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Fenoxaprop-P-ethyl

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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 the respective Volume 4 of the members of the task force.

B.5.1.2. Methods for risk assessment

The behaviour of AE F046360 in aquatic systems had been investigated in detail. The parent compound rapidly disappeared from the water and sediment phase, mainly by hydrolytic transformation to AE F088406 (fenoxaprop-P-acid). Further, a cleavage at the heterocyclic ether bond is observed to form conjugates of the benzoxazole part. The conjugates are subject to hydrolysis under acidic conditions into the AE F054014 (benzoxazolone) as the major portion and other components. The same degradation pathway was observed after the application of the parent compound to plants, resulting in the same degradation products.
Therefore, the relevant residue of AE F046360 in food of plant or animal origin, soil, water, air, and body fluids is defined as the sum of AE F046360 (fenoxaprop-P-ethyl), AE F088406 (fenoxaprop-P-acid) and AE F054014.
The tests were conducted partly with the racemic parent compound AE F033171. This compound was registered and marketed earlier than the pure R-enantiomer AE F046360. However, a testing of the racemic mixture of enantiomers includes the single enantiomer. Such tests are therefore also well able to describe the behaviour of the single isomer. With the S-isomer as a biologically significantly less active component, tests with the racemate had to be conducted generally at higher application rates, in most cases the double rate when compared with the maximum recommended rate for the single isomer in the field. Thus, at least half of the test substance applied consisted of the R-enantiomer AE F046360 in the corresponding test. There was no obvious difference in terms of degradability and behaviour under the conditions of the test for the racemic parent compound and its degradates in comparison to their corresponding single optical isomers.

Methods in support of **environmental fate studies** were conducted with radiolabelled material and therefore no summaries of these methods are required. There were no methods submitted in support of **efficacy studies** and **operator, worker, resident and bystander exposure studies**. Therefore, no summaries are provided for these two sections. Methods in support of **toxicology studies** and **ecotoxicology studies** are summarized together with the respective (eco) toxicology study in MCA section 5 and 8 or MCP section 7 and 10, respectively in this part. They are extracted from the respective sections by RMS. The evaluations are reported below. (B.5.1.2.1 supporting toxicological studies and B.5.1.2.2 ecotoxicological studies). An overview of the available risk assessment analytical methods that allow determination of these **residues in plants and food of animal origin** (B.5.1.2.3) .is given in Table B.5.1.2.3-1. An overview of the available risk assessment analytical methods in support of **physical and chemical properties tests** of AE F046360 and its metabolites is given in Table B.5.1.2.4-1.

Where methods have been previously evaluated as acceptable for the first EU evaluation of AE F046360, this is indicated (writing in grey typeface) and no summary is provided.

B.5.1.2.1. Methods to support toxicological studies

An analytical method to determine fenoxaprop-P-ethyl in rodent feed is submitted in this dossier. The information has already been provided within the evaluation process of the Annex I inclusion but re-evaluated to meet the current data requirements.

Reference:	**Repeated-dose oral toxicity: 28-day feeding study in rats Hoe 046360 technical Code: Hoe 046360 0H ZC96 0002**
Author(s), year:	[redacted] 1987
Report/Doc. number:	A36568 / M-118344-01-1
Guideline(s):	US EPA OPPTS Guideline No. 870.3050
GLP:	yes

The analytical method described in attachment 3 of the report of [redacted] 1987; M-118344-01-1 was developed to determine AE F046360 (HOE 046360) in rodent feed by HPLC-UV in support of this oral toxicity study to rats.

Principle of the method

Feed samples were extracted with water/methanol (1/1 v/v) and n-hexane and centrifuged. The organic layer was evaporated to dryness and redissolved in acetonitrile. This sample was injected into the HPLC-UV instrument and determined at 240 nm.
Column: Lichrosorb RP 18, 5 µm; 250 x 4 mm

Validation

Linearity

Analyte	**Range [mg/L]**	**Regression equation**	**Correlation coefficient r^2**	**n**
AE F046360	0.25 – 2.0	$y = 3.70*10^{-5}\ x - 4.75*10^{-3}$	1.00	4

Specificity
No residues of the substance were detected in the control samples.

Recovery (Accuracy) - Precision
Fortified samples were prepared by adding AE F046360 in acetonitrile to control feed. The following recoveries were determined:

Group 1:	control	
Group 2 (20 mg/L *n*=3):	recovery: 74.0%	RSD: 5.0%
Group 3 (80 mg/L, *n*=3):	recovery: 85.0%	RSD: 3.5%
Group 4 (320 mg/L *n*=3):	recovery: 93.2%	RSD: 4.1%
Group 5 (1280 mg/L *n*=2):	recovery: 86.0%	RSD: -
Group 6 (5120 mg/L *n*=3):	recovery: 86.4%	RSD: 1.4%

LOQ
20 mg/L

Conclusion
According to guidance document SANCO 3029/99 5 determinations at each fortification level are required. However, the results show that the assay method presented above is fit for purpose.

Further analytical methods are submitted in this dossier. The information has already been provided within the evaluation process of the Annex I inclusion but re-evaluated to meet the current data requirements.

Reference:	**Sub-chronic oral toxicity 13-week feeding study in rats Hoe 046360 technical Code: Hoe 046360 0H ZC96 0002**
Author(s), year:	[redacted]; 1987
Report/Doc. number:	A36566 / M-118342-01-1
Guideline(s):	US EPA OPPTS Guideline No. 870.3100
GLP:	yes

The analytical method described in attachment 3 of the report of [redacted]; 1987; M-118342-01-1 was developed to determine AE F046360 (HOE 046360) in rodent feed by HPLC-UV in support of this oral toxicity study to rats.

Principle of the method

Feed samples were extracted with water/methanol (1/1) and n-hexane and centrifuged. The organic layer was evaporated to dryness and redissolved in acetonitrile. This sample was injected into the HPLC-UV instrument and determined at 240 nm.
Column: Lichrosorb RP 18, 5 µm; 250 x 4 mm

Validation

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
AE F046360	0.25 – 5.0	$y = 2.42*10^{-5} x + 0.06$	1.00	5

Specificity
No residues of the substance were detected in the control samples.

Recovery (Accuracy) - Precision
Fortified samples were prepared by adding AE F046360 in acetonitrile to control feed. The following recoveries were determined:

Group 1:	control	
Group 2 (10 mg/L *n*=3):	recovery: 90.1%	RSD: 25.1%
Group 3 (80 mg/L, *n*=3):	recovery: 95.3%	RSD: 2.3%
Group 4 (640 mg/L *n*=3):	recovery: 96.3%	RSD: 8.7%

LOQ
10 mg/L

Conclusion
According to guidance document SANCO 3029/99 5 determinations at each fortification level are required. However, the results show that the assay method presented above is fit for purpose.

Further analytical methods are submitted in this dossier. The information has already been provided within the evaluation process of the Annex I inclusion but re-evaluated to meet the current data requirements.

Reference:	**Sub-chronic oral toxicity 13-week feeding study in mice Hoe 046360 technical Code: Hoe 046360 0H ZC96 0002**
Author(s), year:	[redacted] 1987;
Report/Doc. number:	A36567 / M-118343-01-1
Guideline(s):	US EPA OPPTS Guideline No. 870.3100
GLP:	yes

The analytical method described in attachment 3 of the report of [redacted]; 1987; M-118343-01-1 was developed to determine AE F046360 (HOE 046360) in rodent feed by HPLC-UV in support of this oral toxicity study to mice.

Principle of the method

Feed samples were extracted with water/methanol (1/1) and n-hexane and centrifuged. The organic layer was evaporated to dryness and redissolved in acetonitrile. This sample was injected into the HPLC-UV instrument and determined at 240 nm.
Column: Lichrosorb RP 18, 5 µm; 250 x 4 mm

Validation

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
AE F046360	0.25 – 2.0	$y = 1.933*10^{-5} x + 0.011$	1.00	4

Specificity
No residues of the substance were detected in the control samples.

Recovery (Accuracy) - Precision
Fortified samples were prepared by adding AE F046360 in acetonitrile to control feed. The following recoveries were determined:

Group 1:	control	
Group 2 (10 mg/L *n*=3):	recovery: 87.4% %	RSD: 4.5%
Group 3 (80 mg/L, *n*=3):	recovery: 89.9%	RSD: 7.2%
Group 4 (640 mg/L *n*=3):	recovery: 91.8%	RSD: 5.8%

LOQ
10 mg/L

Conclusion
According to guidance document SANCO 3029/99 5 determinations at each fortification level are required. However, the results show that the assay method presented above is fit for purpose.

Reference:	**Sub-chronic oral toxicity 13-week feeding study in beagle dogs Hoe 046360 technical Code: Hoe 046360 0H ZC96 0002**
Author(s), year:	[redacted] 1987
Report/Doc. number:	A36617 / M-118393-01-1
Guideline(s):	US EPA OPPTS Guideline No. 870.3150
GLP:	yes

The analytical method described in attachment 1 of the report of [redacted]; 1987; M-118393-01-1 was developed to determine AE F046360 (HOE 046360) in dog feed by HPLC-UV in support of this oral toxicity study to dogs.

Principle of the method

Feed samples were extracted with water/methanol (1/1) and n-hexane and centrifuged. The organic layer was evaporated to dryness and redissolved in acetonitrile. This sample was injected into the HPLC-UV instrument and determined at 240 nm.
Column: Lichrosorb RP 18, 5 µm; 250 x 4 mm

Validation

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
AE F046360	0.25 – 2.0	$y = 1.885*10^{-5} x + 0.024$	1.00	4

Specificity
No residues of the substance were detected in the control samples.

Recovery (Accuracy) - Precision
Fortified samples were prepared by adding AE F046360 in acetonitrile to control feed. The following recoveries were determined:

Group 1:	control	
Group 2 (80 mg/L *n*=3):	recovery: 97.2% %	RSD: 7.5%
Group 3 (400 mg/L, *n*=3):	recovery: 97.0%	RSD: 9.7%
Group 4 (2000 mg/L *n*=3):	recovery: 91.3%	RSD: 10.6%

LOQ
80 mg/L

Conclusion
According to guidance document SANCO 3029/99 5 determinations at each fortification level are required. However, the results show that the assay method presented above is fit for purpose.

An analytical method to determine fenoxaprop-P-ethyl in rodent feed is submitted in this dossier. The information has already been provided within the evaluation process of the Annex I inclusion but re-evaluated to meet current data requirements.

Reference:	**Multiple generation study in rats (report part I) on Hoe 033171 substance, technical grade Code: Hoe 033171 0H ZD97 0001**
Author(s), year:	[redacted]; 1987
Report/Doc. number:	A32781 / M-112616-02-1
Guideline(s):	--
GLP:	yes

The analytical method described in attachment 1 of the report of [redacted] 1986 ([redacted] 1987; M-112616-02-1) was developed to determine AE F046360 (HOE 033171) in rodent feed by HPLC-UV in support of this oral toxicity study to rats.

Principle of the method

Feed samples were extracted with water/methanol (1/1) and n-hexane and centrifuged. The organic layer was evaporated to dryness and redissolved in acetonitrile. This sample was injected into the HPLC-UV instrument and determined at 235 nm.
Column: Lichrosorb RP 18, 5 µm; 250 x 4 mm

Validation

Linearity

Analyte	Range [µg/mL]	Regression equation	Correlation coefficient r^2	n
AE F046360	0.25 – 2.0	$y = 1.03*10^{-4} x - 0.00265$	1.00	4

Specificity
No residues of the substance were detected in the control samples.

Recovery (Accuracy) - Precision
Fortified samples were prepared by adding AE F046360 in acetonitrile to control feed. The following recoveries were determined:

Group 1:	control	
Group 2 (10 mg/L *n*=7):	recovery: 93.0% %	RSD: 4.9%
Group 3 (80 mg/L, *n*=7):	recovery: 92.8%	RSD: 3.8%
Group 4 (640 mg/L *n*=7):	recovery: 93.2%	RSD: 4.0%

LOQ
10 mg/L

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**Fenoxaprop-P-ethyl - 28-day immunotoxicity study in the female rat by dietary administration**
Author(s), year:	[redacted] 2011
Report/Doc. number:	SA 10132 / M-404362-01-1
Guideline(s):	U.S.E.P.A., OPPTS Series 870, Health Effects Testing Guidelines, N° 870.7800 (August 1998)
GLP:	yes

The analytical method SA 10132 described in appendix J of the report of [redacted] 2011; M-404362-01-1 was developed to determine AE F046360 (HOE 046360) in ground rodent diet by HPLC-UV in support of this oral toxicity study to rats.

Principle of the method

The substance was extracted from ground rodent diet by simple homogenisation with acetonitrile. The quantification was performed by High Performance Liquid Chromatography (HPLC) using UV detection at 240 nm and external standardisation.

Validation

Limit of validation
The validation limit (VL) was determined to be 10 mg/kg.
The VL corresponds to the lowest AE F046360 concentration in ground rodent diet which was measured accurately.

Spike concentration [mg/kg] Diet mixes	Recovery [%] for method validation	% of nominal concentration
≤ 10	85 to 115	85 to 115
> 10	90 to 110	85 to 115

Linearity
The correlation between the injected amount of substance and the detector response was linear within the calibration range.

Specificity
Blank samples were prepared according to the experimental procedure. The measured concentration was below the VL.

Recovery (Accuracy) - Precision
To confirm method validation, samples were spiked during the study from 10 up to 1300 mg/kg.

Spike concentration [mg/kg]	Measured concentration [mg/kg]	Recovery rate [%]	n
10	10.2	102	1
1300	1327	102	1

Conclusion

The validation data presented are poor. No further information on calibration, no information on sample number of spiked diet mixes ≤ 10 and > 10 mg/kg (VL), a minimum of 5 replicate sample determinations is required according to guidance document SANCO 3029/99.

B.5.1.2.2. Methods to support of ecotoxicological studies

Analytical method 01311:

The method 01311 describes the determination of AE F046360 and its metabolite AE F088406 in test water from aquatic toxicity tests by HPLC-MS/MS in support of aquatic toxicity studies.
The method 01311 is used in support of the following studies:

- "Acute toxicity of fenoxaprop-P-ethyl to fish (*Pimephales promelas*) under flow-through conditions", [redacted] 2011; M-407842-01-2

- "Early-life stage toxicity of fenoxaprop-p-ethyl (techn.) to fish (*Pimephales promelas*)", Bomke, C., [redacted] 2011; M-403248-01-3

Reference:	**Method 01311 for the determination of fenoxaprop-P-ethyl and fenoxaprop-P in test water from aquatic toxicity tests by HPLC-MS/MS**
Author(s), year:	Braune, M.; 2011
Report/Doc. number:	01311 / M-403143-01-1
Guideline(s):	not specified
GLP:	no

Principle of the method

Water samples are added with 0.1 mL/L formic acid and are analysed by direct injection into an HPLC-MS/MS instrument. Identification and quantitative determination are done by means of electrospray MS/MS-detection in the positive mode with two MRM transitions.
Fenoxaprop-p-ethyl: m/z 362 → 244 (quantification) m/z 362 → 288 (confirmation)
Fenoxaprop-p: m/z 334 → 288 (quantification) m/z 334 → 244 (confirmation)
Column: Ascentis Express C18, Length 50 mm, i. d. 2.1 mm, 2.7 µm

Validation

Limit of quantitation
The limit of quantitation (LOQ) is 0.05 µg/L for both substances (quantitation and confirmatory ion)

Linearity

Analyte	Range [µg/L]	Regression equation	Correlation coefficient r^2	n
AE F046360	0.05 – 10.0	y = 34194 x + 173.46	≥0.999	6
AE F088406	0.05 – 10.0	y = 26560 x + 187.58	≥0.999	6

Precision
The repeatability of MS/MS detection of AE F046360and AE F088406 was determined based on 10 injections of a standard solution of 0.05 µg/L and 10 injections of a standard solution of 0.50 µg/L in test water.
The investigation for AE F046360 yielded a relative standard deviation (RSD) for the peak area of the quantitation ion of 4.0% (at 0.05 µg/L) and 8.0% (at 0.50 µg/L) and for the confirmatory ion of 4.3% and 7.7%, respectively. The relative standard deviation (RSD) for the retention time was ≤ 0.4% for both fortification levels and both MRM transitions.
The investigation for AE F088406 yielded a relative standard deviation (RSD) for the peak area of the quantitation ion of 3.0% (at 0.05 µg/L) and 4.5% (at 0.50 µg/L) and for the confirmatory ion of 4.5% and 5.2%, respectively. The relative standard deviation (RSD) for the retention time was ≤ 0.5% for both fortification levels and both MRM transitions.

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection with two MRM transitions. The first MRM transition of AE F046360 (m/z 362) is the quantitation ion with m/z 244 and the second MRM transition is the confirmatory ion with m/z 288. The first MRM transition of AE F088406 (m/z 334) is the quantitation ion with m/z 288 and the second MRM transition is the confirmatory ion with m/z 244.

The MS/MS detection of AE F046360 and AE F088406was not affected by the matrix. The peak areas of the quantitation and confirmatory ion in a test water sample containing 1 µg/L show no significant difference to the corresponding peak areas in deionized water.

Recovery (Accuracy) - Precision

As the water samples were analysed by direct injection into the HPLC-MS/MS system recovery data cannot be calculated as stated in the guidance document on pesticide residue analytical methods (SANCO/825/00 rev.8.1).

Method Validation of Fenoxaprop-P-ethyl in Test Water (Quantitation Ion)

Sample conc. [µg/L]	Peak Areas Single Values	Mean	RSD [%]
0.05	1942 1860 1786 1829 1972 1873 1893 1766 1982 1825	1873	4.0
0.5	19286 18736 16121 14852 17972 17759 18281 17879 16684 16056	17363	8.0

Method Validation of Fenoxaprop-P-ethyl in Test Water (Confirmatory Ion)

Sample conc. [µg/L]	Peak Areas Single Values	Mean	RSD [%]
0.05	15725 14889 14362 15195 15394 14077 15590 13813 14927 15114	14909	4.3
0.5	150225 149011 124179 121023 146383 144641 148556 144288 136862 130668	139585	7.7

Method Validation of Fenoxaprop-P in Test Water (Quantitation Ion)

Sample conc. [µg/L]	Peak Areas Single Values	Mean	RSD [%]
0.05	1444 1483 1513 1473 1511 1418 1555 1438 1466 1424	1473	3.0
0.5	13948 13861 12744 12478 13228 12682 13198 13429 12573 12232	13037	4.5

Method Validation of Fenoxaprop-P in Test Water (Confirmatory Ion)

Sample conc. [µg/L]	Peak Areas Single Values	Mean	RSD [%]
0.05	762 724 684 734 734 754 716 769 762 672	731	4.5
0.5	6835 6473 6328 5999 6264 6483 6406 6167 6137 5625	6272	5.2

Conclusion

The results show that the assay method presented above is fit for purpose.

Reference:	**BCS-AB16188: Acute toxicity to rainbow trout (Oncorhynchus mykiss) in a 96-hour semi static test**
Author(s), year:	[redacted] 2015
Report/Doc. number:	102031230 / M-538851-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) 1107/2009; Commission Regulation (EC) No 440/2008, Annex, Part C, C.1.: Acute Toxicity for Fish, Official Journal of the European Union, May 30, 2008; OECD Guideline for Testing of Chemicals, Section 2, No. 203, Fish, Acute Toxicity Test, adopted July 17, 1992; OECD Series on Testing and Assessment, No. 23, Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, December 15, 2000; SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
GLP:	yes

This method describes the determination of AE F040356 (BCS-AB16188) in test water from aquatic toxicity tests by HPLC-MS/MS in support of the acute toxicity study to rainbow trout.

Principle of the method

The water samples are analysed after appropriate dilution with test water if necessary by injection into an HPLC-MS/MS instrument using electrospray in the positive mode with two MRM transitions.
Fenoxaprop-p-ethyl: m/z 262 → 168 (quantification) m/z 262 → 140 (confirmation)
Column: Kinetex PFP 100 Å 50 x 2.1 mm, 2.6 µm

Validation

Limit of quantitation
The limit of quantitation (LOQ) for AE F040356 is 0.005 mg/L; corresponding to 0.0025 mg/L after dilution by factor 2.
Linearity
Two calibration ranges were used in order to cover the wide concentration range of 0.0005 to 0.25 mg/L

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
AE F040356	0.0005 – 0.25	y = 20622953 x + 23922	0.9996	10
	0.0005 – 0.10	y = 21498868 x + 2221	1.0	10

Precision and accuracy
The repeatability for AE F040356 was determined based on 5 injections of a standard solution of 0.005 mg/L, 0.02 mg/L and 25 mg/L in test water.

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
0.005 dilution factor 2	99	2	5
0.02 dilution factor 2	98	1	5
25 dilution factor 200	90	3	5

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection with two MRM transitions. The first MRM transition of AE F040356 (m/z 262.0) is the quantitation ion with m/z 168.0 and the second MRM transition is the confirmatory ion with m/z 140.1. No interference of total peak area for the target analyte was found.

Conclusion

The results show that the assay method presented above is fit for purpose.

Reference:	**BCS-CY11271: Toxicity to rainbow trout (Oncorhynchus mykiss) in a 96-hour semi static test - Revised final report No. 1 -**
Author(s), year:	[redacted] 2015
Report/Doc. number:	96931230 / M-542854-01-1
Guideline(s):	Commission Regulation (EC) No 440/2008, Annex, Part C, C.1.: Acute Toxicity for Fish, Official Journal of the European Union, May 30, 2008; OECD Guideline for Testing of Chemicals, Section 2, No. 203, Fish, Acute Toxicity Test, adopted July 17, 1992; Additionally the recommendations of the EPA Guideline 712-C-96-118: OPPTS 850.1075, Fish Acute Toxicity Test, Freshwater and Marine April 1996 will be taken into account; SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414; OECD Series on Testing and Assessment, No. 23, Guidance Document on Aquatic Toxicitiy Testing of Difficult Substances and Mixtures, December 15, 2000
GLP:	yes

This method describes the determination of BCS-CY11271 in test media by HPLC-MS/MS in support of an acute toxicity study to rainbow trout (*Oncorhynchus mykiss*).

Principle of the method

The samples are analysed after appropriate dilution with acetonitrile by injection into an HPLC-MS/MS instrument using electrospray in the positive mode with two MRM transitions.
Fenoxaprop-p-ethyl: m/z 316 → 122 (quantification) m/z 316 → 244 (confirmation)
Column: Kinetex C18 100Å

Validation

Limit of quantitation
The limit of quantitation (LOQ) for BCS-CY11271 is 0.04 mg/L after dilution by factor 2, corresponding to a fortification level of nominal 0.08 mg/L.
Linearity
Two calibration ranges were used in order to cover the concentration range of 0.01 to 0.4 mg/L

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
BCS-CY11271	0.01 – 0.4	y = 1782139 x + 18555	0.9977	8
	0.01 – 0.2	y = 1994334 x + 6608	0.9988	8

Precision and Accuracy
The repeatability for BCS-CY11271 was determined based on 5 injections of a standard solution of 0.08 mg/L and 3.0 mg/L in test media.

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
0.08 dilution factor 2	96	3	5
3.0 dilution factor 10	99	3	5

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection with two MRM transitions. The first MRM transition of BCS-CY11271 (316 amu) is the quantitation ion with 122 amu and the second MRM transition is the confirmatory ion with 244 amu.
No interference of total peak area for the target analyte was found.

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**Acute toxicity of (R)-2-(4-hydroxyphenoxy) propionic acid (HPPA) to Daphnia magna in a static 48-hour immobilization test**
Author(s), year:	Pupp, A.; Wydra, V.; 2008
Report/Doc. number:	234 FPE / M-548131-01-1
Guideline(s):	Directive 92/69/EEC, C2, 1996; OECD No. 202, 2004
GLP:	yes

This method describes the determination of AE F096918 ((R)-2-(4-Hydroxyphenoxy) propionic acid) in test water from aquatic toxicity tests by HPLC-UV in support of the acute toxicity study to *Daphnia magna*.

Principle of the method

The water samples are analysed after appropriate dilution with test water if necessary by injection into an HPLC-UV instrument. Determination at 210 nm, column: US ES RP-18, 250*4 mm

Validation

Limit of quantitation
The limit of quantitation (LOQ) for AE F096918 is 5.0 mg/L.

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
AE F096918	2.0 – 40.0	y = 324407 x + 145894	0.9999	6

Recovery (Accuracy) - Precision
Test water was fortified with 5, 20 and 100 mg test item/L for method validation.
The mean recovery in the fortified samples was determined to be 103% (n= 12, RSD 2%).

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
5.0 dilution factor 1	101.5	1.3	5
20.0 dilution factor 1	102.5	1.1	5
100.0 dilution factor 4	105.8	2.9	5

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**BCS-AW69425: Acute toxicity to Daphnia magna in a static 48-hour immobilisation test**
Author(s), year:	Hengsberger, A.; Haertel, C.; 2015
Report/Doc. number:	102041220 / M-538685-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation 1107/2009 (Europe); Commission Regulation (EC) No 440/2008, Annex, Part C, C.2.: Daphnia sp. Acute Immobilisation Test, Official Journal of the European Union (EN), dated May 30, 2008; OECD Guideline for Testing of Chemicals No. 202: Daphnia sp., Acute Immobilisation Test adopted April 13, 2004; SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
GLP:	yes

This method describes the determination of 6-Hydroxybenzoxazolone (BCS-AW69425) in test water from aquatic toxicity tests by HPLC-MS/MS in support of the acute toxicity study to *Daphnia magna*.

Principle of the method

The water samples are analysed after appropriate dilution with test water if necessary by injection into an HPLC-MS/MS instrument using electrospray in the positive mode with two MRM transitions.
Quantifier ion: 152 to 96 amu, qualifier ion: 152 to 81 amu, column: Kinetex PFP 100Å, 50 * 2.1 mm

Validation

Limit of quantitation
The limit of quantitation (LOQ) for 6-Hydroxybenzoxazolone is 20 µg/L after dilution by factor 10, corresponding to a fortification level of nominal 0.2 mg/L.

