solution and sample solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 3 determinations were made at each fortification level (less than the 5 replicates in accordance with the guidance) and RSDs were within the acceptable limit of 20 %. The overall RSD was 5.6 %. The linear range is appropriate for the nominal test concentrations and was determined using solvent-based standards. The LOQ of the method is 0.100 µg/L.

he RMS highlighted to the applicant concerns that only 3 replicates were used to determine recoveries and repeatability at each fortification level and that the suitability of matrix-matched standards had not been addressed. The applicant provided the following justification:

"BASF is the opinion that the matrix effects could not have had an influence on the results, because the fortifications confirmed the expected nominal concentration of the samples. In this study, the source of the analyte was known, therefore, the recoveries can be used as confirmation: if matrix effects have had an effect, then this have been reflected as lower or higher recoveries. Regarding the number of replicates, it should be noted that the same method was used in other two studies (DocID: 2014/7002810 KCA CA 8.2.1/4 and 2015/7000620, KCA 8.2.6.2/1). In each of these studies, 6 fortifications were done at two different levels and the recoveries varied between 83% and 94%. Therefore, enough fortifications are available and the method is suitable for the determination of BAS 750 F."

The RMS considers the applicant's case to be acceptable and, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process..

Report:	KCA 8.2.2.1/2 [redacted] 2015 a BAS 750 F - Early life-stage toxicity test on the zebrafish (Danio rerio) in a flow through system 2014/1262160
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.2.2.1/2* *[redacted] 2015 a* *BAS 750 F - Early life-stage toxicity test on the zebrafish (Danio rerio) in a flow through system* *2014/1262160*

Principle of the method

The mixing water samples were diluted to within the calibration range using an acetonitrile/water mixture and acidified with formic acid.

Analysis was performed by HPLC-MS using a YMC Pro C18 column (50 x 3 mm, 3µm) with a guard column (10 x 3 mm) of the same material at 40 °C with ESI^+ MS detection at *m/z* 398 and external calibration. A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

Validation summary

HPLC-MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the blank solution, standard solution and sample solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 3 determinations were made at each fortification level (less than the 5 replicates in accordance with the guidance) and RSDs were within the acceptable limit of 20 %. The overall RSD was 4.3 %. The linear range is appropriate for the nominal test concentrations. The LOQ of the method is 0.001 mg/L.

The RMS highlighted to the applicant concerns that only 3 replicates were used to determine recoveries and repeatability at each fortification level and that the suitability of matrix matched standards for calibration purposes had not been addressed. The applicant provided the following justification:

"It should be noted that the method was validated with enough replicates in other study (DocID 2014/1098028, KCA 8.2.5.1/1), where 23 fortifications at LOQ (0.001 mg/L) and 100LOQ (0.1 mg/L) were done in M4-Medium. The mean recoveries for these two levels were 89% (RSD:5.5%) and 99% (2.1%) and the relative standard deviations (RSD) were < 5.5 %. This proves that the method is suitable to determine BAS 750 F. Due to matrix comparability a reduced validation data set (3LOQs, 3 10xLOQs) should be considered acceptable for the matrix used in this study (Frankenthaler Water). It should also be noted that the method was also use for the analysis of BAS 750 F in Frankenthaler Water in report 2014/1036951 (KCA 8.2.1/1), in this study, 6 additional fortifications were done. Therefore, sufficient fortifications were run with this method with this matrix and analyte.
In the present study, solvent standards were used and the recoveries varied between 92% and 102% showing that the use of solvent standards is suitable for the determination of BAS 750 F in mixing water, because the source of the analyte was known and the recoveries of the method confirmed the values expected."

The RMS considers the applicant's case to be acceptable and, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.2.1/3 [redacted] 2015 b BAS 750 F - Fish sexual development test on the zebrafish (Danio rerio) 2015/1099093
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.2.2.1/3* *[redacted] 2015 b* *BAS 750 F - Fish sexual development test on the zebrafish (Danio rerio)* *2015/1099093*

Principle of the method

The mixing water samples were diluted to within the calibration range using an acetonitrile/water mixture and acidified with formic acid.

Analysis was performed by HPLC-MS using a YMC Pro C18 column (50 x 3 mm, 3µm) with a guard column (10 x 3 mm) of the same material at 40 °C with ESI^+ MS detection at *m/z* 398 and external calibration. A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

Validation summary

HPLC-MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the blank solution, standard solution and sample solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 3 determinations were made at each fortification level (less than the 5 replicates in accordance with the guidance) and RSDs were within the acceptable limit of 20 %. The overall RSD was 1.5 %. The linear range is appropriate for the nominal test concentrations. The LOQ of the method is 0.001 mg/L.

Although matrix matched standards have not been used for calibration purposes, recoveries upon fortification confirm the expected nominal concentration of the samples. This shows that the matrix does not have an effect on the quantification of the analyte. Therefore, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process. Additional fortifications were conducted in Study 2014/1036951 ([redacted] 2014a) as stated in the justification provided by the applicant in KCA 8.2.1/1. Therefore, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.4.1/2 Backfisch K. Haerthe N., 2015 a Acute toxicity of Reg.No. 6003432 (M750F007; metabolite of BAS 750 F) to Daphnia magna STRAUS in a 48 hour static test 2015/1003915
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.2.4.1/2* *Backfisch K. Haerthe N., 2015 a* *Acute toxicity of Reg.No. 6003432 (M750F007; metabolite of BAS 750 F) to Daphnia magna STRAUS in a 48 hour static test* *2015/1003915*

Principle of the method

The M4-water samples were dissolved with acetonitrile/0.5% formic acid and/or a mixture of water/acetonitrile/formic acid 80:20:0.1 (v/v/v) into the range of the calibration solutions.

Analysis was performed by HPLC-MS using a YMC Pro C18 column (150 x 4.6 mm, 3µm) at 40°C with ESI^+ MS detection at *m/z* 338 and external calibration. A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

Validation summary

HPLC-MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the standard solution, fortified sample, blank matrix and sample solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 4.6 %. The linear range is appropriate for the nominal test concentrations and was determined using solvent-based standards. The LOQ of the method is 0.001 mg/L.

The RMS highlighted to the applicant concerns that the suitability of matrix matched standards for calibration purposes had not been addressed. The applicant provided the following justification:

"The dilution factors of the samples were between 312 and 5200, therefore, matrix effects are not expected due to the high dilution of the samples. Hence, BASF is the opinion that the matrix effects could not have had an influence on the results. The source of the analyte was known and the recoveries of the method confirmed the values expected."

The RMS considers the applicant's case to be acceptable and, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.4.1/3 Haerthe N., 2016 a Acute toxicity of Reg. No. 5924326 (M750F003; metabolite of BAS 750 F) to Daphnia magna Straus in a 48 hour static test 2016/1289876
Guidelines:	SANCO/3029/99
GLP:	Yes
Studies supported:	*KCA 8.2.4.1/3* *Haerthe N., 2016 a* *Acute toxicity of Reg. No. 5924326 (M750F003; metabolite of BAS 750 F) to Daphnia magna Straus in a 48 hour static test* *2016/1289876*

Principle of the method

The test sample in OECD-medium was directly dissolved with acetonitrile and 0.5 % formic acid and if necessary further diluted with a mixture of M4-water/acetonitrile/formic acid 80:20:0.1 (v/v/v) into the range of the calibration solutions.

Analysis was performed by HPLC-MS with a YMC Pro C18 column (150 x 4.6 mm, 3 µm) at 40 °C with ESI^+ detection at *m/z* 288 and external calibration. A gradient elution was used (mobile phase A: water/formic acid 1000/1; mobile phase B: acetonitrile/formic acid 1000/1).

Validation summary

HPLC-MS is a highly specific method and additional confirmation was not necessary. Chromatograms of standard solution, blank solution, fortified solution and sample solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 3 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 4.6 %. The calibration range is appropriate for the nominal test concentrations and was determined using matrix-matched standards. The LOQ of the method is 0.001 mg/L.

Additional fortifications were conducted for the analyte in a water medium in KCA 8.2.5.3/2 **(**Backfisch K., Weltje L., 2015 a). Therefore, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.5.1/1 Janson G.-M., 2014 a Chronic toxicity of the BAS 750 F (Reg.No. 5834378) to Daphnia magna STRAUS in a 21 day semi-static test 2014/1098028
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.2.5.1/1* *Janson G.-M., 2014 a* *Chronic toxicity of the BAS 750 F (Reg.No. 5834378) to Daphnia magna STRAUS in a 21 day semi-static test* *2014/1098028*

Principle of the method

The M4-water samples were diluted to within the calibration range using an acetonitrile/water mixture and acidified with formic acid.

Analysis was performed by HPLC-MS using a YMC Pro C18 column (50 x 3 mm, 3µm) with a guard column (10 x 3 mm) of the same material at 40 °C with ESI^+ MS detection at *m/z* 398 and external calibration. A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

Validation summary

HPLC-MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the blank solution, standard solution and sample solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 8 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 7.1 %. The linear range is appropriate for the nominal test concentrations. The LOQ of the method is 0.001 mg/L.

The RMS highlighted to the applicant concerns that the suitability of matrix matched standards for calibration purposes had not been addressed. The applicant provided the following justification:

"Regarding the suitability of matrix matched standards, no difference was found between the use of solvent or matrix matched standards. In the study DocID (2015/1003912, KCA 8.2.5.2/1), matrix matched standards were used and the recoveries for the two fortification levels were between 101% and 103% for M4-water. In the present study, solvent standards were used and the recoveries varied between 83% and 103%, showing that the use of solvent standards is suitable for the determination of BAS 750 F in M4-water. Furthermore, the source of the analyte in the samples was known and the recoveries of the method confirmed the values expected."

The RMS considers the applicant's case to be acceptable and, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.5.2/1 Janson G.-M., 2015 b Chronic toxicity of BAS 750 F (Reg.No. 5834378) to Daphnia longispina in a 21 day semi-static test 2015/1003912
Guidelines:	SANCO/3029/99
GLP:	Yes
Report:	KCA 8.2.5.2/2 Janson G.-M., 2015 c Report Amendment No.1 - Chronic toxicity of BAS 750 F (Reg.No. 5834378) to Daphnia longispina in a 21 day semi-static test 2015/1251197
Guidelines:	SANCO/3029/99
GLP:	Yes
Studies supported:	*KCA 8.2.5.2/1* *Janson G.-M., 2015 b* *Chronic toxicity of BAS 750 F (Reg.No. 5834378) to Daphnia longispina in a 21 day semi-static test* *2015/1003912* *KCA 8.2.5.2/2* *Janson G.-M., 2015 c* *Report Amendment No.1 - Chronic toxicity of BAS 750 F (Reg.No. 5834378) to Daphnia longispina in a 21 day semi-static test* *2015/1251197*

Principle of the method

The M4-water samples were diluted with 0.5% formic acid in acetonitrile and if necessary further diluted with a mixture of M4-water/acetonitrile/formic acid 80:20:0.1 (v/v/v) into the range of the calibration solutions.

Analysis was performed by HPLC-MS using a YMC Pro C18 column (50 x 3 mm, 3µm) with a guard column (10 x 3 mm) of the same material at 40 °C with ESI^+ MS detection at *m/z* 398 and external calibration. A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

Validation summary

HPLC-MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the standard solution, fortified sample, blank matrix and sample solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 0.9 %. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched standards. The LOQ of the method is 0.001 mg/L. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 8.2.5.2/3 Janson G.-M., 2015 a Chronic toxicity of BAS 750 F (Reg.No. 5834378) to Daphnia pulex in a 21 day semi-static test 2015/1003913
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.2.5.2/3* *Janson G.-M., 2015 a* *Chronic toxicity of BAS 750 F (Reg.No. 5834378) to Daphnia pulex in a 21 day semi-static test* *2015/1003913*

Principle of the method

The M4-water samples were diluted with 0.5% formic acid in acetonitrile or with a mixture of M4-water/acetonitrile/formic acid 80:20:0.1 (v/v/v) into the range of the calibration solutions.

Analysis was performed by HPLC-MS using a YMC Pro C18 column (50 x 3 mm, 3µm) with a guard column (10 x 3 mm) of the same material at 40 °C with ESI^+ MS detection at *m/z* 398 and external calibration. A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

Validation summary

HPLC-MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the standard solution, fortified sample, blank matrix and sample solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 2.0 %. The linear range is appropriate for the nominal test concentrations and was determined using matrix-matched standards. The LOQ of the method is 0.001 mg/L. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 8.2.6.1/2 Backfisch K., 2015 a Effect of Reg.No. 6003432 (M750F007, metabolite of BAS 750 F) on the growth of the green alga Pseudokirchneriella subcapitata 2015/1003914
Guidelines:	SANCO/3029/99
GLP:	Yes
Studies supported:	*KCA 8.2.6.1/2* *Backfisch K., 2015 a* *Effect of Reg.No. 6003432 (M750F007, metabolite of BAS 750 F) on the growth of the green alga Pseudokirchneriella subcapitata* *2015/1003914*

Principle of the method

The OECD-medium samples were dissolved with acetonitrile/0.5% formic acid and/or a mixture of water/acetonitrile/formic acid 80:20:0.1 (v/v/v) into the range of the calibration solutions.

Analysis was performed by HPLC-MS using a YMC Pro C18 column (150 x 4.6 mm, 3µm) at 40°C with ESI^+ MS detection at *m/z* 338 and external calibration. A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

Validation summary

HPLC-MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the standard solution, fortified sample, blank matrix and sample solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 4 determinations were made at the lower fortification level and 5 determinations at the higher level. RSDs were within the acceptable limit of 20 %. The overall RSD was 11.9 %. The linear range is appropriate for the nominal test concentrations and was determined using solvent-based standards. The LOQ of the method is 0.001 mg/L.

The RMS highlighted to the applicant concerns that the suitability of matrix matched standards for calibration purposes had not been addressed. The applicant provided the following justification:

"The dilution factors of the samples were between 312 and 5000, therefore, matrix effects are not expected due to the high dilution of the samples. Hence, BASF is the opinion, that the matrix effects could not have had an influence on the results."

The RMS considers the applicant's case to be acceptable and, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.1/2 [redacted] 2015 b Reg.No. 6003432 (metabolite of BAS 750 F, M750F007) - Rainbow trout, acute toxicity test 2015/1001489
Guidelines:	SANCO/3029/99
GLP:	Yes
Studies supported:	*KCA 8.2.1/2* *[redacted] 2015 b* *Reg.No. 6003432 (metabolite of BAS 750 F, M750F007) - Rainbow trout, acute toxicity test* *2015/1001489*

Principle of the method

The water sample was applied to an ENVI-18 column (3mL, 500 mg) previously washed twice with 5mL methanol and twice with 5 mL water. The column was dried for 5 minutes and eluted with 10 mL methanol. The eluate was dried and redissolved in acetonitrile.

Analysis was performed by HPLC-UV DAD using a Kinetex C18 100A column (150 x 4.6 mm, 5 µm) with UV detection at 220 nm and external calibration. The mobile phase was acetonitrile: 0.05% solution of orthophosphoric acid (55:45 v/v)

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the blank matrix and test sample were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 4.9 %. Acceptable linearity was demonstrated in the range 0.0001 – 0.01 mg/L and was determined used solvent-based standards. The LOQ of the method is 0.002 mg/L.
Although matrix matched standards have not been used for calibration purposes, the method for sample preparation and determination of recoveries mitigates the effects of the matrix. Recoveries upon fortification confirm the expected nominal concentration of the samples which shows that the matrix does not have an effect on the quantification of the analyte. Therefore, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.1/3 [redacted] 2016 a Reg.No. 5863469 (metabolite of BAS 750 F, M750F006) - Rainbow trout, acute toxicity test 2016/1128152
Guidelines:	SANCO/3029/99
GLP:	Yes
Studies supported:	KCA 8.2.1/3 [redacted] *2016 a* *Reg.No. 5863469 (metabolite of BAS 750 F, M750F006) - Rainbow trout, acute toxicity test* *2016/1128152*

Principle of the method

The test sample was applied to an ENVI-18 column (3mL, 500 mg) previously washed twice with 5mL methanol and twice with 5 mL water. The column was dried for 5 minutes and eluted with 10 mL methanol. The eluate was dried and redissolved in acetonitrile:deionised water (1:1, v.v). Samples were diluted/concentrated to bring into the range of the standard curve.

Analysis was performed by HPLC-UV DAD using a Kinetex C18 100A column (150 x 4.6 mm, 5 µm) with UV detection at 220 nm and external calibration. The mobile phase was acetonitrile: 0.05% solution of orthophosphoric acid (58:52, v/v)

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of test sample, fortified samples and control sample were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 1.6 %. The calibration range is appropriate for the nominal test concentrations and was determined using solvent-based standards. The LOQ of the method is 0.005 mg/L. Although matrix matched standards have not been used for calibration purposes, the method for sample preparation and determination of recoveries mitigates the effects of the matrix. Recoveries upon fortification confirm the expected nominal concentration of the samples which shows that the matrix does not have an effect on the quantification of the analyte. Therefore, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.1/4 [redacted] 2015 a BAS 750 F (Reg.No. 5834378) - Zebrafish acute toxicity test 2015/1001581
Guidelines:	SANCO/3029/99
GLP:	Yes
Studies supported:	*KCA 8.2.1/4* *[redacted] 2015 a* *BAS 750 F (Reg.No. 5834378) - Zebrafish acute toxicity test* *2015/1001581*

Principle of the method

The water sample was applied to an ENVI-18 column (3mL, 500 mg) previously washed twice with 5mL methanol and twice with 5 mL water. The column was dried for 5 minutes and eluted with 15 mL methanol. The eluate was dried and redissolved in acetonitrile.

Analysis was performed by HPLC-UV DAD using a Pursuit XRs 3 C8 column (150 x 4.6 mm) with UV detection at 230 nm and external calibration. The mobile phase was acetonitrile: 0.05% solution of orthophosphoric acid (90:10 v/v).

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the control and test items were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 3.8 %. Acceptable linearity was demonstrated in the range 0.0005 – 0.02 mg/L and was determined used solvent-based standards. The LOQ of the method is 0.01 mg/L. Although matrix matched standards have not been used for calibration purposes, the method for sample preparation and determination of recoveries mitigates the effects of the matrix. Recoveries upon fortification confirm the expected nominal concentration of the samples which shows that the matrix does not have an effect on the quantification of the analyte. Therefore, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.6.1/1 Brzozowska K., 2014 b BAS 750 F (Reg.No. 5834378) - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test 2013/1250865
Guidelines:	SANCO/3029/99
GLP:	Yes

Report:	KCA 8.2.7/1 Swierkot A., 2014 a BAS 750 F (Reg.No. 5834378) - Lemna gibba CPCC 310 growth inhibition test 2014/1001322
Guidelines:	SANCO/3029/99
GLP:	Yes

Studies supported:	*KCA 8.2.6.1/1* *Brzozowska K., 2014 b* *BAS 750 F (Reg.No. 5834378) - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test* *2013/1250865* *KCA 8.2.7/1* *Swierkot A., 2014 a* *BAS 750 F (Reg.No. 5834378) - Lemna gibba CPCC 310 growth inhibition test* *2014/1001322*

Principle of the method

The water sample was applied to an ENVI-18 column (3mL, 500 mg) previously washed twice with 5mL methanol and twice with 5 mL water. The column was dried for 5 minutes and eluted with 15 mL methanol. The eluate was dried and redissolved in acetonitrile.

Analysis was performed by HPLC-UV DAD using a Pursuit XRs 3 C8 column (150 x 4.6 mm) with UV detection at 230 nm and external calibration. The mobile phase was acetonitrile: 0.05% solution of orthophosphoric acid (90:10 v/v).

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the blank matrix and test sample were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 2.5 %. Acceptable linearity was demonstrated in the range 0.0005 – 0.02 mg/L and was determined used solvent-based standards. The LOQ of the method is 0.01 mg/L. Although matrix matched standards have not been used for calibration purposes, the method for sample preparation and determination of recoveries mitigates the effects of the matrix. Recoveries upon fortification confirm the expected nominal concentration of the samples which shows that the matrix does not have an effect on the quantification of the analyte. Therefore, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.4.1/4 Rzodeczko H., 2015 c Reg.No. 5863469 (metabolite of BAS 750 F, M750F006) - Daphnia magna, acute immobilization test 2015/1001492
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.2.4.1/4* *Rzodeczko H., 2015 c* *Reg.No. 5863469 (metabolite of BAS 750 F, M750F006) - Daphnia magna, acute immobilization test* *2015/1001492*

Principle of the method

The water sample was applied to an ENVI-18 column (3mL, 500 mg) previously washed twice with 5mL methanol and twice with 5 mL water. The column was dried for 5 minutes and eluted with 10 mL methanol. The eluate was dried and redissolved in a mixture of acetonitrile: water (1:1, v/v).

Analysis was performed by HPLC-UV DAD using a Kinetex C8 100A column (150 x 4.6 mm) with UV detection at 220 nm and external calibration. The mobile phase was acetonitrile: 0.05% solution of orthophosphoric acid (48:52, v/v).

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the control, fortified sample, and solvent control were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 4.6 %. Acceptable linearity was demonstrated in the range 0.0001 – 0.01 mg/L and was determined used solvent-based standards. The LOQ of the method is 0.005 mg/L. Although matrix matched standards have not been used for calibration purposes, the method for sample preparation and determination of recoveries mitigates the effects of the matrix. Recoveries upon fortification confirm the expected nominal concentration of the samples which shows that the matrix does not have an effect on the quantification of the analyte. Therefore, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.4.1/5 Rzodeczko H., 2015 d Reg.No. 6003433 (metabolite of BAS 750 F, M750F005) - Daphnia magna, acute immobilization test 2015/1001490
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.2.4.1/5* *Rzodeczko H., 2015 d* *Reg.No. 6003433 (metabolite of BAS 750 F, M750F005) - Daphnia magna, acute immobilization test* *2015/1001490*

Principle of the method

The water sample was prepared by application to an ENVI-18 column (3mL, 500 mg) previously washed twice with 5mL methanol and twice with 5 mL water. The column was dried for 5 minutes and eluted with 10 mL methanol. The eluate was dried and redissolved in a mixture of acetonitrile: water (1:1, v/v).

Analysis was performed by HPLC-UV DAD using a Kinetex C8 100A column (150 x 4.6 mm, 5µm) with UV detection at 220 nm and external calibration. The mobile phase was acetonitrile: 0.05% solution of orthophosphoric acid (48:52, v/v).

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the control, fortified sample, and solvent control were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 3.5 %. Acceptable linearity was demonstrated in the range 0.0001 – 0.01 mg/L and was determined used solvent-based standards. The LOQ of the method is 0.005 mg/L. Although matrix matched standards have not been used for calibration purposes, the method for sample preparation and determination of recoveries mitigates the effects of the matrix. Recoveries upon fortification confirm the expected nominal concentration of the samples which shows that the matrix does not have an effect on the quantification of the analyte. Therefore, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.6.1/4 Rzodeczko H., 2016 a Reg.No. 5863469 (metabolite of BAS 750 F, M750F006) - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test 2015/1001492
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.2.6.1/4* *Rzodeczko H., 2016 a* *Reg.No. 5863469 (metabolite of BAS 750 F, M750F006) - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test* *2015/1001492*

Principle of the method

The water sample was applied to an ENVI-18 column (3mL, 500 mg) previously washed twice with 5mL methanol and twice with 5 mL water. The column was dried for 5 minutes and eluted with 10 mL methanol. The eluate was dried and redissolved in a mixture of acetonitrile: water (1:1, v/v).

Analysis was performed by HPLC-UV DAD using a Kinetex C8 100A column (150 x 4.6 mm, 5µm) with UV detection at 220 nm and external calibration. The mobile phase was acetonitrile: 0.05% solution of orthophosphoric acid (48:52, v/v).

