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TRITICONAZOLE

Volume 3 – B.5 (AS)

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

Version History

When	What
2003/ September	Initial DAR, first version
2004/ September	Addendum 1
2005/January	Addendum rev. 2
2018/July	DRAR

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Throughout this document the original DAR (referred to as the DAR 2003) and this evaluation, is referred to as the RAR (Renewal assessment report). Studies that were evaluated in the DAR 2003 have not been re-evaluated and the results are presented in this report in **grey typeface**. New information (e.g. historical control data, additional experimental details) or new interpretation of the data has been taken into account or changes compared to the original DAR 2003 are written in black typeface. Letters in **bold** in the overview tables (B.5.1.2.X-1) indicate that the method/validation is evaluated by RMS.

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

Please refer to Volume 4.

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

Table B.5.1.2.1-1 Overview of risk assessment methods in respective environmental fate studies

Annex Point for original study	DocID of original study	Principle of the method	Method Name	Method included in CA 4.1.2
CA 7.1.1.1/1 CA 7.1.2.1.1/1 CA 7.1.2.1.2/1	2014/7000472	HPLC - radio	M-407725	no
CA 7.1.2.1.1/2	**2014/7000471**	**HPLC-MS/MS**	**No. 0051**	**B003339 CA 4.1.2/2**
CA 7.1.2.2.1/1	**2009/1049703**			
CA 7.1.3.1.1/1	2014/3001242	HPLC - radio	n.a.	no
CA 7.1.3.1.2/1	2015/3000503	HPLC - radio	n.a.	no
CA 7.2.1.1/1	2012/1300793	HPLC - radio	n.a.	no
CA 7.2.1.2/1	2007/7001058	HPLC - radio	BAS595F.m	no
CA 7.2.2.2/1	2014/1083345	HPLC - radio	n.a.	no

Reference:	**Triticonazole Method validation for triticonazole, RPA406341 and RPA 404766 in soil**
Author(s), year:	Doran, A.M., McGuire G.M., Charles, E., 2001
Report/Doc. number:	B003339
Guideline(s):	-
GLP:	yes

Principle of the method

The analytical method (CLE 198/120-02R) has been validated for the determination of triticonazole, M595F002 (RPA 406341) and M595F001 (RPA 404766) in soil. Soil is extracted with acetone/ammonium hydroxide using sonication. Extracts are purified using soil solid phase extraction (SPE) applying a C18 phase. Samples are evaporated under a stream of nitrogen and are further analyzed by isocratic reversed phase LC-MS/MS detecting one transition. Triticonazole: m/z 318.1 → 125; M595F001 and M595F002: m/z 334.1 → 125.

HPLC column: Phenomenex Luna Hexyl-Phenyl (100 x 4.6mm, 5µm); Guard column: Kromasil C8 (10 x 4.6mm, 5µm); mobile phase: 0.2% formic acid (methanol/water 70:30 v/v)

Soil specification

Control samples of soil were obtained from Inveresk Research Project No.680045, "Triticonazole Field Soil Dissipation Study in Europe" C021420. The samples were taken from plot numbers 1 and 2 (a sandy silt loam and a silty loam).

Validation

Specificity

According to guidance document SANCO 3029/99 rev.4 no confirmatory analysis is required in this case.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Good linearity was observed in the range of 0.50 ng mL^{-1} to 500 ng mL^{-1} (n=11) for the two mass transitions of triticonazole and its two metabolites. Graphs and equations are available. Correlation coefficients (r) are in all cases >0.996.

Accuracy

The recovery results for all analytes are acceptable according to guidance document SANCO 825/00 rev. 8.1.

Limit of quantification

The limit of quantitation (LOQ) was determined to be 0.002 mg/kg for each analyte.

Precision (repeatability)

The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Validation is summarized in Table B.5.1.2.1-2.

Conclusion

Monitoring method B003339 was taken for analyses in the environmental fate risk assessment part. Please refer as well to B.5.2.3.

The method/validation is considered acceptable according to guidance document SANCO 3029/99 rev.4.

The study was evaluated as monitoring method in the DAR 2003.

Table B.5.1.2.1-2 Validation results in soil

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Doran, A.M., McGuire G.M., Charles, E., 2001	triticonazole	LC-MS/MS m/z 318.1 → 125	Soil, Type 1	0.002	0.002	82.4	4.2	5
					0.020	86.6	2.4	5
	M595F002	LC-MS/MS m/z 334.1 → 125		0.002	0.002	83.7	4.7	5
					0.020	89.3	3.6	5
	M595F001	LC-MS/MS m/z 318.1 → 125		0.002	0.002	82.9	4.3	5
					0.020	87.3	7.2	5
	triticonazole	LC-MS/MS m/z 318.1 → 125	Soil, Type 2	0.002	0.002	81.8	2.8	5
					0.020	89.8	1.8	5
	M595F002	LC-MS/MS m/z 334.1 → 125		0.002	0.002	86.7	4.7	5
					0.020	92.1	3.6	5
	M595F001	LC-MS/MS m/z 318.1 → 125		0.002	0.002	75.8	6.7	5
					0.020	88.3	3.3	5
					0.050	89	2.3	3
					0.050	96	1.0	3
					0.050	89	2.3	3

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

Not relevant.

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

An appropriate monitoring method has been supplied and evaluated see B.5.2.6 below and so the data requirments (283/2013) has been met.

Table B.5.1.2.3-1 Overview of risk assessment methods in respective toxicology studies

Annex Point for original study	DocID of original study	Principle of the method	Method Name	Method included in CA 4.1.2
CA 5.3	R013082 (1993/1003120)	none	none	no
CA 5.3	**C0118959**	**HPLC-UV**	***"MQ271"***	**MQ271**
CA 5.6	C044414 (1990/1004392)	none	none	no
CA 5.6.1	**R013085**	**GC-ECD**	***"MP-RP72_MA"***	**MP-RP72_MA**
CA 5.8.2/1	**2011/1268148**	**LC-UV**	***"Animal Diet"***	**2016/1270666**
…..	**2016/1296133**			**2016/1279220**

Method: Method MQ271
References: Refer to Table B.5.1.2.3-1
Validation: In-study validation

Principle of the method
Method MQ271 was used to test the concentration of BAS 595F in 0.5% methylcellulose matrix.
The total unit sample of known weight was transferred quantitatively into a volumetric flask with acetonitrile. After ensuring complete dissolution, the solution was made to volume and an aliquot further diluted to a known volume with acetonitrile to give a nominal concentration of BAS 595F in the final solution between 200 and 300 µg/mL. The concentration was then determined by isocratic high performance liquid chromatography at 254 nm.

Validation
Specificity
This parameter was not tested in this study.
Linearity
Five accurate standard solutions of BAS 595F in acetonitrile containing approximately 80, 160, 240, 320 and 400 µg/mL were prepared and chromatographed. A graph of BAS 595 F concentration (µg/mL) versus peak area measured by computing integrator was prepared to confirm linearity. The data were regressed linearly and the regression line plotted on the graph. A standard solution of intermediate concentration was injected at intervals throughout the run to monitor the chromatographic performance and update the response factor for computation. Calibration was linear over the concentration range 80 to 400 µg/mL.
No graph, equation parameters and correlation coefficient are reported.
Accuracy
The recovery results are acceptable according to guidance document SANCO 3029/99 rev.4 and summarized in Table B.5.1.2.3-2.
Limit of quantification
The procedure was adequately sensitive for the assay of formulations at concentrations between 1.0 and 100 mg/mL employed in the toxicity studies. The LOQ was therefore set at 1.0 mg/mL.
Precision (repeatability)
For all fortification levels the relative standard deviations (RSD) were below 20%.
Matrix Effects
No matrix effects tested in this study
Reproducibility
Reproducibility of the method was not determined within this validation study.

Conclusion
It could be demonstrated that the method MQ271 fulfilled the requirements and was therefore applicable to correctly determine residues of BAS 595 F in 0.5% methylcellulose matrices.

Table B.5.1.2.3-2: Summary Table of the recoveries of triticonazole in methylcellulose

Matrix	Analyte	LOQ	Forti-fication Level	Mean Recovery [%]	RSD	Overall Recovery [%]	RSD	n
		[mg/mL]	[mg/mL]		[%]		[%]	
0.5% methylcellulose	Triticonazole	1	1.0	92.8	4.0	94.2	3.9	6
			100	95.6	3.0			6

Method: Method MP-RP72_MA
References: Refer to Table B.5.1.2.3-1
Validation: In-study validation

Principle of the method
The method is applicable to the determination of BAS 595F in rat feed samples at 5 to 5000 mg/kg.
Weigh 10 mg of feed sample into a 250 mL Erlenmeyer flask for low level and a 500 mL flask for high level samples. Add acetone and place on a wrist action shaker for 30 minutes. Transfer to a glass centrifuge tube and centrifuge for 10 minutes. Dilute the samples with acetone and inject the extract into a gas chromatograph with detection by ECD.

Validation

Specificity

No analysis of specificity was necessary for this validation.

Linearity

The linear regression is calculated from the four point standard curve in the range of 0.25 – 1.0 µg/mL. No graph is reported.
The coefficient of variation of three identical standards (based on peak area) were less than 2.0% for the results to be considered valid.

Accuracy

The recovery results are acceptable according to guidance document SANCO 3029/99 rev.4 and summarized in Table B.5.1.2.3-3.

Limit of quantification

The analytical method was validated for a limit of detection of 5 mg/kg corresponding to the lowest level validated.

Precision (repeatability)

For all fortification levels the relative standard deviations (RSD) were below 20%.

Matrix Effects

No matrix effects tested in this study

Reproducibility

Reproducibility of the method was not determined within this validation study.

Conclusion
The method proved to be valid for the determination of triticonazole in feed items for rats.

Table B.5.1.2.3-3: Summary Table of the recoveries of triticonazole in feeding items

Analyte	Formu-lation	Fortification Level [mg/kg]	Number of replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
BAS 595F	1	5	8	101.4	5.9	101.6	4.2
		5000	8	101.7	1.9		
	2	5	8	100.0	3.9	101.7	3.6
		5000	8	103.3	2.5		

Method: Animal Diet
References: Refer to Table B.5.1.2.3-1
Validation: 2016/1270666 (Catchpole G.,Hidding B., 2016 b) for Kliba maintenance diet
2016/1279220 (Hidding B., 2016 b) for SSNIFF diet

Principle of the method
The aim of the study was the retrospective validation of a quantitative analytical method for BAS 595 F (Triticonazole) in the vehicle Ground Kliba maintenance diet mouse/rat „GLP“ meal according to control procedure number 05/0622_02. A high performance liquid chromatographic method with UV detection at 262 nm for the quantitative analysis of the test substance in Ground Kliba maintenance diet mouse/rat „GLP“ meal was validated.
The same method was used under the same conditions for a slightly different meal called “Standard powder rodent diet” (SSNIFF®).