Linearity

Analyte	Range [µg/L]	Regression equation	Correlation coefficient r^2	n
6-Hydroxybenzoxazolone	10 – 100	y = 684 x + 2053	0.9989	10

Recovery (Accuracy) - Precision
The repeatability for 6-hydroxybenzoxazolone was determined based on 5 injections of a standard solution of 0.2 mg/L and 14 mg/L in test water.

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
0.2 dilution factor 10	109	4	5
14 dilution factor 200	106	2	5

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection with two MRM transitions. The first MRM transition of 6-Hydroxybenzoxazolone (m/z 152) is the quantitation ion with m/z 96 and the second MRM transition is the confirmatory ion with m/z 81.
No interference of total peak area for the target analyte was found.

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**BCS-AB16188: Acute toxicity to Daphnia magna in a static 48-hour immobilisation test**
Author(s), year:	Boerschig, C.; Emnet, P.; 2015
Report/Doc. number:	102031220 / M-538878-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) 1107/2009; Commission Regulation (EC) No 440/2008, Annex, Part C, C.2.: Daphnia sp. Acute Immobilisation Test, Official Journal of the European Union, May 30, 2008; OECD Guideline for Testing of Chemicals No. 202, Daphnia sp., Acute Immobilisation Test adopted April 13, 2004; OECD Series on Testing and Assessment, No. 23, Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, December 15, 2000; SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
GLP:	yes

This method describes the determination of AE F040356 (BCS-AB16188) in test water from aquatic toxicity tests by HPLC-MS/MS in support of the acute toxicity study to *Daphnia magna*.

Principle of the method

The water samples are analysed after appropriate dilution with test water if necessary by injection into an HPLC-MS/MS instrument using electrospray in the positive mode with two MRM transitions.
Quantifier ion: 262 to 168 amu qualifier ion: 262 to 140 amu, column: Kinetex PFP 100Å, 50 * 2.1 mm, 2.6 µm

Validation

Limit of quantitation
The limit of quantitation (LOQ) for AE F040356 is 0.06 mg/L after dilution by factor 8, corresponding to a fortification level of nominal 0.5 mg/L.

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
AE F040356 (BCS-AB16188)	0.0025 – 0.2	y = 19621977 x + 4995	0.9996	9

Recovery (Accuracy) - Precision
The repeatability for AE F040356 was determined based on 5 injections of a standard solution of 0.5 mg/L and 20 mg/L in test water.

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
0.5 dilution factor 8	96	4	5
20 dilution factor 200	91	6	5

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection with two MRM transitions. The first MRM transition of AE F040356 (m/z 262.0) is the quantitation ion with m/z 168.0 and the second MRM transition is the confirmatory ion with m/z 140.1.
No interference of total peak area for the target analyte was found.

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**BCS-CY11271: Acute toxicity to Daphnia magna in a static 48-hour immobilisation test**
Author(s), year:	Kuhl, R.; Emnet, P.; 2016
Report/Doc. number:	EBFPN010 / M-547464-01-1
Guideline(s):	Commission Regulation (EC) No 440/2008, Annex, Part C, C.2.: Daphnia sp. Acute Immobilisation Test, Official Journal of the European Union (EN), dated May 30, 2008; OECD Guideline for Testing of Chemicals No. 202: Daphnia sp., Acute Immobilisation Test adopted April 13, 2004
GLP:	yes

This method describes the determination of BCS-CY11271 (Ethyl (*R*)-2-{4-[(6-hydroxy-1,3-benzoxazol-2-yl)oxy]phenoxy}propanoate) in test water from aquatic toxicity tests by HPLC-MS/MS in support of the acute toxicity study to *Daphnia magna*.

Principle of the method

The water samples are analysed after appropriate dilution with test water if necessary by injection into an HPLC-MS/MS instrument using electrospray in the positive mode with two MRM transitions.
Quantifier ion: 316 to 122 amu qualifier ion: 316 to 244 amu, column: Kinetex PFP 100Å, 50 * 2.1 mm, 2.6 µm

Validation

Limit of quantitation
The limit of quantitation (LOQ) for BCS-CY11271 is 0.05 mg/L (after a dilution by factor 2; corresponding to fortification level of nominal 0.1 mg/L).
Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
BCS-CY11271	0.1 – 0.5	y = 376571 x + 371	0.9999	8

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection.
No interference of total peak area for the target analyte was found.

Recovery (Accuracy) - Precision
Test water was fortified with 0.10 and 50 mg test item/L for method validation.
The mean recovery in the fortified samples was determined to be 97% (n= 10, RSD 5%).

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
0.10 dilution factor 2	94	5	5
50.0 dilution factor 200	100	2	5

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**96-hour flow-through acute toxicity test with the saltwater mysid (Mysidopsis bahia) Fenoxaprop-P-ethyl technical Code: Hoe 046360 00 ZC97 0002**
Author(s), year:	Murphy, D.; Peters, G. T.; 1991
Report/Doc. number:	A46598 / M-130627-01-1
Guideline(s):	-
GLP:	yes

The analytical method described in the analytical report in Appendix IV of the study Murphy, D.; Peters, G. T.; 1991; M-130627-01-1 was developed to determine AE F046360 (the *R*-ester, *S*-ester, *R*-acid and *S*-acid) in test water in support of this acute toxicity study to saltwater mysid.

Principle of the method

This method describes the determination of AE F046360 in test water by HPLC with diode array detection (DAD).
The method consists of a separatory funnel extraction of a known volume of aqueous sample by partitioning with three portions of methylene chloride. The three extraction volumes were combined and evaporated to dryness. The residue was reconstituted to an appropriate volume with 90:10 n-heptane: reagent alcohol. Afterwards the samples are separated with a Chiralcel OK column (250 x 4.6 mm) at an HPLC instrument and quantified at a wavelength of 240 nm.

Validation

Limit of quantitation
The limit of quantitation (LOQ) was determined to be 50 µg/L as total AE F046360.

Linearity
A good linear correlation between the injected amount of the analytes and the detector response was observed in the concentration ranges displayed below. 8 separate analyses of 5 concentrations were measured each (4 levels for L-acid).
Curves are reported

Analyte	Range [mg/L]	Correlation coefficient r	n
AE F046360 (*R*-ester)	2.88 – 61.0	0.999	5
S-ester	2.15 – 45.7	0.999	5
R-acid	2.76 – 55.1	0.999	5
S-acid	5.45 – 54.6	0.999	5

Specificity
The test matrix was demonstrated to be free of matrix interferences by the analysis of a matrix blank sample. All matrix spike analyses, except one, fell between 110% and 70% recovery.

Recovery (Accuracy) - Precision

AE F046360			
Sample concentration [µg]	**Mean [%]**	**RSD [%]**	**n**
50.3	98.4	-	2*
503	101	2.4	3

* One of the low level fortifications had anomalous results due to possible contamination with DMF and was not used.

Conclusion
According to guidance document SANCO 3029/99 5 determinations at each fortification level are required. However, the results show that the assay method presented above is fit for purpose.

Reference:	**The 28 day chronic toxicity to the mysid, Mysidopsis bahia, in a flow through system Fenoxaprop-P-ethyl technical 88.1 % w/w Code: AE F046360 00 1C97 0002**
Author(s), year:	Boeri, R. L.; Magazu, J. P.; Ward, T. J.; 1999
Report/Doc. number:	C002140 / M-183980-01-1
Guideline(s):	USEPA (=EPA): 72-4 (c)
GLP:	yes

The analytical method (modified version of method BM/01/97) described in the analytical report in Appendix 3 of the study Boeri, R. L.; Magazu, J. P.; Ward, T. J.; 1999; M-183980-01-1 was developed to determine AE F046360 and the degradation product AE F088406 in test water in support of this chronic toxicity study to mysid.

Principle of the method

This method describes the determination of AE F046360 and the degradation product AE F088406 in the same run in test water by HPLC-UV at 248 nm.
Column : LC-ABZ, 250 x 4.6 mm i.d., 5.0 urn particle size.

Validation

Limit of quantitation
The limit of quantitation (LOQ) was determined to be 0.005 mg/L.

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
AE F046360	0.0025 – 0.05	y = 248762 x + 123	0.9998	5
AE F088406	0.0025 – 0.05	y = 247732 x - 49	1.00	5

Recovery (Accuracy) - Precision

AE F088406			
Sample concentration [mg/L]	**Mean [%]**	**RSD [%]**	**n**
0.005	91	8	6
0.4	94	3	6

AE F046360			
Sample concentration [mg/L]	**Mean [%]**	**RSD [%]**	**n**
0.005	90	15	5*
0.4	105	9	6

One value was discarded as outlier by RMS and not considered for calculation

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**BCS-AW69425: Toxicity to Pseudokirchneriella subcapitata in an algal growth inhibition test**
Author(s), year:	Hengsberger, A.; Haertel, C.; 2015
Report/Doc. number:	102041210 / M-538955-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) 1107/2009 (Europe); OECD Guideline for Testing of Chemicals, Section 2, No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test adopted March 23, 2006, corrected July 28, 2011; Commission Regulation (EC) No 761/2009, Annex, Part C, C.3.: Freshwater Algae and Cyanobacteria, Growth Inhibition Test, Official Journal of the European Union (EN), dated August 24, 2009; SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
GLP:	yes

This method describes the determination of 6-Hydroxybenzoxazolone (BCS-AW69425) in test water from aquatic toxicity tests by HPLC-MS/MS in support of a toxicity study to the algae *Pseudokirchneriella subcapitata*.

Principle of the method

The water samples are analysed after appropriate dilution with acetonitrile and/ or acetonitrile/ test water (1/1) and centrifugation by injection into an HPLC-MS/MS instrument using electrospray in the positive mode with two MRM transitions.
Quantifier ion: 152 to 96 amu, qualifier ion: 152 to 81 amu
Column : Synergi 4µ Polar RP 80 A, 150 * 3 mm

Validation

Limit of quantitation
The limit of quantitation (LOQ) for 6-Hydroxybenzoxazolone is 30 µg/L after dilution by factor 20, corresponding to a fortification level of nominal 0.6 mg/L.

Linearity

Analyte	Range [µg/L]	Regression equation	Correlation coefficient r^2	n
6-Hydroxybenzoxazolone	5 – 100	y = 1598 x - 375	0.9997	9

Recovery (Accuracy) - Precision
The repeatability for 6-Hydroxybenzoxazolone was determined based on 5 injections of a standard solution of 0.6 mg/L and 140 mg/L in test water.

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
0.6 dilution factor 20	92	4	5
140 dilution factor 2000	93	3	5

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection with two MRM transitions. The first MRM transition of 6-Hydroxybenzoxazolone (m/z 152) is the quantitation ion with m/z 96 and the second MRM transition is the confirmatory ion with m/z 81.
No interference of total peak area for the target analyte was found.

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**BCS-AB16188: Toxicity to Pseudokirchneriella subcapitata in an algal growth inhibition test**
Author(s), year:	Boerschig, C.; Emnet, P.; 2015
Report/Doc. number:	102031210 / M-538959-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) 1107/2009; OECD Guideline for Testing of Chemicals, Section 2, No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test, adopted March 23, 2006, corrected July 28, 2011; Commission Regulation (EC) No 761/2009, Annex, Part C, C.3.: Freshwater Algae and Cyanobacteria, Growth Inhibition Test, Official Journal of the European Union (EN), dated August 24, 2009; OECD Series on Testing and Assessment, No. 23, Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, December 15, 2000; SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
GLP:	yes

This method describes the determination of AE F040356 (BCS-AB16188) in test water from aquatic toxicity tests by HPLC-MS/MS in support of a toxicity study to the algae *Pseudokirchneriella subcapitata*.

Principle of the method

The water samples are analysed after appropriate dilution with acetonitrile and/ or acetonitrile/ test water (1/1) and centrifugation by injection into an HPLC-MS/MS instrument using electrospray in the positive mode with two MRM transitions.
Quantifier ion: 262 to 168 amu, qualifier ion: 262 to 140 amu
Column : Kinetex PFP 100 Å 50 x 2.1 mm, 2.6 µm

Validation

Limit of quantitation
The limit of quantitation (LOQ) for AE F040356 is 50 µg/L after dilution by factor 2, corresponding to a fortification level of nominal 100 µg/L.

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
AE F040356	0.01 – 0.2	y = 9238022 x - 5166	0.9999	7

Recovery (Accuracy) - Precision
The repeatability for AE F040356 was determined based on 5 injections of a standard solution of 0.1 mg/L and 10 mg/L in test water.

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
0.1 dilution factor 2	104	2	5
10 dilution factor 80	103	1	5

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection with two MRM transitions. The first MRM transition of AE F040356 (m/z 262.0) is the quantitation ion with m/z 168.0 and the second MRM transition is the confirmatory ion with m/z 140.1.
No interference of total peak area for the target analyte was found.

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**Toxicity of fenoxaprop-p-ethyl, technical to *Pseudokirchneriella subcapitata* in an algal growth inhibition test**
Author(s), year:	Meister-Werner, A.; Wydra, V.; 2009
Report/Doc. number:	272 FPE / M-548267-01-1
Guideline(s):	OECD No. 201, 2006
GLP:	yes

This method describes the determination of AE F046360 (fenoxaprop-P-ethyl) and the metabolite AE F088406 (FOP-acid) in test water from aquatic toxicity tests by HPLC-UV in support of the toxicity study to algae (*Pseudokirchneriella subcapitata*).

Principle of the method

The water samples are analysed after adjustment to pH 5, dilution with acetonitrile and filtration by injection into an HPLC-UV instrument. Detection at 210 nm, column: US ES RP 18, 250 * 4 mm, 5µm.

Validation

Limit of quantitation
The limit of quantitation (LOQ) is 0.05mg/L after dilution by factor 2, corresponding to a fortification level of nominal 0.1 mg/L for both analytes.

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
AE F046360	0.05 – 1.0	y = 616652 x + 6630	0.9999	7
AE F088406	0.1 – 1.0	y = 667317 x - 2973	0.9999	6

Recovery (Accuracy) - Precision

AE F046360 in test water			
Sample concentration [mg/L]	**Mean [%]**	**RSD [%]**	**n**
0.1 dilution factor 2	106.8	5.8	4
0.2 dilution factor 2	96.8	1.0	4
0.9 dilution factor 2	97.5	2.0	4

AE F088406 in test water			
Sample concentration [mg/L]	**Mean [%]**	**RSD [%]**	**n**
0.1 dilution factor 2	99.7	2.1	3*
0.2 dilution factor 2	97.5	3.0	4
0.9 dilution factor 2	97.8	3.4	4

* outlier, not considered for calculation

Conclusion
According to guidance document SANCO 3029/99 5 determinations at each fortification level are required. However, the results show that the assay method presented above is fit for purpose.

Reference:	BCS-AW69425: Toxicity to Pseudokirchneriella subcapitata in an algal growth inhibition test
Author(s), year:	Hengsberger, A.; Haertel, C.; 2015
Report/Doc. number:	102041210 / M-538955-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) 1107/2009 (Europe); OECD Guideline for Testing of Chemicals, Section 2, No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test adopted March 23, 2006, corrected July 28, 2011; Commission Regulation (EC) No 761/2009, Annex, Part C, C.3.: Freshwater Algae and Cyanobacteria, Growth Inhibition Test, Official Journal of the European Union (EN), dated August 24, 2009; SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
GLP:	yes

This method describes the determination of 6-Hydroxybenzoxazolone (BCS-AW69425) in test water from aquatic toxicity tests by HPLC-MS/MS in support of a toxicity study to the algae *Pseudokirchneriella subcapitata*.

Principle of the method

The water samples are analysed after appropriate dilution with acetonitrile and/ or acetonitrile/ test water (1/1) and centrifugation by injection into an HPLC-MS/MS instrument using electrospray in the positive mode with two MRM transitions.
Quantifier ion: 152 to 96 amu and qualifier ion: 152 to 81 amu
Column : Synergi 4µ Polar RP 80 A, 150 * 3 mm

Validation

Limit of quantitation
The limit of quantitation (LOQ) for 6-Hydroxybenzoxazolone is 30 µg/L after dilution by factor 20, corresponding to a fortification level of nominal 0.6 mg/L.

Linearity

Analyte	Range [µg/L]	Regression equation	Correlation coefficient r^2	n
6-Hydroxybenzoxazolone	5 – 100	y = 1598 x - 375	0.9997	9

Recovery (Accuracy) - Precision
The repeatability for 6-Hydroxybenzoxazolone was determined based on 5 injections of a standard solution of 0.6 mg/L and 140 mg/L in test water.

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
0.6 dilution factor 20	92	4	5
140 dilution factor 2000	93	3	5

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection with two MRM transitions. The first MRM transition of 6-Hydroxybenzoxazolone (m/z 152) is the quantitation ion with m/z 96 and the second MRM transition is the confirmatory ion with m/z 81.
No interference of total peak area for the target analyte was found.

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**BCS-AB16188: Toxicity to Pseudokirchneriella subcapitata in an algal growth inhibition test**
Author(s), year:	Boerschig, C.; Emnet, P.; 2015
Report/Doc. number:	102031210 / M-538959-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) 1107/2009; OECD Guideline for Testing of Chemicals, Section 2, No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test, adopted March 23, 2006, corrected July 28, 2011; Commission Regulation (EC) No 761/2009, Annex, Part C, C.3.: Freshwater Algae and Cyanobacteria, Growth Inhibition Test, Official Journal of the European Union (EN), dated August 24, 2009; OECD Series on Testing and Assessment, No. 23, Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, December 15, 2000; SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
GLP:	yes

This method describes the determination of AE F040356 (BCS-AB16188) in test water from aquatic toxicity tests by HPLC-MS/MS in support of a toxicity study to the algae *Pseudokirchneriella subcapitata*.

Principle of the method

The water samples are analysed after appropriate dilution with acetonitrile and/ or acetonitrile/ test water (1/1) and centrifugation by injection into an HPLC-MS/MS instrument using electrospray in the positive mode with two MRM transitions, m/z 262 → 168 (quantification) m/z 262 → 140 (confirmation)
Column: Kinetex PFP 100 Å 50 x 2.1 mm, 2.6 µm

Validation

Limit of quantitation
The limit of quantitation (LOQ) for AE F040356 is 50 µg/L after dilution by factor 2, corresponding to a fortification level of nominal 100 µg/L. The limit of detection (LOD) is 2 µg/L.

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
AE F046360	0.01 – 0.2	y = 9238022 x - 5166	0.9999	7

Accuracy - Precision
The repeatability for AE F040356 was determined based on 5 injections of a standard solution of 0.1 mg/L and 10 mg/L in test water.

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
0.1 dilution factor 2	104	2	5
10 dilution factor 80	103	1	5

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection with two MRM transitions. The first MRM transition of AE F040356 (m/z 262.0) is the quantitation ion with m/z 168.0 and the second MRM transition is the confirmatory ion with m/z 140.1.
No interference of total peak area for the target analyte was found.

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**BCS-CY11271: Toxicity to Pseudokirchneriella subcapitata in an algal growth inhibition test - Revised final report No. 1 -**
Author(s), year:	Kuhl, R.; Emnet, P.; 2015
Report/Doc. number:	96931210 / M-542853-01-1
Guideline(s):	OECD Guideline for Testing of Chemicals, Section 2, No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test, adopted March 23, 2006, corrected July 28, 2011; Commission Regulation (EC) No 761/2009, Annex, Part C, C.3.: Freshwater Algae and Cyanobacteria, Growth Inhibition Test, Official Journal of the European Union (EN), dated August 24, 2009; OECD Series on Testing and Assessment, No. 23, Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, December 15, 2000; SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
GLP:	yes

This method describes the determination of BCS-CY11271 in test media by HPLC-MS/MS in support of a toxicity study to *Pseudokirchneriella subcapitata*.

Principle of the method

The samples are analysed after appropriate dilution with acetonitrile by injection into an HPLC-MS/MS instrument using electrospray in the positive mode with two MRM transitions, quantifier ion: 316.0 to 122.0 amu, qualifier ion: 316.0 to 244.0 amu; column: Kinetex C18 100 Å, 50 * 2.1 mm, 2.6 µm

Validation

Limit of quantitation
The limit of quantitation (LOQ) for BCS-CY11271 is 0.05 mg/L after dilution by factor 2, corresponding to a fortification level of nominal 0.1 mg/L.

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
BCS-CY11271	0.005 – 0.75	y = 449850 x + 866	0.9999	8

Precision and Accuracy
The repeatability for BCS-CY11271 was determined based on 5 injections of a standard solution of 0.1 mg/L, 25.0 mg/L and 120.0 mg/L in test media.

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
0.1 dilution factor 2	81	1	5
25.0 dilution factor 80	92	6	3*
120 dilution factor 200	92	5	5

**2 outlier not used for evaluation*

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection with two MRM transitions. The first MRM transition of BCS-CY11271 (316 amu) is the quantitation ion with 122 amu and the second MRM transition is the confirmatory ion with 244 amu.
No interference of total peak area for the target analyte was found.

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**BCS-AC79872: Toxicity to Pseudokirchneriella subcapitata in an Algal Growth Inhibition Test**
Author(s), year:	Boersching, C., Emnet, P.; 2016
Report/Doc. number:	EBFP0001 / M-560630-01-1
Guideline(s):	OECD Guideline 201 (March 2006)
GLP:	yes

The analytical method described in the report of Boerschig, C.; Emnet, P.; 2016; M-560630-01-1, was developed to determine test item BCS-AC79872 (AE F054014) in test water by HPLC-UV in support of this growth inhibition test to *Pseudokirchneriella subcapitata*.

Principle of the method

The samples were shaken well and treated with ultrasound for 1 minute to obtain homogenous samples. The samples were already diluted with acetonitrile by factor 2 directly after sampling. The samples were centrifuged (13,000 rpm, 10 minutes) before analysis.

Fortified samples and analytical blank control samples were shaken well and diluted with acetonitrile by factor 2. The samples were centrifuged (13,000 rpm, 10 minutes) before analysis.

The samples were then injected into the HPLC-UV instrument for analysis at 232 nm. Column: Ultra Sep ESD Pharm RP 18, 125 * 4 mm, 3 µm. Quantification was done by an external standard method.

Validation

Limit of quantitation

The limit of quantitation (LOQ) for BCS-AC79872 (AE F054014) is 0.25 mg/L after dilution by factor 2, corresponding to a fortification level of nominal 0.5 mg/L.

Linearity

Analyte	**Range [mg/L]**	**Regression equation**	**Correlation coefficient r**	**n**
BCS-AC79872 (AE F054014)	0.1 – 40	y = 865577 × x + 27574	1.0000	8

Precision and Accuracy

Sample concentration [mg/L]	**Mean [%]**	**RSD [%]**	**n**
0.5 dilution factor 2	90	1	5
1.5 dilution factor 2	99	1	5
40 dilution factor 2	101	1	5

Specificity

No interference of total peak area for the target analyte was found.

Conclusion

The results show that the assay method presented above is fit for purpose.

Reference:	**Toxicity to Skeletonema costatum (Marine Algae) in a 96-hour algal growth inhibition test**
Author(s), year:	Seyfried, B.; 2008
Report/Doc. number:	248 FPE / M-548169-01-1
Guideline(s):	OECD No. 201, 2006; International Standard ISO 10253, 1995
GLP:	yes

The analytical method described in the analytical report in Appendix IV of the study Seyfried, B.; 2008; M-548169-01-1 was developed to determine AE F046360 (fenoxaprop-P-ethyl) and the metabolite AE F088406 (FOP-acid) in test water in support of this toxicity study to marine algae (*Skeletonema costatum*).

Principle of the method

This method describes the determination of AE F046360 and the metabolite AE F088406 in test water by HPLC with UV detection at 254 nm. Column: Phenomenex Luna CI8 (2); 150 mm x 4.6 mm; 3 µm, Pre-Column: Phenomenex CI8; 4 x 3 mm

Validation

Limit of quantitation

The limit of quantitation (LOQ) is per definition the lowest recovery level and therefore 0.194 mg/L for AE F046360 (pure) and 0.172 mg/L for AE F088406 (pure).

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
AE F046360	0.00499 – 9.98	y = 421973 x – 22.937	0.9991	8
AE F088406	0.0456 – 9.11	y = 420760 x + 712.71	0.9989	7

Specificity

The biological solvent control samples and analysed analytical blanks (test water) did not significantly affect the HPLC-chromatogram at the retention time of the analytes.

Recovery (Accuracy) - Precision

AE F046360 non-centrifuged			
Sample concentration [mg/L]	**Mean [%]**	**RSD [%]**	**n**
0.197	72	-	2
0.903	68	-	2
overall	70	4	4

AE F046360 centrifuged			
Sample concentration [mg/L]	**Mean [%]**	**RSD [%]**	**n**
0.197	67	-	2
0.903	67	-	2
overall	67	0	4

AE F088406 filtrated			
Sample concentration [mg/L]	**Mean [%]**	**RSD [%]**	**n**
0.172	101	-	2
0.810	99	-	2
overall	100	1	4

AE F088406 unfiltrated			
Sample concentration [mg/L]	**Mean [%]**	**RSD [%]**	**n**
0.172	100	-	2
0.810	99	-	2
overall	100	1	4

Conclusion
According to guidance document SANCO 3029/99 5 determinations at each fortification level are required. However, the results show that the assay method presented above is fit for purpose.

Reference:	**Toxicity of fenoxaprop-p-ethyl, technical to Navicula pelliculosa in an algal growth inhibition test**
Author(s), year:	eister-Werner, A.; Wydra, V.; 2008
Report/Doc. number:	269 FPE / M-548208-01-1
Guideline(s):	OECD No. 201, 2006
GLP:	yes

This method describes the determination of AE F046360 (fenoxaprop-P-ethyl) and the metabolite AE F088406 (FOP-acid) in test water from aquatic toxicity tests by HPLC-UV in support of the toxicity study to algae (*Navicula pelliculosa*).