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the control, fortified sample, and solvent control were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 4.6 %. Acceptable linearity was demonstrated in the range 0.0001 – 0.01 mg/L and was determined used solvent-based standards. The LOQ of the method is 0.005 mg/L. Although matrix matched standards have not been used for calibration purposes, the method for sample preparation and determination of recoveries mitigates the effects of the matrix. Recoveries upon fortification confirm the expected nominal concentration of the samples which shows that the matrix does not have an effect on the quantification of the analyte. Therefore, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.6.1/5 Rzodeczko H., 2016 b Reg.No. 6003433 (metabolite of BAS 750 F, M750F005) - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test 2015/1001490
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.2.6.1/5* *Rzodeczko H., 2016 b* *Reg.No. 6003433 (metabolite of BAS 750 F, M750F005) - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test* *2015/1001490*

Principle of the method

The water sample was applied to an ENVI-18 column (3mL, 500 mg) previously washed twice with 5mL methanol and twice with 5 mL water. The column was dried for 5 minutes and eluted with 10 mL methanol. The eluate was dried and redissolved in a mixture of acetonitrile: water (1:1, v/v).

Analysis was performed by HPLC-UV DAD using a Kinetex C8 100A column (150 x 4.6 mm, 5µm) with UV detection at 220 nm and external calibration. The mobile phase was acetonitrile: 0.05% solution of orthophosphoric acid (48:52, v/v).

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the control, fortified sample, and solvent control were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 3.6 %. Acceptable linearity was demonstrated in the range 0.0001 – 0.01 mg/L and was determined used solvent-based standards. The LOQ of the method is 0.005 mg/L. Although matrix matched standards have not been used for calibration purposes, the method for sample preparation and determination of recoveries mitigates the effects of the matrix. Recoveries upon fortification confirm the expected nominal concentration of the samples which shows that the matrix does not have an effect on the quantification of the analyte. Therefore, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.6.1/6 Backfisch K., 2016 a Effect of Reg. No. 5924326 (M750F003, metabolite of BAS 750 F) on the Growth of the Green Alga Pseudokirchneriella subcapitata 2016/1289875
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.2.6.1/6* *Backfisch K., 2016 a* *Effect of Reg. No. 5924326 (M750F003, metabolite of BAS 750 F) on the Growth of the Green Alga Pseudokirchneriella subcapitata* *2016/1289875*

Principle of the method

The test sample of Reg. No. 5924326 (M750F003) in OECD-medium was directly dissolved with acetonitrile and 0.5 %formic acid and if necessary further diluted with a mixture of OECD-medium/acetonitrile/formic acid 80:20:0.1 (v/v/v) into the range of the calibration solutions.

Analysis was performed by HPLC-MS with a YMC Pro C18 column (150 x 4.6 mm, 3 µm) at 40 °C with ESI^+ detection at *m/z* 288 and external calibration. A gradient elution was used (mobile phase A: water/formic acid 1000/1; mobile phase B: acetonitrile/formic acid 1000/1).

Validation summary

HPLC-MS is a highly specific method and additional confirmation was not necessary. Chromatograms of standard solution, blank solution, fortified solution and sample solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 3 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 1.4 %. The calibration range is appropriate for the nominal test concentrations and was determined using matrix-matched standards. The LOQ of the method is 0.001 mg/L. Additional fortifications were performed for the analyte in a water medium in KCA 8.2.5.3/2 **(**Backfisch K., Weltje L., 2015 a). Therefore, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.4.1/6 Rzodeczko H., 2015e Reg.No. 6010286 (metabolite of BAS 750 F, M750F008) - Daphnia magna, acute immobilization test 2015/1001493
Guidelines:	SANCO/3029/99
GLP:	Yes

Report:	KCA 8.2.6.1/3 Brzozowska-Wojoczek K., 2015 a Reg.No. 6010286 (metabolite of BAS 750 F, M750F008) - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test 2015/1001491
Guidelines:	SANCO/3029/99
GLP:	Yes

Studies supported:	*KCA 8.2.4.1/6* *Rzodeczko H., 2015e* *Reg.No. 6010286 (metabolite of BAS 750 F, M750F008) - Daphnia magna, acute immobilization test* *2015/1001493* *KCA 8.2.6.1/3* *Brzozowska-Wojoczek K., 2015 a* *Reg.No. 6010286 (metabolite of BAS 750 F, M750F008) - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test* *2015/1001491*

Principle of the method

The water sample was applied to an ENVI-18 column (3mL, 500 mg) previously washed twice with 5mL methanol and twice with 5 mL water. The column was dried for 5 minutes and eluted with 15 mL methanol. The eluate was dried and redissolved in acetonitrile.

Analysis was performed by HPLC-UV DAD using a Kinetex C8 100A column (150 x 4.6 mm, 5µm) with UV detection at 221 nm and external calibration. The mobile phase was acetonitrile: 0.05% solution of orthophosphoric acid (60:40, v/v).

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of the control and sample solutions were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 2.9 %. Acceptable linearity was demonstrated in the range 0.05 – 20 µg/L and was determined used solvent-based standards. The LOQ of the method is 0.5 µg/L. Although matrix matched standards have not been used for calibration purposes, the method for sample preparation and determination of recoveries mitigates the effects of the matrix. Recoveries upon fortification confirm the expected nominal concentration of the samples which shows that the matrix does not have an effect on the quantification of the analyte. Therefore, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.1/ [redacted] 2015 c BAS 750 F - Acute toxicity study in the common carp (Cyprinus carpio) 2015/1249071
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.2.1/6* *[redacted] 2015 c* *BAS 750 F - Acute toxicity study in the common carp (Cyprinus carpio)* *2015/1249071*

Principle of the method

The mixing water samples were dissolved with 0.5% formic acid in acetonitrile and if necessary further diluted to within the calibration range with a mixture of test water/acetonitrile/formic acid 80:20:0.1 (v/v/v).

Analysis was performed by HPLC-MS using an Acquity UPLC BEH C18 column (2.1 x 50 mm, 1.7 µm) at 40 °C with ESI^+ MS detection at *m/z* 398 and external calibration. A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

Validation summary

HPLC-MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the standard solution, fortified solutions, solvent blank, matrix blank and sample solutions were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 3.1 %. Acceptable linearity was demonstrated in the range 0.00025 – 0.005 mg/L and was determined using matrix-matched standards. The LOQ of the method is 0.001 mg/L. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4.

Report:	KCA 8.2.5.3/1 Clark R., 2015 a BAS 750 F - 10-day toxicity test exposing midge (Chironomus dilutus) to a test substance applied to sediment under static-renewal conditions 2015/7000621
Guidelines:	SANCO/3029/99
GLP:	Yes

Report:	KCA 8.2.5.4/1 Clark R., 2015 b BAS 750 F - 10-Day toxicity test exposing freshwater amphipods (Hyalella azteca) to a test substance applied to sediment under static-renewal conditions 2015/7000622
Guidelines:	SANCO/3029/99
GLP:	Yes

Report:	KCA 8.2.5.4/2 Clark R., 2015 c BAS 750 F - 10-Day toxicity test exposing estuarine amphipods (Leptocheirus plumulosus) to a test substance applied to sediment under static conditions 2015/7000623
Guidelines:	SANCO/3029/99
GLP:	Yes

Studies supported:

KCA 8.2.5.3/1
Clark R., 2015 a
BAS 750 F - 10-day toxicity test exposing midge (Chironomus dilutus) to a test substance applied to sediment under static-renewal conditions
2015/7000621

KCA 8.2.5.4/1
Clark R., 2015 b
BAS 750 F - 10-Day toxicity test exposing freshwater amphipods (Hyalella azteca) to a test substance applied to sediment under static-renewal conditions
2015/7000622

KCA 8.2.5.4/2
Clark R., 2015 c
BAS 750 F - 10-Day toxicity test exposing estuarine amphipods (Leptocheirus plumulosus) to a test substance applied to sediment under static conditions
2015/7000623

Principle of the method

The sediment samples were prepared by addition of 5g of the marine sediment to a 20 mL aliquot of extraction solvent (100 mM ammonium formate in 90:10 (v/v) acetonitrile: water), shaken and centrifuged before being transferred to 50 mL volumetric flasks. This was repeated with another 20 mL aliquot of extraction solvent and the two extracts combined and made to volume (50 mL) with extraction solvent. All samples were then further diluted into the calibration range with 20:80 (v/v) acetonitrile: purified reagent water.

The filtered seawater recovery samples were prepared by dilution of the filtered seawater with 20:80 acetonitrile:purified reagent water (v/v) to a composition of 18:10:72 acetonitrile:filtered seawater:purified reagent water (v/v/v). The high concentration recovery samples were further diluted into calibration standard range with 18:10:72 acetonitrile:filtered seawater:purified reagent water (v/v/v).

Analysis was performed by LC-MS/MS using an XBridge C18 column (2.1 x 50 mm, 2.5 µm) at 40 °C with MRM detection at *m/z* (Q1/Q3) 442.00/124.20 and external calibration. A gradient elution was used (mobile phase A: 10mM ammonium formate in water, mobile phase B: acetonitrile).

Validation summary

A non-linear calibration curve has been used. Good accuracy is observed at a range of fortifications and the calibration curves have a high r^2 value (>0.999)

HPLC-MS/MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the calibration standard, fortified sample and control sample were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 3 determinations were made at each fortification level (less than the 5 replicates in accordance with the guidance) and RSDs were within the acceptable limit of 20 %. The overall RSD was between 0.4 % and 7.2 %. The calibration range is appropriate for the nominal test concentrations and was determined using matrix-matched standards. The LOQ of the method is 0.0972 mg/kg in marine sediment and 11.4 µg/L in filtered seawater.

Although only 3 replicates were used to determine recoveries and repeatability at each fortification level, acceptable procedural recoveries within the range 70 – 110 %were achieved in each study. Therefore, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.5.3/2 Backfisch K., Weltje L., 2015 a Chronic toxicity of Reg.No. 5924326 (M750F003; metabolite of BAS 750 F) to the non-biting midge Chironomus riparius - a spiked sediment study 2015/1003916
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.2.5.3/2* *Backfisch K., Weltje L., 2015 a* *Chronic toxicity of Reg.No. 5924326 (M750F003; metabolite of BAS 750 F) to the non-biting midge Chironomus riparius - a spiked sediment study* *2015/1003916*

Principle of the method

The samples in M4-medium were diluted with 0.5% formic acid in acetonitrile and if necessary further diluted with a mixture of M4-medium/acetonitrile/formic acid 80:20:0.1 (v/v/v) into the range of the calibration solutions.

Sediment samples were extracted twice with 40 mL acetonitrile/water 70:30 (v/v). Extracts were diluted with purified water/acetonitrile/formic acid 80:20:0.1 (v/v/v) depending on nominal concentrations.

Analysis was performed by HPLC-MS using a YMC Pro C18 column (150 x 4.6 mm, 3µm) at 40 °C with ESI^+ MS detection at *m/z* 288 and external calibration. A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

Validation summary

HPLC-MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the standard solution, blank matrix, fortified solutions and sample solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 3 determinations were made at each fortification level (less than the 5 replicates in accordance with the guidance) and RSDs were within the acceptable limit of 20 %. The overall RSD was between 2.7 % and 6.4 %. For M4-medium samples, the calibration range is appropriate for the nominal test concentrations and was determined using matrix-matched standards. For sediment samples, acceptable linearity was demonstrated in the range 0.000125 – 0.005 mg/L and was determined using solvent-based standards. The LOQ of the method is 0.001 mg/L in M4-medium and 50 µg/kg in sediment. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4 for M4-medium.

The RMS highlighted to the applicant concerns that, for validation in sediment, the suitability of matrix matched standards for calibration purposes had not been addressed. The applicant provided the following justification:

"The mean recoveries of the analytical method were between 108-110% indicating that the method is suitable for the determination of M750F003, possible matrix effects would have been reflected in the recoveries."

The RMS considers the applicant's case to be acceptable and, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method for sediment as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.5.3/3 Backfisch K., Weltje L., 2015 a Chronic toxicity of Reg.No. 5834378 to the non-biting midge Chironomus riparius - A spiked sediment study 2014/1243181
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.2.5.3/3* *Backfisch K., Weltje L., 2015 a* *Chronic toxicity of Reg.No. 5834378 to the non-biting midge Chironomus riparius - A spiked sediment study* *2014/1243181*

Principle of the method

The samples in M4-medium were diluted with 0.5% formic acid in acetonitrile or with a mixture of M4-medium/acetonitrile/formic acid 80:20:0.1 (v/v/v) into the range of the calibration solutions.

Sediment samples were extracted twice with 40 mL acetonitrile/water 70:30 (v/v). Extracts were either injected directly or diluted with purified water/acetonitrile/formic acid 80:20:0.1 (v/v/v) depending on nominal concentrations.

Analysis was performed by HPLC-MS using a YMC Pro C18 column (50 x 3 mm, 3µm) with a guard column (10 x 3 mm) of the same material at 40 °C with ESI^+ MS detection at *m/z* 398 and external calibration. A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

Validation summary

HPLC-MS is a highly specific method and additional confirmation was not necessary. Chromatograms of the standard solution, matrix blanks, sample solution and fortified solutions were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 3 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 3 determinations were made at each fortification level (less than the 5 replicates in accordance with the guidance) and RSDs were within the acceptable limit of 20 %. The overall RSD was between 3.9 % and 5.0 %. The calibration range is appropriate for the nominal test concentrations and was determined using matrix-matched standards for M4-medium and non-matrix-matched standards for sediment. The LOQ of the method is 0.001 mg/L in M4-medium and 50 µg/kg in sediment. The method is satisfactorily validated in accordance with SANCO/3029/99 rev.4 for M4-medium.

The RMS highlighted to the applicant concerns that, for validation in sediment, the suitability of matrix matched standards for calibration purposes had not been addressed. The applicant provided the following justification:

"The mean recoveries of the analytical method were between 93-101% indicating that the method is suitable for the determination of BAS 750 F, possible matrix effects would have been reflected in the recoveries."

The RMS considers the applicant's case to be acceptable and, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method for sediment as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.2.6.2/1 Bergfield A., 2015 a BAS 750 F: Growth inhibition test with the marine diatom, Skeletonema costatum 2015/7000620
Guidelines:	SANCO/3029/99
GLP:	Yes

Report:	KCA 8.2.6.2/3 Bergfield A., 2015 b BAS 750 F: Growth inhibition test with the freshwater diatom, Navicula pelliculosa 2015/7000618
Guidelines:	SANCO/3029/99
GLP:	Yes

Report:	KCA 8.2.6.2/5 Bergfield A., 2015 c BAS 750 F: Growth inhibition test with the cyanobacterium, Anabaena flos-aquae 2015/7000617
Guidelines:	SANCO/3029/99
GLP:	Yes

Studies supported:	*KCA 8.2.6.2/1* *Bergfield A., 2015 a* *BAS 750 F: Growth inhibition test with the marine diatom, Skeletonema costatum* *2015/7000620* *KCA 8.2.6.2/3* *Bergfield A., 2015 b* *BAS 750 F: Growth inhibition test with the freshwater diatom, Navicula pelliculosa* *2015/7000618* *KCA 8.2.6.2/5* *Bergfield A., 2015 c* *BAS 750 F: Growth inhibition test with the cyanobacterium, Anabaena flos-aquae* *2015/7000617*

Principle of the method

10 mL of the saltwater algal nutrient medum (SWAM) sample was centrifuged and 5 mL of the supernatant was transferred to a 10 mL culture tube and diluted with 5 mL hexane. The sample was vortexed. After separation, a 1 mL aliquot was removed to a clean 10 mL culture tube and blown to dryness. The evaporated sample was reconstituted in 50:50 water:acetonitrile and further diluted, if necessary, to produce sample concentrations within the linear range.

Analysis was performed by LC-MS/MS using a Waters Acquity HSS T3 column (50 mm x 2.1 mm, 1.8 µm) at 40 °C with TurboIon Spray ionization MRM monitoring Q1 mass 398.0, Q3 mass 182.0 and external calibration. A gradient elution was used (Mobile Phase A: 0.1% formic acid in water; Mobile Phase B: 0.1% formic acid in methanol).

Validation summary

HPLC-MS/MS is a highly specific method and additional confirmation was not necessary. Chromatograms of control solutions, matrix blank, calibration standards, fortified solutions and sample solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 3 determinations were made at each fortification level (less than the 5 replicates in accordance with the guidance) and RSDs were within the acceptable limit of 20 %. The overall RSD was 5.1 %. The calibration range is appropriate for the nominal test concentrations and was determined using solvent-based standards. The LOQ of the method is 0.1 µg/L.

The RMS highlighted to the applicant concerns that only 3 replicates were used to determine recoveries and repeatability at each fortification level and that the suitability of matrix matched standards for calibration purposes had not been addressed. The applicant provided the following justification:

"*BASF is the opinion that the matrix effects could not have had an influence on the results, because the source of the analyte in the the samples was known and the recoveries of the method confirmed the values expected. Regarding the number of replicates, it should be noted that the same method was used in other study (DocID: 2014/7002810 KCA CA 8.2.1/4), in total, 6 fortifications were done at two different levels and the recoveries varied between 83% and 94%. Therefore, the method is suitable for the determination of BAS 750 F."*

The RMS considers the applicant's case to be acceptable and, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Report:	KCA 8.3.1.3/1 Kleebaum K., 2015 b Acute toxicity of BAS 750 F to honeybee larvae (Apis mellifera L.) under laboratory conditions (in vitro) 2013/1235087
Guidelines:	SANCO/3029/99
GLP:	Yes
Study supported:	*KCA 8.3.1.3/1* *Kleebaum K., 2015 b* *Acute toxicity of BAS 750 F to honeybee larvae (Apis mellifera L.) under laboratory conditions (in vitro)* *2013/1235087*

Principle of the method

The diet C sugar solution sample was diluted by a factor of 400 with dilution medium (50:50 (v/v) water: methanol) and then analysed.

Analysis was performed by HPLC-UV DAD with a Phenomenex Kinetex column (2 x 100 mm, 2.6 µm) with UV detection at 231 nm and external calibration. A gradient elution was used (mobile phase A: 0.1% phosphoric acid in water, mobile phase B: 0.1% phosphoric acid in acetonitrile)

Validation summary

HPLC-UV DAD is a highly specific method and additional confirmation was not necessary. Chromatograms of calibration standards, blank solution, fortified solution and sample solution were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSD was 3.7 %. The calibration range is appropriate for the nominal test concentrations and was determined using solvent-based standards. The LOQ of the method is 68.65 mg/L.

The RMS highlighted to the applicant concerns that only 3 replicates were used to determine recoveries and repeatability at each fortification level and that the suitability of matrix matched standards for calibration purposes had not been addressed. The applicant provided the following justification:

"*BASF is the opinion that the evaluation of matrix matched standards are necessary, if a mass spectrometer is used as detector and not an UV or DAD (diode array detector). Matrix effects occur due to co-eluting matrix components, impurities or degradation products, which can affect the ionization process of the target analyte in the MS detector. The most common ionization methods used in MS are ESI (electrospray ionization) and APCI (atmospheric pressure chemical ionization), in these cases a signal enhancement or suppression can be observed. In the case of an UV/DAD detector, the transmission or the extinction coefficient are not changed and any interference is directly apparent and can be observed in the chromatogram of the matrix control sample. Therefore, an assessment of the matrix effects is not necessary in the case of an UV/DAD detector, because any interference would be seen in the matrix control sample."*

The RMS considers the applicant's case to be acceptable and, although the method is not fully validated in accordance with SANCO/3029/99 rev.4, the RMS regards the method as sufficiently validated for the purposes of the regulatory process.

Compilation of validation data for methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

Table 5.1-47: Validation data for methods used in support of ecotoxicology studies

	Matrix	**Analyte**	**LOQ (mg/L unless otherwise stated)**	**Recovery fortification level (mg/L unless otherwise stated)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
CA 8.1.1.2/1 [redacted] 2014 c CA 8.1.1.2/2 [redacted] 2015 a CA 8.1.1.2/3 [redacted] 2014 d	Ground Kliba maintenance diet quail/duck "GLP" meal	BAS 750 F	607 mg/kg	607 mg/kg 1250 mg/kg 9996 mg/kg	96.1 – 101.7 (99.4, 5) 102.9 – 108.4 (106.8, 5) 95.7 – 105.5 (102.0, 5)	2.2 (5) 2.2 (5) 3.8 (5) Overall: 4.0 (15)	0.31 – 7.6 mg/100 mL r^2 = 0.9998, 5 standards, y = 5.466x + 0.1916	Acceptable chromatograms presented for matrix solution and calibration solution. No interference >30% of LOQ
KCA 8.1.1.3/1 [redacted], 2014 a	Avian feed	BAS 750 F	30 mg/kg	30 mg/kg 600 mg/kg	91 – 102 (96, 7) 98 – 106 (100, 7)	4.31 (7) 3.17 (7) Overall: 4.2 (14)	0.5 – 10 mg/L [For samples with concentrations 0 to 150 mg/kg: Approx. 10 – 200 mg/kg For samples with concentrations 285 – 600 mg/kg: Approx. 125 – 2500 mg/kg]	Acceptable chromatograms presented for calibration standard, matrix blank, fortified matrix and sample. No interference >30% of LOQ Identity confirmed by retention time match (10.1 minutes) with reference standard

	Matrix	**Analyte**	**LOQ (mg/L unless otherwise stated)**	**Recovery fortification level (mg/L unless otherwise stated)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
							r^2 = 0.99917, 5 standards, y = 6047x + 947	
KCA 8.1.1.3/2 [redacted] 2015 a	Avian feed	BAS 750 F	30 mg/kg	30 mg/kg 600 mg/kg	99 – 102 (101, 5) 98 – 104 (101, 5)	1.62 (5) 2.36 (5) Overall: 4.2 (10)	0.500 – 5.00 mg/L [For samples with concentrations of 0 to 30.0 mg/kg: Approx. 10 – 100 mg/kg For samples with concentrations of 150 mg/kg: Approx. 25 – 250 mg/kg For samples with concentrations of 300 mg/kg: Approx. 50 – 500 mg/kg For samples with concentrations of 600 mg/kg:	Acceptable chromatograms presented for calibration standard, matrix blank, fortified matrix and sample. No interference >30% of LOQ Identity confirmed by retention time match (10.1 minutes) with reference standard

	Matrix	**Analyte**	**LOQ (mg/L unless otherwise stated)**	**Recovery fortification level (mg/L unless otherwise stated)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
							125 – 1250 mg/kg] r^2 = 1.0000, 5 standards, y = 125.99x-0.1639	
KCA 8.2.1/1 [redacted] 2014 a	Mixing water	BAS 750 F	0.001	0.001 1.5	97 – 99 (98) 100-101 (101)	1.18 (3) 0.57 (3) Overall: 1.8 (6)	0.0005 – 0.005 mg/L r^2 = 0.9999, 4 standards, y = 0.000000806x + 0.00220	Acceptable chromatograms presented for blank solution, standard solution and sample solution. No interference >30% of LOQ Identity confirmed by retention time match (6.3 min) with reference standard
KCA 8.2.1/5 [redacted] 2014 a KCA 8.2.2.1/1 [redacted] 2015 a KCA 8.2.4.2/1 VanHooser	Saltwater	BAS 750 F	0.100 µg/L	0.100 µg/L 1100 µg/L	86 – 97 (90, 3) 84 – 85 (84, 3)	7.02 (3) 0.82 (3) Overall: 5.6 (6)	0.25 – 5 µg/L y = 41089.69x + 399.6636, r = 0.9994, 5 standards	Acceptable chromatograms presented for control sample, matrix control, calibration standards, spiked solutions and treated samples. No interference >30% of LOQ Identity confirmed by

	Matrix	Analyte	LOQ (mg/L unless otherwise stated)	Recovery fortification level (mg/L unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
A., 2014 a KCA 8.2.4.2/2 VanHooser A., 2015 a KCA 8.2.5.2/4 Dinehart S. 2016 a								retention time match (1.0 min) with reference standard
KCA 8.2.2.1/2 [redacted], 2015 a	Mixing water	BAS 750 F	0.001	0.001 0.5	92 – 96 (94, 3) 101 – 102 (102, 3)	2.21(3) 0.57 (3) Overall: 4.3 (6)	0.0005 – 0.005 mg/L r^2 = 0.9999, 4 standards, y = 0.00000208x – 0.00101	Acceptable chromatograms presented for blank solution, standard solution and sample solution. No interference >30% of LOQ Identity confirmed by retention time match (6.3 min) with reference standard
KCA 8.2.2.1/3 [redacted] 2015 b	Mixing water	BAS 750 F	0.001	0.001 0.05	104 – 105 (104, 3) 101- 104 (102, 3)	0.55 (3) 1.49 (3) Overall: 1.5 (6)	0.0005 – 0.005 mg/L r^2 = 0.9996, 4 standards, y = 0.00000167x – 0.00601	Acceptable chromatograms presented for blank solution, standard solution and sample solution.