Validation
Specificity
Chromatograms of a test substance solution, a blank accuracy and a solvent blank were recorded. There was no peak present in the chromatogram corresponding to the analysis of the blank accuracy sample at the retention time of the test substance.
Linearity
There was a linear relationship between the response and the test substance concentration in the range from 0.208 – 2.08 mg/100 mL (n = 6) for Kliba diet and 0.1916 – 1.916 mg/100 mL for SSNIFF diet (n = 6). The absolute bias of all back-calculated values per calibration level never exceeded the value of 15 % and the coefficient of determination (r^2) was > 0.99.
Accuracy
The recovery results are acceptable according to guidance document SANCO 3029/99 rev.4 and summarized in Table B.5.1.2.3-4.
Limit of quantification
The limit of quantification (LOQ) was assessed and confirmed at 50 mg/kg in Ground Kliba maintenance diet mouse/rat „GLP“ meal and as well in SSNIFF diet.
Precision (repeatability)
For all fortification levels the relative standard deviations (RSD) were below 20%.
Matrix Effects
No matrix effects tested in this study
Reproducibility
Reproducibility of the method was not determined within this validation study.

Conclusion
It could be demonstrated that the method *“Kliba diet”* fulfilled the requirements and was therefore applicable to correctly determine residues of BAS 595 F in the vehicles Ground Kliba maintenance diet mouse/rat „GLP“ meal and Standard powder rodent diet (SSNIFF®).

Table B.5.1.2.3-4: Summary Table of the recoveries of triticonazole in diet studies

Matrix	Analyte	Fortification Level [mg/kg]	Number of replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Kliba maintenance diet	BAS 595 F	50	5	103.6	8.4	101.9	6.8
		500	5	100.2	4.7		
SSNIFF diet		80	5	93.8	4.8	96.8	4.4
		750	5	97.6	4.8		
		5000	5	98.9	1.8		

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 exposure studies were conducted with triticonazole. Consequently, such methods of analysis are not required.

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

Table B.5.1.2.5-1 Overview of risk assessment methods in respective residue studies

Annex Point for original study	DocID of original study	Principle of the method	Method Name	Method included in CA 4.1.2
CA 6.2.1/1	2014/1090812	HPLC - radio	n.a.	no
CA 6.2.3/1	2014/1090814	HPLC - radio	n.a.	no
CA 6.5.1/1	2013/1135885	HPLC - radio	n.a.	no
CA 6.3.1/1	**2014/1043281**	**HPLC-MS/MS**	**L0106/01 (562/0)**	**CA 4.1.2/10**
CA 6.3.1/2	2014/1090813			

Reference:	**Validation of the analytical method 562/0: Determination of BAS 595 F (Reg.No. 4378513) in different plant matrices**
Author(s), year:	Weber S., 2006a
Report/Doc. number:	2006/1009635
Guideline(s):	EEC 96/46, SANCO/825/00 rev. 6 (20 June 2000), 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

Principle of the method
BAS 595F is extracted from different plant matrices with a mixture of methanol and water. An aliquot of the extract is centrifuged and partitioned against dichloromethane. The final determination of BAS 595 F is performed by HPLC-MS/MS. Two transitions m/z 318 → 70 and m/z 318 → 125.

Validation
Specificity
LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No significant interferences due to reagents or matrices.

Linearity
Good linearity was observed in the range of 0.025 to 2.5 ng/mL (n = 5) for triticonazole (external reference standard). Corr. coefficient >0.999. Plot and equation are available.

Accuracy
The method proved to be suitable for analysis of Triticonazole in lettuce, bean, lemon, rape and corn (grain, straw and forage). In all matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given in Table B.5.1.2.5-2.

Limit of quantification
The limit of quantitation (LOQ) was determined to be 0.01 mg/kg for Triticonazole in all plant matrices.

Precision (repeatability)
For all commodities and fortification levels the relative standard deviations (RSD) were below 20%.

Validation is summarized in Table B.5.1.2.5-2.

Conclusion
The method is considered acceptable according to guidance document SANCO 3029/99 rev.4.

Table B.5.1.2.5-2 Validation data for the determination of Triticonazole

Reference	Sample Matrix	Test Substance	LOQ [mg/kg]	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	n
Weber S., 2006a	Lettuce	Triticonazole (m/z 318 →70)	0.01	0.01 0.1	80 85.9	4.7 2.2	5 5
		Triticonazole (m/z 318 →125)	0.01	0.01 0.1	88.9 87.1	17.1 3.1	5 5
	Bean	Triticonazole (m/z 318 →70)	0.01	0.01 0.1	89.7 91	2.8 1.9	5 5
		Triticonazole (m/z 318 →125)	0.01	0.01 0.1	95 92.9	9.4 1.6	5 5
	Lemon	Triticonazole (m/z 318 →70)	0.01	0.01 0.1	91.6 88.7	4.1 3.7	5 5
		Triticonazole (m/z 318 →125)	0.01	0.01 0.1	92.5 89.3	18.9 3.9	5 5
	Rape	Triticonazole (m/z 318 →70)	0.01	0.01 0.1	91.6 80.6	7.2 10.4	5 5
		Triticonazole (m/z 318 →125)	0.01	0.01 0.1	85.6 80.1	5.9 10.4	5 5
	Corn grain	Triticonazole (m/z 318 →70)	0.01	0.01 0.1	95.7 86.3	13.4 2.7	5 5
		Triticonazole (m/z 318 →125)	0.01	0.01 0.1	96.5 87.8	11.1 3.3	5 5
	Corn straw	Triticonazole (m/z 318 →70)	0.01	0.01 0.1	89.5 83.6	2.0 1.0	5 5
		Triticonazole (m/z 318 →125)	0.01	0.01 0.1	91.6 86.2	8.7 3.0	5 5
	Corn forage	Triticonazole (m/z 318 →70)	0.01	0.01 0.1	90.3 82.1	2.0 3.1	5 5
		Triticonazole (m/z 318 →125)	0.01	0.01 0.1	91.5 83.1	8.9 5.5	5 5

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

Table B.5.1.2.6-1 Overview of risk assessment methods in respective ecotoxicological studies

Annex Point for original study	DocID of original study	Principle of the method	Method Name	Method included in CA 4.1.2
Confirmatory Data Addendum (July 2009)	**2006/1026908**	**HPLC-UV**	***"Method Support Quails"***	**I. Method Support Quails**
Confirmatory Data Addendum (July 2009)	**2008/1023059**			
CA 8.1.1.3/1	**2011/1269059**			
Confirmatory Data Addendum (July 2009)	**2007/1016397**	**HPLC-MS/MS**	**562/0**	**II. CA 4.1.2/10**
Confirmatory Data Addendum (July 2009)	**2006/1015760**			
CA 8.2.1/3	**R000095**	**HPLC-UV**	***"Method Support Fresh-Seawater"***	**III. Method Support Fresh-Seawater**
CA 8.2.2.1/1	**B003479**			
CA 8.2.2.1/2	**2006/7007245**			
CA 8.2.2.1/3	**C044319**			
CA 8.2.4.1/1	**C044320**			
CA 8.2.4.2/1	**B004421**			
CA 8.2.4.2/2	**C019777**			
CA 8.2.5.1/1	**2006/7007209**			
CA 8.2.5.2/1	**2006/7007246**			
CA 8.2.6.1/1	**R012017**			
CA 8.2.6.2/1	**R012969**			
CA 8.2.6.2/2	**B004429**			
CA 8.2.2.2/1	**2008/1028361**	**HPLC-MS**	**APL0500/02 & 03**	**IV. CA 4.1.2/11**
CA 8.2.2.2/2	**2012/1079000**			
CA 8.2.1/1	**2006/1015993**			
CA 8.2.1/2	**2006/1018146**			
CA 8.2.1/4	**2014/1095638**			
CA 8.2.4.1/2	**2009/1075083**			
CA 8.2.6.1/3	**2009/1050280**			
CP 10.2.1/1	**2009/1072605**			
CP 10.2.1/2	**2009/1072606**			
CA 8.2.5.1/2	**2012/7003660**	**HPLC-UV**	***"Method Support Water Fleas"***	**V. Method Support Water Fleas**
CA 8.2.5.1/3	**R013169**	**HPLC-UV**	**ANL/154-97E**	**VI. Method Support ANL-154-97E**
CA 8.2.6.1/2	**2014/1083347**	**HPLC-UV**	***"Method Support Algal Growth"***	**VII. Method Support Algal Growth**
CP 10.3.1.3/1	**2014/1000024**	**HPLC-MS/MS**	***"Method Support Honeybee"***	**VIII. Method Support Honeybee**
CP 10.6.2/1	**2013/1003205**	**HPLC-UV**	***"Method Support Seeding Growth"***	**IX. Method Support Seeding Growth**
CP 10.2/2	2007/7001058	HPLC - radio	n.a.	no

I.
Method: **"**Method Support Quails"
References: Refer to Table B.5.1.2.6-1
Validation: In-study validation

Principle of the method
A ten gram (10 g) aliquot of each sample was weighed directly into Erlenmeyer flasks. The samples were extracted twice with 45 ml of acetonitrile/formic acid 0.5 % in doubly distilled water 4/1 (v/v) by shaking 30 min at ambient temperature and subsequent sonification (5 min). The combined extracts were filtered and diluted with extraction solvent resulting in a total volume of 100 ml. Aliquots of these extracts were used directly for HPLC analysis at 262 nm.

Validation
Specificity
The analysis of control samples shown no significant interfering substances at the retention time of triticonazole.
Linearity
The method produced linear calibration curves R^2>0.99.
The plot of detector response area for triticonazole demonstrated acceptable linearity over the range 1 to 250 mg/L.
Accuracy
The recovery results are acceptable according to guidance document SANCO 3029/99 rev.4.
Limit of quantification
The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. The method has a limit of quantification (LOQ) of 50 mg/kg.
Precision (repeatability)
For all fortification levels the relative standard deviations (RSD) were below 20%.
Matrix Effects
No matrix effects tested in this study
Stability of Working Solutions and extract stability
Stability of solutions and extracts were not tested in this study.
Reproducibility
Reproducibility of the method was not determined within this validation study.

Validation is summarized in Table B.5.1.2.6-2.

Conclusion
The method/validation is considered acceptable according to guidance document SANCO 3029/99 rev.4.
The method proved to be valid for the determination of triticonazole in feed items for duck and quails.

Table B.5.1.2.6-2: Summary Table of the recoveries of triticonazole in feeding items

Matrix	Analyte	LOQ	Forti-fication Level	Mean Recovery [%]	RSD	Overall Recovery [%]	RSD	n
		[mg/kg]	[mg/kg]		[%]		[%]	
Lab diet for Quail/duck	Triticonazole	50	50	97.4	6.2	96.8	4.2	4
			101	95.5	1.1			4
			250	100.2	3.5			4
			500	94.2	2.5			4

II.

Reference:	**Validation of the analytical method 562/0: Determination of BAS 595 F (Reg.No. 4378513) in different plant matrices**
Author(s), year:	Weber S., 2006a
Report/Doc. number:	2006/1009635
Guideline(s):	EEC 96/46, SANCO/825/00 rev. 6 (20 June 2000), 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

Principle of the method
BAS 595F is extracted from different plant matrices with a mixture of methanol and water. An aliquot of the extract is centrifuged and partitioned against dichloromethane. The final determination of BAS 595 F is performed by HPLC-MS/MS. Two transitions m/z 318 → 70 and m/z 318 → 125.

Validation
Specificity
LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No significant interferences due to reagents or matrices.

Linearity
Good linearity was observed in the range of 0.025 to 2.5 ng/mL (n = 5) for triticonazole (external reference standard). Corr. coefficient >0.999. Plot and equation are available.

Accuracy
The method proved to be suitable for analysis of Triticonazole in lettuce, bean, lemon, rape and corn (grain, straw and forage). In all matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given in Table B.5.1.2.6-3.