Principle of the method

The water samples are analysed after adjustment to pH 4 to 5 and appropriate dilution with acetonitrile if necessary by injection into an HPLC-UV instrument. Detection at 210 nm, column: US ES RP 18, 250 * 4mm.

Validation

Limit of quantitation
The limit of quantitation (LOQ) is 0.03 mg/L after dilution by factor 2, corresponding to a fortification level of nominal 0.06 mg/L for both analytes.

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
AE F046360	0.02 – 0.5	y = 669219 x - 7312	0.9999	8
AE F088406	0.02 – 0.5	y = 809259 x - 4498	0.9978	5

Specificity
Chromatograms demonstrate the lack of interferences.

Recovery (Accuracy) - Precision

AE F046360 in test water			
Sample concentration [mg/L]	**Mean [%]**	**RSD [%]**	**n**
0.06 dilution factor 2	92	2	4
0.1 dilution factor 2	90	4	4
0.9 dilution factor 2	91	2	4

AE F088406 in test water			
Sample concentration [mg/L]	**Mean [%]**	**RSD [%]**	**n**
0.06 dilution factor 2	91	1	4
0.1 dilution factor 2	88	2	4
0.9 dilution factor 2	89	1	4

Conclusion
According to guidance document SANCO 3029/99 5 determinations at each fortification level are required. However, the results show that the assay method presented above is fit for purpose.

Reference:	**Toxicity of fenoxaprop-p-ethyl technical to Anabaena Flos-aquae in an algal growth inhibition test**
Author(s), year:	Pawlowski, S.; Wydra, V.; 2005
Report/Doc. number:	130 FPE / M-548058-01-1
Guideline(s):	GLP compliant study based on the directive 92/69/EEC, C.3, 1992; and the OECD 201, 1984 and OECD draft revised guideline 201, April 2004
GLP:	yes

This method describes the determination of AE F046360 (fenoxaprop-P-ethyl) in test water from aquatic toxicity tests by HPLC-UV in support of the toxicity study to algae (*Anabaena flos-aquae*).

Principle of the method

The water samples are analysed after appropriate dilution with acetonitrile / pure water (55% : 45%, pH3) if necessary by injection into an HPLC-UV instrument. Detection at 200 nm, column: Ultrasep ES Pharm RP 18e (125 * 3 mm)

Validation

Limit of quantitation
The limit of quantitation (LOQ) for AE F046360 is 0.2 mg/L after dilution by factor 2, corresponding to a fortification level of nominal 0.4 mg/L.

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
AE F046360	0.1 – 10.0	y = 142277 x + 1717	≥ 0.9998	9

Specificity
Chromatograms demonstrate the lack of interferences.

Recovery (Accuracy) - Precision

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
0.4 dilution factor 2	90	5	4
1.0 dilution factor 2	97	2	4
3.0 dilution factor 2	95	2	4

Conclusion
According to guidance document SANCO 3029/99 5 determinations at each fortification level are required. However, the results show that the assay method presented above is fit for purpose.

Reference:	**Toxicity of fenoxaprop-P-ethyl to the blue green algae Anabaena flos-aquae**
Author(s), year:	Banman, C. S.; Howerton, J. H.; Lam, C. V.; 2011
Report/Doc. number:	EBFPL010-1 / M-411455-02-1
Guideline(s):	FIFRA Guideline 123-2 (1982); OPPTS Guideline 850.5400 (1996 draft); OECD Guideline 201 (2006)
GLP:	yes

The analytical method in the study Banman, C. S.; Howerton, J. H.; Lam, C. V.; 2011; M-411455-02-1 was developed to determine AE F046360 (fenoxaprop-P-ethyl) and the metabolite AE F088406 (fenoxaprop-P) in test water in support of this toxicity study to the blue-green algae *Anabaena flos-aquae*.

Principle of the method

This method describes the determination of AE F046360 and the metabolite AE F088406 in test water by HPLC-MS/MS instrument using electrospray in the positive mode. The total concentration is determined by adding the measured AE F088406 concentration calculated in AE F046360 equivalent to the measured AE F046360 concentration. This method was validated by spiking dilution water with AE F046360 and AE F088406.
Mass transitions : Fenoxaprop-P m/z: 334.2→ 244.5 and for Fenoxaprop-P-ethyl m/z: 362.2→288.7
Column : Gemini 5µ C18, 50 x 2 mm

Validation

Limit of quantitation
The limit of quantitation (LOQ) was determined to be 8.0 µg/L for AE F046360 and 4.04 µg/L for AE F088406.

Linearity
A calibration curve was generated with each set of sample analysis by plotting the concentration of each standard solution versus the average response of standard peak areas. Separate curves are generated for AE F046360 and AE F088406.

Analyte	Range [µg/L]	Regression equation	Correlation coefficient r^2	n
AE F088406	1.61 – 202	y = 3213.4 x	0.9999	5
AE F046360	4.0 – 500	y = 293.37 x	0.9989	5

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection.

Recovery (Accuracy) – Precision

AE F088406 in test water			
Sample concentration [µg/L]	**Mean [%]**	**RSD [%]**	**n**
4.04	101	6	3
40.4	112	2	3
202	107	-	2
707	-	-	1

AE F046360 in test water			
Sample concentration [µg/L]	**Mean [%]**	**RSD [%]**	**n**
8.0	93	6	3
40.0	100	3	3
200	97	-	2
700	-	-	1

Conclusion
According to guidance document SANCO 3029/99 5 determinations at each fortification level are required. However, the results show that the assay method presented above is fit for purpose.

Reference:	**Toxicity of fenoxaprop-P-ethyl technical to the freshwater diatom Navicula pelliculosa**
Author(s), year:	Banman, C. S.; Howerton, J. H.; Lam, C. V.; 2011
Report/Doc. number:	EBFPL011 / M-411456-01-1
Guideline(s):	FIFRA Guideline 123-2 (1982); OPPTS Guideline 850.5400 (1996 draft); OECD Guideline 201 (2006)
GLP:	yes

The analytical method described in the analytical report in Appendix 5 of the study Banman, C. S.; Howerton, J. H.; Lam, C. V.; 2011; M-411456-01-1 was developed to determine AE F046360 and the metabolite AE F088406 in test water in support of this toxicity study to the freshwater diatom *Navicula pelliculosa*.

Principle of the method

This method describes the determination of AE F046360 and the metabolite AE F088406 in test water by HPLC-MS/MS instrument using electrospray in the positive mode. The total concentration is determined by adding the measured AE F088406 concentration calculated in AE F046360 equivalent to the measured AE F046360 concentration. This method was validated by spiking dilution water with AE F046360 and AE F088406.
Mass transitions : Fenoxaprop-P m/z: 334.2→ 244.5 and for Fenoxaprop-P-ethyl m/z: 362.2→288.7
Column : Gemini 5µ C18, 50 x 2 mm

Validation

Limit of quantitation
The limit of quantitation (LOQ) was determined to be 8.0 ppb for AE F046360 and 4.04 ppb for AE F088406.
Linearity
A calibration curve was generated with each set of sample analysis by plotting the concentration of each standard solution versus the average response of standard peak areas. Separate curves are generated for AE F046360 and AE F088406.

Analyte	Range [µg/L]	Regression equation	Correlation coefficient r^2	n
AE F088406	1.61 – 202	y = 3803.2 x	0.9993	5
AE F046360	4.0 – 500	y = 368.83 x	0.9952	5

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection.

Recovery (Accuracy) – Precision

AE F088406 in test water			
Sample concentration [µg/L]	**Mean [%]**	**RSD [%]**	**n**
4.04	101	6	3
40.4	112	2	3
202	107	-	2
707	-	-	1

AE F046360 in test water			
Sample concentration [µg/L]	**Mean [%]**	**RSD [%]**	**n**
8.0	93	6	3
40.0	100	3	3
200	97	-	2
700	-	-	1

Conclusion
According to guidance document SANCO 3029/99 5 determinations at each fortification level are required. However, the results show that the assay method presented above is fit for purpose.

Reference:	**Toxicity of fenoxaprop-p-ethyl technical to the aquatic plant Lemna Gibba in a semi static growth inhibition test**
Author(s), year:	Pawlowski, S.; 2005
Report/Doc. number:	134 FPE / M-548340-01-1
Guideline(s):	evised porposal for a new OECD Guideline 221; US EPA guideline 712-C-96-156: OPPTS 850.4400, 1996
GLP:	yes

This method describes the determination of AE F046360 (fenoxaprop-P-ethyl) in test water from aquatic toxicity tests by HPLC-UV in support of the toxicity study to algae (*Lemna gibba* G3).

Principle of the method

The water samples are analysed after dilution with acetonitrile/ pure water (55% : 45%, pH3) by injection into an HPLC-UV instrument. Detection at 200 nm, column: Ultrasep ES Pharm RP 18e (125 * 3 mm)

Validation

Limit of quantitation
The limit of quantitation (LOQ) for AE F046360 is 0.5 mg/L after dilution by factor 2, corresponding to a fortification level of nominal 1.0 mg/L.
Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
AE F046360	0.1 – 10.0	y = 148821 x + 1346	≥ 0.9998	≥ 8

Recovery (Accuracy) - Precision
A test water/ acetonitrile mixture was fortified with 1.0 and 3.0 mg test item/L for method validation.
The mean recovery in the fortified samples for AE F046360 was determined to be 94% (n= 16, SD 5%).

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
1.0 dilution factor 2	97	5	8
3.0 dilution factor 2	92	4	8

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**Toxicity of Fenoxaprop-P-ethyl technical to the aquatic macrophyte, Myriophyllum spicatum**
Author(s), year:	Banman, C.S.; Alexander, T. M.; Moore, S.; 2013
Report/Doc. number:	EBFPL025 / M-457254-01-1
Guideline(s):	Higher Tier Study based on OECD 221
GLP:	yes

The analytical method described in the analytical report in Appendix 2 of the study Banman, C.S.; Alexander, T. M.; Moore, S.; 2013; M-457254-01-1 was developed to determine AE F046360 and the metabolite AE F088406 in test water in support of this toxicity study to the aquatic macrophyte *Myriophyllum spicatum*.

Principle of the method

This method describes the determination of AE F046360 and the metabolite AE F088406 in test water by HPLC-MS/MS instrument using electrospray in the positive mode (AE F046360) and in the negative mode (AE F088406). The total concentration is determined by summation of measured AE F046360 and AE F088406 concentrations. This method was validated by spiking control hard water with fenoxaprop-P-ethyl and AE F088406.
Mass transitions : Fenoxaprop-P m/z: 332→ 260 and for Fenoxaprop-P-ethyl m/z: 362→288
Column : Phenomenex Gemini-NX 5 µm, C18, 50 x 2.0 mm

Validation

Limit of quantitation
The limit of quantitation (LOQ) was determined to be 10.0 µg/L.

Linearity
A calibration curve was generated with each set of sample analysis by plotting the concentration of each standard solution versus the average response of standard peak areas. Separate curves are generated for AE F046360 and AE F088406.

Analyte	**Range [µg/L]**	**Regression equation**	**Correlation coefficient r^2**	**n**
AE F088406	2.0-150	graph available	>0.99	7
AE F046360	2.0-150	graph available	>0.99	7

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection.

Recovery (Accuracy) - Precision

AE F088406 in test water			
Sample concentration [µg/L]	**Mean [%]**	**RSD [%]**	**n**
10	97	2	3
50	94	2	3
120	90	2	3

AE F046360 in test water			
Sample concentration [µg/L]	**Mean [%]**	**RSD [%]**	**n**
10	90	1	3
50	92	1	3
120	94	0	3

Conclusion
According to guidance document SANCO 3029/99 5 determinations at each fortification level are required. However, the results show that the assay method presented above is fit for purpose.

Reference:	**Fenoxaprop-P: Toxicity to the aquatic plant Lemna gibba in a static growth inhibition test**
Author(s), year:	Hengsberger, A.; Emnet, P.; 2015
Report/Doc. number:	96921240 / M-548444-01-1
Guideline(s):	Commission Regulation (EC) No 761/2009, Annex, Part C, C.26.: "Lemna sp. Growth Inhibition Test", Official Journal of the European Union (EN), dated August 24, 2009 OECD Guideline 221: "Lemna sp. Growth Inhibition Test", adopted March 23, 2006 SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
GLP:	yes

This method describes the determination of AE F088406 (fenoxaprop-P) in test water from aquatic toxicity tests by HPLC-UV in support of the toxicity study to algae (*Lemna gibba* G3).

Principle of the method

The water samples are analysed after dilution with test water/ acetonitrile (1/1, v/v) by injection into an HPLC-UV instrument. Detection at 220 nm, column: US ES D Pharm RP 18, 125 x 4 mm (3 µm)

Validation

Limit of quantitation
The limit of quantitation (LOQ) is 0.4 mg/L (after dilution by factor 2; corresponding to fortification level of nominal 0.8 mg/L).

Linearity
In order to cover the wide concentration range of 0.25 – 45 mg/L with high accuracy, two calibration curves were used.

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r	n
AE F088406	0.25 – 45	y = 798480 x - 37835	1.0000	8
	0.25 – 10	y= 776317 x + 26879	1.0000	5

Specificity
No significant (< 30%) interference of total peak area for the target analyte was found.

Recovery (Accuracy) - Precision
Test water was fortified with 0.8 and 120 mg/L for method validation.
The mean recovery in the fortified samples was determined to be 100% (n= 10, RSD 5%).

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
0.8 dilution factor 2	98	6	5
120 dilution factor 4	102	1	5

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**BCS-AB16188: Toxicity to the aquatic plant Lemna gibba in a semi-static growth inhibition test**
Author(s), year:	Boerschig, C.; Emnet, P.; 2015
Report/Doc. number:	102031240 / M-536554-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) 1107/2009; Commission Regulation (EC) No 761/2009, Annex, Part C, C.26.: Lemna sp.Growth Inhibition Test, Official Journal of the European Union (EN), dated August 24, 2009; OECD Guideline 221: Lemna sp. Growth Inhibition Test, adopted March 23, 2006; OECD Series on Testing and Assessment, No. 23, Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, December 15, 2000; SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
GLP:	yes

This method describes the determination of AE F040356 (BCS-AB 16188) in test water from aquatic toxicity tests by HPLC-MS/MS in support of a toxicity study to the aquatic plant *Lemna gibba*.

Principle of the method

The water samples are analysed after appropriate dilution with acetonitrile by injection into an HPLC-MS/MS instrument using electrospray in the positive mode with two MRM transitions.
Fenoxaprop-p-ethyl: m/z 262 → 168 (quantification) m/z 262 → 140 (confirmation)
Column: Kinetex PFP 100 Å 50 x 2.1 mm, 2.6 µm

Validation

Limit of quantitation
The limit of quantitation (LOQ) for AE F040356 is 10 µg/L after dilution by factor 2, corresponding to a fortification level of nominal 20 µg/L.

Linearity
In order to cover the wide concentration range of 0.005 – 1.750 mg/L with high accuracy, two calibration curves were used.

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r	n
AE F040356	0.005 – 0.250	y = 3495418 x - 1473	1.00	10
	0.005 – 1.750	y = 2880167 x + 55203	0.9991	

Recovery (Accuracy) - Precision

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
0.02 dilution factor 2	106	11	5
2 dilution factor 4	98	19	5

The repeatability for AE F040356 was determined based on 5 injections of a standard solution of 0.02 mg/L and 2 mg/L in test water.

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection with two MRM transitions. The first MRM transition of AE F040356 (m/z 262.0) is the quantitation ion with m/z 168.0 and the second MRM transition is the confirmatory ion with m/z 140.1.
No interference of total peak area for the target analyte was found.

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**BCS-CY11271: Toxicity to the aquatic plant Lemna gibba in a static growth inhibition test**
Author(s), year:	Kuhl, R.; Emnet, P.; 2015
Report/Doc. number:	EBFPN007 / M-553341-01-1
Guideline(s):	Commission Regulation (EC) No 761/2009, Annex, Part C, C.26.: "Lemna sp. Growth Inhibition Test", Official Journal of the European Union (EN), dated August 24, 2009; OECD Guideline 221: "Lemna sp. Growth Inhibition Test", adopted March 23, 2006; OECD Series on Testing and Assessment, No. 23, "Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures", December 15, 2000; SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
GLP:	yes

This method describes the determination of BCS-CY11271 in test water from aquatic toxicity tests by HPLC-MS/MS in support of this toxicity study to algae (*Lemna gibba* G3).

Principle of the method

The water samples are analysed after dilution with acetonitrile and if necessary with acetonitrile / AAP medium (1/1, v/v) by injection into an HPLC-MS/MS instrument. Quantifier ion: 316.0 to 122.0 amu, qualifier ion: 316.0 to 244.0 amu, column: Kinetex PFP 100Å, 50 * 2.1 mm, 2.6 µm.

Validation

Limit of quantitation
The limit of quantitation (LOQ) is 2.5 µg/L (after dilution by factor 2; corresponding to fortification level of nominal 5 µg/L).

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r	n
BCS-CY11271	0.0005 – 0.06	y = 3045847 x + 269	0.9999	8

Specificity
The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection.
No interference of total peak area for the target analyte was found.

Recovery (Accuracy) - Precision
Test water was fortified with 0.005 and 120 mg/L for method validation.
The mean recovery in the fortified samples was determined to be 95% (n= 10, RSD 4%).

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
0.005 dilution factor 2	96	5	5
120 dilution factor 2000	94	0	5

Conclusion
The results show that the assay method presented above is fit for purpose.

Reference:	**CHA 4960 (fenoxaprop P-ethyl 69 g/L EW): Assessment of Side-Effects on Honeybees (*Apis mellifera* L.) and Determination of residues in a Semi-Field Study in *Phacelia tanacetifolia* in Germany**
Author(s), year:	Rexer, H. U.; 2016
Report/Doc. number:	S15-00053 / M-556072-01-1
Guideline(s):	OECD Guidance Document Number 75 (2007) and current recommendations of the AG Bienenschutz (Pistorius, J. et al., 2012); OEPP/EPPO Guideline No. 170(4), 2010; Guideline 1607/VI/97 (rev. 2) to Directive 91/414/EEC and Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009
GLP:	

The method AGR/MOA/FNX-2 was developed for the determination of the residues of AE F046360 (fenoxaprop-P-ethyl) in flowers, old bee larvae, nectar and pollen using HPLC-MS/MS monitoring two ion transitions in support of a toxicity study to honeybees (*Apis mellifera* L.).

Principle of the method

For nectar and bee larvae, the principle of the method involved the extraction from samples by agitation with ultra-pure water and acetonitrile in presence of magnesium sulfate, sodium chloride, sodium citrate dibasic sesquihydrate and sodium citrate tribasic dehydrate. The extracts were then purified with a mixture of PSA, C18EC and magnesium sulphate and centrifuged before LC-MS/MS determination.
For flowers, the principle of the method involved the extraction from samples by agitation with ultra-pure water and acetonitrile in the presence of magnesium sulfate, sodium chloride, sodium citrate dibasic sesquihydrate, and sodium citrate tribasic dehydrate. The extracts were then centrifuged before LC-MS/MS determination.
For pollen, the principle of the method involved the extraction from samples by agitation with ultra-pure water and acetonitrile. The extracts were then centrifuged before LC-MS/MS determination.
Quantifier ion: m/z 361.8 → 287.7, qualifier ion: m/z 361.8 → 244.0; column: Hypurity Aquastar, 150 mm x 3 mm - 5 µm (Thermo Scientific).

Validation

Limit of quantitation
The limit of quantitation (LOQ) was set at 0.01 mg/kg for all matrices.

Linearity

Analyte	Matrix	Range [ng/mL]	Regression equation quantific. /qual.	Correlation coefficient r^2 all trans.	n
AE F046360	pollen	0.025 – 5.0	y = 73809 x + 899/ y = 14498 x + 449	>0.99	8
	nectar		y = 26467 x + 50/ y = 14426 x + 23		8
	flower		y = 19581 x + 625/ y = 9223 x + 114		8
	larvae		y = 30593 x – 89/ y = 17428 x		8

Specificity
The high selectivity of the method results from the HPLC separation in combination with MS/MS detection.
No significant interference (< 30% LOQ) was detected in any of the blank and unfortified specimens.
No significant matrix effects on the detection of AE F046360 in nectar, bee larvae and flower extracts were found (< ± 20 %) on the LC-MS/MS signal. Matrix effects on the detection of AE F046360 was found to be significant (≥ ± 20 %) in pollen extracts. However, matrix matched calibration standards were used for quantification for all matrices by default.
The AE F046360 stock solution at a target temperature of 4°C was determined to be stable at least 50 days.

Recovery (Accuracy) - Precision

The recovery rates and relative standard deviations (RSD) obtained from matrix fortified with AE F046360 were determined at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg. At each fortification level, the mean recovery for AE F046360 was in the range 70 – 110% and the relative standard deviation ≤ 20%. The overall mean was in the range 70 – 110% with relative standard deviation ≤ 20% for AE F046360.

Analyte	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F046360 (m/z 361.8 → 287.7)	pollen	0.01	0.01	77	14	5
			0.1	82	4	5
	nectar	0.01	0.01	95	5	5
			0.1	84	4	5
	flower	0.01	0.01	76	9	5
			0.1	91	6	5
	larvae	0.01	0.01	99	9	5
			0.1	83	4	4
AE F046360 (m/z 361.8 → 244.0)	pollen	0.01	0.01	93	17	5
			0.1	84	6	5
	nectar	0.01	0.01	102	14	5
			0.1	90	3	5
	flower	0.01	0.01	82	13	5
			0.1	89	9	5
	larvae	0.01	0.01	97	8	5
			0.1	84	5	4

Conclusion

The results show that the assay method presented above is fit for purpose.

Reference:	**Fenoxaprop-P-ethyl EW 69: Chronic oral toxicity test on the honey bee (*Apis mellifera* L.) in the laboratory**
Author(s), year:	Theis, M.; 2017
Report/Doc. number:	E31804950-0 / M-577004-01-1
Guideline(s):	No agreed ring-tested guideline available - Proposal for a New Guideline for the Testing of Chemicals. Honey Bee (*Apis mellifera* L.), Chronic Oral Toxicity Test. 10 Day Feeding Test in the Laboratory, OECD Publishing, Paris, February 2016.
GLP:	yes

Method 01163 (refer to M-354028-01-1)
The analytical method 01163 was developed for the simultaneous determination of pesticide residues in/on plant materials and feeding stuff by HPLC-MS/MS in support of the study.

Principle of the method

For the determination of pesticide residues the sample is weighed into a 50 mL centrifuge tube (5 g of dry sample material, 10 g for samples with high water content). Depending on the water content of the sample water is added to the sample followed by 10 mL acetonitrile. The sample is strongly shaken for approx. 1 minute. 4 g of $MgSO_4$, 1 g NaCl, 1 g Na_3-Citrate dihydrate and 0.5 g Na_2H-Citrate sesquihydrate are added to this mixture and the sample is strongly shaken for 1 minute again. The solution is centrifuged for 5 minutes at 3000 U/min. An aliquot of 1 mL of the supernatant is diluted to 5 mL (if 5 g were weighed) or 10 mL (if 10 g were weighed), respectively, with water (acidified to pH 4 with formic acid). This final solution is filtered and subjected to LC-MS/MS analysis operated in multiple reaction monitoring mode (MRM).
For AE F046360, determination was performed by LC-MS/MS in positive ion mode using the MS/MS transition 362 m/z → 288 m/z for quantification. Column: YMC Triart 30 x 2 mm; 1.9µm, C18 with PreColumn Waters VanGuard Acquity UPLC BEH C18 2.1 x 5 mm; 1.7 µm.

Validation

Limit of quantitation
The limit of quantitation (LOQ) was set at 0.01 mg/kg.

Linearity

Analyte	Range [µg/L]	Regression equation quantific. /qual.	Correlation coefficient r	n
AE F046360	0.025 – 10.0	y = 36284.4x + 2518.083	0.999960	5

Specificity
Apparent residues in control samples were below 0.3 × LOQ. The recoveries can be corrected for interferences in case of the fused presence of an investigated compound. The high selectivity of the method results from the HPLC separation in combination with MS/MS detection. Therefore, the HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary.
Recovery (Accuracy) - Precision

Analyte	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F046360	0.01	0.01	105	1	3
		0.1	107	0.5	3
		3000	96	-	1

Conclusion
The results show that the assay method presented above is fit for purpose.

Analytical method 00972:

Reference:	**Fenoxaprop-P-ethyl & mefenpyr-diethyl; oil in water emulsion; 69+75 g/l - Daphnia magna chronic toxicity test, dosed at two life stages in an uncovered static laboratory test system**
Author(s), year:	Bruns, E.; 2008
Report/Doc. number:	EBFPX008 / M-270407-01-2
Guideline(s):	Only one renewal of exposure media after 10 days of exposure; Exposure vessels remained uncovered during any part of the study; Food amounts were adapted to the actual need of the test animals
GLP:	yes

Reference:	**Method 00972 for the determination of fenoxaprop-p-ethyl (AE F046360), mefenpyr-diethyl (AE F107892), fenoxaprop (AE F053022) and AE F113225 in test water from aquatic toxicity tests by HPLC-MS/MS**
Author(s), year:	Krebber, R.; 2006;
Report/Doc. number:	00972 / M-266218-01-1
Guideline(s):	Not specified
GLP:	yes

The method 00972 describes the determination of AE F046360, mefenpyr-diethyl, AE F053022 and AE F113225in test water from aquatic toxicity tests by HPLC-MS/MS in support of this aquatic toxicity study to *Daphnia magna* (Bruns, E., 2006, M-270407-01-2, see KCP 10.2.2/03).

Principle of the method

Water samples are appropriately diluted with a mixture of test water (acidified to pH 4-5 with formic acid)/acetonitrile, and analysed by direct injection into an HPLC-MS/MS instrument using electrospray. AE F046360 and mefenpyr-diethyl were analysed in the positive mode. AE F053022 and AE F113225 were analysed in the negative mode.