	Matrix	**Analyte**	**LOQ (mg/L unless otherwise stated)**	**Recovery fortification level (mg/L unless otherwise stated)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
								No interference >30% of LOQ Identity confirmed by retention time match (6.3 min) with reference standard
KCA 8.2.4.1/2 Backfisch K. Haerthe N., 2015 a	M4-water	M750F007 (Reg. No. 6003432) (6-(4-chlorophenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one)	0.001	0.001 12	100 – 114 (107, 5) 102 – 110 (106, 5)	6.0 (5) 3.2 (5) Overall: 4.6 (10)	0.00025 – 0.005 mg/L r^2 = 0.9998, 4 standards, y = 0.00000437x – 0.00229	Acceptable chromatograms presented for standard solution, fortified sample, blank matrix and sample solution. No interference >30% of LOQ Identity confirmed by retention time match (7.1 min) with reference standard
KCA 8.2.4.1/3 Haerthe N., 2016	M4-water	Reg. No. 5924326 (M750F003)	0.001	0.001 150	100-112 (106, 3) 101-108 (104, 3)	5.4 (3) 3.8 (3) Overall: 4.6 (6)	0.0002 – 0.004 mg/L 5 standards, r^2=0.9954, y = 0.00000303x – 0.00363	Acceptable chromatograms presented for standard solution, blank solution, fortified solution and sample solution. No interference >30% of LOQ

	Matrix	**Analyte**	**LOQ (mg/L unless otherwise stated)**	**Recovery fortification level (mg/L unless otherwise stated)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
KCA 8.2.5.1/1 Janson G.-M., 2014 a	M4-water	BAS 750 F	0.001	0.001 0.1	83 – 98 (89, 8) 96 – 103 (99, 8)	6.52 (8) 2.11 (8) Overall: 7.1 (16)	0.0005 – 0.003 mg/L r^2 = 0.9998, 4 standards, y = 0.000001484x -0.001116	Acceptable chromatograms presented for blank solution, standard solution and sample solution. No interference >30% of LOQ Identity confirmed by retention time (6.3 min) and MS (m/z 398) match with reference standard
KCA 8.2.5.2/1 Janson G.-M., 2015 b KCA 8.2.5.2/2 Janson G.-M., 2015 c	M4-water	BAS 750 F	0.001	0.001 0.1	101 – 102 (101, 5) 102 – 103 (103, 5)	0.5 (5) 0.5 (5) Overall: 0.9 (10)	0.00025 – 0.005 mg/L r^2 = 0.9995, 5 standards, y = 0.000001368x -0.003165	Acceptable chromatograms presented for standard solution, fortified sample, blank matrix and sample solution. No interference >30% of LOQ Identity confirmed by retention time (6.5 min) and MS (m/z 398) match with reference standard

	Matrix	**Analyte**	**LOQ (mg/L unless otherwise stated)**	**Recovery fortification level (mg/L unless otherwise stated)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
KCA 8.2.5.2/3 Janson G.-M., 2015 a	M4-water	BAS 750 F	0.001	0.001 0.1	98.3 – 103 (99.8, 5) 101 – 103 (102, 5)	2.2 (5) 1.1 (5) Overall: 2.0 (10)	0.00025 – 0.005 mg/L r^2 = 0.9998, 5 standards, y = 0.00000215x – 0.00302	Acceptable chromatograms presented for standard solution, fortified sample, blank matrix and sample solution. No interference >30% of LOQ Identity confirmed by retention time (6.5 min) and MS (m/z 398) match with reference standard
KCA 8.2.6.1/2 Backfisch K., 2015 a	OECD-medium	M750F007 (Reg. No. 6003432) (6-(4-chlorophenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one)	0.001	0.001 12.5	80 – 86 (83, 4) 102 – 106 (104, 5)	2.9 (4 excluding one Dixon outlier) 1.5 (5) Overall: 11.9 (9)	0.00025 – 0.005 mg/L r^2 = 0.9998, 5 standards, y = 0.0000033762x – 0.00269	Acceptable chromatograms presented for standard solution, fortified solutions, blank matrix and sample solution. No interference >30% of LOQ Identity confirmed by retention time (7.1 min) and MS (m/z 338) match with reference standard

	Matrix	Analyte	LOQ (mg/L unless otherwise stated)	Recovery fortification level (mg/L unless otherwise stated)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
KCA 8.2.1/2 [redacted] 2015 b	Water	M750F007 (Reg. No. 6003432) (6-(4-chlorophenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one)	0.002	0.002 5	100 – 110 (105.3, 5) 97 – 99 (98.2, 5)	3.2 (5) 0.9 (5) Overall: 4.9 (10)	0.0001 – 0.01 mg/L r = 0.9995, 7 standards, y = 1.40933x + 0.00344347	Acceptable chromatograms presented for blank matrix and test sample. No interference >30% of LOQ Identity confirmed by retention time match (3.7 min) with reference standard
KCA 8.2.1/3 [redacted] 2015 a	Water	BAS 750 F	0.01	0.01 1	95 – 101 (99.5, 5) 92 – 94 (93.3, 5)	2.4 (5) 0.6 (5) Overall: 3.8 (10)	0.0005 – 0.02 mg/L r = 0.9991, 6 standards, y = 0.62258x	Acceptable chromatograms presented for the control and test items. No interference >30% of LOQ
KCA 8.2.6.1/1 Brzozowska K., 2014 b KCA 8.2.7/1 Swierkot A., 2014 a	Water	BAS 750 F	0.01	0.01 0.1 1	100 – 101 (100.5, 5) 97 – 98 (97.4, 5) 94 – 97 (95.1, 5)	0.3 (5) 0.5 (5) 0.8 (5) Overall: 2.5 (15)	0.0005 – 0.02 mg/L r = 0.9998, 6 standards, y = 0.38819x + 0.05365	Acceptable chromatograms presented for control and test items. No interference >30% of LOQ
KCA 8.2.4.1/4 Rzodeczko H., 2015 c	Water	Reg.No. 5863469 (metabolite M750F006) (6-(4-chlorophenoxy)-3-methyl-	0.005	0.005 0.5	98 – 102 (100.0, 5) 89 – 91	2.4 (5) 1.1 (5)	0.0001 – 0.01 mg/L r = 0.9999, 5	Acceptable chromatograms presented for control, fortified sample, and

	Matrix	**Analyte**	**LOQ (mg/L unless otherwise stated)**	**Recovery fortification level (mg/L unless otherwise stated)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
		3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one)		5	(90.7, 5) 92 – 93 (92.3, 5)	0.6 (5) Overall: 4.6 (15)	standards, y = 113103x + 1648.70	solvent control. No interference >30% of LOQ
KCA 8.2.4.1/45 Rzodeczko H., 2015 d	Water	Reg.No. 6003433 (metabolite M750F005) (4-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenol)	0.005	0.005 0.5 5	90 – 100 (96.7, 5) 100 – 104 (101.6, 5) 97 – 99 (97.9, 5)	5.0 (5) 1.9 (5) 1.0 (5) Overall: 3.5 (15)	0.0001 – 0.01 mg/L r = 0.9999, 5 standards, y = 52752.1x + 2978.97	Acceptable chromatograms presented for control, fortified sample, and solvent control. No interference >30% of LOQ
KCA 8.2.6.1/4 Rzodeczko H. 2016 a	Water	Reg.No. 5863469 (M750F006) (6-(4-chlorophenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one)	0.005	0.005 0.5 5	98 – 102 (100.0, 5) 89 – 91 (90.7, 5) 92 – 93 (92.3, 5)	2.4 (5) 1.1 (5) 0.6 (5) Overall: 4.6 (15)	0.0001 – 0.01 mg/L r = 0.9999, 5 standards, y = 113103x + 1648.70	Acceptable chromatograms presented for control, fortified sample, and solvent control. No interference >30% of LOQ

	Matrix	**Analyte**	**LOQ (mg/L unless otherwise stated)**	**Recovery fortification level (mg/L unless otherwise stated)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
KCA 8.2.6.1/5 Rzodeczko H. 2016 b	Water	Reg.No. 6003433 (metabolite M750F005) (4-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenol)	0.005	0.005 0.5 5	89 – 100 (96.7, 5) 100 – 104 (101.6, 5) 97 – 99 (97.9, 5)	5.0 (5) 1.9 (5) 1.0 (5) Overall: 3.6 (15)	0.0001 – 0.01 mg/L r = 0.9999, 5 standards, y = 52752.1x + 2978.97	Acceptable chromatograms presented for control, fortified sample, and solvent control. No interference >30% of LOQ
KCA 8.2.6.1/6 Backfisch K., 2016 a	OECD-medum	Reg. No. 5924326 (M750F003)	0.001	0.001 120	97.8 – 99.4 (99, 3) 100-102 (101, 3)	0.9 (3) 1.3 (3) Overall: 1.4 (6)	0.0002 – 0.004 mg/L 5 standards, r^2=0.9981, y = 0.00000186x – 0.00125	Acceptable chromatograms presented for standard solution, blank solution, fortified solution and sample solution. No interference >30% of LOQ
KCA 8.2.4.1/6 Rzodeczko H., 2015e KCA 8.2.6.1/3 Brzozowska-Wojoczek K., 2015 a	Water	Reg.No. 6010286 (metabolite M750F008) (6-(5-chloro-2-hydroxyphenyl)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one)	0.5 µg/L	0.5 µg/L 50 µg/L 5000 µg/L	92 – 98 (93.8, 5) 98 – 100 (98.7, 5) 95 – 99 (97.1, 5)	3.5 (5) 0.7 (5) 1.9 (5) Overall: 2.9 (15)	0.05 – 20 µg/L r = 0.9999, 7 standards, y = 104798x +1221.15	Acceptable chromatograms presented for control and sample solutions. No interference >30% of LOQ

	Matrix	**Analyte**	**LOQ (mg/L unless otherwise stated)**	**Recovery fortification level (mg/L unless otherwise stated)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
KCA 8.2.1/6 [redacted] 2015 c	Test water (mixing water)	BAS 750 F	0.001	0.001 5.01	92 – 101 (96, 5) 90 – 98 (94, 5)	3.4 (5) 3.2 (5) Overall: 3.1 (10)	0.00025 – 0.005 mg/L r^2 = 0.9993, 5 standards, y = 710974x + 897.330	Acceptable chromatograms presented for standard solution, fortified solutions, solvent blank, matrix blank and sample solutions. No interference >30% of LOQ Identity confirmed by retention time match (1.25 min) with reference standard
KCA 8.2.5.3/1 Clark R., 2015 a KCA 8.2.5.4/1 Clark R., 2015 b KCA 8.2.5.4/2 Clark R.,	Marine sediment	BAS 750 F	0.0972 mg/kg	1 mg/kg 10 mg/kg 100 mg/kg	115 – 120 (118, 3) 102 – 106 (104, 3) 99.5 – 103 (101.5, 3)	2.14 (3) 2.01 (3) 1.78 (3) Overall: 7.2 (9)	Non-linear calibration curve Polynomial regression 1 – 50 µg/L y = 15.236x2 + 3294.5x – 1295.3, 8 standards, r^2 = 0.99976	Acceptable chromatograms presented for calibration standard, fortified sample and control sample. No interference >30% of LOQ

	Matrix	**Analyte**	**LOQ (mg/L unless otherwise stated)**	**Recovery fortification level (mg/L unless otherwise stated)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
2015 c	Filtered seawater	BAS 750 F	11.4 µg/L	20.0 µg/L 100 µg/L 1000 µg/L	96.4 – 97 (97, 3) 96.8 – 97.2 (97, 3) 96.6 – 97.7 (97, 3)	0.32 (3) 0.21 (3) 0.59 (3) Overall: 0.4 (9)	Non-linear calibration curve Polynomial regression 1 – 50 µg/L y = 15.236x2 + 3294.5x – 1295.3, 8 standards, r^2 = 0.99976	Acceptable chromatograms presented for calibration standard, fortified sample and control sample. No interference >30% of LOQ
KCA 8.2.5.3/2 Backfisch K., Weltje L., 2015 a	M4-medium	Reg.No. 5924326 (metabolite M750F003) (4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenol)	0.001	0.000957 0.478	88.8 – 95.2 (92.9, 5) 99.5 – 107 (104, 5)	2.6 (5) 3.2 (5) Overall: 6.4 (10)	0.00025 – 0.01 mg/L r^2 = 0.9995, y = 0.0000020270x – 0.0031389886, 5 standards	Acceptable chromatograms presented for standard solution, blank matrix, fortified solutions and sample solution. No interference >30% of LOQ Identity confirmed by retention time match (5.7 min) with reference standard
	Sediment	Reg.No. 5924326 (metabolite M750F003) (4-[2-hydroxy-1-(1H-1,2,4-	50 µg/kg	50 µg/kg 100 µg/kg	106 – 112 (109, 5) 104 – 112	2.5 (5) 3.2 (5)	0.000125 – 0.005 mg/L r^2 = 0.9995, y	Acceptable chromatograms presented for standard solution, blank matrix,

	Matrix	**Analyte**	**LOQ (mg/L unless otherwise stated)**	**Recovery fortification level (mg/L unless otherwise stated)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
		triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenol)		4000 µg/kg	(108, 5) 106 – 112 (110, 5)	2.1 (5) Overall: 2.7 (15)	= 0.0000020270x – 0.0031389886, 5 standards	fortified solutions and sample solution. No interference >30% of LOQ Identity confirmed by retention time match (5.7 min) with reference standard
KCA 8.2.5.3/3 Backfisch K., Weltje L., 2015 a	Sediment	BAS 750 F	50 µg/kg	50.5 µg/kg 50.4 µg/kg 1503 µg/kg 1519 µg/kg	96 – 99 (97.8, 5) 90 – 94 (91.8, 5) 104 – 105 (104.8, 5) 100 – 101 (100.2, 5)	1.33 (5) 1.62 (5) 0.43 (5) 0.45 (5) Overall: 5.0 (20)	0.0005 – 0.005 mg/L 4 standards, r^2 = 0.9994, y = 0.000000874x – 0.00316	Acceptable chromatograms presented for standard solution, matrix blanks, sample solution and fortified solutions. No interference >30% of LOQ Identity confirmed by retention time match (6.4 min) with reference standard
	M4-medium	BAS 750 F	0.001	0.001 0.005 0.5	90 – 97 (93, 10) 98 – 100 (99, 5) 99 – 102 (101, 10)	2.7 (10) 0.7 (5) 1.1 (10) Overall:	0.0005 – 0.005 mg/L 4 standards, r^2 = 0.9994, y = 0.000000874x – 0.00316	Acceptable chromatograms presented for standard solution, matrix blank, fortified solution and sample solution. No interference >30% of LOQ

	Matrix	**Analyte**	**LOQ (mg/L unless otherwise stated)**	**Recovery fortification level (mg/L unless otherwise stated)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
						3.9 (25)		Identity confirmed by retention time match (6.5 min) with reference standard
KCA 8.2.6.2/1 Bergfield A., 2015 a KCA 8.2.6.2/2 Bergfield A., 2015 b KCA 8.2.6.2/3 Bergfield A., 2015 c	SWAM	BAS 750 F	0.1 µg/L	0.1 µg/L 1100 µg/L	86 – 94 (89, 3) 82 – 85 (83, 3)	4.55 (3) 2.20 (3) Overall: 5.1 (6)	0.250 – 5 µg/L y = 44034.28x – 134.4762, r = 0.9999, 5 standards	Acceptable chromatograms presented for control solutions, matrix blank, calibration standards, fortified solutions and sample solution. No interference >30% of LOQ Identity confirmed by retention time match (1.1 min) with reference standard
KCA 8.3.1.3/1 Kleebaum K., 2015 b	Diet C sugar solution	BAS 750 F	68.65	68.65 8555	100 – 101 (100, 5) 92 – 103 (96, 5)	0.2 (5) 4.3 (5) Overall: 3.7 (10)	Low range samples: 8.311 – 20.778 mg/L [Approx. 59-146 mg/L before dilution] r^2 = 0.9999, 5 standards, y = 59378.2x +	Acceptable chromatograms presented for calibration standards, blank solution, fortified solution and sample solution. No interference >30% of LOQ Identity confirmed by retention time match

	Matrix	**Analyte**	**LOQ (mg/L unless otherwise stated)**	**Recovery fortification level (mg/L unless otherwise stated)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
							1098.15 High range samples: 7.290 – 24.310 mg/L [Approx. 2916 – 9724 mg/L before dilution] r^2 = 0.9994, 5 standards, y = 60585.8x + 9624.79	(4.0 min) with reference standard

B.5.1.2.7. Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

B.5.1.2.7.1. Determination of active substance in solubility in water and organic solvents tests

HPLC method for the determination of BAS 750 F in solubility in water, buffer solutions and organic solvents

Report:	CA 2.6/1 Wilbrand S., 2013 b Determination of the solubility in organic solvents of Reg.No. 5834378 2013/1391669
Guidelines:	SANCO/3029/99
GLP:	Yes
Report:	CA 2.5/1 Wilbrand S., 2013 a Determination of the solubility in distilled water and in buffer solutions at pH 4 and pH 7 (Column Elution Method) of Reg.No. 5834378 2013/1397136
Guidelines:	SANCO/3029/99
GLP:	Yes
Report:	Sonnenschein L., 2016 a Physical and Chemical Properties of BAS 750 F (Reg.No. 5834378): Additional Validation of Analytical Method Applied for Determination of Solubility in Water and Organic Solvents 2016/1234174
Guidelines:	SANCO/3029/99
GLP:	Yes
Studies supported:	*CA 2.6/1* *Wilbrand S., 2013 b* *Determination of the solubility in organic solvents of Reg.No. 5834378* *2013/1391669* *CA 2.5/1* *Wilbrand S., 2013 a* *Determination of the solubility in distilled water and in buffer solutions at pH 4 and pH 7 (Column Elution Method) of Reg.No. 5834378* *2013/1397136*

Principle of the method

For solubility in water and buffer solutions, preparation was performed in accordance with EEC A.6 and OECD 105. BAS 750 F was coated onto glass beads and transferred to a micro column filled with solvent and left to swell. After elution the test item was swilled down the glass beads with acetonitrile and a sample of the solution was analysed.

For solubility in organic solvents, saturated solutions of BAS 750 F were prepared in accordance with CIPAC method MT 181 and diluted to bring the concentration into the working range of the HPLC method.

Analysis was performed by HPLC-UV using a Nucleosil C18 5 µm, Macherey-Nagel column (250 x 8 mm x 2.1 mm for solubility in organic solvents; 250 x 8 mm x 4 mm for solubility in water and buffer solutions) at 40 °C with UV detection at 230 nm and external calibration. The mobile phase was acetonitrile/water with 0.5% formic acid in the aqueous phase, 60/40 (v/v). No CIPAC methods are available for the determination of BAS 750 F in water, buffer solutions and organic solvents.

Validation summary

HPLC-UV is a highly specific method and additional confirmation was not necessary. Chromatograms of blank matrices, test items and calibration standards were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at a two fortification levels and in all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made and the RSD was within the acceptable limit of 20 %. The overall RSD was 6.9 %. The calibration range is appropriate for the nominal test concentrations and was determined using solvent-based standards. The applicant's reasoning for this choice of standards is that "*the high dilution with acetonitrile results in the fact that each particular solvent has a minimum impact on the chromatographic separation, hence securing that the interaction between BAS 750 F and the tested solvent are negligible against the interaction of BAS 750 F and acetonitrile*". The LOQ of the method is 0.61 mg/L. The method is fit for purpose but not fully validated in accordance with SANCO/3029/99 rev.4 as the suitability of matrix-matched standards has not been fully addressed.

B.5.1.2.7.2. Determination of metabolites in water solubility tests

Method for analysis of metabolite Reg.No. 5924326 in water solubility

Report:	CA 2.14/6 Wilbrand S., 2015 a Reg.No. 5924326: Solubility in water (Flask method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water 2015/1139989
Guidelines:	SANCO/3029/99
GLP:	yes
Report:	CA 2.14/7 Wilbrand S., 2016 a Amendment No. 1 - Reg.No. 5924326: Solubility in water (Flask method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water 2015/1252305
Guidelines:	SANCO/3029/99
GLP:	yes
Report:	CA 2.14/8 Wilbrand S., 2016 b Amendment No. 2 - Reg.No. 5924326: Solubility in water (Flask method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water 2016/1030230
Guidelines:	SANCO/3029/99
GLP:	yes
Studies supported:	*CA 2.14/6* *Wilbrand S., 2015 a* *Reg.No. 5924326: Solubility in water (Flask method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water* *2015/1139989* *CA 2.14/7* *Wilbrand S., 2016 a* *Amendment No. 1 - Reg.No. 5924326: Solubility in water (Flask method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water* *2015/1252305* *CA 2.14/8* *Wilbrand S., 2016 b* *Amendment No. 2 - Reg.No. 5924326: Solubility in water (Flask method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water* *2016/1030230*

Principle of the method

25 mg Reg.No. 5924326 (M750F003) (4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenol) was added to 5 mL water or buffer solutions and stirred. The test item was precipitated, the sample separated and centrifuged. Samples of the satured solutions were diluted with eluent by a factor of 250 (was acetonitrile / 0.1% phosphoric acid in Millipore water, 40:60 (v/v)).

Analysis was performed by HPLC-UV using a Luna-C18 Phenomenex column (250 x 4.6 mm, 5 µm) at 40 °C with UV detection at 225 nm and external calibration. The mobile phase was acetonitrile / 0.1% phosphoric acid in Millipore water, 40:60 (v/v).

Validation summary

HPLC-UV is not a highly specific method however validation has shown that it is specific to the analyte of interest. Chromatograms of blank matrices, calibration standards and test items were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at a single fortification level and the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made and the RSD was within the acceptable limit of 20 %. The calibration range is appropriate for the nominal test concentrations using solvent-based standards. The LOQ of the method is 20 mg/L. The method is fit for purpose but not fully validated in accordance with SANCO/3029/99 rev.4 as the suitability of matrix-matched standards has not been addressed.

Method for analysis of metabolite Reg.No. 6003433 in water solubility

Report:	CA 2.14/9 Wilbrand S., 2015 c Reg.No. 6003433: Solubility in water (Column Elution Method) (double distilled water, ph 4, ph 9), partition coefficient 1-Octanol/Water (distilled water, ph 4, ph 9) and dissociation constant in water (original No. 1 of 2) 2015/1139993
Guidelines:	SANCO/3029/99
GLP:	yes
Study supported:	*CA 2.14/9* *Wilbrand S., 2015 c* *Reg.No. 6003433: Solubility in water (Column Elution Method) (double distilled water, ph 4, ph 9), partition coefficient 1-Octanol/Water (distilled water, ph 4, ph 9) and dissociation constant in water (original No. 1 of 2)* *2015/1139993*

Principle of the method

Reg.No. 6003433 (M750F005) (4-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenol) was coated onto glass beads and transferred to a micro column filled with solvent and left to swell. After elution the test item was rinsed from the glass beads with acetonitrile and diluted (1:20) with HPLC eluent (acetonitrile / 0.1% phosphoric acid in Millipore water, 40:60 (v/v)) a sample of the solution was analysed. Samples of the collected fractions were diluted with acetonitrile (factor 1.25) and analysed.