Limit of quantification
The limit of quantitation (LOQ) was determined to be 0.01 mg/kg for Triticonazole in all plant matrices.

Precision (repeatability)
For all fortification levels the relative standard deviations (RSD) were below 20%.

Validation is summarized in Table B.5.1.2.6-3.

Conclusion
The method is considered acceptable according to guidance document SANCO 3029/99 rev.4.

Table B.5.1.2.6-3 Validation data for the determination of Triticonazole

Reference	Sample Matrix	Test Substance	LOQ [mg/kg]	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	n
Weber S., 2006a	Lettuce	Triticonazole (m/z 318 →70)	0.01	0.01 0.1	80 85.9	4.7 2.2	5 5
		Triticonazole (m/z 318 →125)	0.01	0.01 0.1	88.9 87.1	17.1 3.1	5 5
	Bean	Triticonazole (m/z 318 →70)	0.01	0.01 0.1	89.7 91	2.8 1.9	5 5
		Triticonazole (m/z 318 →125)	0.01	0.01 0.1	95 92.9	9.4 1.6	5 5
	Lemon	Triticonazole (m/z 318 →70)	0.01	0.01 0.1	91.6 88.7	4.1 3.7	5 5
		Triticonazole (m/z 318 →125)	0.01	0.01 0.1	92.5 89.3	18.9 3.9	5 5
	Rape	Triticonazole (m/z 318 →70)	0.01	0.01 0.1	91.6 80.6	7.2 10.4	5 5
		Triticonazole (m/z 318 →125)	0.01	0.01 0.1	85.6 80.1	5.9 10.4	5 5
	Corn grain	Triticonazole (m/z 318 →70)	0.01	0.01 0.1	95.7 86.3	13.4 2.7	5 5
		Triticonazole (m/z 318 →125)	0.01	0.01 0.1	96.5 87.8	11.1 3.3	5 5
	Corn straw	Triticonazole (m/z 318 →70)	0.01	0.01 0.1	89.5 83.6	2.0 1.0	5 5
		Triticonazole (m/z 318 →125)	0.01	0.01 0.1	91.6 86.2	8.7 3.0	5 5
	Corn forage	Triticonazole (m/z 318 →70)	0.01	0.01 0.1	90.3 82.1	2.0 3.1	5 5
		Triticonazole (m/z 318 →125)	0.01	0.01 0.1	91.5 83.1	8.9 5.5	5 5

III.
Method: **"**Method Support Fresh-Seawater"
References: Refer to Table B.5.1.2.6-1
Validation: In-study validation

Principle of the method
Methodology was validated to quantify the amount of triticonazole (RPA 400727) present in freshwater (C044319, C044320, B003479, R012017, R012969, 2006/7007245, 2006/7007246), seawater (R000095, B004421, C019777, B004429, 2006/7007209). Samples containing the test substance were analyzed by automated injection on a high performance liquid chromatographic (HPLC) system equipped with ultraviolet (UV) detection. All method validation samples were extracted twice by liquid-liquid extraction with methylene chloride. After each addition of methylene chloride, the sample was shaken for approximately two minutes by hand and the phases allowed to separate. The methylene chloride extract was drained through funnels containing anhydrous sodium sulfate to remove residual water. The samples were brought to approximately 1 mL by rotary evaporation. The samples were then reduced to dryness with nitrogen. The residues were reconstituted in acetonitrile: reagent grade water (1:1). An aliquot from each sample was collected for analysis by high performance chromatography.

Validation
Recovery findings
The mean assay accuracy (defined as mean % recovery from nominal concentration) and the assay precision (RSD) are summarized in Table B.5.1.2.6-4.

Linearity
The method produced linear calibration curves.
The plot of detector response area for triticonazole demonstrated acceptable linearity over the various range of fortified samples tested. Each calibration was made to contain the samples to be measured.

Specificity
The analysis of control samples demonstrated that there were no significant interfering substances at the retention time of triticonazole.

Matrix Effects
No matrix effects tested in this study.

Limit of Quantification
The limit of quantification was defined independently in each study.

Precision (repeatability)
For all fortification levels the relative standard deviations (RSD) were below 20%.

Stability of Working Solutions and extract stability
Stability of solutions and extracts were not tested in this study.

Reproducibility
Reproducibility of the method was not determined within this validation study.

Conclusion
The method supporting these studies was proven to be valid to quantify the amount of triticonazole in fresh and seawater.

Table B.5.1.2.6-4 Overall summary table of triticonazole recoveries in fresh and seawater

Matrix	Analyte	Forti-fication Level [mg/L]	Number of replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Freshwater	Triticonazole	0.00987	3	99.2	2.0	102.3	4.3
		0.0792	3	99.8	1.1		
		0.396	2	106.0	-		
		0.5	3	101.5	2.7		
		1.25	3	110.3	5.0		
		1.98	3	97.4	1.7		
		7	3	106.0	0.0		
		9.87	3	102.0	1.0		
		15	3	104.0	0.0		
		79.2	3	99.3	0.5		
		396	2	104.5	-		
		1980	3	99.4	0.5		
Seawater	Triticonazole	0.0198	3	100.9	1.1	101.7	1.4
		0.198	3	101.3	0.6		
		0.248	3	101.7	0.6		
		0.99	3	103.7	2.4		
		2.48	3	101.3	0.6		
		4.95	3	101.3	0.6		

IV

Reference:	**Validation of analytical method APL0500/02: Determination of pesticides in water by HPLC/MS**
Author(s), year:	Obermann M., 2006a
Report/Doc. number:	2006/1024332
Guideline(s):	SANCO/825/00 rev. 7 (17 March 2004)
GLP:	Yes

Principle of the method

Analytical method APL0500/02 (based on BASF method no. APL0500/01) was validated for the analysis of several BASF pesticides in aqueous matrices by LC/MS, to support ecotoxicological studies for dose verification. The study was performed by BASF, Limburgerhof, Germany.

The method is based on dilution of the aqueous samples with acetonitrile/water and acidification with formic acid following by final determination by reversed phase HPLC with MS-detection (external calibration). After dissolving in, the samples are quantitated by HPLC-MS using the m/z 318. Analysis was accomplished using an YMC ProC18 column and an acetonitrile-pure water gradient with formic acid as modifier.

The multi-method was further developed for additional analytes and could therefore be found cited under the BASF method no. APL0500/03 too.

Validation

Specificity

HPLC/MS is highly specific for the analyte triticonazole (BAS 595 F). The identification and quantification is based on the selected ion monitoring of ESI MS molecular ion signal M318 characteristic for the analyte. Under the described conditions the method is specific for the determination of BAS 595 F in water.

As the method is only used for dose verification of known substances and known nominal concentrations, no additional confirmatory technique is necessary.

No significant matrix interferences were observed in the investigated blank water samples.

Linearity

The results proved good linearity ($r > 0.999$) of the detector response in the investigated concentration range of approximately 0.0005 mg/L to 0.13 mg/L ($n = 6$) calibration solutions in acetonitrile/water/formic acid.

Accuracy

The method proved to be suitable to determine BAS 595 F in water samples. Samples were fortified at concentrations of 0.001 mg/L (LOQ) and 0.1 mg/L (100xLOQ). The analyses yielded acceptable mean recoveries of 100% and 101%. The detailed results are given in Table B.5.1.2.6-5.

Limit of quantification

The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. The method has a limit of quantification (LOQ) of 0.001 mg/L.

Precision (repeatability)

For all fortification levels the relative standard deviations (RSD) were below 20%.

Validation is summarized in Table B.5.1.2.6-5.

Conclusion

The results of the analytical study proved that analytical method APL0500/02 is suitable for the determination of BAS 595 F in water. It could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, accuracy, repeatability, and limit of quantification.

Table B.5.1.2.6-5 Validation data for the determination of Triticonazole

Matrix	Analyte	LOQ [mg/L]	Fortification level [mg/L]	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]	n
Ft-Mix Water (Tap-Water)	triticonazole Reg.No.4378513	0.001	0.001	100	0.7	100	1.1	5
			0.1	101	1.0			5
AAP-Water			0.001	102	--	--	--	1
M4-Water			0.001	101	--	--	--	1
OECD-Water			0.001	101	--	--	--	1

V
Method: **"**Method Support Support Water Fleas"
Reference: Refer to Table B.5.1.2.6-1
Validation: In-study validation

Principle of the method
Methodology was validated to quantify the amount of triticonazole (BAS 595 F) present in recovery samples prepared in freshwater (reconstituted for hardness). All recovery samples were initially diluted with acetonitrile to a composition of 20:80 acetonitrile: water. The mid and high concentration recovery samples were further diluted into calibration standard range with 20:80 acetonitrile:purified reagent water. Recovery samples were analyzed by automated injection on a high performance liquid chromatographic system equipped with ultraviolet detection (LC/UV).

Validation

Recovery findings
This method was validated by fortification of freshwater (reconstituted for hardness) with BAS 595 F at concentrations of 0.00535, 0.535 and 10.7 mg/L. Recoveries averaged 110% with a limit of quantitation (LOQ) of 0.00244 mg/L. Defined limits for acceptance of quality control sample performance in subsequent studies were set at 80 to 120%.

Linearity
Calibration standards were prepared in 20:80 acetonitrile: water in the range from 0.1 to 1.0 mg/L (n = 5). The obtained linear regression had a regression coefficient $R^2 > 0.999$. Plot and equation of the graph are available.

Specificity
The analysis of control samples demonstrated that there were no significant interfering substances at the retention time of triticonazole.

Matrix Effects
No matrix effects tested in this study.

Limit of Quantification
The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. The method has a limit of quantification (LOQ) of 0.00535 mg/L.

Precision (repeatability)
For all fortification levels the relative standard deviations (RSD) were below 20%.

Stability of Working Solutions and extract stability
Stability of solutions and extracts were not tested in this study.

Reproducibility
Reproducibility of the method was not determined within this validation study.

Conclusion
The analytical method was found to be satisfactory in terms of specificity, linearity and accuracy for the determination of triticonazole in freshwater.

Table B.5.1.2.6-6 Validation data for the determination of Triticonazole

Matrix	Analyte	LOQ	Forti-fication Level	Mean Recovery [%]	RSD	Overall Recovery [%]	RSD	n
		[mg/L]	[mg/L]		[%]		[%]	
Freshwater	Triticonazole	0.00535	0.00535	109.3	2.1	110.4	3.1	3
			0.535	108.7	4.3			3
			10.7	113.3	1.0			3

VI
Method: **"**Method Support Support ANL-154-97E"
Reference: Refer to Table B.5.1.2.6-1
Validation: In-study validation

Principle of the method
Analytical method ANL/154-97E is hereby described for the determination of triticonazole concentrations in freshwater for ecotoxicology. Freshwater samples were either diluted with acetonitrile or concentrated using C18 Empore cartridges and acetonitrile elution.
The quantification was perfomed by High Perfomance Liquid Chromatography (HPLC) using ultraviolet detection at 254 nm and external standardization.

Validation

Recovery findings
The mean assay accuracy (defined as mean % recovery from nominal concentration) and the assay precision (RSD) are summarized in Table B.5.1.2.6-7.

Linearity
The detector response was found to be linear (r^2 > 0.99999) within the calibration range of 20 to 1000 µg/L (n = 5). Plot and equation of the graph are available.

Specificity
No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Matrix Effects
No matrix effects tested in this study.

Limit of Quantification
The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested and covered by the linearity range. Therefore, the method has a limit of quantification (LOQ) of 50µg/L.