Transitions:	AE F046360	m/z 361 → 287
	Mefenpyr-diethyl	m/z 372 → 326
	AE F053022	m/z 331 → 259
	AE F113225	m/z 342 → 253

Column: Luna 5µ C18 100A, length 150; i.d. 2 mm

Validation

Limit of quantitation

The limit of quantitation (LOQ) is 0.05 µg/L for all analytes and the limit of detection is 0.02 µg/L for all analytes.

Linearity

Analyte	Range [µg/L]	Regression equation	Correlation coefficient r	n
AE F046360	0.05 – 10.0	$y = 1.58*10^5 x + 83$	0.9997 – 1.000	7
Mefenpyr-diethyl		$y = 1.06*10^5 x - 69$		7
AE F053022		$y = 5.87*10^4 x – 130$		7
AE F113225		$y = 8.07*10^3 - 90$		7

Specificity

The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection. No residues of the analytes were detected in the test water control samples higher than 0.3 x LOQ.

Recovery (Accuracy) - Precision

As the water samples were analysed by direct injection into the HPLC-MS/MS system recovery data cannot be calculated as stated in the guidance document on pesticide residue analytical methods (SANCO/825/00 rev.8.1). The repeatability of HPLC-MS/MS detection for AE F046360, mefenpyr-diethyl, AE F053022 and AE F113225

was determined based on 10 injections of a standard solution of 0.050 µg/L and 10 injections of a standard solution of 0.50 µg/L. The relative standard deviation for the peak areas ranged between 1.9% and 6.3% at 0.050 µg/L and between 1.8% and 5.4% at 0.50 µg/L. The relative standard deviation for the retention times was ≤ 0.23% in any case.

HPLC-MS/MS Method Validation of Fenoxaprop-p-ethyl

Sample conc. [µg/L]	Peak area			Retention time	
	Single Values [area counts]	Mean [area counts]	RSD [%]	Mean [min]	RSD [%]
0.050	8199 8146 7892 8302 7817 8259 7995 7538 8428 8225	8080	3.3	4.26	0.10
0.50	79853 78315 81248 77138 77765 75828 86691 77557 78692 75819	77891	2.2	4.26	0.11

RSD = Relative standard deviation

HPLC-MS/MS Method Validation of Mefenpyr-diethyl

Sample conc. [µg/L]	Peak area			Retention time	
	Single Values [area counts]	Mean [area counts]	RSD [%]	Mean [min]	RSD [%]
0.050	5433 5294 5405 5406 5492 5500 5394 5505 5685 5543	5466	1.9	4.00	< 0.1
0.50	53649 52822 53409 52890 55173 53717 53376 54098 55956 54189	53928	1.8	4.00	< 0.1

RSD = Relative standard deviation

HPLC-MS/MS Method Validation of AE F053022

Sample conc. [µg/L]	Peak area			Retention time	
	Single Values [area counts]	Mean [area counts]	RSD [%]	Mean [min]	RSD [%]
0.050	2717 2917 2476 3069 2938 3113 2838 2857 2778 2869	2857	6.3	2.80	0.23
0.50	25152 28062 26068 25280 26022 24687 25762 27622 25342 24098	25810	4.8	2.8	< 0.1

RSD = Relative standard deviation

HPLC-MS/MS Method Validation of AE F113225

Sample conc. [µg/L]	Peak area			Retention time	
	Single Values [area counts]	Mean [area counts]	RSD [%]	Mean [min]	RSD [%]
0.050	300 339 302 323 313 296 276 317 289 317	307	5.9	2.52	0.17
0.50	3778 3944 3711 3488 3432 3459 3596 3755 3545 3300	3601	5.4	2.52	0.13

RSD = Relative standard deviation

Conclusion

The results show that the assay method presented above is fit for purpose.

B.5.1.2.3. Methods to support studies of residues or the respective metabolites in plant and animal matrices

Table B.5.1.2.3-1 Analytical risk assessment methods for the determination of AE F046360 residues or the respective metabolites in plant and animal matrices

Method	Doc. No.	Chapter link	Analytes	Matrix (LOQ)[1]	Technique	Year
AL 58/86[2] (evaluated)	M-117453-01-4	KCA 4.1.2/01	AE F046360 AE F088406 AE F054014	Plant matrices: - cereal grain (0.02 mg/kg) - cereal straw (0.05 mg/kg) - cereal shoot (0.02 mg/kg)	GC-ECD	1986
AM01/02 (renamed 00874) (evaluated)	M-213161-01-1	KCA 4.2/11 KCA 4.1.2/03	AE F046360 AE F088406 AE F054014	Plant matrices: - cereal grain (0.01 mg/kg) - cereal straw (0.05 mg/kg) - cereal shoot (0.05 mg/kg)	GC-MS	2003
ILV to AM01/02 (renamed 00874) (evaluated)	M-217415-01-1	KCA 4.2/12 KCA 4.1.2/04	AE F046360 AE F088406 AE F054014	Plant matrices: - cereal grain (0.01 mg/kg)	GC-MS	2003
HRAV-8A (evaluated)	M-127403-02-1	KCA 4.2/07 KCA 4.1.2/11	AE F046360 AE F088406 AE F054014	Animal matrices: - liver (0.05 mg/kg) - kidney (0.05 mg/kg) - muscle (0.05 mg/kg) - milk (0.02 mg/kg) - fat (0.05 mg/kg)	GC-ECD	1991
ILV to HRAV-8A (evaluated)	M-127401-01-1	KCA 4.1.2/12	AE F046360 AE F088406 AE F054014	Animal matrices: - muscle (0.05 mg/kg) - milk (0.02 mg/kg) - liver (0.05 mg/kg) - fat (0.05 mg/kg)	GC-ECD	1990
00874/M001 (new method)	M-234670-02-1	KCA 4.1.2/13	AE F046360 AE F088406 AE F054014	Plant matrices: - cereal grain (0.01 mg/kg) - cereal straw (0.01 mg/kg) - olives (0.01 mg/kg) - citrus (0.01 mg/kg)	LC-MS/MS	2004
ILV to 00874/M001 (new method)	M-091819-02-1	KCA 4.1.2/14	AE F046360 AE F088406 AE F054014	Plant matrices: - cereal grain (0.01 mg/kg) - cereal tomato (0.01 mg/kg)	LC-MS/MS	2004
ENK0020 (new method)	M-552660-01-1	KCA 4.1.2/15	AE F046360 AE F088406 AE F054014	Animal matrices: - chicken egg (0.01 mg/kg) - muscle (0.01 mg/kg) - liver (0.01 mg/kg) - skin/fat (0.01 mg/kg) - blood (0.01 mg/kg)	HPLC-MS/MS	2016

[1] - LOQ : limit of quantification.

[2] – Validation data for method AL 58/86 were provided origin from field trial documents M-187016-01-1, M-142745-01-1 , M-140533-01-1, M-132008-01-2, M-132011-01-2, M-132012-01-2, M-132009-01-2, M-132010-01-2, M-132013-01-2, M-142883-01-1, M-140801-01-1

Additionally with document M-115279-01-1 (see plant metabolism, KCA 6.2.1 /08 in the Baseline dossier) accountability data for the primary extraction via hydrolysation were provided.

Method 00874/M001

Reference:	**Modification M001 to method 00874 for the determination of residues of AE F046360, AE F088406, and AE F054014 in/on matrices of plant origin by HPLC-MS/MS (Method and validation) - Amendment to report no.: 1**
Author(s), year:	Freitag, T.; 2006
Report/Doc. number:	00874/M001 / M-234670-02-1
Guideline(s):	EU Council Directive 91/414/EEC
GLP:	yes

With the presented method 00874/M001 method AM01/02 (for internal reasons renamed to 00874; see summary in Table B.5.1.2.3-1was implemented in the new department test site using the instrumentation available at this site. Some technical modifications and amendments were included and the final determination was switched to LC-MS/MS. A complete new set of validation data in different matrices was performed.

Principle of the method

Residues of AE F046360 are extracted via refluxing with acetonitrile / concentrated hydrochloric acid (90 / 10, v / v). During this process, AE F046360 and AE F088406 are converted to AE F054014. After filtration extracts were applied onto an extrelut cartridge. AE F054014 is eluted from the extrelut cartridge with n-hexane/diethyl ether, the eluate is evaporated to dryness and the residue is reconstituted in acetonitrile/water. Final determination is performed by LC-MS/MS with the fragmentations [m/z] 168 → 132 and [m/z] 168 → 76.
The method determines the sum (total residue) of AE F046360, AE F088406 and AE F054014. The analytical target is AE F054014.
Recovery experiments were conducted at LOQ and 10 times LOQ in cereal grain, straw, olives and citrus. At each fortification level, 5 recoveries were fortified separately with AE F046360, AE F088406 and AE F054014. Samples were analysed according to the method 00874/M001.

Validation

Specificity
No apparent residues (< 1 µg/kg = 10 % of LOQ) were detected in control samples of all matrices. Fragmentation from [m/z] 168 → 132 was used for quantification, [m/z] 168 → 76 is used as qualifier transition. Transition [m/z] 168 → 76 can also be used for quantification but at a higher LOQ (0.04 mg/kg). This LC-MS/MS detection is considered to be highly specific and the development of an additional confirmatory method is not necessary.
Matrix effects were observed, i.e. the peak areas of pure standard solutions were not in the same order than those of matrix-matched standards. Therefore, it is recommended to calibrate against external bracketing matrix-matched standards in these cases.

Linearity:
An excellent linear correlation between injected amount of AE F054014 and detector response was observed for a range from 0.0001 to 0.5 µg/mL for the transition [m/z] 168 → 132 for all sample materials. Correlation coefficients of the 1/x weighted linear regression were always >0.996 for standards in solvent and matrix matched standards.

Linearity data for AE F054014 in different matrices

Matrix	Analyte	Linear range [µg/mL]	Regression equation	Correlation coefficient r	No of standards
solvent	AE F054014	0.0001 to 0.5	$y = 16.45*10^6 x + 723$	0.9982	12
citrus fruit			$y = 7.81*10^6 x + 93$	0.9994	
solvent	AE F054014	0.0001 to 0.5	$y = 11.76*10^6 x + 1702$	0.9974	12
olive fruit			$y = 5.29*10^6 x + 467$	0.9991	
solvent	AE F054014	0.0001 to 0.5	$y = 13.21*10^6 x + 1370$	0.9968	12
wheat grain			$y = 6.15*10^6 x + 536$	0.992	

Matrix	Analyte	Linear range [µg/mL]	Regression equation	Correlation coefficient r	No of standards
solvent	AE F054014 (m/z 132)	0.0001 to 0.5	$y = 12.33*10^6 x + 1095$	0.9974	12
wheat rest of plant			$y = 5.83*10^6 x + 339$	0.9995	

Repeatability:
Relative standard deviations were all well below 20 %. Details are given in the table below.

Limit of quantitation:
The limit of quantitation for AE F046360, AE F088406 and AE F054014 was established and validated at 0.01 mg/kg in wheat grain, wheat straw, olives and citrus.

Recovery and precision:
Most mean recoveries for all three metabolites (AE F046360, AE F088406 and AE F054014) in all four matrices (cereal grain, cereal straw, olives and citrus) at the fortification levels 10 times LOQ were well within the 70–120% range. At the LOQ the mean recoveries are well in the range according to guidance document 825/00 (60-120%).

Recoveries of AE F046360, in cereal grain, cereal straw, olives and citrus fruits

Analyte	Matrix	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F046360 [m/z] 168 → 132	Grain	0.01	73	6	5
		0.1	75	5	5
		Overall	74	5	10
AE F046360 [m/z] 168 → 132	Straw	0.01	75	5	5
		0.1	76	7	5
		Overall	76	6	10
AE F046360 [m/z] 168 → 132	Olives	0.01	86	2	5
		0.1	82	4	5
		Overall	84	4	10
AE F046360 [m/z] 168 → 132	Citrus	0.01	78	5	5
		0.1	78	5	5
		Overall	78	5	10

Recoveries of AE F088406 in cereal grain, cereal straw, olives and citrus fruits

Analyte	Matrix	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F088406 [m/z] 168 → 132	Grain	0.01	73	2	5
		0.1	77	4	5
		Overall	75	4	10
AE F088406 [m/z] 168 → 132	Straw	0.01	82	3	5
		0.1	83	4	5
		Overall	83	3	10
AE F088406 [m/z] 168 → 132	Olives	0.01	69	5	5
		0.1	75	5	5
		Overall	72	6	10
AE F088406 [m/z] 168 → 132	Citrus	0.01	78	2	5
		0.1	76	5	5
		Overall	77	4	10

Recoveries of AE F054014 in cereal grain, cereal straw, olives and citrus fruits

Analyte	Matrix	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F054014 [m/z] 168 → 132	Grain	0.01	72	3	5
		0.1	71	2	5
		Overall	72	2	10
AE F054014 [m/z] 168 → 132	Straw	0.01	78	8	5
		0.1	75	6	5
		Overall	76	7	10
AE F054014 [m/z] 168 → 132	Olives	0.01	72	6	5
		0.1	77	3	5
		Overall	75	5	10
AE F0054014 [m/z] 168 → 132	Citrus	0.01	74	5	5
		0.1	74	2	5
		Overall	74	3	10
AE F054014 [m/z] 168 → 76	Grain	0.01	73	6	5
		0.1	71	2	5
		Overall	72	4	10
AE F054014 [m/z] 168 → 76	Straw	0.01	81	9	5
		0.1	74	6	5
		Overall	78	9	10
AE F054014 [m/z] 168 → 76	Olives	0.01	71	3	5
		0.1	76	3	5
		Overall	74	4	10
AE F0054014 [m/z] 168 → 76	Citrus	0.01	71	4	5
		0.1	75	4	5
		Overall	73	4	10

Conclusion

The residue analytical method 00874/M001 is suitable to determine residues of AE F046360 and its metabolites in cereal grain, cereal straw, olives and citrus and complies with the SANCO guideline 825/00 rev 8.1.

Independent Laboratory validation of analytical method 00874/M001

Reference:	**Independent laboratory validation of Bayer CropScience method 00874/M001 for the determination of residues of fenoxaprop-P-ethyl and two metabolites in plant material**
Author(s), year:	Class, T.; 2004
Report/Doc. number:	C044617 / M-091819-02-1
Guideline(s):	EU (=EEC): 96/46/EC; SANCO: SANCO/825/00 rev. 7
GLP:	yes

Principle of the method

The objective of this study was to independently validate the method 00874/M001 in tomato and wheat grain at a limit of quantitation at 0.01 mg/kg according to the European requirements. Control samples were fortified with AE F046360 (fenoxaprop-P-ethyl) and its metabolites (AE F088406 and AE F054014) at the limit of quantitation and at ten times this limit and analysed according to method 00874/M001.

No contacts to the developers of the original method were necessary.

Validation

Specificity
No apparent residues or interferences were detected in control samples (i.e. apparent residues were < 20% of LOQ). Fragmentation from [m/z] 168 → 132 was used for quantification, [m/z] 168 → 76 is used as qualifier transition. As demonstrated in method 00874/M001 transition [m/z] 168 → 76 can also be used for quantification. Therefore this LC-MS/MS detection is considered to be highly specific and the development of an additional confirmatory method is not necessary.

Repeatability:
At each level the repeatability, expressed as the relative standard deviation (RSD) was ≤ 20%. The overall relative standard deviation (RSD) was lower than 20%.

Limit of quantitation:
The limit of quantitation for AE F046360, AE F088406 and AE F054014 was validated at 0.01 mg/kg in cereal grain and tomato.

Recovery rates:
At each level the mean recovery were between 70% and 110%. Details are given in the following table.

Recoveries of AE F046360, AE F088406 and AE F054014 in tomato

Analyte	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F046360	0.01	93	6	5
	0.1	93	9	5
	Overall	93	7	10
AE F054014	0.01	86	9	5
	0.1	91	13	5
	Overall	88	11	10
AE F088406	0.01	76	6	5
	0.1	87	7	5
	Overall	82	10	10

Recoveries of AE F046360, AE F088406 and AE F054014 in cereal grain

Analyte	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F046360	0.01	84	11	5
	0.1	93	16	5
	Overall	89	14	10
AE F054014	0.01	103	12	5
	0.1	89	2	5
	Overall	96	12	10
AE F088406	0.01	76	6	5
	0.1	86	11	5
	Overall	82	11	10

Linearity:
The quantitative determination was carried out by external standardization with calibration standards in matrix. Calibration functions ranging from 0.4 ng/mL to 25 ng/mL at 6 different concentrations were used to evaluate the extracts. Linear calibration functions with 1/x weighting were calculated and plotted by regression analysis. The correlation coefficients were always > 0.99.

Conclusion

Method 00874/M001 was successfully independently validated according to the European requirements (Council directive 91/414/EEC Annex II (Part A, Section 4.2) and SANCO/825/00 rev. 8.1) for the determination of fenoxaprop-P-ethyl (AE F046360) and its metabolites (AE F088406 and AE F054014) in wheat grain and tomato. Therefore, the method 00874/M001 is considered applicable as a residue analytical method as well as for enforcement purposes.
However, for risk assessment methods no ILV is required.

Reference:	**Fenoxaprop-P-ethyl and fenoxaprop-P: Validation of methodology for the determination of residues as the benzoxazolone metabolite in foodstuff of animal origin**
Author(s), year:	Burton, D.; 2016
Report/Doc. number:	ENK0020 / M-552660-01-1
Guideline(s):	Guidance Document on Residue Analytical Methods; SANCO/825/00 rev. 8.1 of 16 November 2010
GLP:	yes

For the summary of the validation please refer to B.5.2.4 (Methods for the analysis in body fluids and tissues for active substances and relevant metabolites)

B.5.1.2.4. Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

Table B.5.1.2.4-1 Analytical risk assessment methods for the determination of AE F046360 residues or its metabolites in different matrices resulting from physical and chemical properties tests

Method	Doc. No.	Chapter link	Analytes	Matrix (LOQ[1]	Technique	Year
(new method)	M-548748-01-1	KCA 2.5/04	AE F046360	Water: - 0.15 mg/L	HPLC-UV	2005
(new method)	M-527714-01-1	KCA 2.14/02	AE F087795	Water: - 0.97 mg/L	HPLC-UV	2015
(new method)	M-527828-01-1	KCA 2.14/03	AE F054014	Water: - 0.99 mg/L	HPLC-UV	2015
(new method)	M-527831-01-1	KCA 2.14/04	AE F096918	Water: - 4.90 mg/L	HPLC-UV	2015
(new method)	M-547447-01-1	KCA 2.14/05	AE F040356	Water: - 0.10 mg/L	HPLC-UV	2016
(new method)	M-547402-01-1	KCA 2.14/06	AE F088406	Water: - 0.99 mg/L	HPLC-UV	2016
(new method)	M-547452-01-1	KCA 2.14/07	BCS-CY11271	Water: - 4.65 mg/L	HPLC-UV	2016
(new method)	M-547425-01-1	KCA 2.14/08	AE 0316854	Water: - 0.99 mg/L	HPLC-UV	2016
(new method)	M-538121-01-1	KCA 2.7/08	AE F054014	Water: - 0.99 mg/L	HPLC-UV	2015
(new method)	M-547403-01-1	KCA 2.7/09	AE F096918	Water: - 4.90 mg/L	HPLC-UV	2016
(new method)	M-547422-01-1	KCA 2.7/10	AE F088406	Water: - 0.99 mg/L	HPLC-UV	2016
(new method)	M-547427-01-1	KCA 2.7/11	AE 0316854	Water: - 0.99 mg/L	HPLC-UV	2016

[1] - LOQ : limit of quantification

Reference:	**Fenoxaprop-p-ethyl (pure grade): Melting temperature and water solubility**
Author(s), year:	Comb, A. L.; 2005
Report/Doc. number:	CHV0129/053825 / M-548748-01-1
Guideline(s):	European Council Directive 91/414/EEC (Annex l) as amended by European Commission Directive 94/37/EC EEC Method Nos.: A1 and A6 OECD Method No.: 102 and 105
GLP:	yes

The analytical method was developed to determine AE F046360 (fenoxaprop-P-ethyl) in samples from this water solubility determination test using HPLC-UV.

Principle of the method

This procedure describes a method for the quantitative determination of AE F046360 by high performance liquid chromatography using UV detector. The separation is achieved on a Hewlett Packard 1050 Liquid Chromatograph, with hexane : tetrahydrofuran (90:10, v/v) as mobile phase.

Validation

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r	n
AE F046360	0.14 - 1.5	y = 4.12 x + 0.00821	0.9995	6

Specificity

No peaks were observed in the chromatograms of the blank solutions indicating that the analytical method was free from interference.

Recovery (Accuracy) - Precision

The accuracy of the analytical method was investigated by analysing solutions of AE F046360 in each aqueous system, four replicates each. The solutions were prepared by diluting aliquots (0.25 mL) of a 10 mg/L acetone stock solution of AE F046360 into volumes (5 mL) of each solvent. The resulting solutions were then treated in an identical manner to the corresponding definitive test solutions for analysis by the HPLC method.

Slightly lower concentrations of the test substance were found in the pH 10 buffer samples and therefore the verification samples were prepared at a correspondingly lower level. For the verification samples in pH 10 buffer solution, 0.175 ml aliquots of the acetone stock solution were used to fortify 5 ml of the buffer solution.

Mean recoveries of AE F046360 from method verification samples were ≥ 95% with a relative standard deviation (RSD) ≤ 5.1%.

Method verification in different aqueous systems

Concentration of fortified solution [mg/L]	Mean [%]	RSD [%]	n
unbuffered water			
0.4975	99	2.1	4
pH 4 buffer solution			
0.4975	96	0.7	4
pH 7 buffer solution			
0.4975	95	3.5	4
pH 10 buffer solution			
0.3482	96	5.1	4

Conclusion

In conclusion, the results show that the assay method presented above is satisfactory.

Reference:	**AE F087795 (BCS-CA37046): Water solubility at pH 5, pH 7 and pH 9 (flask method)**
Author(s), year:	Sonnenschein, L.; 2015
Report/Doc. number:	VP 009/2015 / M-527714-01-1
Guideline(s):	European Commission Council Regulation (EC) No 440/2008, Annex, Part A, method A.6. OECD Test Guideline 105 US EPA Product Properties Test Guideline OCSPP 830.7840
GLP:	yes

The analytical method was developed to determine AE F087795 in samples from this water solubility determination test using HPLC-UV.

Principle of the method

This procedure describes a method for the quantitative determination of AE F087795 by high performance liquid chromatography using UV detector. The separation is achieved on a Waters Acquity UPLC-System, with acetonitrile/water (each with 0.1% of formic acid, 30/70 (v/v)) as mobile phase. The concentrations of the test item in the aqueous phases were quantified by comparing integrated peak areas to integrated peak areas measured by injection of solutions with known amounts of test sample (external standard method).

Validation

Limit of quantitation
The limit of quantification (LOQ) was defined as the lowest concentration from the linearity test with a signal/noise ratio above 10 and was determined to be 0.97 mg/L.

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r	n
AE F087795	0.97 - 100.77	y = 9506.792 x + 3091.597	0.999923	6

Specificity
No interfering peaks could be detected in the blank aqueous and aqueous buffered solutions. For each condition in this study blank injections were performed prior to the samples of the water solubility testing. Due to the absence of interfering signals in the chromatograms specificity of the method is given.

Recovery (Accuracy) - Precision
The evaluation of accuracy was obtained by the analysis of five sample solutions (five individual sample weights of approximately 30 to 43 mg in 50 mL of acetonitrile, diluted 1 mL to 50 mL with acetonitrile/water (30:70, v/v)). Each sample was injected twice.

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
11.9018	103.51	-	1
15.2721	102.46	-	1
14.9932	105.75	-	1
16.7961	102.16	-	1
13.5870	101.40	-	1
Overall	103.1	1.6	5

Conclusion
In conclusion, the results show that the assay method presented above is satisfactory.

Reference:	**AE F054014 (BCS-AC79872): Water solubility at pH 5, pH 7 and pH 9 (flask method)**
Author(s), year:	Sonnenschein, L.; 2015
Report/Doc. number:	VP 013/2015 / M-527828-01-1
Guideline(s):	European Commission Council Regulation (EC) No 440/2008, Annex, Part A, A.6. OECD Test Guideline 105 EPA Product Properties Test Guideline OCSPP 830.7840
GLP:	**yes**

The analytical method was developed to determine AE F054014 in samples from this water solubility determination test using HPLC-UV.

Principle of the method

This procedure describes a method for the quantitative determination of AE F054014 by high performance liquid chromatography using UV detector. The separation is achieved on a Waters Acquity UPLC-System, with acetonitrile/water (each with 0.1% of formic acid, 30/70 (v/v)) as mobile phase. The concentrations of the test item in the aqueous phases were quantified by comparing integrated peak areas to integrated peak areas measured by injection of solutions with known amounts of test sample (external standard method).

Validation

Limit of quantitation
The limit of quantification (LOQ) was defined as the lowest concentration from the linearity test with a signal/noise ratio above 10 and was determined to be 0.99 mg/L.

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r	n
AE F054014	0.99 – 102.62	y = 19183.89 x + 5341.954	0.999894	6

Specificity
No interfering peaks could be detected in the blank aqueous and aqueous buffered solutions. For each condition in this study blank injections were performed prior to the samples of the water solubility testing. Due to the absence of interfering signals in the chromatograms specificity of the method is given.

Recovery (Accuracy) -Precision
The evaluation of accuracy was obtained by the analysis of five sample solutions (five individual sample weights of approximately 30 to 46 mg in 50 mL of acetonitrile, diluted 1 mL to 50 mL with acetonitrile/water (30:70, v/v)). Each sample was injected twice.

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
13.06	103.78	-	1
11.82	102.24	-	1
12.07	102.38	-	1
16.11	101.96	-	1
18.16	103.07	-	1
Overall	102.7	0.7	5

Conclusion
In conclusion, the results show that the assay method presented above is satisfactory.