Analysis was performed by HPLC-UV using a Luna-C18 Phenomenex column (250 x 4.6 mm, 5 µm) at 40 °C with UV detection at 210 nm and external calibration. The mobile phase was acetonitrile / 0.1% phosphoric acid in Millipore water, 40:60 (v/v).

Validation summary

HPLC-UV is not a highly specific method however validation has shown that it is specific to the analyte of interest. Chromatograms of blank matrices, calibration standards and test items were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at a single fortification level and the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made and the RSD was within the acceptable limit of 20 %. The calibration range is appropriate for the nominal test concentrations using solvent-based standards. The LOQ of the method is 1.01 mg/L. The method is fit for purpose but not fully validated in accordance with SANCO/3029/99 rev.4 as the suitability of matrix-matched standards has not been addressed.

Method for analysis of metabolite Reg.No. 5863469 in water solubility

Report:	CA 2.14/10 Wilbrand S., 2015 d Solubility in water (column elution method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water (original no. 2 of 2) 2015/1139994
Guidelines:	SANCO/3029/99
GLP:	yes
Study supported:	*CA 2.14/10* *Wilbrand S., 2015 d* *Solubility in water (column elution method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water (original no. 2 of 2)* *2015/1139994*

Principle of the method

Reg.No. 5863469 (M750F006) (6-(4-chlorophenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one) was coated onto glass beads and transferred to a micro column filled with solvent and left to swell. After elution the test item was rinsed from the glass beads with acetonitrile and diluted (1:20) with HPLC eluent (acetonitrile / 0.1% phosphoric acid in Millipore water, 40:60 (v/v)) a sample of the solution was analysed. Samples of the collected fractions were diluted with acetonitrile (factor 1.5) and analysed.

Analysis was performed by HPLC-UV using a Luna-C18 Phenomenex column (250 x 4.6 mm, 5 µm) at 40 °C with UV detection at 210 nm and external calibration. The mobile phase was acetonitrile / 0.1% phosphoric acid in Millipore water, 60:40 (v/v).

Validation summary

HPLC-UV is not a highly specific method however validation has shown that it is specific to the analyte of interest. Chromatograms of blank matrices, calibration standards and test items were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at a single fortification level and the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made and the RSD was within the acceptable limit of 20 %. The calibration range is appropriate for the nominal test concentrations using solvent-based standards. The LOQ of the method is 14 mg/L. The method is fit for purpose but not fully validated in accordance with SANCO/3029/99 rev.4 as the suitability of matrix-matched standards has not been addressed.

Method for analysis of metabolite Reg.No. 6003432 in water solubility

Report:	CA 2.14/11 Wilbrand S., 2015 d Solubility in water (flask method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water (original no. 2 of 2) 2015/1139997
Guidelines:	SANCO/3029/99
GLP:	yes
Study supported:	*CA 2.14/11* *Wilbrand S., 2015 d* *Solubility in water (flask method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water (original no. 2 of 2)* *2015/1139997*

Principle of the method

10 mg Reg.No. 6003432 (M750F007) (6-(4-chlorophenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one) was added to 5 mL water or buffer solutions and stirred. The test item was precipitated, the sample separated and centrifuged. Samples of the saturated solutions were diluted (1:5) with eluent (was acetonitrile / 0.1% phosphoric acid in Millipore water, 40:60 (v/v)) and analysed.
Analysis was performed by HPLC-UV using a Luna-C18 Phenomenex column (250 mm x 4.6 mm, 5 µm) at 40 °C with UV detection at 210 nm and external calibration. The mobile phase was acetonitrile / 0.1% phosphoric acid in Millipore water, 40:60 (v/v).

Validation summary

HPLC-UV is not a highly specific method however validation has shown that it is specific to the analyte of interest. Chromatograms of blank matrices, calibration standards and test items were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at a single fortification level and the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made and the RSD was within the acceptable limit of 20 %. The calibration range is appropriate for the nominal test concentrations using solvent-based standards. The LOQ of the method is 10 mg/L. The method is fit for purpose but not fully validated in accordance with SANCO/3029/99 rev.4 as the suitability of matrix-matched standards has not been addressed.

Method for analysis of metabolite Reg.No. 6010286 in water solubility

Report:	CA 2.14/12 Wilbrand S., 2015 e Solubility in water (column elution method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water (Original No. 2 of 2) 2015/1139998
Guidelines:	SANCO/3029/99
GLP:	yes
Study supported:	*CA 2.14/12* *Wilbrand S., 2015 e* *Solubility in water (column elution method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water (Original No. 2 of 2)* *2015/1139998*

Principle of the method

Reg.No. 6010286 (M750F008) (6-(5-chloro-2-hydroxyphenyl)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one) was coated onto glass beads and transferred to a micro column filled with solvent and left to swell. After elution the test item was rinsed from the glass beads with 40 mL acetonitrile and diluted (1:20) with HPLC eluent (acetonitrile / 0.1% phosphoric acid in Millipore water, 40:60 (v/v)) a sample of the solution was analysed. Samples of the collected fractions were diluted with acetonitrile (factor 1.5) and analysed. Analysis was performed by HPLC-UV using a Luna-C18 Phenomenex column (250 mm x 4.6 mm, 5 µm) at 40 °C with UV detection at 220 nm and external calibration. The mobile phase was acetonitrile / 0.1% phosphoric acid in Millipore water, 40:60 (v/v).

Validation summary

HPLC-UV is not a highly specific method however validation has shown that it is specific to the analyte of interest. Chromatograms of blank matrices, calibration standards and test items were provided showing no interference >30% LOQ at the retention time of interest. Accuracy was assessed at a single fortification level and the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made and the RSD was within the acceptable limit of 20 %. The calibration range is appropriate for the nominal test concentrations using solvent-based standards. The LOQ of the method is 0.52 mg/L. The method is fit for purpose but not fully validated in accordance with SANCO/3029/99 rev.4 as the suitability of matrix-matched standards has not been addressed.

Compilation of validation data for methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

Table 5.1-48: Validation data for methods used in support of physical and chemical properties tests studies

	Analyte	**LOQ (mg/L)**	**Recovery fortification level (mg/L)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
CA 2.6/1 Wilbrand S., 2013 b CA 2.5/1 Wilbrand S., 2013 a Sonnenshein L., 2016 a	BAS 750 F	0.61	0.61 5	81.5 – 89.7 (86.0, 5) 97.4 – 100.0 (98.3, 5)	5.3 (5) 1.2 (5) Overall: 6.9 (10)	0.51 – 20.40 mg/L 6 standards, y = 2.00977x – 0.10333, r = 0.9994	Acceptable chromatograms presented for blank matrices, test items and calibration standards. No interference >30% of LOQ Identity confirmed by retention time match with reference standard (c. 7.0 min)
CA 2.14/6 Wilbrand S., 2015 a CA 2.14/7 Wilbrand S., 2016 a CA 2.14/8 Wilbrand S., 2016 b	Reg.No. 5924326 (M750F003) (4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenol)	20	20	100.6 – 101.1 (100.9, 5)	0.1 (5) @ 20 mg/L	5.03 – 51.29 mg/L % of mean concentration from solubility measurements: 51 – 521 % in double distilled water 52 – 530 % in pH 4 (acetate buffered) water 51 – 517 % in pH 9 (borate buffered) water 6 standards, r = 1.0000, y =	Acceptable chromatograms presented for blank matrices, calibration standards and test items. No interference >30% of LOQ

						0.87109x + 0.01276	
CA 2.14/9 Wilbrand S., 2015 c	Reg.No. 6003433 (M750F005) (4-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenol)	500000000000	5	99.2 – 106.5 (103.2, 5)	2.5 (5) @ 5 mg/L	1.04 – 31.19 mg/L % of mean concentration from solubility measurements: Double distilled water: 12 – 345 % Acetate buffer: 13 – 394 % Borate buffer: 9 – 283 % r = 1.0000, 6 standards, y = 1.44914x + 0.01267	Acceptable chromatograms presented for blank matrices, calibration standards and test items. No interference >30% of LOQ
CA 2.14/10 Wilbrand S., 2015 d	Reg.No. 5863469 (M750F006) (6-(4-chlorophenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one)	14	14	98.3 – 100.2 (99.5, 5)	0.7 (5) @ 14 mg/L	1.00 – 41.83 mg/L % of mean concentration from solubility measurements: Double distilled water: 13 - 560% Acetate buffer: 8 - 332 % Borate buffer: 11 - 451% y = 2.26152x – 0.30263, r = 0.9989, 6 standards	Acceptable chromatograms presented for blank matrices, calibration standards and test items. No interference >30% of LOQ
CA 2.14/11 Wilbrand S., 2015 d	Reg.No. 6003432 (M750F007) (6-(4-chlorophenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one)	10	10	99.70 – 100.59 (100.1, 5)	0.3 (5) @ 10 mg/L	0.98 – 29.97 mg/L % of mean concentration from solubility measurements: Double distilled water: 7 – 206 % Acetate buffer: 7 - 204% Borate buffer: 7 - 209% y = 2.47431x + 0.09565, r =	Acceptable chromatograms presented for blank matrices, calibration standards and test items. No interference >30% of LOQ

						0.9997, 6 standards	
CA 2.14/12 Wilbrand S., 2015 e	Reg.No. 6010286 (M750F008) (6-(5-chloro-2-hydroxyphenyl)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one)	25	25	98.0 – 99.4 (98.8, 5)	0.48 (5) @ 25 mg/L	0.52 – 59.15 mg/L % of mean concentration from solubility measurements: Double distilled water: 40 – 4527 % Acetate buffer: 32 - 3651 % Borate buffer: 21 - 2418 % y = 3.04004x – 0.23844, r = 0.9999, 7 standards	Acceptable chromatograms presented for blank matrices, calibration standards and test items. No interference >30% of LOQ

B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES

The following table summarises the methods of analysis available for post-approval control and monitoring purposes for BAS 750 F.

Table 5.2-1: Monitoring methods available

Matrix	Analyte	Method	LOQ	ILV?	Fully validated?	Method number	Reference
Tomato (fruit)	BAS 750 F	LC-MS/MS	0.01 mg/kg	Yes	Yes	L0295/01	Klimmek S. et al., 2015 a (ILV: Richter S.,Schmiedt S., 2015 a)
Orange (whole fruit)							
Dry beans (seeds)							
Wheat (grain)							
Dry soybeans (seeds)							
Bovine meat	BAS 750 F	LC-MS/MS	0.01 mg/kg	Yes	Yes	L0272/01	Devine C., 2015 a (ILV: Richter S.,Djedovic S., 2015 b)
Bovine milk							
Bovine cream							
Bovine fat							
Bovine liver							
Bovine kidney							
Hen eggs							
LUFA 2.2 soil	BAS 750 F	LC-MS/MS	0.002 mg/kg	N/A	Yes	L0214/01	Studenroth S.,Luer D., 2015 a
LUFA 2.3 soil	BAS 750 F						
Surface and drinking water	BAS 750 F	LC-MS/MS	30 ng/kg	Yes	Yes	D1506/01	Malinsky D.S., 2016 a (ILV: Gu G. et al., 2016 a)
Air	BAS 750 F	LC-MS/MS	0.01 ng/L	N/A	Yes	L0327/01	Obermann M., Studenroth S., 2015 b
Urine Blood	BAS 750 F	LC-MS/MS	0.01 mg/L	N/A	Yes	L0339/01	Wiesner, W., Breyer N., 2016

B.5.2.1. Methods for the analysis in food and feed of plant and animal origin

Extraction efficiency is discussed in detail, including full study summaries, in the DAR Volume 3 in Section CA B.7.2.1.4 (plants) and CA B.7.2.3 (animals).

For plants, samples from metabolism studies in wheat, soybean and grapevine were used to investigate extraction efficiency of radiolabelled BAS 750 F. The extraction procedure used in the analytical method to support the residues trials, namely BASF method 535/1, as well as three multi residue methods, were compared to the extraction procedure used in the metabolism studies. When using method 535/1, extraction efficiencies for BAS 750 F were 90% or higher for all matrices investigated, namely wheat forage (98%), wheat straw (111%), soybean green pod (102%) and grapevine grape (93%).

For the multi-methods, extraction efficiency of BAS 750 F was lower for forage (QuEChERS 80%, DFG S 19 63%, SweEt 56%), and for straw (QuEChERS 59%, DFG S 19 52%, SweEt 65%) while similar high extraction efficiency was observed for soybean green pod and grapevine grape (88% or higher). Validation of the QuEChERs method as the method for post approval monitoring of plant commodities is given in section B.5.2.1.1. However, as lower efficiencies were determined for dry commodities (wheat straw), use of method BASF method 535/1 may be more appropriate for such commodities. This method is validated as a pre-registration method for risk assessment in section B.5.1.2.5 (method 535/1 is equivalent to L0076/09, see B.5.1.2.5), and it is also satisfactorily validated as a post approval monitoring method in accordance with SANCO/825/00 rev.8.1 (with the exception of ILV), so could be used for monitoring.

For animals, samples from metabolism studies in hen (fat, liver, muscle and egg) and goat (cream, milk and kidney) were used to investigate extraction efficiency of radiolabelled BAS 750 F. The extraction procedure used in the analytical method to support the feeding studies, L0272/01 was compared to the extraction procedure used in the metabolism studies. Extraction efficiencies were 80% or higher for all matrices except liver which was ~46%. An additional method for the monitoring of BAS 750 F in liver is currently being developed.

The residue definition for monitoring in plants and animals is BAS 750 F. The analytical methods below are consistent with the residue definition.

B.5.2.1.1. Plants

Report:	KCA 4.2/1 Klimmek S. et al., 2015 a Validation of the multi-residue method QuEChERS, BASF method number L0295/01, for the determination of BAS 750 F in different matrices of plant origin 2015/1106708
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07, EPA 860.1340 (1996), SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by Freie und Hansestadt Hamburg, Behoerde fuer Gesundheit und Verbraucherschutz, Hamburg, Germany)

Principle of the method

Each 10 g of tomato (fruit) and orange (whole fruit) or 5 g of dry beans (seeds), wheat (grain), dry soybeans (Seeds) was weighed into a 50 mL Sarstedt centrifuge tube. 10 mL of water was added to dry beans, wheat and dry soybeans. 10 mL acetonitrile was added to all samples and shaken. 4.0 g of magnesium sulfate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate were added and the tube shaken and centrifuged. All extracts, except for those of tomato, were frozen before being centrifuged. 40 mg of PSA (primary secondary amine), 25 mg of ODS (octadecyl silica) and 225 mg of magnesium sulfate was weighed into a 2 mL safe-lock tube. 1.5 mL of the acetonitrile phase was transferred into the tube containing the mixture of PSA, ODS and magnesium sulfate before being shaken and centrifuged. For tomato and orange: 250 µL of the extract and 750 µL of acetonitrile were mixed with 4 mL of water in a test tube. For dry beans, wheat, dry soybeans: 500 µL of the extract and 500 µL of acetonitrile are mixed with 4 mL of water in a test tube.

Analysis was performed by LC-MS/MS using an Ascentis Express C18 column (50 mm x 2.1 mm, 2.7 µm) at 30 °C with external calibration and ESI^+ detection at the following selective ion transitions: *m/z* 398 → 70

(quantification) and *m/z* 398 → 182 (confirmation). A gradient elution was used (mobile phase A: 0.1 % formic acid in acetonitrile; mobile phase B: 0.1% formic acid in water).

Matrix effects

The effect of each matrix on the response of BAS 750 F was determined by comparison of the peak areas of matrix-matched standards and solvent standards were equivalent concentrations.

Table 5.2-2: Matrix effects

Matrix	**Analyte**	**Standard concentration (ng/mL)**	**Mass transition**	**Peak area in matrix matched standards compared to solvent standards (%)***
Tomato (fruit)	BAS 750 F	0.25 – 7.5	398 → 70	100.6
			398 → 182	99.2
Orange (whole fruit)	BAS 750 F	0.25 – 7.5	398 → 70	95.8
			398 → 182	98.4
Dry beans (Seeds)	BAS 750 F	0.25 – 7.5	398 → 70	107.4
			398 → 182	102.2
Wheat (grain)	BAS 750 F	0.25 – 7.5	398 → 70	92.4
			398 → 182	90.4
Dry soybeans (seeds)	BAS 750 F	0.25 – 7.5	398 → 70	98.1
			398 → 182	94.3

*These are mean values calculated from all concentrations tested

Matrix effects on the detection of BAS 750 F in extracts of tomato (fruit), orange (whole fruit), dry beans (seeds) and dry soybeans (seeds) were found to be insignificant (< 20 %). Therefore, solvent standards were used for quantification, respectively. No significant matrix effects (< 20 %) were found in the detection of BAS 750 F in extracts of wheat (grain), nevertheless matrix-matched standards were used for quantification of BAS 750 F in extracts of wheat (grain).

Stability of stock solutions

A stock solution prepared in acetone was stored at 5 °C ± 4 °C for 44 days which covers the length of time it was used in this study. After storage, the stock solution was compared to a freshly prepared stock solution in acetone at the same concentration. One ion mass transition (*m/z* 398 → 70) was evaluated.

Table 5.2-3: Stability of stock solutions

Matrix	Analyte	Concentration (µg/mL)	Storage period (days)	Difference in peak area (%)
Acetone	BAS 750 F	400	44	-3.0

The peak area of the stored solution was within ± 20 % of the peak area of the freshly prepared solution therefore nalytical standards of BAS 750 F were found to be stable for at least 44 days when prepared in acetone and stored refrigerated (1 °C to 10 °C) in the dark.

Stability of calibration solutions

The calibration solutions prepared in solvent mixture (acetonitrile/water, 20/80, v/v) were stored at 5 °C ± 4 °C for 30 days, which was sufficient to cover the length of time they were used in this study. After this time six standards were compared to a freshly prepared solution of the same concentration. One ion mass transition (*m/z* 398→70) was evaluated.

Table 5.2-4: Stability of calibration solutions

Matrix	Analyte	Standard	Storage period	Difference in

		concentration (µg/mL)	(days)	peak area (%)
Acetonitrile/water (20/80, v/v)	BAS 750 F	0.15	30	10.5
		0.50	30	13.3
		1.0	30	1.5
		2.5	30	4.9
		5.0	30	13.4
		7.5	30	7.9

The mean peak area of the stored solutions was within ± 20 % of the peak area of the freshly prepared solutions indicating that standards were stable for the testing period when stored in the tested conditions.

Stability of extracts

Following first analysis, the final extracts of fortified samples at 10xLOQ level together with one control specimen extract were stored at 5 °C ± 4 °C for 6 to 11 days. After this time period, the final extracts were re-analysed against fresh calibration standards comparing one ion mass transition (*m/z* 398→70) of the analyte.

Table 5.2-5: Stability of extracts

Matrix	Analyte	Fortification level (mg/kg)	Days of storage (1st to 2nd injection)	Mean Recovery 1st Injection (%)	Mean Recovery 2nd Injection (%)
Tomato (fruit)	BAS 750 F	0.1	7	81.5	92.5
Orange (whole fruit)	BAS 750 F	0.1	6	86.0	98.5
Dry beans (seeds)	BAS 750 F	0.1	11	97.0	93.8
Wheat (grain)	BAS 750 F	0.1	8	91.9	104
Dry soybeans (seeds)	BAS 750 F	0.1	7	71.8	71.6

Mean recovery values of the re-analysed extracts were in the range of 70 – 120 % and within 20 % of the original result. Therefore, extracts were considered to be stable when stored at 1 °C to 10 °C for at least 6 days.

Validation summary

HPLC-MS/MS is a highly specific technique and two mass transitions (3 ions) were monitored as outlined in the guidance document. Chromatograms of solvent standard, matrix blank and fortified sample have been presented showing no interferences >30% of LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels for the analyte in each matrix of interest corresponding to LOQ and 10xLOQ and in all cases the mean recovery was within the acceptable range of 70 – 120 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSDs were between 2.5 % and 14 %. The linear range is appropriate for the nominal test concentrations, and was determined using solvent-based standards except for wheat (grain) in which matrix-matched standards were used although no matrix effects were observed in any matrix. The LOQ of the method is 0.01 mg/kg. The method is satisfactorily validated in accordance with SANCO/825/00 rev.8.1. The following commodity groups are covered: high water content commodities (tomato), high acid content commodities (orange), high starch/dry commodities (wheat grain), high protein/dry commodities (dry beans) and high oil content commodities (soybean).

Validation data

Table 5.2-6: Validation data

Matrix	Analyte	Mass transition (*m/z*)	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
Tomato (fruit)	BAS 750 F	398 → 70	0.01	0.01 0.10	72.2 – 76.2 (74.8, 5) 76.9 – 85.0 (81.5, 5)	2.2 (5) 3.8 (5) Overall: 5.4 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] r = 0.9983, 7 standards, y = 347024x + 25713	Acceptable chromatograms presented for solvent standard, matrix blank and fortified sample. No interference >30% of LOQ Identity confirmed using LC-MS/MS to monitor two ion mass transitions (*m/z* 398 → 70 and *m/z* 398 → 182)
		398 → 182	0.01	0.01 0.10	79.8 – 85.4 (81.5, 5) 78.2 – 82.1 (80.0, 5)	2.9 (5) 1.9 (5) Overall: 2.5 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] r = 0.9997, 7 standards, y = 35568x + 10.6527	
Orange (whole fruit)	BAS 750 F	398 → 70	0.01	0.01 0.10	77.6 – 91.4 (84.3, 5) 80.9 – 90.1 (86.0, 5)	6.2 (5) 4.2 (5) Overall: 5.1 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] r = 0.9985, 7 standards, y = 292481x + 26810	Acceptable chromatograms presented for solvent standard, matrix blank and fortified sample. No interference >30% of LOQ Identity confirmed using LC-MS/MS to monitor two ion mass transitions (*m/z* 398 → 70 and *m/z* 398 → 182)
		398 → 182	0.01	0.01 0.10	80.6 – 88.5 (83.8, 5) 77.9 – 83.8 (80.8, 5)	3.5 (5) 3.5 (5) Overall: 3.8 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] r = 0.9990, 7 standards, y = 349625x + 5277	

Dry beans (seeds)	BAS 750 F	398 → 70	0.01	0.01 0.10	85.3 – 96.4 (91.1, 5) 63.0 – 110 (97.0, 5)	4.4 (5) 20 (5) Overall: 14 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] r = 0.9972, 7 standards, y = 305862x + 27548	Acceptable chromatograms presented for solvent standard, matrix blank and fortified sample. No interference >30% of LOQ Identity confirmed using LC-MS/MS to monitor two ion mass transitions (*m/z* 398 → 70 and *m/z* 398 → 182)
		398 → 182	0.01	0.01 0.10	87.6 – 96.3 (91.6, 5) 61.9 – 102 (90.4, 5)	3.7 (5) 18 (5) Overall: 12 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] r = 0.9999, 7 standards, y = 34342 + 663	
Wheat (grain)	BAS 750 F	398 → 70	0.01	0.01 0.10	72.9 – 89.1 (83.7, 5) 87.5 – 96.2 (91.9, 5)	7.5 (5) 4.3 (5) Overall: 7.5 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] r = 0.9998, 7 standards, y = 344586x + 12471	Acceptable chromatograms presented for solvent standard, matrix blank and fortified sample. No interference >30% of LOQ Identity confirmed using LC-MS/MS to monitor two ion mass transitions (*m/z* 398 → 70 and *m/z* 398 → 182)
		398 → 182	0.01	0.01 0.10	86.4 – 88.8 (87.5, 5) 87.9 – 96.7 (92.7, 5)	1.1 (5) 4.5 (5) Overall: 4.4 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] r = 0.9972, 7 standards, y = 34403x + 733	
Soybean (seeds)	BAS 750 F	398 → 70	0.01	0.01 0.10	69.6 – 81.2 (75.3, 5) 67.8 – 75.9 (71.8, 5)	6.1 (5) 5.1 (5) Overall: 5.9 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] r = 1.0000, 7 standards, y =	Acceptable chromatograms presented for solvent standard, matrix blank and fortified sample. No interference >30% of LOQ Identity confirmed using LC-

							346017x + 6516	MS/MS to monitor two ion mass transitions (*m/z* 398 → 70 and *m/z* 398 → 182)
		398 → 182	0.01	0.01	67.8 – 74.8 (71.7, 5)	3.9 (5)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg]	
				0.10	64.1 – 77.9 (70.2, 5)	8.0 (5)		
						Overall: 6.0 (10)	r = 0.9999, 7 standards, y = 34571x + 1275	

Report:	KCA 4.2/2 Richter S.,Schmiedt S., 2015 a Independent method validation (ILV) of the QuEChERS method for the determination of BAS 750 F in 5 plant matrices, using LC/MS/MS (BASF Method No. L0295/01) 2015/1240004
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 860.1340 (1996), OECD-ENV/JM/MONO/(2007)17
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the method

The method used was as reported for KCA 4.2/1, except minor modifications were made to the centrifugation regime and an aliquot of 6 mL instead of 1.5 mL was used to add to the PSA (primary secondary amine) clean up step. Matrix-matched standards were also used instead of solvent-based standards as stated in the "matrix effects" section below.