Precision (repeatability)
For all fortification levels the relative standard deviations (RSD) were below 20%.

Stability of Working Solutions and extract stability
Stability of solutions and extracts were not tested in this study.

Reproducibility
Reproducibility of the method was not determined within this validation study.

Conclusion
The analytical method was sufficiently validated for determination of triticonazole in freshwater.

Table B.5.1.2.6-7 Validation data for the determination of Triticonazole

Matrix	Analyte	LOQ [µg/L]	Forti-fication Level [µg/L]	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]	n
Freshwater	Triticonazole	50	5	103.3	5.9	102.3	5.7	3
			50	101.7	6.1			6
			1000	105.3	7.7			3
			20000	99.3	2.1			3

VII
Method: **"**Method Support Support Algal Growth"
Reference: Refer to Table B.5.1.2.6-1
Validation: In-study validation

Principle of the method
Samples were diluted with acetonitrile by factor 2 directly after sampling. After thawing all samples were ultrasonificated for 1 min and finally centrifuged (10 min 13000 rpm) before analysis. The quantification was performed using HPLC-UV at 250 nm.

Validation

Recovery findings
The mean assay accuracy (defined as mean % recovery from nominal concentration) and the assay precision (RSD) are summarized in Table B.5.1.2.6-8.

Linearity
The calibration range was split in a low and a high calibration curve to obtain better linearization. Calibration Range: Low concentration range: 0.025 to 0.25 mg/L and High concentration range: 0.25 to 7.5 mg/L
Regression Coefficients: Low conc. range: r = 0.9994 and high conc. range: r = 1.0000.
Plots and equations of the graphs are available.

Specificity
No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Matrix Effects
No matrix effects tested in this study.

Limit of Quantification
The analytical method was validated at the limit of quantification 0.1 mg/L

Precision (repeatability)
For all fortification levels the relative standard deviations (RSD) were below 20%.

Stability of Working Solutions and extract stability
Stability of solutions and extracts were not tested in this study.

Reproducibility
Reproducibility of the method was not determined within this validation study.

Conclusion
The analytical method was sufficiently validated for determination of triticonazole in freshwater.

Table B.5.1.2.6-8 Validation data for the determination of Triticonazole

Matrix	Analyte	LOQ	Forti-fication Level	Mean Recovery [%]	RSD	Overall Recovery [%]	RSD	n
		[mg/L]	[mg/L]		[%]		[%]	
Testwater	Triticonazole	0.1	0.1	103.4	6.5	105.1	4.2	5
			1	103.8	1.7			5
			11	108.2	1.4			5

VIII
Method: **"**Method Support Support Honeybee"
Reference: Refer to Table B.5.1.2.6-1
Validation: In-study validation

Principle of the method
The purpose of the analytical phase of the study was the verification of the test stock solution's concentration of the active ingredient BAS 595 F (Triticonazole) in sugar solution (18% glucose, 18% fructose, 4% yeast (w/v) prepared in the biological phase of the study. The determination was conducted by an inhouse developed method using reversed phase-high performance liquid chromatography (HPLC) with UV-detection at 254 nm.

Validation

Recovery findings
The mean assay accuracy (defined as mean % recovery from nominal concentration) and the assay precision (RSD) are summarized in Table B.5.1.2.6-9.

Linearity
The correlation coefficient was >0.999. The calibration range was from 11.130 to 33.41 mg/L.
Plots and equations of the graphs are available.

Specificity
The specificity of the method was assured by the following method: UV spectra from 200 to 300 nm were continuously recorded by the diode-array detector. Spectra of the peaks were compared to those of the reference. Similar spectra with approximately equal absorption maxima, a constant chromatographic retention time and no interfering peaks were observed.
No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Matrix Effects
No matrix effects tested in this study.

Limit of Quantification
The limit of quantification (LOQ) was defined in the context of this study as the lowest successfully validated fortification level, i.e. 3489 mg/L of triticonazole.

Precision (repeatability)
For all fortification levels the relative standard deviations (RSD) were below 20%.

Stability of Working Solutions and extract stability
Stability of solutions and extracts were not tested in this study

Reproducibility
Reproducibility of the method was not determined within this validation study.

Conclusion
The analytical method was sufficiently validated for determination of triticonazole in sugar solution.

Table B.5.1.2.6-9 Validation data for the determination of Triticonazole

Matrix	Analyte	LOQ [mg/L]	Forti-fication Level [mg/L]	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]	n
Sugar solution	Triticonazole	3489	3489	106	0.0	100,7	5.6	5
			6959	95.4	0.3			5

IX
Method: **"**Method Support Support Seeding Growth"
Reference: Refer to Table B.5.1.2.6-1
Validation: In-study validation

Principle of the method
The scope of the method was to determine BAS 595 01 F (basis: detector response of its active substance, namely triticonazole) in application solutions. Prior to the chromatographic analysis the laboratory procedural recovery specimens were subsequently diluted using acetonitrile or acetonitrile followed by acetonitrile/water (1: 1; v/v). The prepared specimens/laboratory procedural recovery specimens were subjected to HPLC and UV-detection at 263 nm. The identity of BAS 595 01 F was proven by coincidence of the retention time of the formulated active substance peak with the retention time of the authentic analytical reference item peak (analytical standard of triticonazole).

Validation
Recovery findings
Refer to Table B.5.1.2.6_10
Linearity
The calibration curve ranged from 0.02006 to 1.254 mg/mL (n = 8). The correlation coefficient of the calibration was 0.99989. Plot of the graph and equation are available.
Specificity
The analysis of control samples demonstrated that there were no significant interfering substances at the retention time of triticonazole.
Matrix Effects
No matrix effects tested in this study.
Limit of Quantification
-
Precision (repeatability)
-
Stability of Working Solutions and extract stability
Stability of solutions and extracts were not tested in this study
Reproducibility
Reproducibility of the method was not determined within this validation study.

Conclusion
The validation is not according to guidance document SANCO 3029/99 rev.4 as for determination only one sample instead of five was used. Therefore, no LOQ can be stated.

Table B.5.1.2.6-10 Validation data for the determination of Triticonazole

Matrix	Analyte	Fortification Level [mg/kg]	Recovery [%]	Overall Recovery [%]	n
Tap water	Triticonazole	0.2468	109	101.5	1
		4.7575	94		1

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

B.5.2.1. Plant matrices

Reference:	**Development and validation of an analytical method for the determination of the Triticonazole in various crop types**
Author(s), year:	Stanislowski T., 2014c
Report/Doc. number:	2014/1031578
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	Yes

Reference:	**Independent laboratory validation for the determination of Triticonazole in various crop types**
Author(s), year:	Jarrett H., 2014a
Report/Doc. number:	2014/1031576
Guideline(s):	EU Regulation 544/2011 (10 June 2011) implementing Regulation No 1107/2009, EU Regulation 1107/2009 with Regulation 545/2011 (former Annex III), SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	Yes

Text in cursive is concerning the ILV if different to the primary method.

Principle of the method

Samples of tomato and orange (10 g) and samples of wheat grain and rape seed (5 g) are weighed into 50 mL screw-capped centrifuge vials. Acetonitrile (10 mL) is added to the samples and water (10 mL) is added to the wheat grain and rape seed samples only and the samples are shaken vigorously for 1 minute. Magnesium sulfate (4 g), sodium chloride (1 g), trisodium citrate dehydrate (1 g) and disodium hydrogen citrate sesquihydrate (0.5 g) are added to the samples and the samples are shaken vigorously for 1 minute then centrifuged at 3000 rpm for 5 minutes. The rape seed samples are transferred to a freezer overnight and then centrifuged for 1 minute at 4000 rpm. The raw extracts (6 mL) are then transferred to Dispersive SPE (dSPE) Clean Up Tube containing PSA (150 mg) and magnesium sulphate (900 mg). A spatula of C18 material (150 mg) is added to the rape seed samples only, and the samples are shaken for 30 seconds and centrifuged for 5 minutes at 3000 rpm. An aliquot of the extract (1 mL) is transferred to an autosampler vial and acidified with 5% formic acid in acetonitrile (10 µL) and vortex-mixed. Water containing 0.1% formic acid (1 mL) is added to the samples and the samples vortex-mixed. The orange, wheat grain and rape seed samples are filtered through regenerated cellulose syringe filters (0.45 µm) before analysis. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive ion mode, using a Phenomenex Luna C18 (2) column (50 x 2.0 mm, 5 µm) and gradient elution with mobile phases of methanol + 0.1% formic acid and water + 0.1% formic acid. Quantification is performed using external standards. The ion transition m/z 318 → 70 is used for quantitation and the ion transition for m/z 318 → 125 is used for confirmation.

Validation

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated using six (*seven)* matrices matched external standard solutions across the working range of 1.0 to 60 ng/mL (*0.5 to 53 ng/mL*) for wheat grain and rape seed and across the working range of 0.5 to 30 ng/mL (*0.8 to 69 ng/mL*) for tomato and orange. Graphs and equations are available. Correlation coefficients (r) are in all cases >0.999.

Accuracy

The recovery results for all analytes are acceptable according to guidance document SANCO 825/00 rev. 8.1.

Limit of quantification

The limit of quantitation (LOQ) was determined to be 0.01 mg/kg for Triticonazole in various crop types.

Precision (repeatability)

The results were in all cases <30% for 0.01 mg/kg and < 20% for 0.1 mg/kg.

Extraction efficiency:
No residues above LOQ (0.01 mg/kg) therefore no extraction efficiency is necessary according to guidance document SANCO 825/00 rev. 8.1.

Reproducibility (ILV)
An independent laboratory validation was conducted for method Stanislowski T., 2014c.

Validation is summarized in Table B.5.2.1-1.

Conclusion
The method is considered acceptable according to guidance document SANCO 825/00 rev. 8.1 and is suitable for monitoring purposes.

Table B.5.2.1-1: Summary of validation results –enforcement methods

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Stanislowski T., 2014c **enforcement method**	triticonazole	LC-MS/MS m/z 318 → 70 (quantifier)	Tomato	0.01	0.01	102	2	5
					0.1	95	2	5
			Orange	0.01	0.01	101	1	5
					0.1	101	2	5
			Wheat Grain	0.01	0.01	100	2	5
					0.1	97	1	5
			Rape Seed	0.01	0.01	95	5	5
					0.1	90	3	5
		LC-MS/MS m/z 318 → 125 (qualifier)	Tomato	0.01	0.01	99	3	5
					0.1	94	2	5
			Orange	0.01	0.01	102	3	5
					0.1	100	2	5
			Wheat Grain	0.01	0.01	96	2	5
					0.1	98	2	5
			Rape Seed	0.01	0.01	94	4	5
					0.1	89	4	5

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Jarrett H., 2014a **ILV**	triticonazole	LC-MS/MS m/z 318 → 70 (quantifier)	Tomato	0.01	0.01	108	1.6	5
					0.1	109	1.2	5
			Orange	0.01	0.01	108	2.7	5
					0.1	112	2.0	5
			Wheat Grain	0.01	0.01	101	2.9	5
					0.1	112	4.4	5
			Rape Seed	0.01	0.01	100	1.6	5
					0.1	102	2.0	5
		LC-MS/MS m/z 318 → 125 (qualifier)	Tomato	0.01	0.01	105	1.4	5
					0.1	109	1.8	5
			Orange	0.01	0.01	106	4.1	5
					0.1	113	2.0	5
			Wheat Grain	0.01	0.01	99	1.4	5
					0.1	113	5.6	5
			Rape Seed	0.01	0.01	99	3.0	5
					0.1	102	2.8	5

B.5.2.2. Animal matrices

Reference:	**Development and validation of an analytical method for the determination of the Triticonazole in foodstuffs of animal origin**
Author(s), year:	Stanislowski T., 2014d
Report/Doc. number:	2014/1031579
Guideline(s):	SANCO/825/00 rev. 8.1
GLP:	yes

Reference:	**Independent laboratory validation for the determination of Triticonazole in foodstuffs of animal origin**
Author(s), year:	Chambers J.G. et al., 2014a
Report/Doc. number:	2014/1031577
Guideline(s):	EU Regulation 544/2011 (10 June 2011) implementing Regulation No 1107/2009, EU Regulation 1107/2009 with Regulation 545/2011 (former Annex III), SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes

Text in cursive is concerning the ILV if different to the primary method.