Reference:	**AE F096918 (BCS-CA37065): Water solubility at pH 5, pH 7 and pH 9 (flask method)**
Author(s), year:	Sonnenschein, L.; 2015
Report/Doc. number:	VP 011/2015 / M-527831-01-1
Guideline(s):	Based on European Commission Council Regulation (EC) No 440/2008, Annex, Part A, method A.6. OECD Test Guideline 105 US EPA Product Properties Test Guideline OCSPP 830.7840
GLP:	yes

The analytical method was developed to determine AE F096918 in samples from this water solubility determination test using HPLC-UV.

Principle of the method

This procedure describes a method for the quantitative determination of AE F096918 by high performance liquid chromatography using UV detector. The separation is achieved on a Waters Acquity UPLC-System, with acetonitrile/water (each with 0.1% of formic acid, 30/70 (v/v)) as mobile phase. The concentrations of the test item in the aqueous phases were quantified by comparing integrated peak areas to integrated peak areas measured by injection of solutions with known amounts of test sample (external standard method).

Validation

Limit of quantitation
The limit of quantification (LOQ) was defined as the lowest concentration from the linearity test with a signal/noise ratio above 10 and was determined to be 4.90 mg/L.
Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r	n
AE F096918	0.98 – 200.04	y = 4448.422 x + 4943.202	0.999950	6

Specificity
No interfering peaks could be detected in the blank aqueous and aqueous buffered solutions. For each condition in this study blank injections were performed prior to the samples of the water solubility testing. Due to the absence of interfering signals in the chromatograms specificity of the method is given.

Recovery (Accuracy) -Precision
The evaluation of accuracy was obtained by the analysis of five sample solutions (five individual sample weights of approximately 26 to 44 mg in 50 mL of acetonitrile, diluted 1 mL to 50 mL with acetonitrile/water (15:85, v/v)). Each sample was injected twice.

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
10.4910	100.02	-	1
17.4291	97.41	-	1
11.7125	96.65	-	1
16.6666	96.37	-	1
12.8822	98.33	-	1
Overall	97.8	1.5	5

Conclusion
In conclusion, the results show that the assay method presented above is satisfactory.

Reference:	**AE F040356 (BCS-AB16188): Water solubility at pH 5, pH 7 and pH 9 (column elution method)**
Author(s), year:	Fischer, A.; 2016
Report/Doc. number:	VP 018/2015 / M-547447-01-1
Guideline(s):	European Commission Council Regulation (EC) No 440/2008, Annex, Part A, A.6.; OECD Test Guidelines 105; US EPA Product Properties Test Guidelines 830.7840
GLP:	yes

The analytical method was developed to determine AE F040356 in samples from this water solubility determination test using HPLC-UV.

Principle of the method

This procedure describes a method for the quantitative determination of AE F040356 by high performance liquid chromatography using UV detector. The separation is achieved on a Waters Acquity UPLC-System, with acetonitrile/water (each with 0.1% of formic acid, 50/50 (v/v)) as mobile phase. The concentrations of the test item in the aqueous phases were quantified by comparing integrated peak areas to integrated peak areas measured by injection of solutions with known amounts of test sample (external standard method).

Validation

Limit of quantitation
The limit of quantification (LOQ) was defined as the lowest concentration from the linearity test with a signal/noise ratio above 10 and was determined to be 0.10 mg/L.

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r^2	n
AE F040356	0.10 – 10.20	y = 18709.09 x + 221.7961	0.999949	6

Specificity
No interfering peaks could be detected in the blank diluent and in the blank aqueous buffered solutions. Due to the absence of interfering signals in the chromatograms specificity of the method is given.

Recovery (Accuracy) and precision
The evaluation of accuracy was obtained by the analysis of five sample solutions (five individual sample weights of approximately 29 to 39 mg in 50 mL of acetonitrile/water (50:50, v/v), diluted 1 mL to 100 mL with the same diluent). Each sample was injected twice.

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
5.6027	101.19	-	1
7.2304	96.82	-	1
6.4098	100.82	-	1
7.6145	101.43	-	1
6.2778	95.94	-	1
Overall	99.2	2.7	5

Conclusion
In conclusion, the results show that the assay method presented above is satisfactory.

Reference:	**AE F088406 (BCS-BQ23896): Water solubility at pH 5, pH 7 and pH 9 (flask method)**
Author(s), year:	Fischer, A.; 2016
Report/Doc. number:	VP 014/2015 / M-547402-01-1
Guideline(s):	European Commission Council Regulation (EC) No 440/2008, Annex, Part A, method A.6. OECD Test Guideline 105 US EPA Product Properties Test Guideline OCSPP 830.7840
GLP:	yes

The analytical method was developed to determine AE F088406 in samples from this water solubility determination test using HPLC-UV.

Principle of the method

This procedure describes a method for the quantitative determination of AE F088406 by high performance liquid chromatography using UV detector. The separation is achieved on a Waters Acquity UPLC-System, with acetonitrile/water (each with 0.1% of formic acid, 50/50 (v/v)) as mobile phase. The concentrations of the test item in the aqueous phases were quantified by comparing integrated peak areas to integrated peak areas measured by injection of solutions with known amounts of test sample (external standard method).

Validation

Limit of quantitation
The limit of quantification (LOQ) was defined as the lowest concentration from the linearity test with a signal/noise ratio above 10 and was determined to be 0.99 mg/L.

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r	n
AE F088406	0.99 – 100.38	y = 19769.77 x + 1289.856	0.999986	6

Specificity
No interfering peaks could be detected in the blank aqueous and aqueous buffered solutions. For each condition in this study blank injections were performed prior to the samples of the water solubility testing. Due to the absence of interfering signals in the chromatograms specificity of the method is given.

Recovery (accuracy) and precision
The evaluation of accuracy was obtained by the analysis of five sample solutions (five individual sample weights of approximately 29 to 40 mg in 50 mL of acetonitrile, diluted 1 mL to 50 mL with acetonitrile/water (50:50, v/v)). Each sample was injected twice. For the given method the individual accuracy results expressed as recovery rates and the precision expressed as the relative standard deviation (RSD) were determined to be 100.6% (accuracy) and 0.7% (precision).

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
12.8537	101.30	-	1
12.9402	100.17	-	1
13.8839	99.65	-	1
15.6887	100.75	-	1
11.4461	100.99	-	1
Overall	100.6	0.7	5

Conclusion
In conclusion, the results show that the assay method presented above is satisfactory.

Reference:	**BCS-CY11271: Water solubility at pH 5, pH 7 and pH 9 (flask method)**
Author(s), year:	Fischer, A.; 2016
Report/Doc. number:	VP 033/2015 / M-547452-01-1
Guideline(s):	European Commission Council Regulation (EC) No 440/2008, Annex, Part A, A.6.; OECD Test Guidelines 105; US EPA Product Properties Test Guidelines 830.7840
GLP:	yes

The analytical method was developed to determine BCS-CY11271 in samples from this water solubility determination test using HPLC-UV.

Principle of the method

This procedure describes a method for the quantitative determination of BCS-CY11271 by high performance liquid chromatography using UV detector. The separation is achieved on a Waters Acquity UPLC-System, with acetonitrile/water (each with 0.1% of formic acid, 50/50 (v/v)) as mobile phase. The concentrations of the test item in the aqueous phases were quantified by comparing integrated peak areas to integrated peak areas measured by injection of solutions with known amounts of test sample (external standard method).

Validation

Limit of quantitation
The limit of quantification (LOQ) was defined as the lowest concentration from the linearity test with a signal/noise ratio above 10 and was determined to be 4.65 mg/L.

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r	n
BCS-CY11271	4.65 – 151.62	y = 11622.39 x – 20.389	0.999802	6

Specificity
No interfering peaks could be detected in the blank aqueous and aqueous buffered solutions. For each condition in this study blank injections were performed prior to the samples of the water solubility testing. Due to the absence of interfering signals in the chromatograms specificity of the method is given.

Recovery (Accuracy) and precision
The evaluation of accuracy was obtained by the analysis of five sample solutions (five individual sample weights of approximately 6.3 to 7.8 mg in 100 mL of acetonitrile/water (50:50, v/v)). Each sample was injected twice. For the given method the individual accuracy results expressed as recovery rates and the precision expressed as the relative standard deviation (RSD) were determined to be 99.1% (accuracy) and 0.9% (precision).

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
61.7148	99.65	-	1
66.5496	97.90	-	1
63.8004	100.27	-	1
73.5648	99.02	-	1
59.6292	98.60	-	1
Overall	99.1	0.9	5

Conclusion
In conclusion, the results show that the assay method presented above is satisfactory.

Reference:	**AE 0316854 (BCS-AW69425): Water solubility at pH 5, pH 7 and pH 9 (flask method)**
Author(s), year:	Fischer, A.; 2016
Report/Doc. number:	VP 016/2015 / M-547425-01-1
Guideline(s):	European Commission Council Regulation (EC) No 440/2008, Annex, Part A, A.6., OECD Test Guidelines 105; US EPA Product Properties Test Guidelines 830.7840
GLP:	yes

The analytical method was developed to determine AE 0316854 (6-Hydroxybenzoxazolone) in samples from this water solubility determination test using HPLC-UV.

Principle of the method

This procedure describes a method for the quantitative determination of AE 0316854 by high performance liquid chromatography using UV detector. The separation is achieved on a Waters Acquity UPLC-System, with acetonitrile/water (each with 0.1% of formic acid, 15/85 (v/v)) as mobile phase. The concentrations of the test item in the aqueous phases were quantified by comparing integrated peak areas to integrated peak areas measured by injection of solutions with known amounts of test sample (external standard method).

Validation

Limit of quantitation
The limit of quantification (LOQ) was defined as the lowest concentration from the linearity test with a signal/noise ratio above 10 and was determined to be 0.99 mg/L.

Linearity

Analyte	Range [mg/L]	Regression equation	Correlation coefficient r	n
AE 0316854	0.99 – 99.56	y = 4251.034 x + 537.1571	0.999993	6

Specificity
No interfering peaks could be detected in the blank aqueous and aqueous buffered solutions. For each condition in this study blank injections were performed prior to the samples of the water solubility testing. Due to the absence of interfering signals in the chromatograms specificity of the method is given.

Recovery (Accuracy) and precision
The evaluation of accuracy was obtained by the analysis of five sample solutions (five individual sample weights of approximately 30 to 47 mg in 50 mL of acetonitrile, diluted 1 mL to 50 mL with acetonitrile/water (15:85, v/v)). Each sample was injected twice. For the given method the individual accuracy results expressed as recovery rates and the precision expressed as the relative standard deviation (RSD) were determined to be 105% (accuracy) and 0.8% (precision).

Sample concentration [mg/L]	Mean [%]	RSD [%]	n
12.4409	105.09	-	1
17.7012	105.26	-	1
13.6894	104.85	-	1
11.9876	103.20	-	1
18.7230	104.86	-	1
Overall	105	0.8	5

Conclusion
In conclusion, the results show that the assay method presented above is satisfactory.

Reference:	**AE F054014 (BCS-AC79872): Partition coefficients 1-octanol/water at pH 5, pH 7 and pH 9 (HPLC method and shake flask method)**
Author(s), year:	Fischer, A.; 2015
Report/Doc. number:	VP 037/2015 / M-538121-01-1
Guideline(s):	European Commission Council Regulation (EC) No 440/2008, Annex, Part A, A.8.; OECD Test Guidelines 117 and 107; US EPA Product Properties Test Guidelines OCSPP 830.7570 and 830.7550
GLP:	yes

The analytical method was developed to determine AE F054014 in samples from this test for the determination of the partition coefficient (log Kow) using HPLC-UV.

Principle of the method

This procedure describes a method for the quantitative determination of AE F054014 by high performance liquid chromatography using UV detector. The validation of the HPLC method was performed within the study of Sonnenschein, L., 2015 (M-527828-01-1) and a summary is already provided above.

Reference:	**AE F096918 (BCS-CA37065): Partition coefficients 1-octanol/water at pH 5, pH 7 and pH 9 (shake flask method)**
Author(s), year:	Fischer, A.; 2016
Report/Doc. number:	VP 012/2015 / M-547403-01-1
Guideline(s):	European Commission Council Regulation (EC) No 440/2008, Annex, Part A, A.8., OECD Test Guidelines 107 US EPA Product Properties Test Guidelines 830.7550
GLP:	yes

The analytical method was developed to determine AE F096918 in samples from this test for the determination of the partition coefficient (log Kow) using HPLC-UV.

Principle of the method

This procedure describes a method for the quantitative determination of AE F096918 by high performance liquid chromatography using UV detector. The validation of the HPLC method was performed within the study of Sonnenschein, L., 2015 (M-527831-01-1) and a summary is already provided above.

Reference:	**AE F088406 (BCS-BQ23896): Partition coefficients 1-octanol/water at pH 5, pH 7 and pH 9 (shake flask method)**
Author(s), year:	Fischer, A.; 2016
Report/Doc. number:	VP 015/2015 / M-547422-01-1
Guideline(s):	European Commission Council Regulation (EC) No 440/2008, Annex, Part A, A.8., OECD Test Guidelines 107; US EPA Product Properties Test Guidelines 830.7550
GLP:	yes

The analytical method was developed to determine AE F088406 in samples from this test for the determination of the partition coefficient (log Kow) using HPLC-UV.

Principle of the method

This procedure describes a method for the quantitative determination of AE F088406 by high performance liquid chromatography using UV detector. The validation of the HPLC method was performed within the study of Fischer, A., 2016 (M-547402-01-1) and a summary is already provided above.

Reference:	**AE 0316854 (BCS-AW69425): Partition coefficients 1-octanol/water at pH 5, pH 7 and pH 9 (shake flask method)**
Author(s), year:	Fischer, A.; 2016
Report/Doc. number:	VP 017/2015 / M-547427-01-1
Guideline(s):	European Commission Council Regulation (EC) No 440/2008, Annex, Part A, A.8., OECD Test Guidelines 107; US EPA Product Properties Test Guidelines 830.7550
GLP:	yes

The analytical method was developed to determine 6-Hydroxybenzoxazolone (AE 0316854) in samples from this test for the determination of the partition coefficient (log Kow) using HPLC-UV.

Principle of the method

This procedure describes a method for the quantitative determination of 6-Hydroxybenzoxazolone by high performance liquid chromatography using UV detector. The validation of the HPLC method was performed within the study of Fischer, A., 2016 (M-547425-01-1) and a summary is already provided above.

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

Where methods have been previously evaluated as acceptable for the first EU evaluation of AE F046360, this is indicated (writing in grey typeface) and no summary is provided.

B.5.2.1. Methods for the determination of all components included in the monitoring residue definition as submitted in accordance with the provision of point 6.7.1 in order to enable Member States to determine compliance with established maximum residue levels (MRLs); they shall cover residues in or on food and feed of plant and animal origin

The EFSA conclusion 2007 revealed:
The methods are not enantio-selective and are not specific to fenoxaprop-P-ethyl.
It should be noted that the residue definition is unspecific not only with respect to the racemicmixture fenoxaprop-ethyl but also with respect to other chemicals, as eg. insecticide phosalone, that may potentially be converted to 6-chloro-2,3-dihydrobenzoxazol-2-one upon hydrolysis and give false positive results when determined with these methods.

Remark RMS: Phosalone is no longer approved since December 2006 in the EU.

Table B.5.2.1-1 Summary of analytical monitoring methods for the determination of AE F046360 residues in plant and animal matrices

Method	Doc. No.	Chapter link	Analytes	Matrix (LOQ)[1]	Technique	Year
01163 (QuEChERS) multi residue (new method)	M-354028-01-1	KCA 4.2/16	AE F046360	Plant matrices: - rape, whole plant (0.01 mg/kg) - wheat, green material (0.01 mg/kg)	LC-MS/MS	2009
AM01/02 (renamed 00874) (evaluated in the DAR 2005)	M-213161-01-1	KCA 4.2/11 KCA 4.1.2/02	AE F046360 AE F088406 AE F054014	Plant matrices: - cereal grain (0.01 mg/kg) - cereal straw (0.05 mg/kg) - cereal shoot (0.05 mg/kg)	GC-MS	2003
ILV to AM01/02 (renamed 00874) (evaluated)	M-217415-01-1	KCA 4.2/12 KCA 4.1.2/04	AE F046360 AE F088406 AE F054014	Plant matrices: - cereal grain (0.01 mg/kg)	GC-MS	2003
BM/02/95 (equivalent to HRAV-8A) (evaluated)	M-139128-01-1	KCA 4.2/09	AE F046360 AE F088406 AE F054014	Animal matrices: - egg (0.05 mg/kg) - muscle (0.05 mg/kg) - fat (0.05 mg/kg) - liver (0.05 mg/kg) - skin (0.05 mg/kg)	GC-ECD	1995
BM/02/95 (equivalent to HRAV-8A) (evaluated)	M-139131-02-1	KCA 6.1/05	AE F046360 AE F088406 AE F054014	Animal matrices: - egg (0.05 mg/kg) - muscle (0.05 mg/kg) - liver (0.05 mg/kg)	GC-ECD	1995
HRAV-8A (evaluated)	M-127403-02-1	KCA 4.2/07 KCA 4.1.2/11	AE F046360 AE F088406 AE F054014	Animal matrices: - liver (0.05 mg/kg) - kidney (0.05 mg/kg) - muscle (0.05 mg/kg) - milk (0.02 mg/kg) - fat (0.05 mg/kg)	GC-ECD	1991

Method	Doc. No.	Chapter link	Analytes	Matrix (LOQ)[1]	Technique	Year
HRAV-8A (evaluated)	M-139130-01-1	KCA 4.2/08	AE F046360 AE F088406 AE F054014	Animal matrices: - liver (0.05 mg/kg) - fat (0.05 mg/kg) - skin (0.05 mg/kg) - breast meat (0.05 mg/kg) - egg (0.05 mg/kg)	GC-ECD	1995
ILV to HRAV-8A (evaluated)	M-127401-01-1	KCA 4.1.2/12	AE F046360 AE F088406 AE F054014	Animal matrices: - muscle (0.05 mg/kg) - milk (0.02 mg/kg) - liver (0.05 mg/kg) - fat (0.05 mg/kg)	GC-ECD	1990
00874/M001 (new method)	M-234670-02-1	KCA 4.2/17	AE F046360 AE F088406 AE F054014	Plant matrices: - cereal grain (0.01 mg/kg) - cereal straw (0.01 mg/kg) - olives (0.01 mg/kg) - citrus (0.01 mg/kg)	LC-MS/MS	2004
ILV to 00874/M001 (new method)	M-091819-02-1	KCA 4.2/18	AE F046360 AE F088406 AE F054014	Plant matrices: - cereal grain (0.01 mg/kg) - cereal tomato (0.01 mg/kg)	LC-MS/MS	2004
01316 (new method)	M-404091-01-1	KCA 4.2/19	AE F046360 AE F088406 AE F054014	Animal matrices: - liver (0.01 mg/kg) - kidney (0.01 mg/kg) - muscle (0.01 mg/kg) - milk (0.01 mg/kg) - fat (0.01 mg/kg) - egg (0.01 mg/kg)	LC-MS/MS	2011
ILV to 01316 (new method)	M-411523-01-1	KCA 4.2/20	AE F046360 AE F088406 AE F054014	Animal matrices: - liver (0.01 mg/kg) - kidney (0.01 mg/kg) - meat (0.01 mg/kg) - milk (0.01 mg/kg) - fat (0.01 mg/kg) - egg (0.01 mg/kg)	LC-MS/MS	2011

[1] - LOQ: limit of quantification.

Method 01163

Reference:	**Description of the multi-residue analytical method 01163 for the simultaneous determination of pesticides by HPLC-MS/MS in plant materials and feeding stuff based on the official QuEChERS method**
Author(s), year:	Schmeer, K.; Stuke, S.; 2009
Report/Doc. number:	01163 / M-354028-01-1
Guideline(s):	91/414/EEC, 96/68/EC SANCO/825/00 rev. 7, Test Guideline OPPTS 860.1340:
GLP:	no

Principle of the method

Bayer CropScience analytical method 01163 describes the simultaneous determination of pesticide residues in/on plant materials based on the official multi-residue method according to M. Anastassiades *et al*. [QuEChERS A Mini-Multiresidue Method for the Analysis of Pesticide Residues in Low-Fat Products, 2005]. When written, multi-residue method 01163 covered approximately 150 different compounds and will be extended continuously in the future as appropriate. The report refers on numerous occasions to validation results given in [www.quechers.com] which were determined and published by M. Anastassiades *et al*.
For the determination of pesticide residues the sample is weighed into a 50 mL centrifuge tube (5 g of dry sample material, 10 g for samples with high water content). Depending on the water content of the sample, water is added followed by 10 mL acetonitrile. The sample is shaken for approximately 1 minute. To the extract, 4 g of

$MgSO_4$, 1 g NaCl, 1 g Trisodium citrate dihydrate and 0.5 g disodium hydrogen citrate sesquihydrate are added to this mixture and the sample is shaken again for 1 minute. The resulting solution is centrifuged for 5 minutes at 3000 G/min. An aliquot of 1 mL of the supernatant is diluted to 5 mL (if 5 g were used) or 10 mL (if 10 g were used), respectively, with water (acidified to pH 4 with formic acid). This final solution is filtered and subjected to LC-MS/MS analysis operated in multiple reaction monitoring mode (MRM).
For AE F046360, determination was performed by LC-MS/MS in positive ion mode using the MS/MS transition 362 m/z → 288 m/z for quantification. A Phenomenex Luna C18 column was used for chromatographic separation. A solvent gradient of methanol and water, both acidified with formic acid, is used for elution.

Validation

Calibration
The concentration of AE F046360 in the extracts is calculated against matrix-matched external standards, either using a calibration curve or according to the bracketing standard procedure. The calibration data obtained justified using the single point calibration method for the calculation of residues. However, an appropriate bracketing standard concentration prepared in matrix, corresponding to the order of magnitude of the residues should be used for quantification.
Quantification of residue samples was performed against matrix-matched standards.
During the method validation linear calibration curves for both MS/MS transitions were established in the nominal range of 0.5 to 50 µg/L, tested for oilseed rape whole plant or wheat green plant material. Duplicate injections at minimum of 5 concentrations, correlation coefficients r = 0.9975, $y = 2.37*10^4 x + 3.47*10^3$.

Specificity
LC-MS/MS using two characteristic MS/MS transitions for detection and quantification of AE F046360 in the final extracts can provide a high level of specificity. The chromatograms of control samples of the investigated matrices showed no significant signal at the retention time of AE F046360 (< 30% LOQ).

Limit of quantitation
The procedure allows determination of AE F046360 in plant matrices with a limit of quantitation of 0.01 mg/kg.

Repeatability (precision)
The procedure was shown to yield repeatable results. For each of the investigated matrices the overall relative standard deviations were <20% (n=3). The SANCO guideline 825 requires 5 repetitions for the determination of the precision but as this QuECHERS method was published and the method already validated from different laboratories the validation of this method is sufficiently shown.

Reproducibility
Not evaluated for this method. Refer to the reported results using the QuEChERS method.

Confirmatory method
Quantitative determination of AE F046360 residues in plant matrices may be performed by monitoring either of two MS/MS transitions 362 m/z → 288 m/z or 362 m/z → 121 m/z. The results determined with any of the two transitions may be confirmed by using the signal of the other transition. The data in this report were determined using the first quantification MRM.

Validation (recovery)
The procedure for AE F046360 was validated in oilseed rape whole plant and wheat plant green matter. For each matrix there were three recovery experiments with AE F046360 at the LOQ (0.01 mg/kg, n=2) and the 10-fold LOQ (0.10 mg/kg, n=1). The results of the validation are summarised in the table below. Overall, the mean recoveries for each matrix and each level were within the acceptable range of 70 to 110% and the relative standard deviations were <20%. Thus, the method was successfully validated according to the European guidance document SANCO/825/00 for post-registration monitoring methods.

Summary of validation data for AE F046360 in plant matrices using method 01163 (QuEChERS); Primary quantification conditions (m/z 362 → 288)

Reference	Sample Matrix	Analyte	LOQ [mg/kg]	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
Schmeer, K.; Stuke, S.; 2009	oilseed rape (whole plant)	Fenoxaprop-P-ethyl	0.01	0.01	99	-	2
				0.1	96	-	1
	wheat plant (green matter)	Fenoxaprop-P-ethyl	0.01	0.01	95.5	-	2
				0.1	92	-	1
				overall	96	3.0	6

Conclusion
The validation data are not compliant with the requirements of SANCO/825/00 rev. 8.1 as the number of samples is <5. However, the study demonstrates that a QuEChERS method works for the determination of residues at a LOQ of 0.01 mg/kg for crops with high water content without hydrolysis of fenoxaprop-P-ethyl to the common moiety compound AE F054014.

Method 00874/M001

Reference:	**Modification M001 to method 00874 for the determination of residues of AE F046360, AE F088406, and AE F054014 in/on matrices of plant origin by HPLC-MS/MS (Method and validation) - Amendment to report no.: 1**
Author(s), year:	Freitag, T.; 2006
Report/Doc. number:	00874/M001 / M-234670-02-1
Guideline(s):	EU Council Directive 91/414/EEC
GLP:	yes

Reference:	**Independent laboratory validation of Bayer CropScience method 00874/M001 for the determination of residues of fenoxaprop-P-ethyl and two metabolites in plant material**
Author(s), year:	Class, T.; 2004
Report/Doc. number:	C044617 / M-091819-02-1
Guideline(s):	EU (=EEC): 96/46/EC; SANCO: SANCO/825/00 rev. 7
GLP:	yes

The presented method 00874/M001 and the corresponding ILV are summarized in chapter B.5.1.2.3 (risk assessment methods) as well.

With the presented method 00874/M001 method AM01/02 (for internal reasons renamed to 00874; see summary in Table B.5.1.2.3-1 was implemented in the new department test site using the instrumentation available at this site. Some technical modifications and amendments were included and the final determination was switched to LC-MS/MS. A complete new set of validation data in different matrices was performed.