Matrix effects

The response of each analyte in matrix-matched standards compared to solvent based standards was compared. Concentrations ranging from 0.15 ng/mL to 7.5 ng/mL were prepared. The matrix effect was the ratio of the slope of the calibration curve in matrix-matched standards to the slope of the calibration curve in solvent standards, expressed as a percentage (with the slope for the solvent standards set to 100 %).

Table 5.2-7: Matrix effects

Matrix	**Analyte**	**Mass transition (*m/z*)**	**Slope in matrix matched standards compared to solvent standards (%)**
Tomato fruit	BAS 750 F	398 → 70	103
		398 → 182	103
Wheat grain	BAS 750 F	398 → 70	111
		398 → 182	113
Dried broad beans	BAS 750 F	398 → 70	111
		398 → 182	110
Dried soybeans	BAS 750 F	398 → 70	105
		398 → 182	104
Whole orange	BAS 750 F	398 → 70	100
		398 → 182	100

*These are mean values calculated from all concentrations tested

No significant matrix effects (<20% suppression or enhancement) in the LC-MS/MS response were observed for any matrix. However, for the evaluation of the results, matrix-matched standards were still used.

Stability of standards

The stability of stock solution (44 days of refrigerated storage) and calibration solutions (30 days of refrigerated storage) was demonstrated in the validation of the original method. The stability of the analyte in fortification solutions (solvent: acetonitrile) was shown in the present study for 11 days when stored refrigerated at 3 - 8 °C. The percentage difference in the peak area between a stored solution and a freshly prepared solution was calculated at a fortification of 1.0 ng/mL was determined.

Table 5.2-8: Stability of standards

Matrix	Analyte	Storage period (days)	Mass transition (*m/z*)	Concentration (ng/mL)	% difference in peak area (n)
Acetonitrile	BAS 750 F	11	398 → 70	1.0	10 (6)
			398 → 182	1.0	11 (6)

Stability of extracts

The stability of extract solutions (for at least 6 days of refrigerated storage) was demonstrated in the validation of the original method.

Validation summary

HPLC-MS/MS is a highly specific technique and two mass transitions (3 ions) were monitored as outlined in the guidance document. Chromatograms of standard solution, blank matrix and fortified sample have been presented showing no interferences >30% of LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels for the analyte in each matrix of interest corresponding to LOQ and 10xLOQ and in all cases the mean recovery was within the acceptable range of 70 – 120 %. To assess method precision, 5 determinations were made at each fortification level (with the exception of two fortification levels where only 4 determinations were made) and RSDs were within the acceptable limit of 20 %. It is noted that two recovery results which were previously excluded from the mean recovery and RSD were found to be not significant outliers and have now been included (for the lowest fortification for dried soybeans for both transitions). The overall RSDs were between 3.6 % and 7.1 %. The linear range is appropriate for the nominal test concentrations, and was determined using solvent-based standards as no matrix effects were observed. The ILV confirms the LOQ of the method is 0.01 mg/kg. The ILV employs a sample preparation technique and method of analysis which are sufficiently similar to the original study.The reproducibility of the method has therefore been successfully shown. The ILV of the analytical method is therefore satisfactorily validated in accordance with SANCO/825/00 rev.8.1. The following commodity groups are covered: high water content commodities (tomato), high acid content commodities (orange), high starch/dry commodities (wheat grain), high protein/dry commodities (dry beans) and high oil content commodities (soybean).

Validation data

Table 5.2-9: Validation data

Matrix	Analyte	Mass transition (*m/z*)	**LOQ (mg/kg)**	**Recovery fortification level (mg/kg)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	Linearity	Specificity
Tomato fruit	BAS 750 F	398 → 70	0.01	0.01 0.1	83.2 – 86.4 (84.5, 5) 75.4 – 96.4 (85.4, 5)	1.5 (5) 10 (5) Overall: 7.1 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] 7 standards, y = 5.5×10^5 + 414, r= 0.9998	Acceptable chromatograms presented for standard solution, blank matrix and fortified sample. No interference >30% of LOQ Identity confirmed using LC-MS/MS to monitor two ion mass transitions (*m/z* 398 → 70 and *m/z* 398 → 182)
		398 → 182	0.01	0.01 0.1	82.6 – 86.0 (84.6, 5) 74.6 – 94.2 (84.8, 5)	1.7 (5) 9.6 (5) Overall: 6.5 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] 7 standards, y = 1.01×10^5 - 158, r= 0.9999	
Wheat grain	BAS 750 F	398 → 70	0.01	0.01 0.1	105 – 117 (110, 5) 108 – 113 (110, 5)	4.2 (5) 1.9 (5) Overall: 3.1 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] 7 standards, y = 5.17×10^5 + 7.76×10^3, r= 0.9999	Acceptable chromatograms presented for standard solution, blank matrix and fortified sample. No interference >30% of LOQ Identity confirmed using LC-MS/MS to monitor two ion mass transitions (*m/z* 398 → 70 and *m/z* 398 → 182)
		398 → 182	0.01	0.01 0.1	101 – 118 (107, 5) 107 – 113 (110, 5)	6.1 (5) 2.1 (5) Overall: 4.5 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] 7 standards, y = 9.66×10^4 + 2.71×10^3, r=	

							0.9998	
Dried broad beans	BAS 750 F	398 → 70	0.01	0.01 0.1	103 – 118 (108, 5) 101 – 109 (105, 5)	5.2 (5) 2.9 (5) Overall: 4.2 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] 7 standards, y = 9.72×10^4 + 1.54×10^3, r= 0.9996	Acceptable chromatograms presented for standard solution, blank matrix and fortified sample. No interference >30% of LOQ Identity confirmed using LC-MS/MS to monitor two ion mass transitions (*m/z* 398 → 70 and *m/z* 398 → 182)
		398 → 182	0.01	0.01 0.1	102 – 116 (107, 5) 103 – 109 (105, 5)	4.8 (5) 2.2 (5) Overall: 3.7 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] 7 standards, y = 9.72×10^4 + 1.54×10^3, r= 0.9996	
Dried soybeans	BAS 750 F	398 → 70	0.01	0.01 0.1	106 – 142 (117, 5) 108 – 115 (110, 5)	12.7 (5) 2.9 (5) *Dixon's outlier excluded Overall: 3.6 (9)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] 7 standards, y = 4.87×10^5 + 1.15×10^4, r= 0.9998	Acceptable chromatograms presented for standard solution, blank matrix and fortified sample. No interference >30% of LOQ Identity confirmed using LC-MS/MS to monitor two ion mass transitions (*m/z* 398 → 70 and *m/z* 398 → 182)
		398 → 182	0.01	0.01 0.1	104 – 118 (116, 5) 108 – 115 (110, 5)	14.7 (5) 2.6 (5) *Dixon's outlier excluded	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] 7 standards, y = 9.11×10^4 + 1.51×10^3, r= 0.9997	

						Overall: 4.0 (9)		
Whole orange	BAS 750 F	398 → 70	0.01	0.01 0.1	106 – 111 (109, 5) 106 – 120 (110, 5)	1.7 (5) 5.1 (5) Overall: 3.7 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] 7 standards, y = 4.78×10^5 + 1.12×10^4, r= 0.9998	Acceptable chromatograms presented for standard solution, blank matrix and fortified sample. No interference >30% of LOQ Identity confirmed using LC-MS/MS to monitor two ion mass transitions (*m/z* 398 → 70 and *m/z* 398 → 182)
		398 → 182	0.01	0.01 0.1	108 – 111 (110, 5) 104 – 123 (110, 5)	1.1 (5) 6.9 (5) Overall: 4.6 (10)	0.15 – 7.5 ng/mL [approx. 0.003 - 0.15 mg/kg] 7 standards, y = 8.94×10^4 + 2.35×10^3, r= 0.9998	

B.5.2.1.2. Animals

Report:	KCA 4.2/3 Devine C., 2015 a Validation of the BASF analytical method L0272/01 for BAS 750 F in animal matrices 2015/1106707
Guidelines:	EPA 860.1340 (1996), SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010), OECD-ENV/JM/MONO/(2007)17
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

This method has been evaluated in the Methods for Risk Assessment Section B.5.1.2.5 under Report KCA 4.1.2/27 and found to be successfully validated in accordance with SANCO/825/00 rev.8.1 with an LOQ of 0.01 mg/kg.

Report:	KCA 4.2/4 Richter S.,Djedovic S., 2015 b Independent method validation (ILV) of a method for the determination of BAS 750 F in various foodstuffs of animal origin, using LC/MS/MS - (BASF Method No. L0272/01) 2015/1240005
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 860.1340 (1996), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Principle of method
As for KCA 4.2/3. No deviations to the original method were made.

Matrix effects
Matrix effects were tested by preparing matrix matched standards for the analyte in each matrix and injecting with the samples in the same injection series. Concentrations ranging from 0.10 ng/mL to 10 ng/mL (for milk, cream, fat) and 0.040 ng/mL to 5.0 ng/mL (for egg, meat, kidney, liver) were prepared. The matrix effect is the ratio of the slope of the calibration curve in matrix-matched standards to the slope of the calibration curve in solvent standards, expressed as a percentage (with the slope for the solvent standards set to 100 %).

Table 5.2-10: Matrix effects

Matrix	**Analyte**	**Mass transition (*m/z*)**	**Slope in matrix matched standards compared to solvent standards (%)**
Milk	BAS 750 F	398 → 182	101
		298 → 133	96.5
Cream	BAS 750 F	398 → 182	103
		298 → 133	101
Fat	BAS 750 F	398 → 182	102
		298 → 133	101
Egg	BAS 750 F	398 → 182	115
		298 → 133	111
Meat	BAS 750 F	398 → 182	106
		298 → 133	108
Kidney	BAS 750 F	398 → 182	109
		298 → 133	106
Liver	BAS 750 F	398 → 182	107
		298 → 133	108

*These are mean values calculated from all concentrations tested

No significant matrix effects for all matrices were observed. According to the original method, calibration standards in solvent were used for the evaluation of residues in milk and cream and matrix matched standards were used for the evaluation of residues in meat, fat, liver and hen egg, however these were not necessary as no significant matrix effects were observed in the original method. Thus for these matrices calibration solutions in solvent were used for the evaluation of the results.

Stability of standards
Stock solutions prepared in methanol were tested in the original study. The stability of solution was shown for 30 days when stored refrigerated. The stability of fortification solutions (stored refrigerated) prepared in ACN or MeOH and of the calibration solutions prepared in MeOH/H_2O (1/1, v/v) was shown for 7 days in the original study.

Stability of extracts
The stability of the analyte in extracts was already tested in the original method validation. The extracts were shown to be stable for a storage period of 7 days when stored refrigerated.

Validation summary

HPLC-MS/MS is a highly specific technique and two mass transitions (3 ions) were monitored as outlined in the guidance document. Chromatograms of calibration standards, blank matrix and fortified sample have been presented showing no interferences >30% of LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels for the analyte in each matrix of interest corresponding to LOQ and 10xLOQ and in all cases the mean recovery was within the acceptable range of 70 – 120 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSDs were between 2.6 % and 18 %. The linear range is appropriate for the nominal test concentrations, and was determined using solvent-based. The ILV confirms the LOQ of the method is 0.01 mg/kg. The ILV employs a sample preparation technique and method of analysis which are sufficiently similar to the original study.The reproducibility of the method has therefore been successfully shown. The ILV of the analytical method is satisfactorily validated in accordance with SANCO/825/00 rev.8.1.

Validation data

Table 5.2-11: Validation data

Matrix	Analyte	Mass transition (*m/z*)	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
Milk	BAS 750 F	398 → 182	0.01	0.01 0.1	81.3 – 94.6 (90.5, 5) 87.2 – 92.2 (89.6, 5)	3.1 (5) 2.2 (5) Overall: 2.6 (10)	0.10 – 10 ng/mL [Approx. 0.002 – 0.2 mg/kg] 7 standards, r = 0.9999, y = 28100x - 212	Acceptable chromatograms presented for calibration standards, blank matrix and fortified sample. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions (*m/z* 398 → 182, 398 → 133)
		298 → 133	0.01	0.01 0.1	88.6 – 96.6 (92.5, 5) 86.0 – 92.0 (90.0, 5)	3.5 (5) 3.3 (5) Overall: 3.5 (10)	0.10 – 10 ng/mL [Approx. 0.002 – 0.2 mg/kg] 7 standards, r = 0.9998, y = 11400x - 111	
Cream	BAS 750 F	398 → 182	0.01	0.01 0.1	81.0 – 90.0 (85.2, 5) 79.0 – 89.8 (83.0, 5)	4.4 (5) 5.2 (5) Overall: 4.7 (10)	0.10 – 10 ng/mL [Approx. 0.002 – 0.2 mg/kg] 7 standards, r = 0.9990, y = 28200x + 4.64	Acceptable chromatograms presented for calibration standards, blank matrix and fortified sample. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two
		298 → 133	0.01	0.01 0.1	79.0 – 86.2 (82.7, 5) 71.6 – 83.8 (77.1, 5)	3.7 (5) 6.8 (5) Overall:	0.10 – 10 ng/mL [Approx. 0.002 – 0.2 mg/kg]	

						6.3 (10)	7 standards, r = 0.9996, y = 11800x - 210	mass transitions (*m/z* 398 → 182, 398 → 133)
Fat	BAS 750 F	398 → 182	0.01	0.01 0.1	73.4 – 83.2 (79.2, 5) 74.3 – 91.6 (81.9, 5)	5.9 (5) 8.2 (5) Overall: 7.0 (10)	0.10 – 10 ng/mL [Approx. 0.002 – 0.2 mg/kg] 7 standards, r = 0.9999, y = 34500x + 288	Acceptable chromatograms presented for calibration standards, blank matrix and fortified sample. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions (*m/z* 398 → 182, 398 → 133)
		298 → 133	0.01	0.01 0.1	73.0 – 93.4 (83.9, 5) 72.2 – 91.6 (82.0, 5)	9.2 (5) 10 (5) Overall: 9.2 (10)	0.10 – 10 ng/mL [Approx. 0.002 – 0.2 mg/kg] 7 standards, r = 0.9998, y = 13600x – 14.7	
Egg	BAS 750 F	398 → 182	0.01	0.01 0.1	69.0 – 84.8 (76.7, 5) 68.8 – 110 (92.8, 5)	8.0 (5) 19 (5) Overall: 18 (10)	0.04 – 5 ng/mL [Approx. 0.002 – 0.25 mg/kg] 7 standards, r = 0.9999, y = 32900x + 52.5	Acceptable chromatograms presented for calibration standards, blank matrix and fortified sample. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions (*m/z* 398 → 182, 398 → 133)
		298 → 133	0.01	0.01 0.1	72.5 – 90.0 (80.9, 5) 65.0 – 104 (88.4, 5)	9.7 (5) 19 (5) Overall: 15 (10)	0.04 – 5 ng/mL [Approx. 0.002 – 0.25 mg/kg] 7 standards, r = 0.9986, y = 13500x – 17.2	

Meat	BAS 750 F	398 → 182	0.01	0.01 0.1	62.5 – 102 (79.8, 5) 93.5 – 104 (99.9, 5)	19 (5) 4.0 (5) Overall: 17 (10)	0.04 – 5 ng/mL [Approx. 0.002 – 0.25 mg/kg] 7 standards, r = 0.9998, y = 33200x + 138	Acceptable chromatograms presented for calibration standards, blank matrix and fortified sample.
		298 → 133	0.01	0.01 0.1	61.0 – 99.5 (83.0, 5) 92.5 – 104 (97.8, 5)	19 (5) 4.8 (5) Overall: 15 (10)	0.04 – 5 ng/mL [Approx. 0.002 – 0.25 mg/kg] 7 standards, r = 0.9995, y = 12800x + 18.6	No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions (*m/z* 398 → 182, 398 → 133)
Kidney	BAS 750 F	398 → 182	0.01	0.01 0.1	92.0 – 105 (100, 5) 67.5 – 103 (92.4, 5)	5.3 (5) 15 (5) Overall: 11 (10)	0.04 – 5 ng/mL [Approx. 0.002 – 0.25 mg/kg] 7 standards, r = 0.9990, y = 37800x + 193	Acceptable chromatograms presented for calibration standards, blank matrix and fortified sample.
		298 → 133	0.01	0.01 0.1	95.5 – 107 (101, 5) 67.5 – 102 (91.5, 5)	4.7 (5) 15 (5) Overall: 11 (10)	0.04 – 5 ng/mL [Approx. 0.002 – 0.25 mg/kg] 7 standards, r = 0.9994, y = 15200x – 31.6	No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions (*m/z* 398 → 182, 398 → 133)
Liver	BAS 750 F	398 → 182	0.01	0.01	94.8 – 103 (98.4, 5)	3.8 (5)	0.04 – 5 ng/mL [Approx. 0.002	Acceptable chromatograms presented for

				0.1	93.5 – 107 (100, 5)	4.8 (5) Overall: 4.2 (10)	– 0.25 mg/kg] 7 standards, r = 0.9996, y = 36800 + 241	calibration standards, blank matrix and fortified sample.
		298 → 133	0.01	0.01 0.1	89.5- 109 (97.4, 5) 93.0 – 105 (98.9, 5)	8.0 (5) 4.4 (5) Overall: 6.1 (10)	0.04 – 5 ng/mL [Approx. 0.002 – 0.25 mg/kg] 7 standards, r = 0.9995, y = 14500x + 87.8	No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions (*m/z* 398 → 182, 398 → 133)

Report:	KCA 4.2/6 Bendig P.,Wabbel C., 2015 a Independent method validation (ILV) of BASF method no. L0309/01 for the determination of the BAS 750 F diol metabolite in various foodstuffs of animal origin, using GC/MS 2015/1240006
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 860.1340 (1996), OECD-ENV/JM/MONO/(2007)17
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

This ILV of BASF method no. L0309/01 is not currently required as the residue definition for enforcement for products of animal origin does not include metabolite M750F022. However, as it has been submitted by the applicant, it has been included here for completeness.

Principle of the method
As for CA 4.1.2/28 and CA 4.1.2/32.

Matrix effects
The response of each analyte in matrix-matched standards compared to solvent based standards was compared. Concentrations ranging from 2.5 ng/mL to 125 ng/mL were prepared. The matrix effect was the ratio of the slope of the calibration curve in matrix-matched standards to the slope of the calibration curve in solvent standards, expressed as a percentage (with the slope for the solvent standards set to 100 %).

Table 5.2-12: Matrix effects

Matrix	Analyte	Fragment ion (*m/z*)	Slope in matrix matched standards compared to solvent* (%)
Milk	BAS 750 F	295	124
		297	122
		317	119
Fat	BAS 750 F	295	288
		297	274
		317	162
Kidney	BAS 750 F	295	129
		297	130
		317	90
Liver	BAS 750 F	295	101
		297	100
		317	106
Egg	BAS 750 F	295	135
		297	141
		317	111
Muscle	BAS 750 F	295	96.4
		297	102
		317	100

*These are mean values calculated from all concentrations tested

No significant matrix effects (> 20 % suppression or enhancement) in the GC-MS response were observed for liver and muscle. Significant matrix effects (>20% suppression or enhancement) in the GC-MS response were observed for milk, egg, kidney and fat. For all matrices, matrix-matched standards were used for the evaluation of the results.

Stability of standards
The stability of stock solutions, calibration solutions and AP-Mix solutions was demonstrated in the validation of the original study (CA 4.1.2/28 and CA 4.1.2/32).

Stability of extracts

The stability of M750F022 in extracts and final volumes was demonstrated in the validation of the original study (CA 4.1.2/28 and CA 4.1.2/32).

Validation summary

GC-MS is a highly specific technique and 3 fragment ions were monitored as outlined in the guidance document. Chromatograms of standards solutions in matrix, control and fortification samples have been presented showing no interferences >30% of LOQ at the retention time of interest. Accuracy was assessed at 2 fortification levels for the analyte in each matrix of interest corresponding to LOQ and 10xLOQ and in all cases the mean recovery was within the acceptable range of 70 – 120 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSDs were between 6.2 % and 14 %. The linear range is appropriate for the nominal test concentrations, and was determined using solvent-based. The LOQ of the method is 0.01 mg/kg. The reproducibility of the method has therefore been successfully shown. The ILV of the analytical method is satisfactorily validated in accordance with SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1.