Principle of the method

Samples of whole milk, eggs, bovine meat and liver (5.0 g) and samples of fat (2.5 g) are weighed into 50 mL screw-capped centrifuge vials. The fat sample is warmed/ melted in a water bath at 40°C. Water (milk: 5 mL, eggs, meat and liver: 6 mL) is added along with acetonitrile (10 mL) and the samples are shaken vigorously for 1 minute. Water (10 mL) is added to the fat sample along with acetonitrile (10 mL) and the samples are warmed in a water bath set at 40°C before being shaken vigorously for 1 minute. Magnesium sulfate (4 g), sodium chloride (1 g), trisodium citrate dehydrate (1 g) and disodium hydrogen citrate sesquihydrate (0.5 g) are added to the samples and the samples are shaken vigorously for 1 minute then centrifuged at 3000 rpm for 5 minutes. The egg and fat samples are transferred to a freezer for 4 hours and then centrifuged for 1 minute at 4000 rpm. The raw extract (6 mL) is transferred to Dispersive SPE (dSPE) Clean Up Tube containing PSA (150 mg) and magnesium sulphate (900 mg). A spatula of C18 material (150 mg) is added to the egg and fat samples only, and the samples are shaken for 30 seconds and centrifuged for 5 minutes at 3000 rpm. An aliquot of the extract (1.0 mL) is transferred to an autosampler vial and acidified with 5% formic acid in acetonitrile (10 µL). The milk, egg, meat and liver samples are diluted by a factor of 5 using acetonitrile/water (2/6, v/v), containing 0.1% formic acid. The fat samples are diluted by a factor of 2.5 using water containing 0.1% formic acid. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive ion mode, using a Phenomenex Luna C18 (2) column (50 x 2.0 mm, 5 µm particle size) and gradient elution with mobile phases of methanol + 0.1% formic acid and water + 0.1% formic acid. Quantification is performed using external standards. The ion transition m/z 318 → 70 is used for quantitation and the ion transition for m/z 318 → 125 is used for confirmation.

Validation

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated using six *(five)* matrices matched external standard solutions across the working range of 0.20 to 20.0 ng/mL. Graphs and equations are available. Correlation coefficients (r) are in all cases >0.99.

Accuracy

The recovery results for all analytes are acceptable according to guidance document SANCO 825/00 rev. 8.1.

Limit of quantification
The limit of quantitation (LOQ) was determined to be 0.01 mg/kg for Triticonazole in foodstuffs of animal origin.
Precision (repeatability)
The results were in all cases <30% for 0.01 mg/kg and < 20% for 0.1 mg/kg.
Extraction efficiency:
No residues above LOQ (0.01 mg/kg) therefore no extraction efficiency is necessary according to guidance document SANCO 825/00 rev. 8.1.
Reproducibility (ILV)
An independent laboratory validation was conducted for method.

Validation is summarized in Table B.5.2.2-1.

Conclusion
The method is considered acceptable according to guidance document SANCO 825/00 rev. 8.1 and is suitable for monitoring purposes.

Table B.5.2.2-1: Validation results animal matrices –enforcement methods

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Stanislowski T., 2014d **enforcement method**	triticonazole	LC-MS/MS m/z 318 → 70 (quantifier)	Bovine Meat	0.01	0.01	104	6	5
					0.1	90	7	5
			Bovine Liver	0.01	0.01	98	2	5
					0.1	88	9	5
			Whole Milk	0.01	0.01	92	13	5
					0.1	90	19	5
			Egg	0.01	0.01	96	4	5
					0.1	86	5	5
			Fat	0.01	0.01	91	6	5
					0.1	90	2	5
		LC-MS/MS m/z 318 → 125 (qualifier)	Bovine Meat	0.01	0.01	104	3	5
					0.1	90	8	5
			Bovine Liver	0.01	0.01	98	4	5
					0.1	88	9	5
			Whole Milk	0.01	0.01	89	15	5
					0.1	90	19	5
			Egg	0.01	0.01	97	3	5
					0.1	86	5	5
			Fat	0.01	0.01	89	6	5
					0.1	90	1	5

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Chambers J.G. et al., 2014a **ILV**	triticonazole	LC-MS/MS m/z 318 → 70 (quantifier)	Bovine Meat	0.01	0.01	105	4.0	7
					0.1	95	7.1	6
			Bovine Liver	0.01	0.01	91	5.2	6
					0.1	83	1.1	6
			Whole Milk	0.01	0.01	116	4.1	6
					0.1	95	3.8	6
			Eggs	0.01	0.01	113	4.9	6
					0.1	99	4.9	5
			Fat	0.01	0.01	97	2.1	6
					0.1	73	2.5	6
		LC-MS/MS m/z 318 → 125 (qualifier)	Bovine Meat	0.01	0.01	97	9.8	5
					0.1	95	7.9	5
			Bovine Liver	0.01	0.01	93	5.1	5
					0.1	83	1.3	5
			Whole Milk	0.01	0.01	116	4.3	5
					0.1	96	3.6	5
			Eggs	0.01	0.01	112	5.2	5
					0.1	99	4.4	5
			Fat	0.01	0.01	98	2.2	5
					0.1	73	2.3	5

B.5.2.3. Soil

Reference:	**Triticonazole Method validation for triticonazole, RPA406341 and RPA 404766 in soil**
Author(s), year:	Doran, A.M., McGuire G.M., Charles, E., 2001
Report/Doc. number:	B003339
Guideline(s):	-
GLP:	yes

Reference:	**Triticonazole: Validation of analytical method to determine residues of triticonazole and its metabolite (RPA406341) in soil**
Author(s), year:	Wright, D.R. 1999
Report/Doc. number:	R004193
Guideline(s):	--
GLP:	yes

Principle of the method

The analytical method (CLE 198/120-02R) has been validated for the determination of triticonazole, M595F002 (RPA 406341) and M595F001 (RPA 404766) in soil. Soil is extracted with acetone/ammonium hydroxide using sonication. Extracts are purified using soil solid phase extraction (SPE) applying a C18 phase. Samples are evaporated under a stream of nitrogen and are further analyzed by isocratic reversed phase LC-MS/MS detecting one transition. Triticonazole: m/z 318.1 → 125; M595F001 and M595F002: m/z 334.1 → 125.

HPLC column: Phenomenex Luna Hexyl-Phenyl (100 x 4.6mm, 5µm); Guard column: Kromasil C8 (10 x 4.6mm, 5µm); mobile phase: 0.2% formic acid (methanol/water 70:30 v/v)

Soil specification

Control samples of soil were obtained from Inveresk Research Project No.680045, "Triticonazole Field Soil Dissipation Study in Europe" C021420. The samples were taken from plot numbers 1 and 2 (a sandy silt loam and a silty loam).

Validation

Specificity

Only one transition is determined in study Doran, A.M., McGuire G.M., Charles, E., 2001.

However, study "Triticonazole: Validation of analytical method to determine residues of Triticonazole and its metabolite (RPA406341) in soil" D R Wright (1999) determines a second transition for triticonazole: m/z 318.1 → 70 and M595F002 (RPA406341): m/z 334.1 → 70 and can be taken for confirmation. Metabolite M595F001 (RPA 404766) was not covered by study D R Wright (1999). Both studies are reported and peer reviewed in the DAR 2003.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Good linearity was observed in the range of 0.50 ng/mL to 500 ng/mL (n=11) for the two mass transitions of triticonazole and its two metabolites. Graphs and equations are available. Correlation coefficients (r) are in all cases >0.996.

Accuracy

The recovery results for all analytes are acceptable according to guidance document SANCO 825/00 rev. 8.1.

Limit of quantification

The limit of quantitation (LOQ) was determined to be 0.002 mg/kg for each analyte.

Precision (repeatability)

For all fortification levels the relative standard deviations (RSD) were below 20%.

Validation is summarized in Table B.5.2.3-1.

Conclusion

The method/validation is considered acceptable according to guidance document SANCO 825/00 rev. 8.1 and is suitable for monitoring purposes for triticonazole and metabolite M595F002 (RPA406341).

Reference:	**Validation of the method of analysis for Triticonazole (RPA 400727) and its metabolites in soil and turf**
Author(s), year:	Bruns G.,Tauber R., 1998a
Report/Doc. number:	R012011
Guideline(s):	EPA 164-1
GLP:	yes

Principle of the method

Method MS 90.01 (modified) was developed for the determination of triticonazole and its metabolites M595F002 (RPA 406341) and M595F014 (RPA 406203) in soil and turf by LC-MS. Residues of triticonazole and its metabolites are extracted from soil or turf using a double extraction with an acetone/NH_4OH solution. An aliquot of the combined extracts are concentrated by rotary evaporation and the concentrated extract is cleaned-up on a C18 SPE cartridge. The eluent is concentrated and analyzed by gradient LC-MS and confirmed by LC-MS/MS.

Soil specification

Soil (0-18") (i.e. 0-0.45 m) from 3 sites: Washington, Carolina, and North Carolina. The North Carolina soil is of higher organic consistence.

Validation

Specificity

Residues of triticonazole and its metabolites M595F002 (RPA 406341) and M595F014 (RPA 406203) in soil and turf were confirmed by LC-MS/MS analysis. The analysis gave similar results for selected extracts as reported in the table below. Under the described conditions, the method is specific for the determination of triticonazole and its metabolites M595F002 (RPA 406341) and M595F014 (RPA 406203) in soil.

Linearity

Linearity (r^2 > 0.989) was observed in the range of 5 ng/mL to 100 ng/mL for the reported mass transitions of triticonazole and its two metabolites. Plots and equations of the graph are available.

Accuracy

The recovery results are summarized in Table B.5.2.3-1.

Limit of quantification

The limit of quantitation (LOQ) was determined to be 0.005 mg/kg for each analyte.

Precision (repeatability)

The results were in all cases <20.

Validation is summarized in Table B.5.2.3-1.

Conclusion

The method is according to guidance document SANCO 825/00 rev.8.1 and can be used as enforcement method for triticonazole and its metabolites M595F002 (RPA 406341) and M595F014 (RPA 406203) in soil.