Principle of the method

Residues of AE F046360 are extracted via refluxing with acetonitrile / concentrated hydrochloric acid (90 / 10, v / v). During this process, AE F046360 and AE F088406 are converted to AE F054014. After filtration extracts were applied onto an extrelut cartridge. AE F054014 is eluted from the extrelut cartridge with n-hexane/diethyl ether, the eluate is evaporated to dryness and the residue is reconstituted in acetonitrile/water. Final determination is performed by LC-MS/MS with the fragmentations [m/z] 168 → 132 and [m/z] 168 → 76.
The method determines the sum (total residue) of AE F046360, AE F088406 and AE F054014. The analytical target is AE F054014.
Recovery experiments were conducted at LOQ and 10 times LOQ in cereal grain, straw, olives and citrus. At each fortification level, 5 recoveries were fortified separately with AE F046360, AE F088406 and AE F054014. Samples were analysed according to the method 00874/M001.

Validation

Specificity
No apparent residues (< 1 µg/kg = 10 % of LOQ) were detected in control samples of all matrices. Fragmentation from [m/z] 168 → 132 was used for quantification, [m/z] 168 → 76 is used as qualifier transition. Transition [m/z] 168 → 76 can also be used for quantification but at a higher LOQ (0.04 mg/kg). This LC-MS/MS detection is considered to be highly specific and the development of an additional confirmatory method is not necessary.
Matrix effects were observed, i.e. the peak areas of pure standard solutions were not in the same order than those of matrix-matched standards. Therefore, it is recommended to calibrate against external bracketing matrix-matched standards in these cases.

Linearity:
An excellent linear correlation between injected amount of AE F054014 and detector response was observed for a range from 0.0001 to 0.5 µg/mL for the transition [m/z] 168 → 132 for all sample materials. Correlation coefficients of the 1/x weighted linear regression were always >0.996 for standards in solvent and matrix matched standards.

Linearity data for AE F054014 in different matrices

Matrix	Analyte	Linear range [µg/mL]	Regression equation	Correlation coefficient r	No of standards
solvent	AE F054014	0.0001 to 0.5	$y = 16.45*10^6 x + 723$	0.9982	12
citrus fruit			$y = 7.81*10^6 x + 93$	0.9994	
solvent	AE F054014	0.0001 to 0.5	$y = 11.76*10^6 x + 1702$	0.9974	12
olive fruit			$y = 5.29*10^6 x + 467$	0.9991	
solvent	AE F054014	0.0001 to 0.5	$y = 13.21*10^6 x + 1370$	0.9968	12
wheat grain			$y = 6.15*10^6 x + 536$	0.992	
solvent	AE F054014 (m/z 132)	0.0001 to 0.5	$y = 12.33*10^6 x + 1095$	0.9974	12
wheat rest of plant			$y = 5.83*10^6 x + 339$	0.9995	

Repeatability:
Relative standard deviations were all well below 20 %. Details are given in the table below.

Limit of quantitation:
The limit of quantitation for AE F046360, AE F088406 and AE F054014 was established and validated at 0.01 mg/kg in wheat grain, wheat straw, olives and citrus.

Extraction efficiency
Not required as the MRLs are equal to the LOQ of the method.

Recovery rates:
Most mean recoveries for all three metabolites (AE F046360, AE F088406 and AE F054014) in all four matrices (cereal grain, cereal straw, olives and citrus) at the fortification levels 10 times LOQ were well within the 70–120% range. At the LOQ the mean recoveries are well in the range according to guidance document 825/00 (60-120%). The recovery rates for AE F046360, AE F088406, and AE F054014 were assayed for the transition [m/z] 168 → 132. The qualifier transition [m/z] 168 → 76 can also be used for accurate quantitation as demonstrated for AE F054014.

Recoveries of AE F046360, in cereal grain, cereal straw, olives and citrus fruits

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F046360 [m/z] 168 → 132	Grain	0.01	0.01	73	6	5
			0.1	75	5	5
AE F046360 [m/z] 168 → 132	Straw	0.01	0.01	75	5	5
			0.1	76	7	5
AE F046360 [m/z] 168 → 132	Olives	0.01	0.01	86	2	5
			0.1	82	4	5
AE F046360 [m/z] 168 → 132	Citrus	0.01	0.01	78	5	5
			0.1	78	5	5

Recoveries of AE F088406 in cereal grain, cereal straw, olives and citrus fruits

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F088406 [m/z] 168 → 132	Grain	0.01	0.01	73	2	5
			0.1	77	4	5
AE F088406 [m/z] 168 → 132	Straw	0.01	0.01	82	3	5
			0.1	83	4	5
AE F088406 [m/z] 168 → 132	Olives	0.01	0.01	69	5	5
			0.1	75	5	5
AE F088406 [m/z] 168 → 132	Citrus	0.01	0.01	78	2	5
			0.1	76	5	5

Recoveries of AE F054014 in cereal grain, cereal straw, olives and citrus fruits

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F054014 [m/z] 168 → 132	Grain	0.01	0.01	72	3	5
			0.1	71	2	5
AE F054014 [m/z] 168 → 76			0.01	73	6	5
			0.1	71	2	5
AE F054014 [m/z] 168 → 132	Straw	0.01	0.01	78	8	5
			0.1	75	6	5
AE F054014 [m/z] 168 → 76			0.01	81	9	5
			0.1	74	6	5
AE F054014 [m/z] 168 → 132	Olives	0.01	0.01	72	6	5
			0.1	77	3	5
AE F054014 [m/z] 168 → 76			0.01	71	3	5
			0.1	76	3	5
AE F0054014 [m/z] 168 → 132	Citrus	0.01	0.01	74	5	5
			0.1	74	2	5
AE F0054014 [m/z] 168 → 76		0.01	0.01	71	4	5
			0.1	75	4	5

Conclusion

The residue analytical method 00874/M001, a common moiety method, is suitable to determine residues of AE F046360 and its metabolites in cereal grain, cereal straw, olives and citrus. The hydrolysis product (AE F0054014) of AE F046360 and AE F088406 is only covered by one transition. However, two transitions are performed determining AE F0054014 after hydrolysis.

The method is considered as common moiety. The final analyte AE F054014 is not enantio-selective and not only specific to fenoxaprop-P-ethyl.

Independent Laboratory validation of analytical method 00874/M001

Principle of the method

The objective of this study was to independently validate the method 00874/M001 in tomato and wheat grain at a limit of quantitation at 0.01 mg/kg according to the European requirements. Control samples were fortified in the same matter with AE F046360 (fenoxaprop-P-ethyl) and its metabolites (AE F088406 and AE F054014) at the limit of quantitation and at ten times this limit and analysed according to method 00874/M001.

No contacts to the developers of the original method were necessary.

Validation

Specificity
No apparent residues or interferences were detected in control samples (i.e. apparent residues were < 20% of LOQ). Fragmentation from [m/z] 168 → 132 was used for quantification, [m/z] 168 → 76 is used as qualifier transition. As demonstrated in method 00874/M001 transition [m/z] 168 → 76 can also be used for quantification. Therefore this LC-MS/MS detection is considered to be highly specific and the development of an additional confirmatory method is not necessary.

Repeatability:
At each level the repeatability, expressed as the relative standard deviation (RSD) was ≤ 20%. The overall relative standard deviation (RSD) was lower than 20%.

Limit of quantitation:
The limit of quantitation for AE F046360, AE F088406 and AE F054014 was validated at 0.01 mg/kg in cereal grain and tomato.

Recovery rates:
At each level the mean recovery were between 70% and 110%. Details are given in the following table.

Recoveries of AE F046360, AE F088406 and AE F054014 in tomato

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F046360 [m/z] 168 → 132	Tomato	0.01	0.01	93	6	5
			0.1	93	9	5
AE F054014 [m/z] 168 → 132		0.01	0.01	86	9	5
			0.1	91	13	5
AE F088406 [m/z] 168 → 132		0.01	0.01	76	6	5
			0.1	87	7	5

Recoveries of AE F046360, AE F088406 and AE F054014 in cereal grain

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F046360 [m/z] 168 → 132	Grain	0.01	0.01	84	11	5
			0.1	93	16	5
AE F054014 [m/z] 168 → 132		0.01	0.01	103	12	5
			0.1	89	2	5
AE F088406 [m/z] 168 → 132		0.01	0.01	76	6	5
			0.1	86	11	5

Linearity:
The quantitative determination was carried out by external standardization with calibration standards in matrix. Calibration functions ranging from 0.4 ng/mL to 25 ng/mL at 6 different concentrations were used to evaluate the extracts. Linear calibration functions with 1/x weighting were calculated and plotted by regression analysis. The correlation coefficients were always > 0.99.

Conclusion

Method 00874/M001 was successfully independently validated according to the European requirements (Council directive 91/414/EEC Annex II (Part A, Section 4.2) and SANCO/825/00 rev. 8.1) for the determination of fenoxaprop-P-ethyl (AE F046360) and its metabolites (AE F088406 and AE F054014) in wheat grain and tomato at a limit of quantitation (LOQ) of 0.010 mg/kg and at ten times this limit. Therefore, the method 00874/M001 is considered applicable as a residue analytical method as well as for enforcement purposes.

Only one transition is validated for the common moiety product AE F054014. However, an additional ion is monitored at 76 m/z and can be observed in the chromatograms.

The method is considered as common moiety. The final analyte AE F054014 is not enantio-selective and not only specific to fenoxaprop-P-ethyl.

In addition a sufficiently validated GC-MS method for grain (LOQ 0.01 mg/kg) was provided for the first inclusion procedure following the guidance document SANCO 825/00 rev.7.

Method 01316

Reference:	**Validation of analytical BCS method 01316 for the determination of residues of fenoxaprop-P-ethyl and its metabolites fenoxaprop-P and AE F054014 (using LC-MS/MS in animal tissues: liver, kidney, muscle, milk, egg, fat)**
Author(s), year:	Konrad, St.; Neuland, M.; 2011
Report/Doc. number:	P603117507 / M-404091-01-1
Guideline(s):	91/414/EEC, SANCO/3029/99, SANCO/825/00, OECD, ENV/JM/Mono
GLP:	yes

Reference:	**Independent method validation of BCS method 01316 for the determination of residues of fenoxaprop-p-ethyl and its metabolites in animal tissues**
Author(s), year:	Lakaschus, S.; Amann, S.; 2011
Report/Doc. number:	01316 / M-411523-01-1
Guideline(s):	91/414/EEC, 96/68/EC, SANCO/3029/99, SANCO/825/00 rev. 8.1 ,OPPTS 860.1340
GLP:	yes

Principle of the method

The analytical BCS method 01316 was validated for the determination of the residues of AE F046360, AE F088406 and AE F054014 in whole milk, muscle, kidney, liver, fat and whole egg.
Specimen material was extracted under acidic organic conditions (acetonitrile / concentrated hydrochloric acid (9+1, v+v)) by refluxing for 5 hours. At these conditions AE F046360 and AE F088406 are converted into AE F054014. After clean up the residue extract was then analysed for residues of AE F054014 by HPLC-MS/MS for two mass transitions. Mass transition m/z 168 → 132 was used for quantitation, mass transition m/z 168 → 76 was used for confirmation.

Validation

Limit of quantitation
The limit of detection (LOD) was 0.003 mg/kg and the limit of quantitation (LOQ) was 0.01 mg/kg (expressed as AE F046360, valid for both mass transitions) in whole milk, muscle, kidney, liver, fat and whole egg.

Linearity
The LC-MS/MS system was tested to be linear between 0.200 ng/mL (m/z 168→132), 0.500 ng/mL (m/z 168→76), respectively, to 100 ng/mL for AE F054014 in solvent (expressed as AE F046360).

Matrix-matched standards and standards in solvent (acetonitrile / water (1+1, v+v)) were compared.
The tests showed a signal depression for all matrices (except fat). It was decided to use matrix-matched standards (at 2 concentration levels, 4.0 and 20.0 ng/mL) for all matrices in order to compensate all possible matrix effects.

The calibration data were provided for the solvent based standards only:

Linearity data for AE F046360 in solvent

Matrix	Analyte	Linear range [ng/mL]	Regression equation	Correlation coefficient r	No of standards
Solvent	AE F046360 m/z 168→132	0.200 – 100.0	y =2847.5 x + 2047.2	1.000	9
Solvent	AE F046360 m/z 168→76	0.500 – 100.0	y =710.1 x + 659.0	1.000	8

Specificity
No signals interfered with the analytical target AE F054014 allowing a specific determination of residues of AE F046360 (AE F046360, AE F088406 and AE F054014 including conjugates) in the tested matrices.
Further, the control samples (two-fold determination of each matrix) contain no residues of AE F046360, AE F088406 and AE F054014 above the limit of detection.
Matrix effects were observed for all matrices (except fat). It was decided to use matrix-matched standards for all matrices in order to compensate all possible matrix effects.

Extraction efficiency

Feeding study *Residues of Fenoxaprop-P-ethyl and its Metabolites in Eggs and Tissues of Laying Hens* (KCA 6.4.1/01 Bull, A. D. 2016) reveals in the analytical method that "the analytical method comprised of extraction and hydrolysis of the sub-sample with acetonitrile:hydrochloric acid (90:10 v:v). During this process Fenoxaprop-P-ethyl, Fenoxaprop-P and conjugates are extracted and hydrolysed to produce an extract of the common moiety benzoxazolone."

The extraction solvent is the same as used in Method 01316 therefore this issue is addressed.

Recovery and precision

A series of recovery experiments were performed by fortifying control (untreated) specimens of the matrices whole milk, muscle, kidney, liver, fat and whole egg. AE F046360, AE F088406 and AE F054014 were fortified separately. Fortifications were performed at the limits of quantitation and ten times that level. The results are displayed in the tables below for AE F046360, AE F088406 and AE F054014.

Mean recovery values obtained for both fortification levels (LOQ and ten times LOQ) are in the range of 70 – 110% as recommended by SANCO Guideline 825/00 with two exceptions. For AE F046360 in fat at 10 times LOQ a mean recovery rate of 68% (quantitation mass transition) was obtained. For AE F046360 in muscle at LOQ level a mean recovery rate of 69% (quantitation mass transition) was obtained. When corrected for one outlier (AE F046360 in muscle at LOQ; recovery in one sample was 62% (168 --> 132; quantitation) the recovery rate in muscle is 71% with RSD = 1%. The overall mean recoveries for AE F046360 per sample material were ≥ 70% and the overall RSDs ≤ 1 % (n=10).

With respect to the complex sample preparation procedure the mean recovery for AE F046360 68% in fat at 10 times LOQ level was accepted because the mean value is just below 70%, overall mean recoveries with n=10 for both fortification levels are ≥ 70% for AE F046360 and because of a high precision with relative standard deviation < 10%. The method was therefore considered to be valid for the determination of AE F046360, AE F088406 and AE F54014 residues in whole milk, fat, muscle, kidney, whole egg and liver.

Recoveries obtained for fortified AE F046360

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F046360 [m/z] 168 → 132	whole milk	0.01	0.01	70	9	5
			0.1	72	1	5
AE F046360 [m/z] 168 → 76		0.01	0.01	67	10	5
			0.1	73	2	5
AE F046360 [m/z] 168 → 132	muscle	0.01	0.01	69 (71*)	6 (1*)	5 (4*)
			0.1	76	6	5
AE F046360 [m/z] 168 → 76		0.01	0.01	68	5	5
			0.1	76	5	5
AE F046360 [m/z] 168 → 132	kidney	0.01	0.01	72	3	5
			0.1	79	6	5
AE F046360 [m/z] 168 → 76		0.01	0.01	73	5	5
			0.1	79	7	5
AE F046360 [m/z] 168 → 132	liver	0.01	0.01	75	4	5
			0.1	72	4	5
AE F046360 [m/z] 168 → 76		0.01	0.01	73	2	5
			0.1	73	4	5
AE F046360 [m/z] 168 → 132	fat	0.01	0.01	72	3	5
			0.1	68	6	5
AE F046360 [m/z] 168 → 76		0.01	0.01	72	2	5
			0.1	67	7	5
AE F046360 [m/z] 168 → 132	whole egg	0.01	0.01	82	9	5
			0.1	71	5	5
AE F046360 [m/z] 168 → 76		0.01	0.01	81	7	5
			0.1	72	6	5

* Values in brackets are corrected for one outlier according to Grubbs test.

Recoveries obtained for fortified AE F088406

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F088406 [m/z] 168 → 132	whole milk	0.01	0.01	73	1	5
			0.1	81	2	5
AE F088406 [m/z] 168 → 76		0.01	0.01	70	4	5
			0.1	81	3	5
AE F088406 [m/z] 168 → 132	muscle	0.01	0.01	82	3	5
			0.1	80	6	5
AE F088406 [m/z] 168 → 76		0.01	0.01	80	3	5
			0.1	81	6	5
AE F088406 [m/z] 168 → 132	kidney	0.01	0.01	81	3	5
			0.1	76	6	5
AE F088406 [m/z] 168 → 76		0.01	0.01	81	5	5
			0.1	78	7	5
AE F088406 [m/z] 168 → 132	liver	0.01	0.01	97	11	5
			0.1	83	4	5
AE F088406 [m/z] 168 → 76		0.01	0.01	97	11	5
			0.1	85	6	5
AE F088406 [m/z] 168 → 132	fat	0.01	0.01	82	3	5
			0.1	82	1	5
AE F088406 [m/z] 168 → 76		0.01	0.01	82	4	5
			0.1	82	1	5
AE F088406 [m/z] 168 → 132	whole egg	0.01	0.01	86	6	5
			0.1	87	2	5
AE F088406 [m/z] 168 → 76		0.01	0.01	88	3	5
			0.1	86	4	5

Recoveries obtained for AE F054014

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F054014 [m/z] 168 → 132	whole milk	0.01	0.01	80	2	5
			0.1	73	3	5
AE F054014 [m/z] 168 → 76		0.01	0.01	82	5	5
			0.1	72	2	5
AE F054014 [m/z] 168 → 132	muscle	0.01	0.01	80	2	5
			0.1	81	2	5
AE F054014 [m/z] 168 → 76		0.01	0.01	78	1	5
			0.1	81	3	5
AE F054014 [m/z] 168 → 132	kidney	0.01	0.01	73	11	5
			0.1	72	14	5
AE F054014 [m/z] 168 → 76		0.01	0.01	73	11	5
			0.1	72	14	5
AE F054014 [m/z] 168 → 132	liver	0.01	0.01	81	3	5
			0.1	85	3	5
AE F054014 [m/z] 168 → 76		0.01	0.01	81	5	5
			0.1	85	5	5
AE F054014	fat	0.01	0.01	83	3	5

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
[m/z] 168 → 132			0.1	82	2	5
AE F054014 [m/z] 168 → 76		0.01	0.01	84	7	5
			0.1	82	3	5
AE F054014 [m/z] 168 → 132	whole egg	0.01	0.01	84	7	5
			0.1	82	3	5
AE F054014 [m/z] 168 → 76		0.01	0.01	100	8	5
			0.1	83	2	5

Conclusion
The data presented demonstrate that the method permits the determination of residues of AE F046360, AE F088406 and AE F054014 in/on whole milk, fat, muscle, kidney, whole egg and liver with satisfactory specificity, accuracy, precision and repeatability according to the guideline SANCO/825/00 rev. 8.1. However, the calibration is not adequate. The solvent based standards showed signal depressions for all matrices (except fat). The matrix matched standards were performed only at 2 concentration levels (4.0 and 20.0 ng/mL) which is not guidance conform.
Due to insufficient calibration data of the primary method (Konrad, St.; Neuland, M.; 2011) RMS suggests that ILV (Lakaschus, S.; Amann, S.; 2011) is used as primary method (and vice versa).

The method is considered as common moiety. The final analyte AE F054014 is not enantio-selective and not only specific to fenoxaprop-P-ethyl.

Independent Laboratory validation of analytical method 01316

Principle of the method

The analytical method 01316 was validated in an independent method validation for the determination of AE F046360 and its metabolites AE F088406 and AE F054014 in animal tissues. The analytes were extracted from liver, kidney, meat, milk, fat and egg under acidic organic conditions (acetonitrile/concentrated hydrochloric acid (9/1, v/v)) by refluxing for 5 hours. An aliquot of the extract was taken and cleaned up. The solution was subjected to LC-MS/MS and the residues were quantified using one-point external standard calibration.
No contact with the developer of the method was necessary. Minor adjustments to adapt the method to the equipment of the preforming laboratory were done but had no influence on the results of the study. For confirmation of the individual residues a 2nd MRM transition was used.

Validation

Limit of quantitation
The limit of quantitation (LOQ) for each single analyte expressed as AE F046360 was 0.01 mg/kg in all matrices tested. The limit of determination (LOD) was fixed at 0.003 mg/kg.

Linearity
During the ILV both, solvent matched linearity and matrix matched linearity curves, were determined for each matrix. Matrix effects were found to be minor. Nevertheless, to ensure comparability with the original method, matrix matched standards were used for quantitation within this study.
The correlation between the injected amount of AE F054014 (calculated as AE F046360) and the detector response was linear for solvent standards ranging from 1.07 ng/mL to 107 ng/mL. The correlation coefficients were >0.9980.

Linearity data for AE F054014 in solvent

Matrix	Analyte	Linear range [ng/mL]	Regression equation	Correlation coefficient r	No of standards
Solvent	AE F054014 m/z 168→132 (quantitation)	1.07 – 107.0	y =3056 x + 1753.2	0.9997	6
Solvent	AE F054014	1.07 – 107.0	y =744.77 x + 178.69	0.9999	6

	m/z 168→76 (confirmation)				

Linearity data for AE F046360 in different matrices

Matrix	Analyte	Linear range [ng/mL]	Regression equation	Correlation coefficient r	No of standards
liver	AE F054014 m/z 168→132 (quantitation)	1.07 – 107.0	y =2547.7 x - 5030.1	0.997	6
kidney			y =2687.2 x + 842.92	0.9996	
meat			y =3333.7 x – 1832.4	0.9997	
milk			y =2796.7 x + 375.32	0.9999	
fat			y =2932.6 x + 64.058	1.00	
egg			y =2926.7 x + 950.39	0.9997	
liver	AE F054014 m/z 168→76 (confirmation)	1.07 – 107.0	y =601.85 x – 1108.5	0.9977	6
kidney			y =647 x + 3.5742	0.9995	
meat			y =793.33 x – 274.42	0.9999	
milk			y =649.51 x – 102.39	1.00	
fat			y =682.87 x + 124.38	1.00	
egg			y =691.12 x + 311.43	0.9997	

Precision

As a measure for the precision of the method, the intra-laboratory repeatability (n = 5) is given as relative standard deviation (% RSD) for all sample materials at fortification levels of 0.01 and 0.1 mg/kg. The RSD of the repeatability tests at each recovery set were < 20%, except for the confirmation of liver where the overall RSD for AE F054014 was 21%.

Specificity

The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection. Apparent residues in control samples were below 0.3 × LOQ. The recoveries were not corrected for interferences. Two MRM transitions were monitored for AE F054014 and each matrix tested. Therefore, the HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary.

Recovery

Mean recoveries for each fortification level were within the 70 - 110% range for all matrices and each transition.

Recoveries obtained for AE F046360 (quantitation and confirmation)

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F046360 [m/z] 168 → 132	Liver	0.01	0.01	101	6.2	5
			0.1	95	4.0	5
AE F046360 [m/z] 168 → 76		0.01	0.01	105	6.6	5
			0.1	94	4.3	5
AE F046360 [m/z] 168 → 132	Kidney	0.01	0.01	97	4.6	5
			0.1	97	3.0	5
AE F046360 [m/z] 168 → 76		0.01	0.01	97	5.0	5
			0.1	96	4.2	5
AE F046360 [m/z] 168 → 132	Meat	0.01	0.01	89	7.2	5
			0.1	88	1.9	5
AE F046360 [m/z] 168 → 76		0.01	0.01	91	9.6	5
			0.1	89	3.8	5
AE F046360 [m/z] 168 → 132	Milk	0.01	0.01	89	1.7	5
			0.1	83	6.0	5
AE F046360 [m/z] 168 → 76		0.01	0.01	89	5.0	5
			0.1	84	7.0	5
AE F046360 [m/z] 168 → 132	Egg	0.01	0.01	99	3.4	5
			0.1	96	0.7	5
AE F046360		0.01	0.01	100	7.1	5

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
[m/z] 168 → 76			0.1	95	1.7	5
AE F046360 [m/z] 168 → 132	Fat	0.01	0.01	100	3.3	5
			0.1	104	1.6	5
AE F046360 [m/z] 168 → 76		0.01	0.01	101	5.5	5
			0.1	105	2.6	5

Recoveries obtained for AE F088406 (quantitation and confirmation)

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F088406 [m/z] 168 → 132	Liver	0.01	0.01	104	7.7	5
			0.1	81	14	5
AE F088406 [m/z] 168 → 76		0.01	0.01	105	7.5	5
			0.1	80	15	5
AE F088406 [m/z] 168 → 132	Kidney	0.01	0.01	72	6.7	5
			0.1	72	10	5
AE F088406 [m/z] 168 → 76		0.01	0.01	73	4.8	5
			0.1	71	12	5
AE F088406 [m/z] 168 → 132	Meat	0.01	0.01	76	2.2	5
			0.1	75	4.7	5
AE F088406 [m/z] 168 → 76		0.01	0.01	81	11	5
			0.1	74	7.0	5
AE F088406 [m/z] 168 → 132	Milk	0.01	0.01	71	6.4	5
			0.1	72	7.0	5
AE F088406 [m/z] 168 → 76		0.01	0.01	71	2.5	5
			0.1	70	4.5	5
AE F088406 [m/z] 168 → 132	Egg	0.01	0.01	79	5.3	5
			0.1	86	3.0	5
AE F088406 [m/z] 168 → 76		0.01	0.01	78	6.4	5
			0.1	86	2.2	5
AE F088406 [m/z] 168 → 132	Fat	0.01	0.01	82	6.0	5
			0.1	88	6.3	5
AE F088406 [m/z] 168 → 76		0.01	0.01	80	6.4	5
			0.1	88	7.4	5

Recoveries obtained for AE F054014 (quantitation and confirmation)

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F054014 [m/z] 168 → 132	Liver	0.01	0.01	103	12	5
			0.1	79	9.2	5
AE F054014 [m/z] 168 → 76		0.01	0.01	108	14	5
			0.1	78	9.5	5
AE F054014 [m/z] 168 → 132	Kidney	0.01	0.01	91	11	5
			0.1	85	5.9	5
AE F054014 [m/z] 168 → 76		0.01	0.01	89	8.2	5
			0.1	85	5.7	5
AE F054014 [m/z] 168 → 132	Meat	0.01	0.01	84	12	5
			0.1	95	3.0	5
AE F054014 [m/z] 168 → 76		0.01	0.01	84	12	5
			0.1	94	4.8	5

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F054014 [m/z] 168 → 132	Milk	0.01	0.01	107	5.8	5
			0.1	103	9.2	5
AE F054014 [m/z] 168 → 76		0.01	0.01	110	5.4	5
			0.1	100	9.5	5
AE F054014 [m/z] 168 → 132	Egg	0.01	0.01	109	3.5	5
			0.1	101	14	5
AE F054014 [m/z] 168 → 76		0.01	0.01	108	1.3	5
			0.1	98	14	5
AE F054014 [m/z] 168 → 132	Fat	0.01	0.01	90	3.1	5
			0.1	85	7.5	5
AE F054014 [m/z] 168 → 76		0.01	0.01	91	4.7	5
			0.1	85	7.1	5

Conclusion

The method meets all guideline criteria (SANCO/825/00) to determine residues of AE F046360 and its metabolites AE F088406 and AE F054014 in animal tissues at 0.01 mg/kg.