Validation data

Table 5.2-13: Validation data

Matrix	**Analyte**	**Fragment ion (*m/z*)**	**LOQ (mg/kg)**	**Recovery fortification level (mg/kg)**	**Recoveries % range (mean, n)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
Milk	M750F022	295	0.01	0.01 0.1	72.5 – 85.1 (79.3, 5) 69.0 – 89.1 (77.1, 5)	6.1 (5) 10 (5) Overall: 7.8 (10)	2.5 – 125 ng/mL [Approx. 0.0025 – 0.125 mg/kg] 6 standards, r^2 = 0.9996, y = 96557 + 87454x	Acceptable chromatograms provided for standards solutions in matrix, control and fortification samples. No interference >30% of LOQ Identity confirmed by GC-MS monitoring 3 ion fragments (*m/z* 295, 297, 317)
		297	0.01	0.01 0.1	76.1 – 85.4 (80.3, 5) 61.8 – 84.7 (72.4, 5)	4.5 (10) 12 (10) Overall: 10(10)	2.5 – 125 ng/mL [Approx. 0.0025 – 0.125 mg/kg] 6 standards, r^2 = 0.9994, y = 4532.7 + 26446.1x	
		317	0.01	0.01 0.1	88.5 – 97.2 (91.8, 5) 68.1 – 87.3 (76.9, 5)	4.4 (10) 11 (10) Overall: 12 (10)	2.5 – 125 ng/mL [Approx. 0.0025 – 0.125 mg/kg] 6 standards, r^2 = 0.9985, y = 9259.84 + 8569.11x	

Fat	M750F022	295	0.01	0.01 0.1	97.7 – 105 (100, 5) 72.0 – 93.5 (82.3, 5)	3.1 (5) 11 (5) Overall: 12 (10)	2.5 – 125 ng/mL [Approx. 0.0025 – 0.125 mg/kg] 7 standards, r^2 = 0.9988, y = 55427x + 113706	Acceptable chromatograms provided for standards solutions in matrix, control and fortification samples. No interference >30% of LOQ Identity confirmed by GC-MS monitoring 3 ion fragments (*m/z* 295, 297, 317)
		297	0.01	0.01 0.1	82.1 – 97.3 (91.8, 5) 74.7 – 88.6 (88.6, 5)	6.3 (5) 12 (5) Overall: 8.9 (10)	2.5 – 125 ng/mL [Approx. 0.0025 – 0.125 mg/kg] 7 standards, r^2 = 0.9979, y = 17178x + 29935	
		317	0.01	0.01 0.1	89.4 – 102 (96.6, 5) 77.6 – 114 (99.4, 5)	4.9 (5) 15 (5) Overall: 11 (10)	2.5 – 125 ng/mL [Approx. 0.0025 – 0.125 mg/kg] 7 standards, r^2 = 0.9992, y = 5381x + 22903	
Kidney	M750F022	295	0.01	0.01 0.1	67.3 – 78.5 (73.2, 5) 71.1 – 103 (83.2, 5)	7.6 (5) 16 (10) Overall: 14 (10)	2.5 – 125 ng/mL [Approx. 0.0025 – 0.125 mg/kg] 6 standards, r^2 = 0.9992, y = 12321x - 2327	Acceptable chromatograms provided for standards solutions in matrix, control and fortification samples. No interference >30% of LOQ Identity confirmed by GC-MS monitoring 3 ion fragments (*m/z* 295, 297, 317)
		297	0.01	0.01 0.1	70.8 – 87.0 (77.4, 5) 71. – 103 (84.6, 5)	8.4 (5) 18 (10) Overall: 14 (10)	2.5 – 125 ng/mL [Approx. 0.0025 – 0.125 mg/kg] 6 standards, r^2 = 0.9996, y = 4109x + 1657	
		317	0.01	0.01 0.1	70.4 – 81.8 (76.6, 5) 71.9 – 101	6.5 (5) 15 (5)	2.5 – 125 ng/mL [Approx. 0.0025 – 0.125 mg/kg]	

					(84.6, 5) Overall: 13 (10)		6 standards, r^2 = 0.9984, y = 1433x + 992		
Liver	M750F022	295	0.01	0.01 0.1	81.8 – 94.7 (89.4, 5) 70.4 – 93.9 (83.6, 5)	5.6 (5) 13 (5) Overall: 10 (10)	2.5 – 125 ng/mL [Approx. 0.0025 – 0.125 mg/kg] 7 standards, r^2 = 0.9991, y = 8540x + 3201		Acceptable chromatograms provided for standards solutions in matrix, control and fortification samples. No interference >30% of LOQ Identity confirmed by GC-MS monitoring 3 ion fragments (*m/z* 295, 297, 317)
		297	0.01	0.01 0.1	83.0 – 94.3 (88.3, 5) 70.3 – 93.7 (82.0, 5)	4.8 (5) 13 (10) Overall: 10 (10)	2.5 – 125 ng/mL [Approx. 0.0025 – 0.125 mg/kg] 7 standards, r^2 = 0.9994, y = 2835x + 2807		
		317	0.01	0.01 0.1	76.4 – 99.6 (89.7, 10) 71.2 – 96.3 (81.0, 5)	10 (10) 15 (10) Overall: 13 (10)	2.5 – 125 ng/mL [Approx. 0.0025 – 0.125 mg/kg] 7 standards, r^2 = 0.9979, y = 958x + 3023		
Egg	M750F022	295	0.01	0.01 0.1	79.5 – 101 (91.2, 5) 95.0 – 109 (99.4, 5)	9.5 (5) 5.8 (5) Overall: 8.6 (10)	2.5 – 50 ng/mL [Approx. 0.0025 – 0.05 mg/kg] 7 standards, r^2 = 0.9933, y = 54278 - 3050	10 – 125 ng/mL [Approx. 0.01 – 0.125 mg/kg] 7 standards, r^2 = 0.9974, y = 33140x + 502821	Acceptable chromatograms provided for standards solutions in matrix, control and fortification samples. No interference >30% of LOQ Identity confirmed by GC-MS monitoring 3 ion fragments (*m/z* 295, 297, 317)
		297	0.01	0.01	86.8 – 105 (93.3, 5)	7.7 (5)	2.5 – 50 ng/mL	10 – 125 ng/mL	

				0.1	91.8 – 108 (98.4, 5)	6.3 (5) Overall: 7.2 (10)	[Approx. 0.0025 – 0.05 mg/kg] 7 standards, r^2 = 0.9975, y = 13086x + 3391	[Approx. 0.01 – 0.125 mg/kg] 7 standards, r^2 = 0.9960, y = 11218x + 128746	
		317	0.01	0.01 0.1	87.8 – 107 (97.9, 5) 95.1 – 109 (101, 5)	7.0 (5) 5.7 (5) Overall: 6.2 (10)	2.5 – 50 ng/mL [Approx. 0.0025 – 0.05 mg/kg] 7 standards, r^2 = 0.9935, y = 4321x - 2146	10 – 125 ng/mL [Approx. 0.01 – 0.125 mg/kg] 7 standards, r^2 = 0.9964, y = 3540x + 32610	
Muscle	M750F022	295	0.01	0.01 0.1	74.3 – 98.2 (84.8, 5) 71.9 – 97.9 (80.6, 5)	11 (5) 14 (5) Overall: 12 (10)	2.5 – 125 ng/mL [Approx. 0.0025 – 0.125 mg/kg] 7 standards, r^2 = 0.9993, y = 6937x + 4338		Acceptable chromatograms provided for standards solutions in matrix, control and fortification samples. No interference >30% of LOQ Identity confirmed by GC-MS monitoring 3 ion fragments (*m/z* 295, 297, 317)
		297	0.01	0.01 0.1	73.5 – 98.5 (82.3, 5) 72.9 – 101 (82.6, 5)	12 (5) 14 (5) Overall: 12 (10)	2.5 – 125 ng/mL [Approx. 0.0025 – 0.125 mg/kg] 7 standards, r^2 = 0.9992, y = 2205x + 4455		
		317	0.01	0.01 0.1	74.6 – 90.5 (83.5, 5) 70.1 – 96.6	8.3 (5) 14 (5)	2.5 – 125 ng/mL [Approx. 0.0025 – 0.125 mg/kg]		

					(79.1, 5)	Overall: 11 (10)	7 standards, r^2 = 0.9989, y = 669x +1021	

B.5.2.2. Methods for the analysis in soil

The residue definitions for monitoring in soil is BAS 750 F. The analytical method below is consistent with the residue definition.

Report:	KCA 4.2/8 Studenroth S.,Lueer D., 2015 b Validation of analytical method L0214/01 for the determination of BAS No. 750 F (Reg.No. 5834378) and metabolites of Reg.No. 5924326 and 1,2,4-Triazole (Reg.No. 87084) in soil by LC-MS/MS 2015/1039006
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Report:	KCA 4.2/9 Lueer D., 2016 a Report Amendment No. 1: Validation of analytical method L0214/01 for the determination of BAS No. 750 F (Reg.No. 5834378) and its metabolites Reg.No. 5924326 and 1,2,4-Triazole (Reg.No. 87084) in soil by LC-MS/MS 2016/1030227
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Report:	KCA 4.2/10 Obermann M., 2016 a Report Amendment No. 2: Validation of analytical method L0214/01 for the determination of BAS No. 750 F (Reg.No. 5834378) and its metabolites Reg.No. 5924326 and 1,2,4-Triazole (Reg.No. 87084) in soil by LC-MS/MS 2016/1215646
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

This method has been evaluated in the Methods for Risk Assessment Section B.5.1.2.1 under Report KCA 4.1.2/1 and found to be satisfactorily validated in accordance with SANCO/825/00 rev.8.1 with an LOQ of 0.002 mg/kg.

B.5.2.3. Methods for the analysis in water

The residue definitions for monitoring in water are:

Groundwater: BAS 750 F
Surface water: BAS 750 F

The analytical method below is consistent with the residue definition.

Report:	KCA 4.1.2/5 Malinsky D.S., 2016 a Validation of analytical method D1506/01: Method for the determination of Mefentrifluconazole (BAS 750 F, Reg. No.5834378) and its metabolites M750F003 (Reg. No.5924326), M750F005 (Reg. No.6003433), M750006 (Reg. No.5863469), M750F007 (Reg. No.6003432) and M750F008 (Reg. No.6010286) in surface and drinking water by LC-MS/MS 2015/7001125
Guidelines:	EPA 850.6100, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by United States Environmental Protection Agency)

Report:	KCA 4.1.2/6 Malinsky D.S., 2016 a Amended Report: Validation of analytical method D1506/01: Determination of Mefentrifluconazole (BAS 750 F, Reg. No.5834378) and its metabolites M750F003 (Reg. No.5924326), M750F005 (Reg. No.6003433), M750006 (Reg. No.5863469), M750F007 (Reg. No.6003432) and M750F008 (Reg. No.6010286) in surface and drinking water by LC-MS/MS 2016/7010048
Guidelines:	EPA 850.6100, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by United States Environmental Protection Agency)

Report:	KCA 4.2/11 Malinsky D.S., 2016 a Validation of analytical method D1506/01: Method for the determination of Mefentrifluconazole (BAS 750 F, Reg. No.5834378) and its metabolites M750F003 (Reg. No.5924326), M750F005 (Reg. No.6003433), M750006 (Reg. No.5863469), M750F007 (Reg. No.6003432) and M750F008 (Reg. No.6010286) in surface and drinking water by LC-MS/MS 2015/7001125
Guidelines:	EPA 850.6100, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by United States Environmental Protection Agency)

Report:	KCA 4.2/12 Malinsky D.S., 2016 a Amended Report: Validation of analytical method D1506/01: Determination of Mefentrifluconazole (BAS 750 F, Reg. No.5834378) and its metabolites M750F003 (Reg. No.5924326), M750F005 (Reg. No.6003433), M750006 (Reg. No.5863469), M750F007 (Reg. No.6003432) and M750F008 (Reg. No.6010286) in surface and drinking water by LC-MS/MS 2016/7010048
Guidelines:	EPA 850.6100, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes

(certified by United States Environmental Protection Agency)

Principle of the method

The method has been developed for the determination of BAS 750 F (Reg.No. 5834378) and the following analytes in surface and drinking water by LC-MS/MS:

Table 5.2-14: Identity of metabolites

Metabolite	Reg.No.	Chemical name
M750F003	5924326	4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenol
M750F005	6003433	4-{4-[2-hydroxy-1-(1H-1,2,4-triazol-1-yl)propan-2-yl]-3-(trifluoromethyl)phenoxy}phenol
M750F006	5863469	6-(4-chlorophenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one
M750F007	6003432	6-(4-chlorophenoxy)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one
M750F008	6010286	6-(5-chloro-2-hydroxyphenyl)-3-methyl-3-(1H-1,2,4-triazol-1-ylmethyl)-2-benzofuran-1(3H)-one

10 mL of each water sample was diluted with 2.5 mL acetonitrile.

Analysis was performed by LC-MS/MS using an an Xbridge BEH C18 column (50 x 2.1 mm, 2.5µm) and, for confirmatory purposes for M750F003, M750F005 and M750F007, an Xbridge BEH phenyl column (100 x 2.1mm, 2.5µm particle size) at 50 °C with external calibration and detection by ESI^+ MS/MS monitoring the following ion transitions:

- BAS 750 F *m/z* 398→70 (quantification)
  m/z 400→70 (confirmation)
- M750F003 *m/z* 288→70 (confirmation of identity achieved using a second column)
- M750F005 *m/z* 380→70 (confirmation of identity achieved using a second column)
- M750F006 *m/z* 356→259 (quantification)
  m/z 356→217 (confirmation)
- M750F007 *m/z* 338→241 (confirmation of identity achieved using a second column)
- M750F008 *m/z* 356→259 (quantification)
  m/z 356→241 (confirmation)

A gradient elution was used (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile).

Water characteristics

The drinking water (well water) was obtained from 8026 Lowell Valley Drive, Bahama, North Carolina 27503 (Durham County). The surface water (lake water) was collected under BASF Study 722850 and was obtained from Golden Lake, near Northwood, North Dakota (Steele County).

Table 5.2-15: Water characteristics

	Drinking water	Surface water
pH	7.5	8.3
Calcium (mg/kg)	42	111
Magnesium (mg/kg)	2.8	99
Sodium (mg/kg)	6.1	96
Hardness (mg equivalent $CaCO_3$/L)	116	693
Conductivity (mmhos/com)	0.24	1.36
Sodium Adsorption Ratio	0.25	1.60
Total Dissolved Solids (mg/kg)	156	1270
Turbidity (NTU)	0.41	10.7
Total Organic Carbon (mg/kg)	0.8	14.4
Nitrate-Nitrogen	-	<LOD (0.1mg/kg)
Phosphate-P	-	<LOD (0.1mg/kg)
Bacteria	-	1750 CFU 46.3 hours
Actinomycetes	-	85 CFU 41.8 hours

Matrix effects

The influence of matrix effects on LC-MS/MS analysis was determined by preparation of calibration standards in matrix water: acetonitrile (80: 20, v/v) and calibration standards prepared with HPLC water: acetonitrile (80:20, v/v). The matrix matched standards were prepared by diluting standards of each analyte with control drinking or surface water to 0.015 and 0.025 ng/mL for drinking water; or 0.015 and 0.060 ng/mL for surface water.

The average area response of the standards for three injections for the two standard concentration levels with and without matrix and expressed the Mean Area Change (%) calculated across the two tested concentrations.

Matrix	Analyte	Mass transition	Area of response in matrix matched standards compared to solvent standards* (%)
Drinking (well) water	BAS 750 F	398→70	5
		400→70	4
	M750F003	288→70 (C18)	2
		288→70 (Phenyl)	1
	M750F005	380→70 (C18)	25
		380→70 (Phenyl)	27
	M750F006	356→259	3
		356→217	10
	M750F007	338→241 (C18)	34
		338→241 (Phenyl)	28
	M750F008	356→259	17
		356→241	16
Surface (lake) water	BAS 750 F	398→70	3
		400→70	6
	M750F003	288→70 (C18)	1
		288→70 (Phenyl)	3
	M750F005	380→70 (C18)	21
		380→70 (Phenyl)	10
	M750F006	356→259	5
		356→217	8
	M750F007	338→241 (C18)	28
		338→241 (Phenyl)	10
	M750F008	356→259	10
		356→241	16

Table 5.2-16: Matrix effects
*These are mean values calculated from the two concentrations tested

No significant matrix effects were observed with the exception of M750F005 and M750F007 in both water types. Therefore, the validation samples were analysed primarily using solvent-based standards with matrix-matched standards used for M750F005 and M750F007 in both water types.

Stability of standards

Stock and fortification solutions of the BAS 750 F, M750F003, M750F005, M750F006, M750F007 and M750F008 were prepared in acetonitrile, and calibration standards prepared by dilution of the intermediate standards using water:acetonitrile (80:20, v/v).

Each analyte was stable in the stock and fortification solutions in acetonitrile when refrigerated for at least 3 months. Each analyte was stable in the standards prepared in acetonitrile:water (20:80, v/v) when refrigerated for 1 month.

Stability of extracts

The stability of extracts was determined as the stability of the "final volume" prepared for HPLC analysis. The stability was measured through reanalysing a control and recovery sample in surface water stored under refrigeration. Quantification was performed for the primary mass transitions.

The recoveries from stored solutions demonstrate that each analyte was stable in extracts for at least 5 days in water.

Validation summary

HPLC-MS/MS is a highly specific technique and two mass transitions (3 ions) were monitored for BAS 750 F, M750F006 and M750F008 whereas a single mass transition was monitored for M750F003, M750F005 and

M750F007 with confirmation of identity achieved with a second column. Chromatograms of standards, reagent blank, control water samples and fortified water samples were presented showing no interferences >30% of LOQ at the retention times of interest. Accuracy was assessed at 2 fortification levels for each analyte of interest corresponding to LOQ and 10xLOQ. The mean recovery was within the acceptable range of 70 – 110 % in all cases except for M750F005 in drinking water and M750F007 in drinking and surface water. To assess method precision, between 5 and 15 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 % (except for M750F007 monitoring 338→241 (C18 column) in drinking water at both fortification levels (23% and 27%)). The overall RSDs were between 6 and 18 (except for M750F007 monitoring 338→241 (C18 column) in drinking water (24 %)). The linear range is appropriate for the nominal test concentrations and was determined using matrix matched standards for M750F005 and M750F007, and using solvent-based standards for BAS 750 F, M750F003, M750F006 and M750F008. The LOQ of the method is 30 ng/L. The method is satisfactorily validated in accordance with SANCO/825/00 rev.8.1 except for M750F005 and M750F007 in drinking water and where it is fit for purpose.

Validation data

Matrix	Analyte	LOQ (ng/L)	Mass transition (m/z)	Recovery fortification level (ng/L)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
Drinking water	BAS 750 F	30	398→70	30 300	67 – 126 (93, 10) 69 – 106 (95, 10)	18 (10) 12 (10) Overall: 15 (20)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9993, y = 1.77×10^6 + 4985	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by additional mass transition
		30	400→70	30 300	66 – 98 (77, 10) 69 – 101 (89, 10)	13 (10) 11 (10) Overall: 14 (20)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9888, y = 5.30×10^5 + 3100	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by additional mass transition
	M750F003 (The recoveries for M750F003 in drinking water includes one set run with matrix-matched standards; however, the response of M750F003 was not influenced by matrix effects based on the extensive testing in this study.)	30	288→70 (C18)	30 300	88 – 131 (107, 9) Anomalous result excluded (Dixon's outlier) 70 – 118 (99, 10)	16 (9) 19 (10)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9939, y =	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ

						Overall: 17 (19)	1.14×10^6 - 4862	Identity confirmed a different LC-MS/MS column
		30	288→70 (Phenyl)	30 300	84 – 106 (97, 10) 80 – 106 (95, 10)	7 (10) 10 (10) Overall: 9 (20)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9995, y = 1.26×10^6 - 999	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by a different LC-MS/MS column
	M750F005	30	380→70 (C18)	30 300	99 – 133 (112, 5) 112 – 162 (130, 5)	11 (5) 15 (5) Overall: 15 (10)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9935, y = 1.58×10^6 - 472	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed a different LC-MS/MS column
		30	380→70 (Phenyl)	30 300	67 – 111 (98, 15) 115 – 135 (123, 10)	18 (15) 7 (10) Overall: 18 (25)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r =0.9999, y =	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ

							1.67×10^6 + 505	Identity confirmed by a different LC-MS/MS column
	M750F006 (The recoveries for M750F006 in drinking water includes one set run with matrix-matched standards; however, the response of M750F006 was not influenced by matrix effects based on the extensive testing in this study.)	30	356→259	30 300	88 – 113 (102, 10) 91 – 119 (105, 10)	8 (10) 10 (10) Overall: 9 (20)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9886, y = 6.73×10^5 - 2396	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by additional mass transition
		30	356→217	30 300	79 – 120 (91, 10) 84 – 120 (99, 10)	15 (10) 13 (10) Overall: 14 (28)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9980, y = 4.05×10^5 - 391	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by additional mass transition
	M750F007	30	338→241 (C18)	30 300	68 – 129 (112, 5) 71 – 143 (118, 5)	23 (5) 27 (5) Overall: 24 (10)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9972, y = 4.21×10^5 – 28.6	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed a

								different LC-MS/MS column
		30	338→241 (Phenyl)	30 300	80 – 113 (99, 15) 109 – 125 (116, 10)	13 (15) 5 (10) Overall: 13 (25)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9987, y = 4.89×10^5 - 455	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed a different LC-MS/MS column
	M750F008	30	356→259	30 300	73 – 118 (97, 10) 74 – 118 (94, 10)	16 (10) 16 (10) Overall: 16 (20)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9968, y = 3.58×10^5 + 659	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by additional mass transition
		30	356→241	30 300	61 – 108 (90, 10) 88 – 142 (105, 10)	15 (10) 15 (10) Overall: 17 (20)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9957, y = 1.98×10^5 + 167	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by additional mass transition

Surface water	BAS 750 F	30	398→70	30 300	78 – 101 (91, 10) 80 – 104 (96, 10)	9 (10) 15 (10) Overall: 13 (20)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9993, y = 1.77×10^6 + 4985	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by additional mass transition
		30	400→70	30 300	67 – 112 (93, 10) 79 – 118 (98, 10)	18 (10) 12 (10) Overall: 15 (20)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9888, y = 5.30×10^5 + 3100	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by additional mass transition
	M750F003	30	288→70 (C18)	30 300	87 – 110 (93, 10) 89 – 134 (105, 10)	7 (10) 18 (10) Overall: 15 (20)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9939, y = 1.14×10^6 - 4862	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed a different LC-MS/MS column
		30	288→70 (Phenyl)	30	88 – 107 (98, 10)	7 (10)	0.006 – 0.3 ng/mL	Acceptable chromatograms presented

				300	94 – 113 (101, 10)	6 (10) Overall: 7 (20)	[Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9995, y = 1.26×10^6 - 999	for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by a different LC-MS/MS column
	M750F005	30	380→70 (C18)	30 300	82 – 127 (104, 10) 85 – 141 (112, 10)	15 (10) 15 (10) Overall: 15 (20)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9935, y = 1.58×10^6 - 472	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed a different LC-MS/MS column
		30	380→70 (Phenyl)	30 300	90 – 106 (100, 10) 93 – 125 (108, 10)	6 (10) 11 (10) Overall: 9 (20)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r =0.9999, y = 1.67×10^6 + 505	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by a different LC-MS/MS column
	M750F006	30	356→259	30	85 – 114 (98, 10)	9 (10)	0.006 – 0.3 ng/mL	Acceptable chromatograms presented

				300	92 – 117 (105, 10)	7 (10) Overall: 8 (20)	[Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9886, y = 6.73×10^5 - 2396	for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by additional mass transition
		30	356→217	30 300	76 – 122 (90, 10) 78 – 120 (98, 10)	15 (10) 12 (10) Overall: 14 (20)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9980, y = 4.05×10^5 - 391	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by additional mass transition
	M750F007	30	338→241 (C18)	30 300	85 – 127 (112, 10) 98 – 152 (117, 10)	10 (10) 16 (10) Overall: 13 (20)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9972, y = 4.21×10^5 – 28.6	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed a different LC-MS/MS column
		30	338→241 (Phenyl)	30 300	87 – 113 (102, 10) 95 – 115 (105,	7 (10) 7 (10)	0.006 – 0.3 ng/mL	Acceptable chromatograms presented for standards, reagent blank, control water

					10)	Overall: 7 (20)	[Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9987, y = 4.89×10^5 - 455	samples and fortified water samples. No interference >30% of LOQ Identity confirmed a different LC-MS/MS column
	M750F008	30	356→259	30 300	68 – 105 (92, 15) 86 – 135 (103, 15)	13 (15) 19 (15) Overall: 18 (30)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9968, y = 3.58×10^5 + 659	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by additional mass transition
		30	356→241	30 300	55 – 113 (92, 15) 73 – 119 (95, 15)	19 (15) 15 (15) Overall: 17 (30)	0.006 – 0.3 ng/mL [Approx. 7.5 – 375 ng/L] 5 standards, r = 0.9957, y = 1.98×10^5 + 167	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by additional mass transition

Report:	KCA 4.2/13 Gu G. et al., 2016 a Independent laboratory validation of BASF analytical method D1506/01: Method for the determination of BAS 750 F (Reg. No. 5834378) and its metabolites M750F003 (Reg. No. 5924326), M750F005 (Reg. No. 6003433), (Reg. No. 5863469), M750F007 (Reg. No. 6003432) and M750F008 (Reg. No. 6010286) in surface and drinking water by LC-MS/MS 2015/7006199
Guidelines:	EPA 850.6100, SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by United States Environmental Protection Agency)

Principle of the method
As described in KCA 4.1.2/5.

Water characteristics
As described in KCA 4.1.2/5.

Matrix effects
As described in KCA 4.1.2/5.
Solvent-based standards were used for all analytes in all matrices except for BAS 750 F in surface water, for which matrix-matches standards were used due to a significant matrix effect.

Stability of standards
As described in KCA 4.1.2/5.

Stability of extracts
As described in KCA 4.1.2/5.