Reference:	**Method validation for the determination of Triticonazole in soil**
Author(s), year:	Chambers J. et al., 2014c
Report/Doc. number:	2014/1036830
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010), EU Regulation 544/2011 (10 June 2011) implementing Regulation No 1107/2009, EU Regulation 1107/2009 with Regulation 545/2011 (former Annex III)
GLP:	yes

Principle of the method

Samples of soil (10 g) are weighed into plastic extraction bottles (125 mL) and acetone/water, 4/1, v/v (100 mL) is added. The samples are shaken on a mechanical wrist-action shaker for 1 hour then centrifuged at 4000 rpm for 2 minutes. The supernatant is decanted into a volumetric flask (200 mL). The soil sample is extracted again using acetone/water, 4/1, v/v (100 mL). After the sample has been shaken on a mechanical wrist-action shaker for 1 hour and centrifuged at 4000 rpm for 2 minutes, the supernatant is combined with the first extract in the volumetric flask and the final volume adjusted to 200 mL with acetone/water, 4:1, v/v. An aliquot (0.1 mL) is diluted with acetone/water, 1:1, v/v (0.9 mL). The samples are analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS), in positive ion mode, using a Phenomenex Luna C18(2) column (50 mm x 2.0 mm, 5 µm particle size) and gradient elution with mobile phases of water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid. Quantification is performed using external standards. The ion transition m/z 318 → 70 is used for quantitation and the ion transition m/z 318 → 125 is used for confirmation.

Soil specification

LUFA 2.2

Validation

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.
No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated using six matrix matched external standard solutions across the working range of 0.0510 ng/mL to 3.67 ng/mL. Correlation Coefficient (r) is for each transition >0.99. Graphs and equations are available.

Accuracy

The recovery results for all analytes are acceptable according to guidance document SANCO 825/00 rev. 8.1.

Limit of quantification

The limit of quantitation (LOQ) was determined to be 0.05 mg/kg.

Precision (repeatability)

For all fortification levels the relative standard deviations (RSD) were below 20%.

Validation is summarized in Table B.5.2.3-1.

Conclusion

The method is sufficiently validated according to guidance document SANCO 825/00 rev.8.1 and suitable for monitoring triticonazole.

Reference:	**Validation of Analytical Method L0353/01 for the Determination of BAS 595 F (Triticonazole) and its Metabolites M595F001, M595F002 and M595F014 in Soil by LC-MS/MS**
Author(s), year:	Obermann M., Langenbach S., 2016
Report/Doc. number:	2016/1190727
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4 OCSPP 850.6100 Environmental Chemistry Methods and Associated ILV
GLP:	yes

Method L0353/01 was validated for the determination of triticonazole and its metabolites M595F001, M595F002 and M595F014 in soil by LC-MS/MS with a limit of quantification (LOQ) of 0.002 mg kg-1. The principle of the existing analytical method, Aventis method no. 0051, BASF analytical method no. L0353/01 stayed unchanged, however, it was downscaled by a factor of 10 to reduce the weigh in, as well as the amount of solvents and other consumables.

Principle of the method

A 5 g soil samples is extracted by solid-liquid extraction using sonication and shaking with 0.1M ammonium hydroxide and acetone. After each extraction, the suspension is centrifuged and decanted over cotton wool into a graduated cylinder. Then, an aliquot of the combined extracts is evaporated until the aqueous phase remained and subsequent redissolved in water. A sample clean-up by solid-phase extraction using a C18-SPE column is followed. The residues are eluted with acetonitrile/methanol (95/5, v/v) from the C18-SPE column. Afterwards, the liquid phase is evaporated to nearly dryness using a nitrogen evaporator and the remaining liquid phase is redissolved in water and acetonitrile.

The samples are analyzed by LC-MS/MS, in positive ion mode, using a Phenomenex Luna Phenyl Hexyl column and a Phenomenex SecurityGuard C18 and gradient elution with mobile phases of water containing 0.2% formic acid and methanol containing 0.2% formic acid. The ion transitions m/z 318 → 70 (triticonazole and M595F014) and m/z 334 → 70 (M595F001 and M595F002) are used for quantification and the ion transitions m/z 318 → 125 (triticonazole and M595F014) and m/z 334 → 125 (M595F001 and M595F002) are used for confirmation.

Soil specification

LUFA 2.2

LUFA 2.4

Validation

Specificity

LC-MS/MS is a highly specific self-confirmatory technique. Under the described conditions the method is specific for the determination of triticonazole and its metabolites M595F001, M595F002 and M595F014 in soil. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of the analytes.

Due to the high selectivity and specificity of LC-MS/MS, monitoring two mass transitions, an additional confirmatory technique was not necessary.

Linearity

Linearity ($r > 0.999$) was observed in the range of 0.2 ng/mL to 20 ng/mL ($n = 7$) for triticonazole and and each analyzed mass transition. Graphs and equations are available.

Accuracy

The recovery results for all analytes are acceptable according to guidance document SANCO 825/00 rev. 8.1.

Limit of quantification

The limit of quantitation (LOQ) resulting from the lowest fortification level successfully tested is 0.002 mg/kg.

Precision (repeatability)
The relative standard deviations (RSD, %) for all fortification levels were below 10%.

Validation is summarized in Table B.5.2.3-1.

Conclusion
The method is sufficiently validated according to guidance document SANCO 825/00 rev.8.1 and suitable for monitoring triticonazole and its metabolites M595F001, M595F002 and M595F014.

Table B.5.2.3-1: Validation results in soil

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Doran, A.M., McGuire G.M., Charles, E., 2001	triticonazole	LC-MS/MS m/z 318.1 → 125	Soil, Type 1	0.002	0.002	82.4	4.2	5
					0.020	86.6	2.4	5
	M595F002 (RPA406341)	LC-MS/MS m/z 334.1 → 125		0.002	0.002	83.7	4.7	5
					0.020	89.3	3.6	5
	M595F001 (RPA404766)	LC-MS/MS m/z 318.1 → 125		0.002	0.002	82.9	4.3	5
					0.020	87.3	7.2	5
	triticonazole	LC-MS/MS m/z 318.1 → 125	Soil, Type 2	0.002	0.002	81.8	2.8	5
					0.020	89.8	1.8	5
	M595F002 (RPA406341)	LC-MS/MS m/z 334.1 → 125		0.002	0.002	86.7	4.7	5
					0.020	92.1	3.6	5
	M595F001 (RPA404766)	LC-MS/MS m/z 318.1 → 125		0.002	0.002	75.8	6.7	5
					0.020	88.3	3.3	5
Wright, 1999	triticonazole	LC-MS/MS m/z 318.1 → 70	Soil Maningtree (England)	0.002	0.002	94	8.7	3
					0.050	91	1.3	3
		LC-MS/MS m/z 318.1 → 70	Soil Goch (Germany)	0.002	0.002	87	6.5	3
					0.050	89	2.3	3
	M595F002 (RPA406341)	LC-MS/MS m/z 334.1 → 70	Soil Maningtree (England)	0.002	0.002	100	7.6	3
					0.050	96	1.0	3
		LC-MS/MS m/z 334.1 → 70	Soil Goch (Germany)	0.002	0.002	77	9.7	3
					0.050	89	2.3	3

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Bruns G.,Tauber R., 1998a	triticonazole	LC-MS/MS m/z 318.1 → 70	LUCAMA, NC soil	0.005	0.005	77	7.0	5
					0.025	88	11	5
	M595F002 (RPA406341)	LC-MS/MS m/z 334.1 → 70		0.005	0.005	91	9.9	5
					0.025	109	10	5
	M595F014 (RPA406203)	LC-MS/MS m/z 318.1 → 70		0.005	0.005	85	13	5
					0.025	81	12	5
	triticonazole	LC-MS/MS m/z 318.1 → 70	WATSONVILLE, CA soil	0.005	0.005	80	6.6	3
					0.050	77	14	3
	M595F002 (RPA406341)	LC-MS/MS m/z 334.1 → 70		0.005	0.005	105	7.7	3
					0.050	93	5.4	3
	M595F014 (RPA406203)	LC-MS/MS m/z 318.1 → 70		0.005	0.005	91	8.4	3
					0.050	80	15	3
	triticonazole	LC-MS/MS m/z 318.1 → 70	EPHARATA, WA soil	0.005	0.005	96	10	3
					0.025	84	17	3
	M595F002 (RPA406341)	LC-MS/MS m/z 334.1 → 70		0.005	0.005	120	8.8	3
					0.025	91	11	3
	M595F014 (RPA406203)	LC-MS/MS m/z 318.1 → 70		0.005	0.005	106	3.8	3
					0.025	76	14	3
Chambers J. et al., 2014c	triticonazole	LC-MS/MS m/z 318 → 70 (quantifier)	LUFA 2.2 soil	0.05	0.0541	102	6.1	5
					0.541	102	3.0	5
		LC-MS/MS m/z 318 → 125 (confirmation)		0.05	0.0541	98	4.5	5
					0.541	99	3.0	5

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Obermann M., Langenbach S., 2016 **enforcement method**	triticonazole	LC-MS/MS m/z 318 → 70	LUFA 2.4 soil	0.002	0.002	104	6.9	5
					0.02	101	7.8	5
		LC-MS/MS m/z 318 → 125		0.002	0.002	105	7.3	5
					0.02	100	7.8	5
	M595F001	LC-MS/MS m/z 334 → 70		0.002	0.002	106	2.5	5
					0.02	99	6.2	5
		LC-MS/MS m/z 334 → 125		0.002	0.002	107	2.2	5
					0.02	97	5.9	5
	M595F002	LC-MS/MS m/z 334 → 70		0.002	0.002	108	8.1	5
					0.02	101	6.3	5
		LC-MS/MS m/z 334 → 125		0.002	0.002	108	9.2	5
					0.02	99	6.6	5
	M595F014	LC-MS/MS m/z 318 → 70		0.002	0.002	94	7.5	5
					0.02	100	7.1	5
		LC-MS/MS m/z 318 → 125		0.002	0.002	100	8.3	5
					0.02	99	7.1	5
	triticonazole	LC-MS/MS m/z 318 → 70	LUFA 2.2 soil	0.002	0.002	103	3.7	5
					0.02	101	9.3	5
		LC-MS/MS m/z 318 → 125		0.002	0.002	102	3.4	5
					0.02	99	9.2	5
	M595F001	LC-MS/MS m/z 334 → 70		0.002	0.002	99	2.2	5
					0.02	94	8.4	5
		LC-MS/MS m/z 334 → 125		0.002	0.002	102	1.6	5
					0.02	94	8.3	5
	M595F002	LC-MS/MS m/z 334 → 70		0.002	0.002	98	2.3	5
					0.02	95	8.5	5
		LC-MS/MS m/z 334 → 125		0.002	0.002	98	4.4	5
					0.02	94	8.9	5
	M595F014	LC-MS/MS m/z 318 → 70		0.002	0.002	98	2.6	5
					0.02	100	8.8	5
		LC-MS/MS m/z 318 → 125		0.002	0.002	106	7.6	5
					0.02	99	9.2	5

B.5.2.4. Water

Reference:	**Determination of Triticonazole in drinking and in surface water**
Author(s), year:	Class T., 2014d
Report/Doc. number:	2014/1036831
Guideline(s):	SANCO/825/00 rev. 8.1
GLP:	yes

Reference:	**Report amendment No. 1 - Determination of Triticonazole in drinking and in surface water**
Author(s), year:	Class T., 2015b
Report/Doc. number:	2015/1173435
Guideline(s):	SANCO/825/00 rev. 8.1
GLP:	yes

Reference:	**Independent laboratory validation for the determination of Triticonazole in drinking water**
Author(s), year:	Chambers J. et al., 2014b
Report/Doc. number:	2014/1036832
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010), EU Regulation 1107/2009 with Regulation 544/2011, EU Regulation 1107/2009 with Regulation 545/2011
GLP:	yes

Reference:	**Battelle UK report amendment certification - Independent laboratory validation for the determination of Triticonazole in drinking water**
Author(s), year:	Chambers J., 2015a
Report/Doc. number:	2015/1177041
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010), EU Regulation 1107/2009 with Regulation 544/2011, EU Regulation 1107/2009 with Regulation 545/2011
GLP:	yes

Text in cursive is concerning the ILV if different to the primary method.