The ILV was performed successfully and the method was applied as described by the method developers. Therefore method 01316 is applicable to determine residues of AE F046360 in animal tissue monitoring samples.

The method is considered as common moiety. The final analyte AE F054014 is not enantio-selective and not only specific to fenoxaprop-P-ethyl.

Due to insufficient calibration data of the primary method (Konrad, St.; Neuland, M.; 2011) RMS suggests that ILV (Lakaschus, S.; Amann, S.; 2011) should be used as primary method (and vice versa).

B.5.2.2. Methods for the determination of all components included for monitoring purposes in the residue definitions for soil and water

Table B.5.2.2-2 Analytical monitoring methods for the determination of AE F046360 residues in environmental matrices

Method	Doc. No.	Chapter link	Analytes	Matrix (LOQ)[1]	Technique	Year
ENC-8/91 ENC-4/92 (evaluated)	M-132776-01-1	KCA 4.2/01 KCA 4.1.2/05	AE F046360 AE F088406 AE F054014	- soil (0.01 or 0.02 mg/kg)	HPLC-UV	1993
01056 (evaluated but not peer reviewed: addendum B.5 rev.2 of DAR 2005)	M-291683-01-1	KCA 4.2/15	AE F046360 AE F088406 AE F054014	- soil (0.01 mg/kg)	HPLC-MS/MS	2007
EM F10/99-0 (evaluated)	M-192979-01-1	KCA 4.2/03 KCA 4.1.2/07	AE F046360 AE F088406 AE F054014	- drinking water (0.1 µg/L)	HPLC-UV	1999
validation to EM F10/99-0 (evaluated)	M-197369-01-1	KCA 4.2/04 KCA 4.1.2/08	AE F046360 AE F088406 AE F054014	- drinking water (0.1 µg/L) - surface water (1 µg/L)	HPLC-UV	2000
(evaluated)	M-133552-01-2	KCA 4.2/05 KCA 4.1.2/09	AE F046360	- air (5.05 ng/L)	HPLC-UV	1994
(evaluated)	M-247508-01-1 addendum to M-133552-01-2	KCA 4.2/06 KCA 4.1.2/10	AE F046360	addition information to calibration was provided	HPLC-UV	2005
(evaluated)	M-261234-01-1	KCA 4.2/14	AE F046360	- air (1.0 µg/m^3)	HPLC-MS/MS	2005
(evaluated)	M-261230-01-1	KCA 4.2/13	AE F088406	- air (1.0 µg/m^3)	HPLC-MS/MS	2005
01357 (new method)	M-445048-01-1	KCA 4.2/21	AE F046360 AE F088406	- surface water (0.05 µg/L)	HPLC-MS/MS	2013
ILV to 01357 (new method)	M-533252-01-1	KCA 4.2/22	AE F046360 AE F088406	- surface water (0.05 µg/L)	HPLC-MS/MS	2015

[1] - LOQ : limit of quantification.

Method 01357

Reference:	**Analytical method 01056 for the determination of fenoxaprop-P-ethyl (AE F046360) and its metabolites AE F088406 and AE F054014 in soil using LC/MS/MS**
Author(s), year:	Freitag T., (2007)
Report/Doc. number:	M-291683-01-1
Guideline(s):	EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC -European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 -Guidance document on residue analytical methods; SANCO/825/00 rev. 7, -European Commission, Directorate General Health and Consumer Protection, 2004-03-17 -US EPA Residue Chemistry Test Guideline OPPTS 860.1340: Residue Analytical Method
GLP:	yes

Principle of the method

Soil samples of 20 g are extracted in a microwave extractor with 40 mL of a mixture of water/acetonitrile (1/1 v/v). Then a subsample is centrifuged to remove fine particles of soil and evaporated to dryness at 45 °C for 30 minutes and redissolved in 1 mL methanol/water (containing 10 mmol/L ammoniumformiate and formic acid) 10/90 v/v. Identification and quantitation of the test items is done by high performance liquid chromatography [column: Phenomenex Luna C18(2)-HST 50 x 2 mm 2.5 µm, with guardcolumn; gradient mobile phase A: methanol/water(containing 10 mmol/L ammonium formiate and formic acid) (10/90 v/v) and mobile phase B: methanol/water (containing 10 mmol/L ammonium formiate and formic acid) (90/10 v/v)] using MS/MS detection in the Multiple Reaction Monitoring mode (two transitions).

Determined analyte	**Q1 [m/z]**	**Q3 [m/z]** for quantification	**Q3 [m/z]** for confirmation
Fenoxaprop-ethyl (AE F033171)	362	288	77
Fenoxaprop (AE F053022)	332	260	152
Chlorobenzoxazolone (AE F054014)	168	132	76

Due to the fact that no enatio-selective LC-column was used the analytes determined are fenoxaprop-ethyl and fenoxaprop.

Characteristics of the soils used:

Description	**Soil Höfchen** Plot 4011 0-30 cm soil layer	**Soil Laacher Hof** Plot 712/718 0-30 cm soil layer
pH (in $CaCl_2$ solution)	6.7	6.8
pH (in H_2O)	7.4	7.4
Organic Carbon [%]	0.92	1.20
Organic Matter [%] *	1.58	2.06
Cation Exchange Capacity [meq / 100 g dry soil]	12.4	9.8
max. Water Holding Capacity [g / 100 g dry soil]	39.5	37.9
Textural Description according to USDA [Fraction %]	**Fraction [%]**	**Fraction [%]**
Clay (<0.002 mm)	19.4	12.0
Silt (0.002-0.050 mm)	76.3	18.3
Sand (0.050-2.000 mm)	4.3	69.7
Soil type	Silt loam	Sandy loam

* Organic matter = Organic carbon x 1.72

Validation

Specificity

The blank values of all analytes tested in each soil type were below 30 % of the LOQ. Chromatograms of the control samples are available. No confirmatory technique is required since LC-MS/MS is highly specific.

Linearity
The chromatographic response was shown to be linear over the range of concentrations from 1 to 100 µg/L at 5 levels for each compound (r > 0.99).

Recovery
Mean recoveries are between 70-110 % at each fortification level with a RSD < 20 %.

Analyte	**Matrix**	**LOQ [mg/kg]**	**Fortification level [mg/kg]**	**Mean recovery [%]**	**RSD [%]**	**n**
Fenoxaprop-ethyl (AE F033171) m/z 362 ➔ m/z 288	Soil (silt loam)	0.01	0.01	85	2.8	5
			0.1	78	4.9	5
	Soil (sandy loam)	0.01	0.01	88	2.7	5
			0.1	77	1.5	5
Fenoxaprop-ethyl (AE F033171) m/z 362 ➔ m/z 77	Soil (silt loam)	0.01	0.01	83	3.2	5
			0.1	75	4.7	5
	Soil (sandy loam)	0.01	0.01	84	3.4	5
			0.1	74	1.0	5
Fenoxaprop (AE F053022) m/z 332 ➔ m/z 260	Soil (silt loam)	0.01	0.01	93	5.2	5
			0.1	94	4.3	5
	Soil (sandy loam)	0.01	0.01	92	2.1	5
			0.1	96	1.1	5
Fenoxaprop (AE F053022) m/z 332 ➔ m/z 152	Soil (silt loam)	0.01	0.01	93	3.8	5
			0.1	92	6.0	5
	Soil (sandy loam)	0.01	0.01	93	4.5	5
			0.1	96	2.6	5
Chlorobenzoxazolone (AE F054014) m/z 168 ➔ m/z 132	Soil (silt loam)	0.01	0.01	87	3.2	5
			0.1	88	4.1	5
	Soil (sandy loam)	0.01	0.01	91	2.4	5
			0.1	93	3.3	5
Chlorobenzoxazolone (AE F054014) m/z 168 ➔ m/z 76	Soil (silt loam)	0.01	0.01	88	7.1	5
			0.1	87	3.0	5
	Soil (sandy loam)	0.01	0.01	95	3.2	5
			0.1	94	1.2	5

LOQ
0.01mg/kg (10 µg/kg) for all soil types and analytes.

Conclusion
The LC/MS/MS based analytical method was successfully validated for the determination of fenoxaprop-ethyl and the metabolites fenoxaprop and AE F054014 in two types of soil with a limit of quantification of 0.01 mg/kg.

The method is in accordance with the current residue definition for soil [i.e. Fenoxaprop-ethyl and the sum of all components which give chlorobenzoxazolone (AE F054014) metabolites expressed as fenoxaprop-P-ethyl].

Method 01357

Reference:	**Analytical method 01357 for the determination of fenoxaprop-p-ethyl and its metabolite fenoxaprop-p in drinking and surface water by HPLC-MS/MS**
Author(s), year:	Braune, M.; 2013
Report/Doc. number:	MR-12/103 / M-445048-01-1
Guideline(s):	not specified
GLP:	no

Reference:	**Independent laboratory validation of the BCS analytical method 01357 for the determination of fenoxaprop-P-ethyl and its metabolite fenoxaprop-P in surface water by HPLC-MS/MS**
Author(s), year:	Thies, S.; 2015
Report/Doc. number:	M-533252-01-1
Guideline(s):	REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99. Guidance document on residue analytical methods; SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection; 2010-11-16. OECD Guidance Document on Pesticide Residue analytical Methods; ENV/JM/Mono (2007); 2007-08-13 US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method
GLP:	yes

The method 01357 describes the determination of AE F046360 and its metabolite AE F088406 in drinking and surface water by HPLC-MS/MS using two MRM transitions.

Principle of the method

The water samples are analysed by direct injection into an HPLC-MS/MS instrument or injected after appropriate dilution with test water using the positive ion mode for both substances without further clean-up. Concentrations were quantified using external matrix-matched standard solutions to eliminate matrix effects. A validation for drinking water was not necessary because the limit of quantitation for surface water is below the drinking water limit of 0.1 µg/L.

Characteristics of the Surface Water From River Rhine, Sampled on 2009-07-29 in Leverkusen-Hitdorf (Germany):

Parameter	Value
Total organic carbon (TOC)	2 mg/L
Dissolved organic carbon (DOC)	2 mg/L
Conductivity	448 µS/cm
pH	7.3
Water hardness	9.9 °dH
Filterable solids	14 mg/L
Dry residue after filtration	290 mg/L

Validation

Limit of quantitation
The limit of quantitation (LOQ) for AE F046360 and AE F088406 is 0.05 µg/L in surface water.

Linearity
A good linear correlation between the injected amount of the respective analyte in surface water and the detector response was observed in the concentration ranges mentioned below.

Linearity data for AE F046360 and its metabolite in surface water

Matrix	Analyte	Linear range [µg/L]	Regression equation	Correlation coefficient r (1/x weighted)	No of standards
surface water	AE F046360 m/z 362→244 (quantitation MRM)	0.016 – 10.0	$y = 7.79*10^4 x - 62.8$	0.9994	≥ 5
	AE F046360 m/z 362→288 (confirmation MRM)	0.016 – 1.0	$y = 7.86*10^5 x – 0.0045$	0.9994	
	AE F088406 m/z 334→288 (quantitation MRM)	0.016 – 10.0	$y = 6.51*10^4 x + 155$	0.9996	
	AE F088406 m/z 334→244 (confirmation MRM)	0.016 – 10.0	$y = 3.18*10^4 x – 74.1$	0.9995	

Precision

The repeatability for both substances was determined based on 10 injections of a standard solution of 0.05 µg/L and 10 injections of a standard solution of 0.5 µg/L in test water. The relative standard deviations for the peak areas and the relative standard deviations for the retention times are displayed below.

Method validation of AE F046360 and AE F088406 in test water

Fortification level [µg/L]	Peak area		Retention time	
	Mean value	RSD [%]	Mean [min]	RSD [%]
AE F046360 m/z 362 --> 244 (quantitation)				
0.05	3923	6.1	3.32	0.2
0.5	41391	3.8	3.32	0.1
AE F046360 m/z 362 --> 288 (confirmation)				
0.05	36132	6.1	3.31	0.1
0.5	390816	4.8	3.32	0.2
AE F088406 m/z 334 --> 288 (quantitation)				
0.05	3516	5.2	2.68	0.2
0.5	34258	5.5	2.68	0.2
AE F088406 m/z 334 --> 244 (confirmation)				
0.05	1488	4.7	2.68	0.2
0.5	16610	5.3	2.68	0.1

Specificity

The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection. Apparent concentrations in control samples were below 0.3 × LOQ. Two MRM transitions were monitored for AE F046360 (m/z 362 → m/z 244 for quantitation and m/z 362 → m/z 288 for confirmation) and for AE F088406 (m/z 334 → m/z 288 for quantitation and m/z 334 → m/z 244 for confirmation). Therefore, the HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary.

The MS/MS detection is affected by the matrix. Peak area decrease for the 1st and 2nd mass transition down to 65% compared to the peak area in deionizied water was observed for fenoxaprop-P-ethyl and down to 92% for fenoxaprop-P. Matrix effects can be eliminated by using matrix-matched standard solutions.

Recovery (Accuracy)

As the water samples were analysed by direct injection into the HPLC-MS/MS system recovery data cannot be calculated as stated in the guidance document on pesticide residue analytical methods (SANCO/825/00 rev.8.1).

Conclusion

In conclusion, the results show that the assay method presented above is satisfactory and further fulfils the requirements of the SANCO/825/00 rev. 8.1.

Independent Laboratory validation of analytical method 01357

The analytical method 01357 was validated in an independent method validation for the determination of AE F046360 and its metabolite AE F088406 in surface water by HPLC-MS/MS using two MRM transitions.

Principle of the method

Water samples were determined by direct injection into the HPLC-MS/MS instrument after appropriate dilution using the positive ion mode for AE F046360 and AE F088406 without further clean-up.

Characteristics of the Surface Water From River Rhine, Sampled on 2015-01-13 inLeverkusen (Germany)

Parameter	Value
Total organic carbon (TOC)	3 mg/L
Dissolved organic carbon (DOC)	3 mg/L
Conductivity	453 µS/cm
pH	7.8
Water hardness	9.3 °dH
Filterable solids	33 mg/L
Dry residue after filtration	290 mg/L

Validation

Limit of quantitation
The limit of quantitation (LOQ) for AE F046360 and AE F088406 is 0.05 µg/L and the limit of detection (LOD) is 0.016 µg/L in surface water.

Linearity
Linearity data for AE F046360 and its metabolite in surface water

Matrix	Analyte	Linear range [µg/L]	Regression equation	Correlation coefficient r (1/x weighted)	No of standards
surface water	AE F046360 m/z 362→244 (quantitation)	0.016 – 10.0	$y = 2.05*10^5 x - 969$	0.9993	≥ 5
	AE F046360 m/z 362→288 (confirmation)		$y = 2.59*10^6 x – 1.51*10^4$	0.9996	
	AE F088406 m/z 334→288 (quantitation)		$y = 1.12*10^5 x - 645$	0.9994	
	AE F088406 m/z 334→244 (confirmation)		$y = 4.31*10^4 x - 107$	0.9993	

Precision
For method validation surface water was fortified with AE F046360 and AE F088406 (fortification levels 0.05 µg/L and 0.5 µg/L).
From each test solution five aliquots were taken and injected into the HPLC-MS/MS instrument. The relative standard deviations for the peak areas of AE F046360 for the quantification ion were 3.7% at 0.05 µg/L and 1.5% at 0.5 µg/L. The relative standard deviations for the peak areas of AE F088406 for the quantification ion were 6.5% at 0.05 µg/L and 2.0% at 0.5 µg/L. The relative standard deviations for the retention times were ≤ 0.2% for all analytes.
The relative standard deviations for the peak areas of AE F046360 for the confirmatory ion were 3.6% at 0.05 µg/L and 1.0% at 0.5 µg/L. The relative standard deviations for the peak areas of AE F088406 for the confirmatory ion were 5.5% at 0.05 µg/L and 4.6% at 0.5 µg/L. The relative standard deviations for the retention times were ≤ 0.2% for all analytes.

Precision data for AE F046360 and its metabolite in surface water

Fortification level [µg/L]	Peak area		Retention time	
	Mean value	RSD [%]	Mean [min]	RSD [%]
AE F046360 m/z 362 --> 244 (quantitation)				
0.05	9672	3.7	2.97	< 0.01
0.5	106200	1.5	2.97	< 0.01
AE F046360 m/z 362 --> 288 (confirmation)				
0.05	121000	3.6	2.97	< 0.01
0.5	1326000	1.0	2.97	< 0.01
AE F088406 m/z 334 --> 288 (quantitation)				
0.05	5160	6.5	2.38	≤ 0.2
0.5	57200	2.0	2.38	≤ 0.2
AE F088406 m/z 334 --> 244 (confirmation)				
0.05	1776	5.5	2.38	≤ 0.2
0.5	22020	4.6	2.38	≤ 0.2

Specificity

The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection. Apparent concentrations in control samples were below 0.3 × LOQ. Two MRM transitions were monitored for AE F046360 (m/z 362 → m/z 244 for quantitation and m/z 362 → m/z 288 for confirmation) and AE F088406 (m/z 334 → m/z 288 for quantitation and m/z 334 → m/z 244 for confirmation). Therefore, the HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary.

Recovery (Accuracy)

As the water samples were analysed by direct injection into the HPLC-MS/MS system recovery data cannot be calculated as stated in the guidance document on pesticide residue analytical methods (SANCO/825/00 rev.8.1).

Conclusion

In conclusion, the results show that the assay method presented above is satisfactory and further fulfils the requirements of the SANCO/825/00 rev. 8.1.

B.5.2.3. Methods for the analysis in air of the active substance and relevant breakdown products formed during or after application, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

There are no new methods submitted.

B.5.2.4. Methods for the analysis in body fluids and tissues for active substances and relevant metabolites

Table B.5.2.4-1 monitoring methods for the determination of AE F046360 residues in body fluids and tissues

Method	Doc. No.	Chapter link	Analytes	Matrix (LOQ)[1]	Technique	Year
EKN0020 (new method)	M-552660-01-1	KCA 4.1.2/15; KCA 4.2/23	AE F046360 AE F088406 AE F054014	- chicken egg (0.01 mg/kg) - muscle (0.01 mg/kg) - liver (0.01 mg/kg) - skin/fat (0.01 mg/kg) - blood (0.01 mg/kg)	HPLC-MS/MS	2016

[1] - LOQ : limit of quantification.

Reference:	**Fenoxaprop-P-ethyl and fenoxaprop-P: Validation of methodology for the determination of residues as the benzoxazolone metabolite in foodstuff of animal origin**
Author(s), year:	Burton, D.; 2016
Report/Doc. number:	ENK0020 / M-552660-01-1
Guideline(s):	Guidance Document on Residue Analytical Methods; SANCO/825/00 rev. 8.1 of 16 November 2010
GLP:	yes

The method was developed for the determination of the residues of AE F046360 (fenoxaprop-P-ethyl) and its metabolite AE F088406 as benzoxazolone metabolite (AE F054014) in foodstuff of animal origin (chicken eggs, tissue (muscle, liver, skin/fat) and blood) using HPLC-MS/MS monitoring two ion transitions.

Principle of the method

The analytical method comprised of extraction and hydrolysis with acetonitrile:hydrochloric acid (90:10 v:v) to produce an extract of the common moiety benzoxazolone (AE F054014). This was diluted with water and centrifuged. An aliquot of clear extract was washed with hexane, further diluted with water, then subjected to solid-phase-extraction (spe) clean-up using an HLB cartridge. Extracts of skin/fat were subjected to further spe clean-up using a PSA cartridge. Quantitation was performed using liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) monitoring two ion transitions to satisfy the confirmatory analysis requirement.
The analytical method was validated using the three reference materials AE F046360, AE F088406 and AE F054014 by fortifying at concentrations of 0.01 and 0.1 mg/kg in chicken egg, muscle, liver, skin/fat and blood and then subjecting these to the analytical procedure.
The ion transitions monitored for AE F054014 were *m/z* 168 > 132 and 170 > 132 for quantitation and confirmation purposes, respectively. The analysis of the validation samples using the confirmatory transition produced data comparable to that acquired monitoring the quantitation ion transition.
As the chosen technique for AE F054014 (LC-MS/MS with 2 ion transitions) is highly specific this method also addresses the confirmatory requirement and thus no separate confirmatory method is required.

Validation

Limit of quantitation (LOQ)
The limit of quantitation (LOQ) was determined to be 0.01 mg/kg in chicken egg, muscle, liver, skin/fat and blood for all analytes.
The limit of detection (LOD) is defined as the lowest calibration standard chromatographed having a measurable response. The LOD of the analytical system for AE F054014 was shown to be 0.01 ng/mL AE F054014 (approximately 0.001 mg/kg in chicken egg, muscle and liver and 0.002 mg/kg in skin/fat and blood expressed as AE F046360 equivalents).

Linearity

Linearity data for AE F054014 in different matrices

Matrix	Analyte	Linear range [ng/mL]	Regression equation	Correlation coefficient r	No of standards
egg	AE F054014 m/z 168→ 132 (quantitation)	0.01 - 1	y =18496.1 x + 101.82	0.9972	9
muscle			y =15679.6 x + 36.1019	0.9998	
liver			y =25740.7 x + 98.5468	1.00	
skin/fat			y =69667.6 x -116.504	0.9995	
blood			y =37492.6 x – 166.48	0.9991	
egg	AE F054014 m/z 170→ 132 (confirmation)	0.01 - 1	y =6227.46 x + 71.8269	0.9964	9
muscle			y =5446.14 x – 0.887783	0.9998	
liver			y =8429.48 x + 51.4559	0.9998	
skin/fat			y = 22953.8 x – 2.61731	0.9995	
blood			y = 12345.1 x – 58.8977	0.9993	

Specificity

The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection. Two untreated sub-samples of each matrix type per analytical batch were subjected to the analytical procedures. There were no interferences from these matrices observable under the monitoring conditions (blank values <30% of the limit of quantitation (LOQ)).

Matrix effects were assessed by comparing fortified extracts of control matrix to calibration standards prepared in solvent. Matrix effects were significant (>20%) for AE F054014 in the final extracts of each matrix type. Consequently chromatographic runs were calibrated using matrix matched calibration standards throughout.

Recovery (accuracy) and precision

The analytical method was validated by fortifying sub-samples of chicken egg, muscle, liver, skin/fat and blood with known concentrations of the analytes (0.01 mg/kg (LOQ) and 0.1 mg/kg).