Validation summary

HPLC-MS/MS is a highly specific technique and two mass transitions (3 ions) were monitored for BAS 750 F, M750F006 and M750F008 whereas a single mass transition was monitored for M750F003, M750F005 and M750F007 with confirmation of identity achieved with a second column as outlined in the guidance document. Chromatograms of standards, reagent blank, control water samples and fortified water samples were presented showing no interferences >30% of LOQ at the retention times of interest. Accuracy was assessed at 2 fortification levels for each analyte of interest corresponding to LOQ and 10xLOQ. In almost all cases the mean recovery was within the acceptable range of 70 – 110 % (except for surface water: BAS 750 F 398→70 and 400→70 at 300 ng/L fortification; M750F003 288→70 (phenyl) at 30 ng/L fortification; and M750F007 338→241 (C18 and phenyl) at 30 ng/L fortification). To assess method precision, between 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 % (except for BAS 750 F monitoring 398→70 in surface water at the lower fortification level (22.6%)). The overall RSDs were between 2.6 and 20.0. Acceptable linearity was demonstrated in the range 0.006 – 0.2 ng/mL in the extract (approx.. 7.5 – 250 ng/L in the matrix) and was determined using solvent-based standards were for all analytes in all matrices except for BAS 750 F in drinking water, for which matrix-matches standards were used due to a significant matrix effect. The LOQ of the method is 30 ng/L which is equivalent to 30 ng/kg as stated in the original method. The reproducibility of the method has therefore been successfully shown. The ILV of the analytical method is therefore satisfactorily validated in accordance with SANCO/825/00 rev.8.1.

Validation data

Table 5.2-17: Validation data

Matrix	Analyte	Mass transition (*m/z*)	LOQ (ng/L)*	Recovery fortification level (ng/L)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
Drinking water	BAS 750 F	398→70	30	30 300	99.7 – 111.2 (104.1, 5) 98.2 – 105.0 (101.7, 5)	4.2 (5) 2.8 (5) Overall: 3.6 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9989, y = 1.64×10^{6}x – 1.06×10^{3}	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 398 → 70 and 400 → 70
		400→70	30	30 300	98.1 – 105.7 (101.7, 5) 98.0 – 109.7 (105.6, 5)	2.7 (5) 4.7 (5) Overall: 4.2 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9941, y = 5.07×10^{5}x + 1.47×10^{3}	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 398 → 70 and 400 → 70
	M750F003	288→70 (C18)	30	30 300	90.7 – 97.5 (94.1, 5) 86.4 – 95.4 (91.0, 5)	2.7 (5) 3.7 (5) Overall: 3.5 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9933, y =	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by monitoring a

							1.21×10^6x – 253	single mass transition (*m/z* 288→70) using two different chromatographic columns
		288→70 (Phenyl)	30	30 300	96.3 – 11.2 (99.5, 5) 91.2 – 100.3 (97.3, 5)	1.9 (5) 3.6 (5) Overall: 3.0 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9985, y = 1.79×10^5x + 171	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by monitoring a single mass transition (*m/z* 288→70) using two different chromatographic columns
	M750F005	380→70 (C18)	30	30 300	90.5 – 98.2 (95.6, 5) 90.4 – 97.9 (94.9, 5)	3.5 (5) 3.3 (5) Overall: 3.2 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9963, y = 1.97×10^6x – 209	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by monitoring a single mass transition (*m/z* 380→70) using two different chromatographic columns
		380→70 (Phenyl)	30	30 300	98.7 – 103.0 (101.6, 5) 96.8 – 102.9 (99.4, 5)	2.4 (5) 2.5 (5) Overall: 2.6 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9994, y = 4.31×10^5x – 76.1	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by monitoring a single mass transition (*m/z* 380→70) using two different chromatographic columns
	M750F006	356→259	30	30	96.1 – 108.4 (103.7, 5)	4.8 (5)	0.006 – 0.2 ng/mL	Acceptable chromatograms presented for standards, reagent blank, control

				300	99.8 – 110.8 (104.6, 5)	4.2 (5) Overall: 4.3 (10)	[Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9987, y = 3.35×10^5x – 251	water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 356 → 259 and 356 → 217
		356→217	30	30 300	100.8 – 110.5 (105.2) 96.4 – 109.2 (102.6, 5)	4.4 (5) 4.8 (5) Overall: 4.5 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9993, y = 3.27×10^5x – 234	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 356 → 259 and 356 → 217
	M750F007	338→241 (C18)	30	30 300	97.6 – 109.1 (102.0, 5) 94.8 – 106.1 (99.8, 5)	4.2 (5) 4.1 (5) Overall: 4.1 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9952, y = 3.89×10^5x + 302	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by monitoring a single mass transition (*m/z* 338→241) using two different chromatographic columns
		338→241 (Phenyl)	30	30 300	87.6 – 109.6 (101.2, 5) 95.1 – 104.0 (99.6, 5)	8.4 (5) 3.6 (5) Overall: 6.2 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9972, y = 1.16×10^5x – 57.1	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by monitoring a single mass transition (*m/z* 338→241) using two different

								chromatographic columns
	M750F008	356→259	30	30 300	91.6 – 118.0 (101.8, 5) 97.3 – 108.0 (103.4, 5)	10.7 (5) 4.5 (5) Overall: 4.9 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9977, y = 2.14×10^5x + 36.1	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 356 → 259 and 356 → 241
		356→241	30	30 300	100.4 – 116.8 (106.5, 5) 100.0 – 103.7 (102.3, 5)	6.4 (5) 1.6 (5) Overall: 10.8 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9929, y = 1.68×10^5x + 501	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 356 → 259 and 356 → 241
Surface water	BAS 750 F	398→70	30	30 300	73.9 – 124.5 (89.3, 5) 110.3 – 125.2 (118.5, 5)	22.6 (5) 4.8 (5) Overall: 20.0 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9989, y = 1.64×10^6x – 1.06×10^3	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 398 → 70 and 400 → 70
		400→70	30	30 300	76.0 – 112.5 (92.0, 5) 114.0 – 127.6 (118.9, 5)	14.6 (5) 7.2 (5) Overall: 16.8 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L]	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ

							5 standards, r = 0.9941, y = 5.07×10^5x + 1.47×10^3	Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 398 → 70 and 400 → 70
	M750F003	288→70 (C18)	30	30 300	84.7 – 95.2 (90.0, 5) 81.5 – 95.0 (88.6, 5)	4.1 (5) 6.0 (5) Overall: 4.9 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9933, y = 1.21×10^6x – 253	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by monitoring a single mass transition (*m/z* 288→70) using two different chromatographic columns
		288→70 (Phenyl)	30	30 300	109.5 – 119.5 (115.3, 5) 93.0 – 103.7 (96.6, 5)	3.5 (5) 4.4 (5) Overall: 10.0 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9985, y = 1.79×10^5x + 171	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by monitoring a single mass transition (*m/z* 288→70) using two different chromatographic columns
	M750F005	380→70 (C18)	30	30 300	90.5 – 98.2 (95.6, 5) 83.8 – 87.8 (85.1, 5)	3.2 (5) 1.9 (5) Overall: 4.7 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9963, y = 1.97×10^6x – 209	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by monitoring a single mass transition (*m/z* 380→70) using two different chromatographic columns

		380→70 (Phenyl)	30	30 300	87.7 – 100.9 (95.6, 5) 83.5 – 93.1 (97.7, 5)	5.6 (5) 4.1 (5) Overall: 6.5 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9994, y = 4.31×10^5x – 76.1	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by monitoring a single mass transition (*m/z* 380→70) using two different chromatographic columns
	M750F006	356→259	30	30 300	85.6 – 99.9 (94.2, 5) 78.2 – 92.0 (85.2, 5)	7.2 (5) 6.0 (5) Overall: 8.2 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9987, y = 3.35×10^5x – 251	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 356 → 259 and 356 → 217
		356→217	30	30 300	85.1 – 95.6 (91.8, 5) 79.7 – 93.5 (85.8, 5)	4.3 (5) 5.9 (5) Overall: 6.0 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9993, y = 3.27×10^5x – 234	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 356 → 259 and 356 → 217
	M750F007	338→241 (C18)	30	30 300	110.2 – 122.4 (117.1, 5) 82.2 – 88.9 (85.0, 5)	4.4 (5) 3.1 (5) Overall: 17.1 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r =	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by monitoring a

							0.9952, y = 3.89×10^5x + 302	single mass transition (*m/z* 338→241) using two different chromatographic columns
		338→241 (Phenyl)	30	30 300	106.9 – 129.5 (117.9, 5) 84.6 – 94.2 (90.2, 5)	7.4 (5) 4.2 (5) Overall: 15.3 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9972, y = 1.16×10^5x – 57.1	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by monitoring a single mass transition (*m/z* 338→241) using two different chromatographic columns
	M750F008	356→259	30	30 300	82.4 – 104.1 (91.6, 5) 73.2 – 88.9 (80.9, 5)	9.3 (5) 8.9 (5) Overall: 10.8 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9977, y = 2.14×10^5x + 36.1	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 356 → 259 and 356 → 241
		356→241	30	30 300	85.9 – 99.3 (92.9, 5) 76.0 – 80.0 (78.5, 5)	5.9 (5) 2.1 (5) Overall: 9.9 (10)	0.006 – 0.2 ng/mL [Approx. 7.5 – 250 ng/L] 5 standards, r = 0.9929, y = 1.68×10^5x + 501	Acceptable chromatograms presented for standards, reagent blank, control water samples and fortified water samples. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 356 → 259 and 356 → 241

*ng/L is equivalent to ng/kg in the original method validation

B.5.2.4. Methods for the analysis in air

The residue definition for monitoring in air is BAS 750 F. The analytical method below is consistent with the residue definition.

Report:	KCA 4.2/14 Obermann M.,Studenroth S., 2015 b Validation of analytical method L0327/01, for the determination of BAS 750 F in air by LC-MS/MS 2015/1111330
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4, EPA 850.6100, OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

This method has been evaluated in the Methods for Risk Assessment Section B.5.1.2.1 and found to be satisfactorily validated in accordance with SANCO/825/00 rev.8.1 with an LOQ of 0.01 ng/L.

B.5.2.5. Methods for the analysis in body fluids and tissues

Since BAS 750 F is not classified as toxic or very toxic, methods of analysis for parent or metabolites in human body fluids are not required according to SANCO/825/00 rev. 8.1 but are required in accordance with Regulation (EC) 283/2013. Analytical methods for body tissues for BAS 750 F and M750F022 (Reg.No. 6011210) (2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]propane-1,2-diol) can be found in KCA 4.2/3 [2015/1240005; the LOQ for the method being 0.01 mg/kg. This method for body tissues were found to be satisfactorily validated in accordance with SANCO/825/00 rev.8.1. The following method is for the determination of BAS 750 F in urine and blood and has been found to be satisfactorily validated in accordance with SANCO/825/00 rev.8.1.

Report: CA 4.2/7
Wiesner, W., Breyer N., 2016
Validation of BASF Analytical Method L0339/01 for the Determination of BAS 750 F
2016/1148911

Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010); SANCO/3029/99 rev. 4 of 11-Jul-2000, OECDENV/JM/MONO(2007)17, OECD; EPA OPPTS 860.1340, Aug 1996

GLP: yes
(certified by Behörde für Gesundheit und Verbraucherschutz, Hamburg, Germany)

Principle of the method
10 mL samples of body fluid matrices (blood, urine) were extracted with 10 mL acetonitrile (in case of blood after addition of 5 mL water too). A salt mixture containing magnesium sulphate, sodium chloride and sodium citrate was added, and the extract was shaken. After centrifugation, a 1.5 mL aliquot of the acetonitrile phase was cleaned up using primary secondary amine (PSA). A 250 µL aliquot of the acetonitrile phase was diluted with 750 µL acetonitrile and made to a final volume of 5.0 mL with water.

Analysis was performed by LC-MS/MS using an Ascentis Express C18 column (50 mm x 2.1 mm, 2.7 µm) at 30 °C with external calibration and ESI^+ monitoring the following mass transitions: 398→70 (quantification); 398 →182 (confirmation). A gradient elution was used (mobile phase A: 0.1 % formic acid in acetonitrile; mobile phase B: 0.1 % formic acid in water).

Matrix effects
To check possible ion enhancement or suppression effects in LC/MS-MS analysis, final extracts from urine and blood blank samples were spiked with defined concentrations of BAS 750 F. The peak areas of matrix-matched standards prepared in blank matrix extracts with solvent standards at equivalent concentrations.

Table 5.2-18: Matrix effects

Matrix	**Standard concentration (ng/mL)**	**Mass transition (*m/z*)**	**Mean matrix effect for BAS 750 F (%)**
Urine	0.5-10	398→70	102.7
		398→182	99.2
Blood	0.5-10	398→70	97.7
		398→182	100.6

Matrix effects on the detection of BAS 750 F in extracts of blood and urine were found to be insignificant (< 20 %). Therefore, solvent standards were used for quantification.

Stability of standards
The solvent calibration solutions prepared in acetonitrile/water (20+80, v+v) and fortification solutions prepared in acetonitrile were stored at 1 – 10 °C. One ion mass transition (*m/z* 398→70) was monitored.

Matrix	Analyte	Standard concentration (ng/mL)	Storage period (days)	Difference in peak area (%)

Acetonitrile/water (20/80, v/v)	BAS 750 F	0.25	8	-7.8
		0.50	8	+6.5
		1.0	8	-2.8
		2.5	8	+4.2
		5.0	8	-3.2
		7.5	8	-0.5
		10	8	0.0
Acetonitrile	BAS 750 F	1.0	8	-4.0
		10	8	-3.9

The mean peak area of the stored solutions was within ± 20 % of the mean peak area of the freshly prepared solutions indicating that standards are stable for the testing period when stored under the tested conditions.

Stability of extracts

Following first analysis, the final extracts of fortified samples at LOQ level together with one control specimen extract were stored at 1 - 10 °C for 9 days. After this time period, the final extracts were re-analysed against fresh calibration standards comparing one ion mass transition (*m/z* 398→70) of the analyte.

Table 5.2-19: Stability of extracts

Matrix	Analyte	Fortification level (mg/L)	Days of storage (1st to 2nd injection)	Mean Recovery 1st Injection (%)	Mean Recovery 2nd Injection (%)
Urine	BAS 750 F	0.01	9	103	104
Blood	BAS 750 F	0.01	9	110	110

Mean recovery values of the re-analysed extracts were in the range of 60 – 120 % and within 20 % of the original result. Therefore, extracts were considered to be stable when stored at 1 °C to 10 °C for at least 9 days.

Validation summary

HPLC-MS/MS is a highly specific technique and two mass transitions (3 ions) were monitored for BAS 750 F. Chromatograms of standard solutions, control samples and fortified samples were presented showing no interferences >30% of LOQ at the retention times of interest. Accuracy was assessed at 2 fortification levels corresponding to LOQ and 10xLOQ. In almost all cases the mean recovery was within the acceptable range of 70 – 110 %. To assess method precision, 5 determinations were made at each fortification level and RSDs were within the acceptable limit of 20 %. The overall RSDs were between 3.2 and 7.2. Acceptable linearity was demonstrated in the range 0.006 – 0.4 mg/L for urine and 0.0075 – 0.5 mg/L for blood and was determined using solvent-based standards in all matrices. The LOQ of the method is 0.01 mg/L. The method is satisfactorily validated in accordance with SANCO/825/00 rev.8.1.

Validation data

Table 5.2-20: Validation data

Matrix	Analyte	Mass transition (*m/z*)	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % range (mean, n)	Repeatability % RSD (n)	Linearity	Specificity
Urine	BAS 750 F	398 → 70	0.01	0.01 0.10	99.1 – 106 (103, 5) 89.6 – 94.0 (91.4, 5)	2.8 (5) 1.8 (5) Overall: 6.7 (10)	Solvent based standards used: 0.150 – 10 ng/mL [Approx. 0.006 – 0.4 mg/L] 8 standards, r^2 = 0.9971, y = 105498x + 461838	Acceptable chromatograms provided for standard solutions, control samples and fortified samples. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 398 → 70 and 398 → 182
		398 → 182	0.01	0.01 0.10	97.0 – 103 (100, 5) 93.9 – 100 (96, 5)	2.2 (5) 2.5 (5) Overall: 3.2 (10)	Solvent based standards used: 0.150 – 10 ng/mL [Approx. 0.006 – 0.4 mg/L] 8 standards, r^2 = 0.9998, y = 105498x + 461838	Acceptable chromatograms provided for standard solutions, control samples and fortified samples. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 398 → 70 and 398 → 182
Blood	BAS 750 F	398 → 70	0.01	0.01 0.10	107 – 112 (110, 5) 94.0 – 99.1 (96.0, 5)	1.9 (5) 2.1 (5) Overall: 7.2 (10)	Solvent based standards used: 0.150 – 10 ng/mL [Approx. 0.0075 – 0.5 mg/L] 8 standards, r^2 = 0.9971, y = 105498x + 461838	Acceptable chromatograms provided for standard solutions, control samples and fortified samples. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 398 → 70 and 398 → 182

		398 → 182	0.01	0.01 0.10	101 – 109 (105, 5) 99.3 – 104 (102, 5)	2.7 (5) 2.6 (5) Overall: 3.2 (10)	Solvent based standards used: 0.150 – 10 ng/mL [Approx. 0.0075 – 0.5 mg/L] 8 standards, r^2 = 0.9998, y = 105498x + 461838	Acceptable chromatograms provided for standard solutions, control samples and fortified samples. No interference >30% of LOQ Identity confirmed by LC-MS/MS monitoring two mass transitions: *m/z* 398 → 70 and 398 → 182

B.5.3. REFERENCES RELIED ON

The following databases were searched :

ANABSTR	- Analytical Abstracts	1980 – to present
CAPLUS	- Chemical Abstracts Plus	1907 – to present

Search criteria :

- BAS 750 F, synonyms and CAS numbers were used
- Appropriate metabolites, synonyms and CAS numbers were used
- 1,2,4-triazole, synonyms and CAS numbers were used

A two-step process for selection of relevant scientific peer-reviewed open literature was undertaken:

First Selection step for relevance based on summary records (e.g. titles, abstracts, index terms, keywords)

- Obviously irrelevant records were tagged as "Ballast" and not further processed.
- Records which appeared to be relevant and those of unclear relevance were tagged for further evaluation ("Hits")

Second Detailed Assessment for records requiring further information.
"Hits" were reviewed based on the **title and the abstract** with regard to relevance for the regulatory endpoints. Those records which were clearly not assignable to any regulatory endpoint were categorised as **"no relevant endpoint"**. All remaining records were assessed in detail based on the **complete report** and separated into relevant and non-relevant reports.

Criteria to assign records as **"evaluated - not-relevant"** were:

- Records which did not provide any new relevant data or information
- Records which were not assignable to the substance of interest
- Secondary literature linking to primary literature already discussed under relevant records
- Records with limited reliability of grade 3 or 4 based on the 'Klimisch' scoring system.

Any remaining records were assigned to the category **"used for dossier"**.

3 records relating to BAS 750 F and metabolites and 43 records relating to 1,2,4-triazole were identified under a consideration of analytics. Of these, 23 records (3 BAS 750 F/20 1,2,4-T) were considered to be "hits".

The 3 records relating to BAS 750 F and metabolites and 20 records relating to 1,2,4-triazole were assessed further and considered not to be relevant to the risk assessment for analytics and have therefore not been included in the dossier.

The methodology used in the search, and determination of records as non-relevant is considered acceptable.

Table 5.3-1

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 1.11/3	Harsch M.	2015 b	Determination of the enantiomeric ratio of BAS 750 F in TGAI and formulations 2015/1180118 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 2.5/1	Wilbrand S.	2013 a	Determination of the solubility in distilled water and in buffer solutions at pH 4 and pH 7 (Column Elution Method) of Reg.No. 5834378 2013/1397136 Allessa GmbH, Frankfurt/Main, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 2.5/3	Sonnensch ein L.	2016 a	Physical and chemical properties of BAS 750 F (Reg.No. 5834378): Additional validation of analytical method applied for determination of solubility in water and organic solvents Allessa GmbH,	No	Yes	Data for first Approval	KCA 2.5/3	N.A

			Frankfurt/Main, Germany Fed.Rep. 2016/1234174 yes Unpublished					
KCA 2.6/1	Wilbrand S.	2013 b	Determination of the solubility in organic solvents of Reg.No. 5834378 2013/1391669 Allessa GmbH, Frankfurt/Main, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 2.14/6	Wilbrand S.	2015 a	Reg.No. 5924326: Solubility in water (Flask method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water 2015/1139989 Allessa GmbH, Frankfurt/Main, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 2.14/7	Wilbrand S.	2016 a	Amendment No. 1 - Reg.No. 5924326: Solubility in water (Flask method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water	No	Yes	Data for first Approval	BASF	N.A

			2015/1252305 Allessa GmbH, Frankfurt/Main, Germany Fed.Rep. yes Unpublished					
KCA 2.14/8	Wilbrand S.	2016 b	Report amendment No. 2 to final report - Reg.No. 5924326: Solubility in water (Flask method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water (Original No. 2 of 2) 2016/1030230 Allessa GmbH, Frankfurt/Main, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 2.14/9	Wilbrand S.	2015 b	Reg.No. 6003433: Solubility in water (Column Elution Method) (double distilled water, ph 4, ph 9), partition coefficient 1-Octanol/Water (distilled water, ph 4, ph 9) and dissociation constant in water (original No. 1 of 2) 2015/1139993 Allessa GmbH, Frankfurt/Main, Germany Fed.Rep. yes	No	Yes	Data for first Approval	BASF	N.A

			Unpublished					
KCA 2.14/10	Wilbrand S.	2015 c	Solubility in water (column elution method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water (original no. 2 of 2) 2015/1139994 Allessa GmbH, Frankfurt/Main, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 2.14/11	Wilbrand S.	2015 d	Solubility in water (flask method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water (original no. 2 of 2) 2015/1139997 Allessa GmbH, Frankfurt/Main, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 2.14/12	Wilbrand S.	2015 e	Reg.No. 6010286: Solubility in water (column elution method) (double distilled water, pH 4, pH 9), partition coefficient 1-octanol/water (distilled water, pH 4, pH 9) and dissociation constant in water	No	Yes	Data for first Approval	BASF	N.A

			(Original No. 2 of 2) 2015/1139998 Allessa GmbH, Frankfurt/Main, Germany Fed.Rep. yes Unpublished					
KCA 4.1.1/1	Bentz A.	2013 a	Analytical method APL0669/01 - Determination of the active ingredient Reg.No.5834378 in Reg.No. 5834378 TGAI 2013/1140545 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF	N.A
KCA 4.1.1/2	Bentz A. Harsch M.	2013 a	Validation of the analytical method APL0669/01: Determination of the active ingredient Reg.No. 5834378 in Reg.No. 5834378 TGAI 2013/1140546 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.1/9	Harsch M.	2014 b	Analytical method APL0685/01 - Determination of [redacted] in Reg.No. 5834378 TGAI (Technical Grade Active Ingredient)	No	No	Not applicable	BASF	N.A

			by GC 2014/1010801 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished					
KCA 4.1.1/10	Harsch M.	2014 c	Validation of the analytical method APL0685/01: Determination of [redacted] in Reg.No. 5834378 TGAI (Technical Grade Active Ingredient) by GC 2014/1010802 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/1	Studenroth S. Lueer D.	2015 a	Validation of analytical method L0214/01 for the determination of BAS No. 750 F (Reg.No. 5834378) and metabolites of Reg.No. 5924326 and 1,2,4-Triazole (Reg.No. 87084) in soil by LC-MS/MS 2015/1039006 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/2	Lueer D.	2016 a	Report Amendment No. 1: Validation of analytical method L0214/01 for the determination of	No	Yes	Data for first Approval	BASF	N.A