Principle of the method

Samples of acidified drinking and surface water (0.1% formic acid) are transferred to an autosampler vial and analysed by direct injection high performance liquid chromatography with tandem mass specific detection ((DI-) LC-MS/MS) in positive polarity mode, using a Phenomenex Aqua C18 column (50 x 2 mm, 5µm particle size) and gradient elution with mobile phases of water containing 0.1% formic acid and methanol containing 0.1% formic acid. Quantification is performed using external standards. The ion transition m/z 318 → 70 is used for quantification and the ion transition for 318 → 125 is used for confirmation.

Characteristics of water

Drinking water source	Tap water (Illertissen, Southern Germany)
Total hardness	2.32 mmol/L (calculated from Mg and Ca)
Deutsche Härtegrade	13 °d
TOC	1.0 mg/L
DOC	1.0 mg/L
pH	7.45
Silt content	< 0.1 mg/L

Surface water source	River Danube (Ulm, Southern Germany)
Total hardness	3.20 mmol/L (calculated from Mg and Ca)
Deutsche Härtegrade	17.9 °d
TOC	1.9 mg/L
DOC	1.7 mg/L
pH	8.16
Silt content	3.1 mg/L

Validation

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated using six matrices matched external standard solutions across the working range of 0.010 to 1.5 ng/mL *(0.00984 to 1.51 ng/mL)* and all transitions. Graphs and equations are available. Correlation coefficients (r) are in all cases >0.999.

Accuracy

The recovery results for all analytes are acceptable according to guidance document SANCO 825/00 rev. 8.1.

Limit of quantification

The limit of quantitation (LOQ) was determined to be 0.05 µg/L for Triticonazole in drinking and surface water.

Precision (repeatability)

For all fortification levels the relative standard deviations (RSD) were below 20%.

Reproducibility (ILV)

An independent laboratory validation was conducted.

Validation is summarized in Table B.5.2.4-1.

Conclusion

The method is considered acceptable according to guidance document SANCO 825/00 rev. 8.1 and is suitable for monitoring purposes.

Table B.5.2.4-1: Validation results in drinking and surface water

References	Analyte	Detection method	Matrix	LOQ [µg/L]	Fortification level [µg/L]	Mean recovery [%]	RSD [%]	n
Class T., 2014d Class T., 2015b **enforcement method**	triticonazole	LC-MS/MS m/z 318 → 70 (quantifier)	Drinking water	0.05	0.05	114	1.0	5
					0.5	111	0.4	5
		LC-MS/MS m/z 318 → 125 (confirmation)		0.05	0.05	113	2.1	5
					0.5	110	1.3	5
		LC-MS/MS m/z 318 → 70 (quantifier)	Surface water	0.05	0.05	113	1.7	5
					0.5	112	2.2	5
		LC-MS/MS m/z 318 → 125 (confirmation)		0.05	0.05	114	2.8	5
					0.5	109	1.7	5
Chambers J. et al., 2014b Chambers J., 2015a **ILV**	triticonazole	LC-MS/MS m/z 318 → 70 (quantifier)	Drinking water	0.05	0.0493	81	4.2	6
					0.493	94	2.5	6
		LC-MS/MS m/z 318 → 125 (confirmation)		0.05	0.0493	81	12.0	6
					0.493	92	3.2	6

B.5.2.5. Air

Reference:	**Development and validation of an analytical method for the determination of Triticonazole in air**
Author(s), year:	Stanislowski T., 2014b
Report/Doc. number:	2014/1036833
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes

Principle of the method

ORBO^{TM}-43 absorbent (XAD) tubes fortified with triticonazole are flushed with air (36°C, 94% relative humidity) at 1 mL/min for 6 hours. The analyte is extracted with ethyl acetate (3 mL) by ultra-sonicating the samples for approximately 3 minutes then transferring the extract to a graduated centrifuge vial. The extraction process is repeated a further two times and the extracts are combined in the graduated centrifuge vial and diluted to 10 mL with ethyl acetate. The samples are analysed by gas chromatography with tandem mass specific detection (GC-MS/MS) in positive ion mode, using an Agilent VF-5ms column (30 m x 0.25 mm x 0.25 µm) and an oven temperature program. Quantification is performed using external standards. The ion transition m/z 235 → 182 is used for quantitation and the ion transition for 235 → 217 is used for confirmation.

Validation

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated using six matrices matched external standard solutions across the working range of 50 to 3500 ng/mL and both transitions (n=8). Graphs and equations are available. Correlation coefficients (r) are >0.99.

Accuracy

The recovery results are acceptable according to guidance document SANCO 825/00 rev. 8.1.

Limit of quantification

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was determined to be 2.7 µg per adsorption tube for Triticonazole in air (corresponding to about 7.5 µg/m^3).

Precision (repeatability)

For all fortification levels the relative standard deviations (RSD) were below 20%.

Breakthrough

No significant breakthrough of triticonazole was observed on the back sections of the absorbent tubes.

Validation is summarized in Table B.5.2.5-1.

Conclusion

The method is considered acceptable according to guidance document SANCO 825/00 rev. 8.1 and is suitable for monitoring purposes.

Table B.5.2.5-1: Validation results in air

References	Analyte	Detection method	Matrix	LOQ [µg/m³]	Fortification level [µg/L]	Mean recovery [%]	RSD [%]	n
Stanislowski T., 2014b **enforcement method**	triticonazole	LC-MS/MS m/z 235 → 188 (quantifier)	Air	7.5	7.5	80	9	5
					75	89	11	5
		LC-MS/MS m/z 235 → 217 (confirmation)		7.5	7.5	80	9	5
					75	90	11	5

B.5.2.6. Methods for the analysis in body fluids and tissues for active substances and relevant metabolites

Reference:	**Validation of BASF analytical method L0345/01 for the determination of BAS 595 F (Triticonazole) in body fluids**
Author(s), year:	Richter S., 2016a
Report/Doc. number:	2016/1152029
Guideline(s):	SANCO/825/00 rev. 8.1 (16/11/2010), SANCO/3029/99 rev. 4 (11/07/00)
GLP:	yes

Principle of the method

The analytical method is derived from the QuEChERS multi-residue method. Homogenized specimens are extracted with acetonitrile. After addition of $MgSO_4$, NaCl and buffering citrate salts, the mixture is shaken intensively and centrifuged. For urine samples, an extract aliquot is diluted prior to LC-MS/MS analysis. For blood samples, an aliquot of the organic extract is cleaned-up by addition of PSA and $MgSO_4$. After shaking and centrifugation, an extract aliquot is diluted followed by LC-MS/MS analysis. Quantitation was achieved by gradient liquid chromatography using tandem mass spectrometric detection (LC-MS/MS), monitoring two mass transitions, m/z 318 ⇨ m/z 70 for quantitation and m/z 318 ⇨ m/z 125 for confirmation.
LC column: Thermo Betasil C18, 100 mm x 2.1 mm ID; 5.0 µm

Validation

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.
The method L0345/01 determines residues of BAS 595 F in body fluid matrices. The interferences/residues of the analyte measured at the retention time in the control samples were below 20 % of the limit of quantification (LOQ) for each matrix and each mass transition.

Linearity

Linear calibration curves in the range of 0.01 to 1.0 ng/mL (n = 7) were calculated and plotted by regression analysis. Correlation coefficients (r) were always ≥ 0.99.

Accuracy

The recovery results are acceptable according to guidance document SANCO 825/00 rev. 8.1.

Limit of quantification

LOQ is 0.01 mg/kg corresponding to a concentration in the extract of 0.050 ng/mL

Precision (repeatability)

For all fortification levels the relative standard deviations (RSD) were below 20%.

Matrix Effect

The matrix effect was tested for each matrix. No significant matrix effect was observed.

Stability of Working Solutions

BAS 595 F indicated sufficient stability (less than 13% difference) in stock / spike (acetonitrile) for 29 days as well as in calibration solutions (less than 6% difference) in FV when stored refrigerated in the dark.

Extract Stability

Final sample extracts in acetonitrile/water (2/8, v/v) + 0.1 % FA were re-injected after 8 days of storage under refrigerated conditions. No significant decrease (90.7 to 106 % of initial value) in recovery in the stored final extracts was observed when the results were evaluated with freshly prepared calibration solutions in matrix. Thus stability of final extracts is considered sufficiently proven for at least 8 days under refrigerated storage conditions. When selected raw extracts in acetonitrile were re-analyzed after 8 days of storage under refrigerated conditions no significant decrease in recovery in the stored raw extracts was observed (90.2 to 110 % of initial value).

Validation is summarized in Table B.5.2.6-1.

Conclusion

The method is considered acceptable according to guidance document SANCO 825/00 rev. 8.1 and is suitable for monitoring purposes.

Table B.5.2.6-1: Validation results in body fluids

References	Analyte	Detection method	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Richter S., 2016 **enforcement method**	triticonazole	LC-MS/MS m/z 318 → 70 (quantifier)	Urine	0.01	0.01	102	2.5	5
					0.1	100	1.8	5
		LC-MS/MS m/z 318 → 125 (confirmation)			0.01	93.5	5.7	5
					0.1	101	2.8	5
		LC-MS/MS m/z 318 → 70 (quantifier)	Blood	0.01	0.01	103	1.9	5
					0.1	106	1.2	5
		LC-MS/MS m/z 318 → 125 (confirmation)			0.01	95.5	3.5	5
					0.1	105	0.8	5

Overall conclusion B.5.2

Methods for post control and monitoring purposes

Matrix group / crop group	Analyte	LOQ	Methods		
			Primary method	**Confirmatory method**	**Independent lab validation**
Wheat grain = Dry commodity (high protein/high starch content)	Triticonazole	0.01 mg/kg	**Stanislowski T., 2014c** 2014/1031578 LC-MS/MS using two transitions	**Not necessary**	**Jarrett H., 2014a** 2014/1031576 LC-MS/MS using two transitions covering all crop groups
Tomato = Commodity with high water content		0.01 mg/kg			
Rape seed = Commodity with high oil content		0.01 mg/kg			
Orange fruit = Commodity with high acid content		0.01 mg/kg			
Bovine Meat	Triticonazole	0.01 mg/kg	**Stanislowski T., 2014d** 2014/1031579 LC-MS/MS using two transitions	**not necessary**	**Chambers J.G. et al., 2014a** 2014/1031577 LC-MS/MS using two transitions covering all matrices
Bovine Liver		0.01 mg/kg			
Whole Milk		0.01 mg/kg			
Eggs		0.01 mg/kg			
Fat		0.01 mg/kg			
Soil LUFA 2.2 LUFA 2.4	Triticonazole M595F001 M595F002 M595F014	0.002 mg/kg each	**Obermann M., Langenbach S., 2016** 2016/1190727	**not necessary**	**not necessary**
Water (drinking and surface)	Triticonazole	0.05 µg/L	**Class T., 2014d** 2014/1036831 **Class T., 2015b** 2015/1173435 LC-MS/MS using two transitions	**not necessary**	drinking water**: Chambers J. et al., 2014b** 2014/1036832 **Chambers J., 2015a** 2015/1177041 LC-MS/MS using two transitions
Air	Triticonazole	7.5 µg/m^3	**Stanislowski T., 2014b** LC-MS/MS using two transitions	**not necessary**	**not necessary**
Body fluids Urine, blood	Triticonazole	0.01 mg/kg	**Richter S., 2016** LC-MS/MS using two transitions	**not necessary**	**not necessary**
Body tissues	Triticonazole	0.01 mg/kg	refer to animal matrices above	refer to animal matrices above	**not necessary**