			BAS No. 750 F (Reg.No. 5834378) and its metabolites Reg.No. 5924326 and 1,2,4-Triazole (Reg.No. 87084) in soil by LC-MS/MS BASF SE, Limburgerhof, Germany Fed.Rep. 2016/1030227 yes Unpublished					
KCA 4.1.2/3	Obermann M.	2016 a	Report Amendment No. 2: Validation of analytical method L0214/01 for the determination of BAS No. 750 F (Reg.No. 5834378) and its metabolites Reg.No. 5924326 and 1,2,4-Triazole (Reg.No. 87084) in soil by LC-MS/MS BASF SE, Limburgerhof, Germany Fed.Rep. 2016/1215646 yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/4	Geschke S.	2014 a	Validation of an analytical method for determination of BAS 555 F (Metconazole) and its metabolite 1,2,4-(1H)-Triazole in soil 2013/1377001 Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/5	Malinsky D.S.	2016 a	Validation of analytical method D1506/01: Method for the	No	Yes	Data for first Approval	BASF	N.A

			determination of Mefentrifluconazole (BAS 750 F, Reg. No.5834378) and its metabolites M750F003 (Reg. No.5924326), M750F005 (Reg. No.6003433), M750006 (Reg. No.5863469), M750F007 (Reg. No.6003432) and M750F008 (Reg. No.6010286) in surface and drinking water by LC-MS/MS 2015/7001125 BASF Crop Protection, Research Triangle Park NC, United States of America yes Unpublished					
KCA 4.1.2/6	Malinsky D.S.	2016 a	Amended Report: Validation of analytical method D1506/01: Determination of Mefentrifluconazole (BAS 750 F, Reg. No.5834378) and its metabolites M750F003 (Reg. No.5924326), M750F005 (Reg. No.6003433), M750006 (Reg. No.5863469), M750F007 (Reg. No.6003432) and M750F008 (Reg. No.6010286) in surface and drinking water by LC-MS/MS 2016/7010048 BASF Crop Protection,	No	Yes	Data for first Approval	BASF	N.A

			Research Triangle Park NC, United States of America yes Unpublished					
KCA 4.1.2/7	Penning H. et al.	2013 a	Validation of analytical method L0199/01 for the determination of 1,2,4-Triazole (Reg.No. 87084) in water by LC-MS/MS 2012/1297158 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/8	Obermann M. Studenroth S.	2015 a	Validation of analytical method L0327/01, for the determination of BAS 750 F in air by LC-MS/MS 2015/1111330 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/9	Baltussen E.	2013 a	Development and validation of an analytical method for the analysis of BAS 750 F in diet 2015/1189151 WIL Research Europe BV, s-Hertogenbosch, Netherlands yes	No	Yes	Data for first Approval	BASF	N.A

			Unpublished					
KCA 4.1.2/10	Baltussen E.	2016 a	Report Amendment Number 1: Development and validation of an analytical method for the analysis of BAS 750 F in diet 2016/1041496 WIL Research BV, s-Hertogenbosch, Netherlands yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/11	Becker M Kamp H.	2015 i	BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in diet using HPLC-UV 2015/1174512 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/12	Becker M. Kamp H.	2015 j	BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in diet using HPLC-UV 2015/1175541 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/13	Becker M. Kamp H.	2015 a	BAS 750 F - Validation of an analytical method	No	Yes	Data for first Approval	BASF	N.A

			for the analysis of BAS 750 F in diet using LC-MS/MS 2015/1175542 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished					
KCA 4.1.2/14	Becker M. Kamp H.	2015 b	BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in aqueous Carboxymethylcellulose (CMC) using HPLC-UV 2015/1177605 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/15	Becker M. Kamp H.	2015 c	BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in a mixture of Dimethyl Sulfoxide and corn oil using HPLC-UV 2015/1185311 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/16	Becker M. Kamp H.	2015 d	BAS 750 F - Validation of an analytical method	No	Yes	Data for first Approval	BASF	N.A

			for the analysis of BAS 750 F in corn oil using HPLC-UV 2015/1184812 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished					
KCA 4.1.2/17	Becker M. Kamp H.	2015 e	BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in Dimethyl sulfoxide using HPLC-UV 2015/1184813 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/18	Becker M. Kamp H.	2015 f	BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in plasma using LC-MS/MS 2015/1186912 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/19	Becker M. Kamp H.	2015 g	BAS 750 F - Validation of an analytical method for the analysis of BAS 750 F in Paraffinum	No	Yes	Data for first Approval	BASF PS	N.A

			subliquidum using HPLC-UV 2015/1186913 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished					
KCA 4.1.2/20	Becker M. Kamp H.	2015 k	Reg.No. 6011210 - Validation of an analytical method for the analysis of Reg.No. 6011210 in diet using HPLC-UV 2015/1188594 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/21	Hedrich R.	2015 a	Validation of an analytical method for the analysis of Reg.No. 6011210 in corn oil using HPLC 2015/1189154 Institut Kuhlmann GmbH Analytik-Zentrum Ludwigshafen, Ludwigshafen, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/22	Becker M. Kamp H.	2015 l	Reg.No. 6011210 - Validation of an analytical method for the analysis of Reg.No. 6011210 in Dimethyl sulfoxide	No	Yes	Data for first Approval	BASF	N.A

			using HPLC-UV 2015/1188599 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished					
KCA 4.1.2/23	Mallat E.	2015 a	The validation of the determination of Reg.No. 6011210 in mouse EDTA plasma samples using LC-MS/MS 2015/1186930 ABL - Analytical Biochemical Laboratory B.V., AJ Assen, Netherlands yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/25	Paula Jose W.F. de	2015 a	Validation of BASF Method Number L0076/09 for the determination of BAS 750 F in citrus (whole fruit), coffee (grain), dry beans (seed), soybeans (grain), tomato (whole fruit), wheat (grain) and wheat (straw) using LC-MS/MS 2015/3001681 BASF SA, Guaratingueta, Brazil yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/26	Class T.	2011 a	Modification M004 of BCS residue analytical method 01062 for the	No	Yes	Data for first Approval	TDMG	N.A

			determination of 1,2,4-Triazole, Triazolylalanine, Triazole acetic acid and Triazole lactic acid by LC/DMS/MS/MS in plant materials 2012/1294644 PTRL Europe GmbH, Ulm, Germany Fed.Rep. yes Unpublished					
KCA 4.1.2/27	Devine C.	2015 a	Validation of the BASF analytical method L0272/01 for BAS 750 F in animal matrices 2015/1106707 CEMAS - CEM Analytical Services Ltd., Wokingham Berkshire RG41 2FD, United Kingdom yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/28	Heger N. Taraschewski I.	2016 b	Validation of the BASF analytical method L0309/01: For the determination of M750F022 (Reg.No. 6011210) in animal matrices 2015/1106706 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA	Heger N.	2017 a	Revalidation of the BASF analytical	No	Yes	Data for first	BASF	N.A

4.1.2/32			method L0309/01: For the determination of M750F022 (Reg.No. 6011210) in cow fat and milk 2017/1002385 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished			approval		
KCA 4.1.2/29	Guedez Orozco A.A. Heger N.	2016 a	Determination of the fatty conjugates metabolites of M750F022 (Reg. No. 6011210) in animal matrices 2016/1001326 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.1.2/30	Billian P. Druskus M.	2009 a	Residue analytical method 01132 for the determination of 1,2,4-Triazole, Triazole Alanine, Triazole Acetic Acid and Triazole Lactic Acid in/on milk, egg, muscle, fat, liver and kidney by HPLC-MS/MS (including amendment No. 1) 2010/1230632 Bayer CropScience AG, Monheim, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	TF	N.A

KCA 4.2/1	Klimmek S. et al.	2015 a	Validation of the multi-residue method QuEChERS, BASF method number L0295/01, for the determination of BAS 750 F in different matrices of plant origin 2015/1106708 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.2/2	Richter S. Schmiedt S.	2015 a	Independent method validation (ILV) of the QuEChERS method for the determination of BAS 750 F in 5 plant matrices, using LC/MS/MS (BASF Method No. L0295/01) 2015/1240004 PTRL Europe, Ulm, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.2/3	Devine C.	2015 a	Validation of the BASF analytical method L0272/01 for BAS 750 F in animal matrices 2015/1106707 CEMAS - CEM Analytical Services Ltd., Wokingham Berkshire RG41 2FD, United Kingdom	No	Yes	Data for first Approval	BASF	N.A

			yes Unpublished					
KCA 4.2/4	Richter S. Djedovic S.	2015 b	Independent method validation (ILV) of a method for the determination of BAS 750 F in various foodstuffs of animal origin, using LC/MS/MS - (BASF Method No. L0272/01) 2015/1240005 PTRL Europe, Ulm, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.2/6	Bendig P. Wabbel C.	2015 a	Independent method validation (ILV) of BASF method no. L0309/01 for the determination of the BAS 750 F diol metabolite in various foodstuffs of animal origin, using GC/MS 2015/1240006 PTRL Europe, Ulm, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.2/7	Wiesner F.,Breyer N.	2016 a	Validation of BASF Analytical Method No. L0339/01 for the determination of BAS 750 F in body fluids 2016/1148911 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany Fed.Rep.	No	Yes	Data for first approval	BASF	

			yes Unpublished					
KCA 4.2/8	Studenroth S. Lueer D.	2015 b	Validation of analytical method L0214/01 for the determination of BAS No. 750 F (Reg.No. 5834378) and metabolites of Reg.No. 5924326 and 1,2,4-Triazole (Reg.No. 87084) in soil by LC-MS/MS 2015/1039006 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.2/9	Lueer D.	2016 a	Report Amendment No. 1: Validation of analytical method L0214/01 for the determination of BAS No. 750 F (Reg.No. 5834378) and its metabolites Reg.No. 5924326 and 1,2,4-Triazole (Reg.No. 87084) in soil by LC-MS/MS BASF SE, Limburgerhof, Germany Fed.Rep. 2016/1030227 yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.2/10	Obermann M.	2016 a	Report Amendment No. 2: Validation of analytical method L0214/01 for the determination of BAS No. 750 F (Reg.No. 5834378) and its metabolites Reg.No. 5924326 and 1,2,4-Triazole (Reg.No. 87084) in soil by LC-MS/MS BASF SE, Limburgerhof, Germany Fed.Rep.	No	Yes	Data for first Approval	BASF	N.A

			2016/1215646 yes Unpublished					
KCA 4.2/11	Malinsky D.S.	2016 a	Validation of analytical method D1506/01: Method for the determination of Mefentrifluconazole (BAS 750 F, Reg. No.5834378) and its metabolites M750F003 (Reg. No.5924326), M750F005 (Reg. No.6003433), M750006 (Reg. No.5863469), M750F007 (Reg. No.6003432) and M750F008 (Reg. No.6010286) in surface and drinking water by LC-MS/MS 2015/7001125 BASF Crop Protection, Research Triangle Park NC, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.2/12	Malinsky D.S.	2016 a	Amended Report: Validation of analytical method D1506/01: Determination of Mefentrifluconazole (BAS 750 F, Reg. No.5834378) and its metabolites M750F003 (Reg. No.5924326), M750F005 (Reg. No.6003433), M750006 (Reg. No.5863469), M750F007 (Reg. No.6003432) and M750F008 (Reg. No.6010286) in	No	Yes	Data for first Approval	BASF	N.A

			surface and drinking water by LC-MS/MS 2016/7010048 BASF Crop Protection, Research Triangle Park NC, United States of America yes Unpublished					
KCA 4.2/13	Gu G. et al.	2016 a	Independent laboratory validation of BASF analytical method D1506/01: Method for the determination of BAS 750 F (Reg. No. 5834378) and its metabolites M750F003 (Reg. No. 5924326), M750F005 (Reg. No. 6003433), M750F006 (Reg. No. 5863469), M750F007 (Reg. No. 6003432) and M750F008 (Reg. No. 6010286) in surface and drinking water by LC-MS/MS 2015/7006199 Alliance Pharma Inc., Malvern PA, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 4.2/14	Obermann M. Studenroth S.	2015 b	Validation of analytical method L0327/01, for the determination of BAS 750 F in air by LC-MS/MS	No	Yes	Data for first Approval	BASF	N.A

			2015/1111330 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished					
KCA 7.1.2.2.1/1	Schaeufele M.	2015 d	Field soil dissipation study of Reg.No. 5834378 in the formulation EXP 5834378 F-AV on bare soil at six sites in Europe, 2013 2015/1046920 Envigo CRS Limited, Suffolk IP23 7PX, United Kingdom yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 7.1.2.2.1/2	Schaeufele M.	2015 e	Final report amendment No. 1: Field soil dissipation study of Reg.No. 5834378 in the formulation EXP 5834378 F-AV on bare soil at six sites in Europe, 2013 2015/1242234 Envigo CRS Limited, Suffolk IP23 7PX, United Kingdom yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 7.1.2.2.1/3	Jacobson B. et al.	2016 a	Terrestrial field dissipation of the fungicide BAS 750 F following broadcast applications of BAS 750 01 F (EC) or	No	Yes	Data for first Approval	BASF	N.A

			BAS 750 UA F (SC) 2015/7006396 Waterborne Environmental Inc., Leesburg VA, United States of America yes Unpublished					
KCA 7.1.2.2.1/6	Brewin S.	2015 a	Storage stability of residues of BAS 750 F- Reg.No. 5834378 and its metabolite Reg.No. 5924326 in soil when stored at approximately -20°C for 540 days - Interim Report 2015/1050221 Envigo CRS Limited, Suffolk IP23 7PX, United Kingdom yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 7.1.2.2.1/7	Brewin S.	2015 b	Interim report Amendment No. 1: Storage stability of residues of BAS 750 F- Reg.No. 5834378 and its metabolite Reg.No. 5924326 in soil when stored at approximately -20°C for 540 days - Interim Report 2015/1249072 Envigo CRS Limited, Suffolk IP23 7PX, United Kingdom yes Unpublished	No	Yes	Data for first Approval	BASF	N.A

KCA 7.1.2.2.1/8	Brewin S.	2016 a	Storage stability of residues of BAS 750 – Reg.No. 5834378 and its metabolite Reg.No.5924326 in soil when stored at approximately -20°C for 650 days 2015/1106725 Envigo CRS Limited, Suffolk IP23 7PX, United Kingdom yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	N.A
KCA 7.1.2.2.1/9	Geschke S.	2015 a	Determination of storage stability of BAS 555 F (Metconazole) and its metabolite 1,2,4-Triazole in soil 2015/1204922 Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 7.1.4/1	Sandt H.J. van de	2015 a	Determination of foliar DT50 of Triazole (BAS 750 F) after application of BAS 750 01 F to wheat surfaces 2015/1130156 De Bredelaar BV, Elst, Netherlands yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA	[redacted]	2014 c	BAS 750 F - Avian dietary toxicity test	Yes	Yes	Data for first	BASF	N.A

8.1.1.2/1			in chicks of the bobwhite quail (Colinus virginianus) 2014/1127963 [redacted] yes Unpublished			Approval		
KCA 8.1.1.2/2	[redacted]	2015 a	Amendment No. 1 - BAS 750 F - Avian dietary toxicity test in chicks of the bobwhite quail (Colinus virginianus) 2015/1223324 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	N.A
KCA 8.1.1.2/3	[redacted]	2014 d	BAS 750 F - Avian dietary toxicity test in ducklings of the mallard duck (Anas platyrhynchos) 2014/1117035 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	N.A
KCA 8.1.1.3/1	[redacted]	2014 a	BAS 750 F: A reproduction study with the Northern bobwhite 2013/1281276	Yes	Yes	Data for first Approval	BASF	N.A

			[redacted] yes Unpublished					
KCA 8.1.1.3/2	[redacted]	2015 a	BAS 750 F: A reproduction study with the mallard 2015/7005819 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	N.A
KCA 8.2.1/1	[redacted]	2014 a	BAS 750 F - Acute toxicity study in the rainbow trout (Oncorhynchus mykiss) 2014/1036951 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	N.A
KCA 8.2.1/2	[redacted]	2015 b	Reg.No. 6003432 (metabolite of BAS 750 F, M750F007) - Rainbow trout, acute toxicity test 2015/1001489 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	N.A
KCA	[redacted]	2016 a	Reg.No. 5863469 (metabolite of BAS	Yes	Yes	Data for first	BASF	

8.2.1/3	H.		750 F, M750F006) - Rainbow trout, acute toxicity test 2016/1128152 [redacted] yes Unpublished			Approval		
KCA 8.2.1/4	[redacted]	2015 a	BAS 750 F (Reg.No. 5834378) - Zebrafish acute toxicity test 2015/1001581 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	N.A
KCA 8.2.1/5	[redacted]	2014 a	BAS 750 F: Acute toxicity to the sheepshead minnow, Cyprinodon variegatus, determined under static-renewal test conditions 2014/7002810 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	N.A
KCA 8.2.1/6	[redacted]	2015 c	BAS 750 F - Acute toxicity study in the common carp (Cyprinus carpio) 2015/1249071 [redacted]	Yes	Yes	Data for first Approval	BASF	N.A

			[redacted] yes Unpublished					
KCA 8.2.2.1/1	[redacted]	2015 a	BAS 750 F: Early life-stage toxicity test with the sheepshead minnow, Cyprinodon variegatus, under flow-through conditions 2015/7000619 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	N.A
KCA 8.2.2.1/2	[redacted]	2015 a	BAS 750 F - Early life-stage toxicity test on the zebrafish (Danio rerio) in a flow through system 2014/1262160 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF	N.A
KCA 8.2.2.1/3	[redacted]	2015 b	BAS 750 F - Fish sexual development test on the zebrafish (Danio rerio) 2015/1099093 [redacted] yes	Yes	Yes	Data for first Approval	BASF	N.A

			Unpublished					
KCA 8.2.4.1/2	Backfisch K. Haerthe N.	2015 a	Acute toxicity of Reg.No. 6003432 (M750F007; metabolite of BAS 750 F) to Daphnia magna STRAUS in a 48 hour static test 2015/1003915 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.4.1/3	Haerthe N.	2016 a	Acute toxicity of Reg.No. 5924326 /M750F003; metabolite of BAS 750 F) to Daphnia magna STRAUS in a 48 hour static test BASF SE, Limburgerhof, Germany Fed.Rep. 2016/1289876 yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.4.1/4	Rzodeczko H.	2015 c	Reg.No. 5863469 (metabolite of BAS 750 F, M750F006) - Daphnia magna, acute immobilization test 2015/1001492 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.4.1/5	Rzodeczko H.	2015 d	Reg.No. 6003433 (metabolite of BAS 750 F, M750F005) - Daphnia magna, acute immobilization test	No	Yes	Data for first Approval	BASF	N.A

			2015/1001490 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished					
KCA 8.2.4.1/6	Rzodeczko H.	2015 e	Reg.No. 6010286 (metabolite of BAS 750 F, M750F008) - Daphnia magna, acute immobilization test 2015/1001493 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.4.2/1	VanHooser A.	2014 a	BAS 750 F: Acute toxicity test with the saltwater mysid, Americamysis bahia, determined under flow-through test conditions 2014/7002845 ABC Laboratories Inc., Columbia MO, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.4.2/2	VanHooser A.	2015 a	BAS 750 F: Effect on new shell growth of the eastern oyster (Crassostrea virginica) 2015/7000021 ABC Laboratories Inc., Columbia MO, United States of	No	Yes	Data for first Approval	BASF	N.A

			America yes Unpublished					
KCA 8.2.5.1/1	Janson G.-M.	2014 a	Chronic toxicity of the BAS 750 F (Reg.No. 5834378) to Daphnia magna STRAUS in a 21 day semi-static test 2014/1098028 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.5.2/1	Janson G.-M.	2015 b	Chronic toxicity of BAS 750 F (Reg.No. 5834378) to Daphnia longispina in a 21 day semi-static test 2015/1003912 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.5.2/2	Janson G.-M.	2015 c	Report Amendment No.1 - Chronic toxicity of BAS 750 F (Reg.No. 5834378) to Daphnia longispina in a 21 day semi-static test 2015/1251197 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A

KCA 8.2.5.2/3	Janson G.-M.	2015 a	Chronic toxicity of BAS 750 F (Reg.No. 5834378) to Daphnia pulex in a 21 day semi-static test 2015/1003913 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.5.2/4	Dinehart S.	2016 a	BAS 750 F: Life-cycle toxicity test of the saltwater mysid, Americamysis bahia, conducted under flow-through conditions 2016/7001293 ABC Laboratories Inc., Columbia MO, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.5.3/1	Clark R.	2015 a	BAS 750 F - 10-day toxicity test exposing midge (Chironomus dilutus) to a test substance applied to sediment under static-renewal conditions 2015/7000621 Smithers Viscient LLC, Wareham MA, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA	Backfisch K.	2015 a	Chronic toxicity of Reg.No. 5924326	No	Yes	Data for first	BASF	N.A

8.2.5.3/2	Weltje L.		(M750F003; metabolite of BAS 750 F) to the non-biting midge Chironomus riparius - a spiked sediment study 2015/1003916 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished			Approval		
KCA 8.2.5.3/3	Backfisch K. Weltje L.	2015 a	Chronic toxicity of Reg.No. 5834378 to the non-biting midge Chironomus riparius - A spiked sediment study 2014/1243181 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.5.4/1	Clark R.	2015 b	BAS 750 F - 10-Day toxicity test exposing freshwater amphipods (Hyalella azteca) to a test substance applied to sediment under static-renewal conditions 2015/7000622 Smithers Viscient LLC, Wareham MA, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.5.4/2	Clark R.	2015 c	BAS 750 F - 10-Day toxicity test exposing estuarine	No	Yes	Data for first Approval	BASF	N.A

			amphipods (Leptocheirus plumulosus) to a test substance applied to sediment under static conditions 2015/7000623 Smithers Viscient LLC, Wareham MA, United States of America yes Unpublished					
KCA 8.2.6.1/1	Brzozowska K.	2014 b	BAS 750 F (Reg.No. 5834378) - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test 2013/1250865 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.6.1/2	Backfisch K.	2015 a	Effect of Reg.No. 6003432 (M750F007, metabolite of BAS 750 F) on the growth of the green alga Pseudokirchneriella subcapitata 2015/1003914 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A

KCA 8.2.6.1/3	Brzozowska-Wojoczek K.	2015 a	Reg.No. 6010286 (metabolite of BAS 750 F, M750F008) - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test 2015/1001491 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.6.1/4	Rzodeczko H.	2016 a	Reg.No. 5863469 (metabolite of BAS 750 F, M750F006) - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test 2015/1184815 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.6.1/5	Rzodeczko H.	2016 b	Reg.No. 6003433 (metabolite of BAS 750 F, M750F005) - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test 2015/1184816 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.6.1/6	Backfisch K.	2016 a	Effect of Reg.No. 5924326 (M750F003,	No	Yes	Data for first Approval	BASF	N.A

			metabolite of BAS 750 F) on the growth of the green alga Pseudokirchneriella subcapitata BASF SE, Limburgerhof, Germany Fed.Rep. 2016/1289875 yes Unpublished					
KCA 8.2.6.2/1	Bergfield A.	2015 a	BAS 750 F: Growth inhibition test with the marine diatom, Skeletonema costatum 2015/7000620 ABC Laboratories Inc., Columbia MO, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.6.2/3	Bergfield A.	2015 b	BAS 750 F: Growth inhibition test with the freshwater diatom, Navicula pelliculosa 2015/7000618 ABC Laboratories Inc., Columbia MO, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.2.6.2/5	Bergfield A.	2015 c	BAS 750 F: Growth inhibition test with the cyanobacterium, Anabaena flos-aquae 2015/7000617 ABC Laboratories Inc., Columbia MO, United States of	No	Yes	Data for first Approval	BASF	N.A

			America yes Unpublished					
KCA 8.2.7/1	Swierkot A.	2014 a	BAS 750 F (Reg.No. 5834378) - Lemna gibba CPCC 310 growth ihibition test 2014/1001322 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first Approval	BASF	N.A
KCA 8.3.1.3/1	Kleebaum K.	2015 b	Acute toxicity of BAS 750 F to honeybee larvae (Apis mellifera L.) under laboratory conditions (in vitro) 2013/1235087 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF	N.A