B.5.3. REFERENCES RELIED ON

Data Point	Author(s)	Year	Title Compagny 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 4.1.2	Guillet M., Simonin B.	1994a	Triticonazole - Analytical method for the determination of residues in cereals Rhone-Poulenc - Secteur Agro; Lyon; France R013117 Yes unpublished	N	Y	Not applicable	BCS	Yes
KCA 4.1.2	Muller M.-A	1996a	Triticonazole - Iprodione - Formulation EXP 80524C (FS) - Trials Italy 1994 - 1995 - Residues in winter barley (grain - straw) Rhone-Poulenc - Secteur Agro; Lyon; France C014712 Yes unpublished	N	Y	Not applicable	BASF	Yes
KCA 4.1.2	Kretschmer S.	2001a	Formulation EXP 80525D and EXP 81044A (FS) - North - United Kingdom - 1999-2000 - 4 trials (per crop) - Harvest study - Residues in winter wheat (soil, grain and straw), rape (soil and grain), potato (soil and tuber) - Following crop study PTRL Europe GmbH; Ulm; Germany Fed.Rep. C017298 Yes unpublished	N	Y	Not applicable	BASF	Yes
KCA 4.1.2	Kretschmer S.	2001b	Formulation EXP 80525D and EXP 81044A (FS) - North - United Kingdom - 1999-2000 - 4 trials (per crop) - Harvest study - Residues in winter wheat (soil, grain and straw), rape (soil and grain), potato (soil and tuber) - Following crop study PTRL Europe GmbH; Ulm; Germany Fed.Rep. C017299 Yes unpublished	N	Y	Not applicable	BASF	Yes
KCA 4.1.2	Class T.	1998a	Multi-residue enforcement method (DFG S19) for the determination of Triticonazole in peas and wheat PTRL Europe GmbH; Ulm; Germany Fed.Rep. R004100 Yes unpublished	N	Y	Not applicable	BCS	Yes
KCA 4.1.2	Le Gren I.	1994a	Triticonazole - Analytical method for the	N	Y	Not applicable	BCS	Yes

Data Point	Author(s)	Year	Title Compagny 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
			determination of residues in beef and poultry tissues, in milk and in eggs Rhone-Poulenc - Secteur Agro; Lyon; France R013119 Yes unpublished					
KCA 4.1.2	Le Gren I.	1995a	Triticonazole: Analytical method for the determination of residues in beef and poultry tissues, in milk and in eggs Rhone-Poulenc - Secteur Agro; Lyon; France R013148 Yes unpublished	N	Y	Not applicable	BCS	Yes
KCA 4.1.2	Kieken J.L., Claviere B.	1993a	Flurtamone - Triticonazole - Validation de la methode AR 96-93 modifiee pour les etudes de dissipation P92/028 et P92/029 Rhone-Poulenc - Secteur Agro; Lyon; France C015600 Yes unpublished	N	Y	Not applicable	BCS	Yes
KCA 4.1.2	Kieken J.L.	2001a	Analysis of Triticonazole (RPA 400727) and Flurtamone (RE40885) residues in soil and petri dish samples from field dissipation studies (P92/028 and P92/029) Aventis CropScience; Lyon; France C016752 Yes unpublished	N	Y	Not applicable	BCS	Yes
KCA 4.1.2	Wright D.R.	1999a	Triticonazole: Validation of analytical method to determine residues of Triticonazole and its metabolite (RPA406341) in soil Covance Laboratories; Harrogate North Yorkshire HG3 1PY; United Kingdom R004193 Yes unpublished	N	Y	Not applicable	BCS	Yes
KCA 4.1.2	Doran A.M. et al.	2001a	Triticonazole - Method validation for Triticonazole, RPA 406341 and RPA 404766 in soil Inveresk Research; Tranent East Lothian EH33 2NE; United Kingdom B003339 Yes	N	Y	Not applicable	BCS	Yes

Data Point	Author(s)	Year	Title Compagny 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
			unpublished					
KCA 4.1.2	Fuchsbichler G.	1999b	Validation of an analytical method for the determination of Triticonazole in surface water (river, pond) Bayerische Hauptversuchsanstalt fuer Landwirtschaft; Freising; Germany Fed.Rep. R005064 Yes unpublished	N	Y	Not applicable	BCS	Yes
KCA 4.1.2	Fuchsbichler G.	2001a	Validation of Aventis CropScience method study No. 99-141 for the determination of Triticonazole in drinking water Bayerische Hauptversuchsanstalt fuer Landwirtschaft; Freising; Germany Fed.Rep. B003504 Yes unpublished	N	Y	Not applicable	BCS	Yes
KCA 4.1.2	Maestracci M.M., Turier G.P.	1995a	Analytical method for the determination of Triticonazole in air Rhone-Poulenc - Secteur Agro; Lyon; France R013125 Yes unpublished	N	Y	Not applicable	BCS	Yes
KCA 4.1.2	Laporte F.	2001a	Determination of residues of Triticonazole in air: Use of GC/MS as confirmatory technique Aventis CropScience GmbH; Frankfurt/Main; Germany Fed.Rep. C014405 No unpublished	N	Y	Not applicable	BCS	Yes
KCA 4.1.2/1	Bruns G., Tauber R.	1998 b	Validation of the method of analysis for Triticonazole (RPA 400727) and its metabolites in soil and turf R012011 Enviro-Test Laboratories, Edmonton Alberta T6E 0P5, Canada yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No
KCA 4.1.2/2	Doran A.M. et al.	2001 a	Triticonazole - Method validation for Triticonazole, RPA 406341 and RPA 404766 in soil B003339 Inveresk Research, Tranent East Lothian EH33 2NE, United	No	Yes	New data for AIR3 renewal	BCS	No

Data Point	Author(s)	Year	Title Compagny 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
			Kingdom yes Unpublished					
KCA 4.1.2/3	Obermann M., Langenbach S.	2016 a	Validation of analytical method L0353/01 for the determination of BAS 595 F (Triticonazole) and its metabolites M595F001, M595F002 and M595F014 in soil by using LC-MS/MS 2016/1190727 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No
KCA 4.1.2/4	Beck I.-C.	2007 a	Development and validation of an analytical method for the determination of Triticonazole and its dihydroxy metabolites in drinking and in surface water, using LC/MS/MS 2007/1035039 PTRL Europe GmbH, Ulm, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No
KCA 4.1.2/5	Chambers J. et al.	2014 c	Method validation for the determination of Triticonazole in soil 2014/1036830 Battelle UK Ltd., Chelmsford Essex CM2 5LB, United Kingdom yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No
KCA 4.1.2/6	Class T.	2014 d	Determination of Triticonazole in drinking and in surface water 2014/1036831 PTRL Europe, Ulm, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No
KCA 4.1.2/7	Class T.	2015 b	Report amendment No. 1 - Determination of Triticonazole in drinking and in surface water 2015/1173435 PTRL Europe GmbH, Ulm, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No
KCA 4.1.2/8	Stanislowski T.	2014 b	Development and validation of an analytical method for the determination of Triticonazole in air 2014/1036833 PTRL Europe, Ulm, Germany Fed.Rep. yes	No	Yes	New data for AIR3 renewal	BASF	No

Data Point	Author(s)	Year	Title Compagny 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
			Unpublished					
KCA 4.1.2/9	Catchpole G., Hidding B.	2016 a	BAS 595 F (Triticonazole) - Validation of an alnalytical method for the analysis of BAS 595 F (Triticonazole) in ground Kliba maintenance diet mouse/rat GLP meal using HPLC-UV 2016/1270666 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No
KCA 4.1.2/10	Catchpole G., Hidding B.	2016 b	BAS 595 F - Homogeneity and concentration control analyses in standard powder rodent diet (SSNIFF) 2016/1279220 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No
KCA 4.1.2/11	Stanislowski T.	2014 c	Development and validation of an analytical method for the determination of the Triticonazole in various crop types 2014/1031578 PTRL Europe, Ulm, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No
KCA 4.1.2/12	Stanislowski T.	2014 d	Development and validation of an analytical method for the determination of the Triticonazole in foodstuffs of animal origin 2014/1031579 PTRL Europe, Ulm, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No
KCA 4.1.2/13	Spangler C., Taraschewski I.	2017 a	Validation of analytical method L0106/03 for the determination of BAS 595 F and M595F014 residues in plant matrices by LC-MS/MS 2016/1204847 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No
KCA 4.1.2/14	Weber S.	2006 a	Validation of the analytical method 562/0: Determination of BAS 595 F (Reg.No. 4378513) in different plant matrices	No	Yes	New data for AIR3 renewal	BASF	No

Data Point	Author(s)	Year	Title Compagny 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
			2006/1009635 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished					
KCA 4.1.2/15	Obermann M.	2006 a	Validation of analytical method APL0500/02: Determination of pesticides in water by HPLC/MS 2006/1024332 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No
KCA 4.2	Fuchsbichler G.	1999a	Independent laboratory validation of the multi residue method (DFG S19) for the analysis of Triticonazole in pea (pod, seed) and wheat (grain, straw) Bayerische Hauptversuchsanstalt fuer Landwirtschaft; Freising; Germany Fed.Rep. R005050 Yes unpublished	N	Y	Not applicable	BCS	Yes
KCA 4.2	Wouters G.A.J.M.	2000a	Final analytical report - Independent laboratory validation of Rhone-Poulenc analytical method AR 104-94 for the determination of Triticonazole in products of animal origin Analytico Medinet BV; Breda; Netherlands R012078 Yes unpublished	N	Y	Not applicable	BCS	Yes
KCA 4.2/1	Jarrett H.	2014 a	Independent laboratory validation for the determination of Triticonazole in various crop types 2014/1031576 Battelle UK Ltd., Chelmsford Essex CM2 5KB, United Kingdom yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No
KCA 4.2/2	Chambers J.G. et al.	2014 a	Independent laboratory validation for the determination of Triticonazole in foodstuffs of animal origin 2014/1031577 Battelle UK Ltd.,	No	Yes	New data for AIR3 renewal	BASF	No

Data Point	Author(s)	Year	Title Compagny 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
			Chelmsford Essex CM2 5LB, United Kingdom yes Unpublished					
KCA 4.2/3	Chambers J. et al.	2014 b	Independent laboratory validation for the determination of Triticonazole in drinking water 2014/1036832 Battelle UK Ltd., Chelmsford Essex CM2 5LB, United Kingdom yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No
KCA 4.2/4	Chambers J.	2015 a	Battelle UK report amendment certification - Independent laboratory validation for the determination of Triticonazole in drinking water 2015/1177041 Battelle UK Ltd., Chelmsford Essex CM2 5LB, United Kingdom yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No
KCA 4.2/5	Richter S., Djedovic S.	2016 a	Validation of BASF analytical method L0345/01 for the determination of BAS 595 F (Triticonazole) in body fluids 2016/1152029 PTRL Europe, Ulm, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF	No