Recovery and precision data obtained for AE F046360 (quantitation and confirmation)

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F046360 [m/z] 168 → 132	egg	0.01	0.01	92	3.0	5
			0.1	92	3.8	5
AE F046360 [m/z] 170 → 132		0.01	0.01	93	10.3	5
			0.1	87	1.7	5
AE F046360 [m/z] 168 → 132	muscle	0.01	0.01	102	4.5	5
			0.1	91	2.4	5
AE F046360 [m/z] 170 → 132		0.01	0.01	109	10.7	5
			0.1	91	4.6	5
AE F046360 [m/z] 168 → 132	liver	0.01	0.01	101	5.8	5
			0.1	102	2.3	5
AE F046360 [m/z] 170 → 132		0.01	0.01	105	5.5	5
			0.1	102	1.1	5
AE F046360 [m/z] 168 → 132	skin/fat	0.01	0.01	102	5.8	5
			0.1	97	5.7	5
AE F046360 [m/z] 170 → 132		0.01	0.01	103	7.3	5
			0.1	97	3.0	5
AE F046360 [m/z] 168 → 132	blood	0.01	0.01	96	4.9	5
			0.1	105	3.6	5
AE F046360 [m/z] 170 → 132		0.01	0.01	99	10.8	5
			0.1	108	2.9	5

Recovery and precision data obtained for AE F088406 (quantitation and confirmation)

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F088406 [m/z] 168 → 132	egg	0.01	0.01	91	8.8	5
			0.1	97	5.2	5
AE F088406 [m/z] 170 → 132		0.01	0.01	95	8.2	5
			0.1	97	3.9	5
AE F088406 [m/z] 168 → 132	muscle	0.01	0.01	86	6.4	5
			0.1	91	2.4	5
AE F088406 [m/z] 170 → 132		0.01	0.01	90	13.3	5
			0.1	90	4.6	5
AE F088406 [m/z] 168 → 132	liver	0.01	0.01	101	3.7	5
			0.1	104	7.1	5
AE F088406 [m/z] 170 → 132		0.01	0.01	101	6.1	5
			0.1	104	6.6	5
AE F088406 [m/z] 168 → 132	skin/fat	0.01	0.01	93	6.0	5
			0.1	97	5.4	5
AE F088406 [m/z] 170 → 132		0.01	0.01	102	5.2	5
			0.1	99	4.7	5
AE F088406 [m/z] 168 → 132	blood	0.01	0.01	75	5.7	5
			0.1	75	5.8	5
AE F088406 [m/z] 170 → 132		0.01	0.01	76	2.0	5
			0.1	77	6.2	5

Recovery and precision data obtained for AE F054014 (quantitation and confirmation)

Analyte	Matrix	LOQ	Fortification level [mg/kg]	Mean recovery [%]	RSD [%]	n
AE F054014 [m/z] 168 → 132	egg	0.01	0.01	95	6.4	5
			0.1	93	8.4	5
AE F054014 [m/z] 170 → 132		0.01	0.01	97	7.6	5
			0.1	95	4.6	5
AE F054014 [m/z] 168 → 132	muscle	0.01	0.01	103	3.2	5
			0.1	103	2.6	5
AE F054014 [m/z] 170 → 132		0.01	0.01	101	8.7	5
			0.1	104	4.2	5
AE F054014 [m/z] 168 → 132	liver	0.01	0.01	96	3.4	5
			0.1	97	2.0	5
AE F054014 [m/z] 170 → 132		0.01	0.01	97	6.8	5
			0.1	94	4.0	5
AE F054014 [m/z] 168 → 132	skin/fat	0.01	0.01	100	1.8	5
			0.1	98	3.7	5
AE F054014 [m/z] 170 → 132		0.01	0.01	101	6.1	5
			0.1	98	2.8	5
AE F054014 [m/z] 168 → 132	blood	0.01	0.01	104	18.3	5
			0.1	98	4.0	5
AE F054014 [m/z] 170 → 132		0.01	0.01	108	16.9	5
			0.1	97	4.5	5

Conclusion

The analytical method for the determination of AE F046360, AE F088406 and AE F054014 each quantified as AE F054014 in chicken egg, muscle, liver, skin/fat and blood was found to be satisfactory in terms of linearity, specificity, accuracy, and precision and therefore fulfils the requirements according to guidance document SANCO/825/00 rev. 8.1.

Overall conclusion methods for post control and monitoring purposes

Matrix group / crop group	**Analyte**	**LOQ**	**Methods**		
			Primary method	**Confirmatory method**	**Independent lab validation**
Grain = Dry commodity (high protein/high starch content) Tomato = Commodity with high water content Olive = Commodity with high oil content Citrus = Commodity with high acid content	AE F46360 [1]: >**AE F054014** (one transition) AE F088406 [2] >**AE F054014** (one transition) **AE F054014** [3] >**AE F054014** (two transitions)	0.01 mg/kg	**Freitag, T.;** LC-MS/MS AE F46360 [1]: >AE F054014 (one transition) AE F088406 [2] >AE F054014 (one transition) AE F054014 [3] >AE F054014 (two transitions	**Not necessary**	**Class, T.; 2004** LC-MS/MS
Whole Milk Muscle Kidney Liver Fat Whole Egg	AE F46360 [1]: >**AE F054014** (two transitions) AE F088406 [2] >**AE F054014** (two transitions) **AE F054014** [3] >**AE F054014** (two transitions)	0.01 mg/kg	**Lakaschus, S.; Amann, S.; 2011** LC-MS/MS two transitions	**not necessary**	**Konrad, St.; Neuland, M.; 2011** LC-MS/MS
Soil (silt loam, sandy loam)	AE F033171 [4] AE F053022 [5] AE F054014 [3]	0.01 mg/kg	**Freitag T., (2007)** LC-MS/MS two transitions	**not necessary**	**not necessary**
Water (surface)	AE F046360 [1] AE F088406 [2]	0.05 µg/L	**Braune, M.; 2013** LC-MS/MS two transitions	**not necessary**	**Thies, S.; 2015** LC-MS/MS two transitions
Air	AE F46360 [1] AE F088406 [2]	1.0 µg/m^3	**Bacher R. (2005)** LC-MS/ two transitions	**not necessary**	**not necessary**
Body fluids Urine, blood	AE F46360 [1]: >**AE F054014** (two transitions) AE F088406 [2] >**AE F054014** (two transitions) **AE F054014** [3] >**AE F054014** (two transitions)	0.01 mg/kg	**Burton, D.** LC-MS/MS two transitions	**not necessary**	**not necessary**
Body tissues				**not necessary**	**not necessary**

[1] AE F046360 Fenoxaprop-P-ethyl not enantio-selective detected

[2] AE F088406 Fenoxaprop-P (free acid) not enantio-selective detected

[3] AE F054014 6-chloro-2,3-dihydro-benzoxazol-2-one

[4] AE F33171 Fenoxaprop-ethyl

[5] AE F053022 Fenoxaprop

B.5.3. REFERENCES RELIED ON

Please note:

- References written in grey typeface have been provided for Annex I inclusion (Baseline Dossier).
- References written in black typeface are submitted for the renewal of approval (Supplementary Dossier)

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP status, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.1 /01	Bogdoll, B.; Eichelmann, C.; Kloeckner, C.	2003	Determination of AE F046360 (fenoxaprop-P-ethyl) in technical grade and pure active ingredient and also determination of AE F046360 (fenoxaprop-P-ethyl) and AE F107892 (mefenpyr-diethyl) in formulations by liquid chromatography (HPLC) (anal Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C030496, Edition Number: M-219452-01-1 Date: 2003-03-24 GLP/GEP: no, unpublished	N	N		Bayer CropScience
KCA 4.1.1 /02	Dobrat, W.; Martijn, A.	2000	Analysis of technical and formulated pesticides (Fenoxaprop-ethyl) -public data-, Report No.: CIPAC 484, Edition Number: M-211540-01-1 Date: 2000-01-01 GLP/GEP: no, unpublished	N	N		-public data-

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP status, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.1 /07	Mueller, T.	1998	Validation of the analytical method AL009/94-3 for the determination of AE F046360 (fenoxaprop-P-ethyl) and AE F107892 (mefenpyr-diethyl) and AL005/91-1 for the determination of AE F066606 (MCPA-isooctyl) in AE F046360 50 EC51 A2 Code: AE F Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C001740, Edition Number: M-183264-01-1 Date: 1998-12-02 GLP/GEP: yes, unpublished	N	N		Bayer CropScience
KCA 4.1.1 /08	Mueller, T.	1998	Validation of the analytical method AL048/96-2 for the determination of AE F046360 (fenoxaprop-P-ethyl) and AE F122006 in AE F046360 42 EC14 A3 Code: AE F046360 42 EC14 A3 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: A67435, Report includes Trial Nos.: PA98/005V Edition Number: M-147819-01-1 Date: 1998-04-27 GLP/GEP: yes, unpublished	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP status, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.1 /09	Bogdoll, B.; Kloeckner, C.	2004	First addendum to the validation report PA03/012: Validation of the analytical method AL009/94-4 for the determination of AE F046360 (fenoxaprop-P-ethyl) in technical grade and pure active ingredient by liquid chromatography (HPLC) Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C045835, Edition Number: M-237436-01-1 Date: 2004-12-07 GLP/GEP: no, unpublished	N	N		Bayer CropScience
KCA 4.1.1 /11	Bogdoll, B.; Eichelmann, C.; Kloeckner, C.	2004	Determination of AE F046360 (fenoxaprop-P-ethyl) in technical grade and pure active ingredient and also determination of AE F046360 (fenoxaprop-P-ethyl) and AE F107892 (mefenpyr-diethyl) in formulations by liquid chromatography (HPLC) Code: Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C040369, Edition Number: M-228772-01-1 Date: 2004-03-09 GLP/GEP: no, unpublished	N	N		Bayer CropScience
KCA 4.1.1 /12	Weilbaecher, R.; Schaefer, C.	2006	Analytical method - Determination of the enantiomeric purity of AE F046360 (Fenoxaprop-P-ethyl) in technical grade and pure active ingredient by liquid chromatography (HPLC) Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: AM011506FP1, Edition Number: M-274639-01-1 Date: 2006-07-04 GLP/GEP: no, unpublished	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP status, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.1 /13	Weilbaecher, R.; Schaefer, C.	2006	Validation of HPLC-method AM011506FP1: Determination of the enantiomeric purity of AE F046360 (Fenoxaprop-P-ethyl) in technical grade and pure active ingredient by liquid chromatography (HPLC) Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: AF06/002, Edition Number: M-274643-01-1 Date: 2006-07-04 GLP/GEP: no, unpublished	N	N		Bayer CropScience
KCA 4.1.2 /01	Idstein, H.; Werner, H. J.; Johnson, J. H.	1986	Residue determination in biological materials and soil of Hoe 046360 and its metabolites Hoe 088406 and Hoe 054014 Hoechst AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: A35596, Edition Number: M-117453-01-4 Date: 1986-10-31 GLP/GEP: no, unpublished	N	N		Bayer CropScience
KCA 4.1.2 /02	Helgers, A.; Idstein, H.; Junker, H.	1996	Fenoxaprop-P-ethyl and Hoe 107892 oil in water emulsion 69 and 18.75 g/I Code: Hoe 046360 24 EWOB A503 Determination of residues of Hoe 046360 and Hoe 107892 to establish a maximum residue level following two (2) applications in cereals un Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: A56746, Edition Number: M-140533-01-1 Date: 1996-08-27 GLP/GEP: yes, unpublished **...also filed: KCA 4.2 /10**	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP status, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2 /03	Kaune, A.	2003	Description and validation of an analytical method to determine AE F046360, AE F088406 and AE F054014 in cereal shoot, straw and grain Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C027353, Edition Number: M-213161-01-1 Date: 2003-02-07 GLP/GEP: yes, unpublished **...also filed: KCA 4.2 /11**	N	N		Bayer CropScience
KCA 4.1.2 /04	Bourgade, C.; Rosati, D.	2003	Independent laboratory validation of analytical method AM01/02 for the determination of residue in wheat grain of AE F046360 and its metabolites AE F088406 and AE F054014. Bayer CropScience S.A., Lyon, France Bayer CropScience, Report No.: C029562, Report includes Trial Nos.: 02-139 Edition Number: M-217415-01-1 Date: 2003-03-21 GLP/GEP: yes, unpublished **...also filed: KCA 4.2 /12**	N	N		Bayer CropScience
KCA 4.1.2 /05	Clayton, B.; Parkes, R. L.	1993	Method validation for determinationation in soil, water and application cards of Fenoxaprop-P-ethyl and its metabolites, Fenoxaprop-P-acid and benzoxazolone EN-CAS Analytical Laboratories, Winston-Salem, NC, USA Bayer CropScience, Report No.: A51877, Edition Number: M-132776-01-1 Date: 1993-12-21 GLP/GEP: yes, unpublished **...also filed: KCA 4.2 /01**	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP status, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2 /06	Buettner, B.; Schweighoefer, U.; Kuenzler, K.	1992	Aerobic soil metabolism study at 11 and 21 C Hoe 046360-chlorophenyl-U-14C Hoechst AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: A47274, Edition Number: M-135697-01-1 EPA MRID No.: 43400602 Date: 1992-01-23 GLP/GEP: yes, unpublished **...also filed: KCA 4.2 /02** **...also filed: KCA 7.1.1.1 /02** **...also filed: KCA 7.1.2.1.1 /02** **...also filed: KCA 7.1.2.1.2 /02**	N	N		Bayer CropScience
KCA 4.1.2 /07	Neuss, B.	1999	Enforcement method for water by HPLC-UV/VIS AE F046360 and its metabolites AE F088406 and AE F054014 Fenoxaprop-P-ethyl Code: AE F046360 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C005936, Edition Number: M-192979-01-1 Date: 1999-11-03 GLP/GEP: no, unpublished **...also filed: KCA 4.2 /03**	N	N		Bayer CropScience
KCA 4.1.2 /08	Neuss, B.	2000	Validation of method EM F10/99-0 AE F046360 and its metabolites AE F088406 and AE F054014 Fenoxaprop-P-ethyl Code: AE F046360 Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C008304, Edition Number: M-197369-01-1 Date: 2000-04-17 GLP/GEP: yes, unpublished **...also filed: KCA 4.2 /04**	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP status, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2 /09	Reichert, N.	1994	Development and validation of a method in air for determining Fenoxaprop-P-ethyl Code: Hoe 046360 RCC Umweltchemie GmbH & Co. KG, Rossdorf, Germany Bayer CropScience, Report No.: A56140, Edition Number: M-133552-01-2 Date: 1994-07-26 GLP/GEP: yes, unpublished **...also filed: KCA 4.2 /05**	N	N		Bayer CropScience
KCA 4.1.2 /10	Glaenzel, A.	2005	Methodenentwicklung und -Validierung zur Bestimmung von Fenoxaprop-P-ethyl (HOE 046360) in Luft Addendum to final report RCC Ltd., Itingen, Switzerland Bayer CropScience, Report No.: C046678, Report includes Trial Nos.: 461500 Edition Number: M-247508-01-1 Date: 2005-02-03 GLP/GEP: no, unpublished **...also filed: KCA 4.2 /06**	N	N		Bayer CropScience
KCA 4.1.2 /11	Czarnecki, J. J.	1990	Gas chromatographic determination of Fenoxaprop-ethyl (Hoe 033171): ethyl-2-(4-(6-chloro-2-benzoxazolyloxy)-phenoxy)-propanoate and its metabolites (Hoe 053022): 2-(4-(6-chloro-2-benzoxazolyloxy)-phenoxy)-propanoic acid and Hoe 054014: Hoechst AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: A45545, Edition Number: M-127403-02-1 Date: 1990-09-10 **...Amended: 1991-03-05** GLP/GEP: no, unpublished **...also filed: KCA 4.2 /07**	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP status, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2 /12	Morris, A. D.; Clayton, F. B.	1990	Method validation ruggedness trial for Hoechst-Roussel Agri-Vet (HRAV) method (HRAV-8, gas chromatographic determination of Fenoxaprop-ethyl (Hoe 033171): ethyl-2-(4-(6-chloro-2-benzoxazolyloxy)-phenoxy)-propanoate and its metabolites Ho EN-CAS Analytical Laboratories, Winston-Salem, NC, USA Bayer CropScience, Report No.: A44532, Edition Number: M-127401-01-1 EPA MRID No.: 41654404 Date: 1990-10-04 GLP/GEP: yes, unpublished	N	N		Bayer CropScience
KCA 4.1.2 /13	Freitag, T.	2004	Modification M001 to method 00874 for the determination of residues of AE F046360, AE F088406, and AE F054014 in/on matrices of plant origin by HPLC-MS/MS (Method and validation) - Amendment to report no.: 1 TF- BCS-CHA, Report No.: 00874/M001, Report includes Trial Nos.: P602041803 Edition Number: M-234670-02-1 Date: 2004-08-20 **...Amended: 2006-01-27** GLP/GEP: yes, unpublished **...also filed: KCA 4.2 /17**	N	Y	Modification of analytical method used for residue trials	TF- BCS-CHA

Annex point / reference number	Author(s)	Year	Title Source Company name, Report No., Date, GLP status, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2 /14	Class, T.	2004	Independent laboratory validation of Bayer CropScience method 00874/M001 for the determination of residues of fenoxaprop-P-ethyl and two metabolites in plant material PTRL Europe GmbH, Ulm, Germany TF- BCS-CHA, Report No.: C044617, Edition Number: M-091819-02-1 Method Report No.: P/B 786 G Date: 2004-09-24 **...Amended: 2004-10-21** GLP/GEP: yes, unpublished **...also filed: KCA 4.2 /18**	N	Y	ILV to adapted method	TF- BCS-CHA
KCA 4.1.2 /15	Burton, D.	2016	Fenoxaprop-P-ethyl and fenoxaprop-P: Validation of methodology for the determination of residues as the benzoxazolone metabolite in foodstuff of animal origin Envigo CRS Limited, Eye, Suffolk, United Kingdom TF- BCS-CHA, Report No.: ENK0020, Edition Number: M-552660-01-1 Date: 2016-03-30 GLP/GEP: yes, unpublished **...also filed: KCA 4.2 /23**	N	Y	Analytical method used in new hen feeding study	TF- BCS-CHA
KCA 4.1.2 /16	Krebber, R.	2006	Method 00972 for the determination of Fenoxaprop-P-ethyl (AE F046360), mefenpyr-diethyl (AE F107892), fenoxaprop (AE F053022) and AE F113225 in test water from aquatic toxicity tests by HPLC-MS/MS TF- BCS-CHA, Report No.: 00972, Edition Number: M-266218-01-1 Date: 2006-02-02 GLP/GEP: yes, unpublished	N	Y		TF- BCS-CHA

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KCA 4.1.2 /17	Braune, M.	2011	Method 01311 for the determination of fenoxaprop-P-ethyl and fenoxaprop-P in test water from aquatic toxicity tests by HPLC-MS/MS TF- BCS-CHA Report No.: 01311 Edition Number: M-403143-01-1 Method Report No.: MR-10/172 Date: 2011-03-04 GLP/GEP: No, unpublished	N	N		TF- BCS-CHA
KCA 4.2 /01	Clayton, B.; Parkes, R. L.	1993	Method validation for determinationation in soil, water and application cards of Fenoxaprop-P-ethyl and its metabolites, Fenoxaprop-P-acid and benzoxazolone EN-CAS Analytical Laboratories, Winston-Salem, NC, USA Bayer CropScience, Report No.: A51877, Edition Number: M-132776-01-1 Date: 1993-12-21 GLP/GEP: yes, unpublished **...also filed: KCA 4.1.2 /05**	N	N		Bayer CropScience
KCA 4.2 /02	Buettner, B.; Schweighoefer, U.; Kuenzler, K.	1992	Aerobic soil metabolism study at 11 and 21 C Hoe 046360-chlorophenyl-U-14C Hoechst AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: A47274, Edition Number: M-135697-01-1 EPA MRID No.: 43400602 Date: 1992-01-23 GLP/GEP: yes, unpublished **...also filed: KCA 4.1.2 /06** **...also filed: KCA 7.1.1.1 /02** **...also filed: KCA 7.1.2.1.1 /02** **...also filed: KCA 7.1.2.1.2 /02**	N	N		Bayer CropScience

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KCA 4.2 /03	Neuss, B.	1999	Enforcement method for water by HPLC-UV/VIS AE F046360 and its metabolites AE F088406 and AE F054014 Fenoxaprop-P-ethyl Code: AE F046360 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C005936, Edition Number: M-192979-01-1 Date: 1999-11-03 GLP/GEP: no, unpublished **...also filed: KCA 4.1.2 /07**	N	N		Bayer CropScience
KCA 4.2 /04	Neuss, B.	2000	Validation of method EM F10/99-0 AE F046360 and its metabolites AE F088406 and AE F054014 Fenoxaprop-P-ethyl Code: AE F046360 Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C008304, Edition Number: M-197369-01-1 Date: 2000-04-17 GLP/GEP: yes, unpublished **...also filed: KCA 4.1.2 /08**	N	N		Bayer CropScience
KCA 4.2 /05	Reichert, N.	1994	Development and validation of a method in air for determining Fenoxaprop-P-ethyl Code: Hoe 046360 RCC Umweltchemie GmbH & Co. KG, Rossdorf, Germany Bayer CropScience, Report No.: A56140, Edition Number: M-133552-01-2 Date: 1994-07-26 GLP/GEP: yes, unpublished **...also filed: KCA 4.1.2 /09**	N	N		Bayer CropScience

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KCA 4.2 /06	Glaenzel, A.	2005	Methodenentwicklung und -Validierung zur Bestimmung von Fenoxaprop-P-ethyl (HOE 046360) in Luft Addendum to final report RCC Ltd., Itingen, Switzerland Bayer CropScience, Report No.: C046678, Report includes Trial Nos.: 461500 Edition Number: M-247508-01-1 Date: 2005-02-03 GLP/GEP: no, unpublished **...also filed: KCA 4.1.2 /10**	N	N		Bayer CropScience
KCA 4.2 /07	Czarnecki, J. J.	1990	Gas chromatographic determination of Fenoxaprop-ethyl (Hoe 033171): ethyl-2-(4-(6-chloro-2-benzoxazolyloxy)-phenoxy)-propanoate and its metabolites (Hoe 053022): 2-(4-(6-chloro-2-benzoxazolyloxy)-phenoxy)-propanoic acid and Hoe 054014: Hoechst AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: A45545, Edition Number: M-127403-02-1 Date: 1990-09-10 **...Amended: 1991-03-05** GLP/GEP: no, unpublished **...also filed: KCA 4.1.2 /11**	N	N		Bayer CropScience
KCA 4.2 /08	Kulkarni, T. S.; Huff, D. K.; Winkler, D. A.	1995	Method validation for determinationation in poultry tissues: (fat, liver, breast meat, skin) and eggs of Fenoxaprop-ethyl and its metabolites EN-CAS Analytical Laboratories, Winston-Salem, NC, USA Bayer CropScience, Report No.: A55152, Edition Number: M-139130-01-1 Date: 1995-06-05 GLP/GEP: yes, unpublished	N	N		Bayer CropScience

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KCA 4.2 /09	Czarnecki, J. J.; Brady, S. S.	1995	Magnitude of residues in the tissues and eggs of chickens of Fenoxaprop-ethyl dosed at 1, 3, and 10 times the estimated maximum daily intake, for 28 consecutive days, USA, 1994 with Fenoxaprop-ethyl AgrEvo USA Company, Pikeville, NC, USA Bayer CropScience, Report No.: A55150, Edition Number: M-139128-01-1 Date: 1995-11-28 GLP/GEP: yes, unpublished	N	N		Bayer CropScience
KCA 4.2 /10	Helgers, A.; Idstein, H.; Junker, H.	1996	Fenoxaprop-P-ethyl and Hoe 107892 oil in water emulsion 69 and 18.75 g/I Code: Hoe 046360 24 EWOB A503 Determination of residues of Hoe 046360 and Hoe 107892 to establish a maximum residue level following two (2) applications in cereals un Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: A56746, Edition Number: M-140533-01-1 Date: 1996-08-27 GLP/GEP: yes, unpublished **...also filed: KCA 4.1.2 /02**	N	N		Bayer CropScience
KCA 4.2 /11	Kaune, A.	2003	Description and validation of an analytical method to determine AE F046360, AE F088406 and AE F054014 in cereal shoot, straw and grain Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C027353, Edition Number: M-213161-01-1 Date: 2003-02-07 GLP/GEP: yes, unpublished **...also filed: KCA 4.1.2 /03**	N	N		Bayer CropScience

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KCA 4.2 /12	Bourgade, C.; Rosati, D.	2003	Independent laboratory validation of analytical method AM01/02 for the determination of residue in wheat grain of AE F046360 and its metabolites AE F088406 and AE F054014. Bayer CropScience S.A., Lyon, France Bayer CropScience, Report No.: C029562, Report includes Trial Nos.: 02-139 Edition Number: M-217415-01-1 Date: 2003-03-21 GLP/GEP: yes, unpublished **...also filed: KCA 4.1.2 /04**	N	N		Bayer CropScience
KCA 4.2 /13	Bacher, R.	2005	Analytical method for the determination of Fenoxarpop-P in air PTRL Europe GmbH, Ulm, Germany Bayer CropScience, Report No.: B939-1, Edition Number: M-261230-01-1 Method Report No.: B939-1 Method Report No.: B939-1G Date: 2005-11-18 GLP/GEP: yes, unpublished	N	Y		Bayer CropScience
KCA 4.2 /14	Bacher, R.	2005	Analytical method for the determination of Fenoxarpop-P-ethyl in air PTRL Europe GmbH, Ulm, Germany Bayer CropScience, Report No.: B939-2, Edition Number: M-261234-01-1 Method Report No.: B939-2 Method Report No.: B939-2G Date: 2005-11-18 GLP/GEP: yes, unpublished	N	Y		Bayer CropScience

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KCA 4.2 /15	Freitag, T.	2007	Analytical method 01056 for the determination of fenoxaprop-p-ethyl (AE F046360) and its metabolites AE F088406 and AE F054014 in soil using LC/MS/MS Bayer CropScience, Report No.: 01056, Edition Number: M-291683-01-1 Method Report No.: MR-07/288 Date: 2007-08-15 GLP/GEP: yes, unpublished	N	Y		Bayer CropScience
KCA 4.2 /16	Schmeer, K.; Stuke, S.	2009	Description of the multi-residue analytical method 01163 for the simultaneous determination of pesticides by HPLC-MS/MS in plant materials and feeding stuff based on the official QuEChERS method Bayer CropScience, Report No.: 01163, Edition Number: M-354028-01-1 Method Report No.: MR-09/104 Date: 2009-08-11 GLP/GEP: no, unpublished	N	N		Bayer CropScience
KCA 4.2 /17	Freitag, T.	2004	Modification M001 to method 00874 for the determination of residues of AE F046360, AE F088406, and AE F054014 in/on matrices of plant origin by HPLC-MS/MS (Method and validation) - Amendment to report no.: 1 TF- BCS-CHA, Report No.: 00874/M001, Report includes Trial Nos.: P602041803 Edition Number: M-234670-02-1 Date: 2004-08-20 **...Amended: 2006-01-27** GLP/GEP: yes, unpublished **...also filed: KCA 4.1.2 /13**	N	Y	Modification of analytical method used for residue trials	TF- BCS-CHA

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KCA 4.2 /18	Class, T.	2004	Independent laboratory validation of Bayer CropScience method 00874/M001 for the determination of residues of fenoxaprop-P-ethyl and two metabolites in plant material PTRL Europe GmbH, Ulm, Germany TF- BCS-CHA, Report No.: C044617, Edition Number: M-091819-02-1 Method Report No.: P/B 786 G Date: 2004-09-24 **...Amended: 2004-10-21** GLP/GEP: yes, unpublished **...also filed: KCA 4.1.2 /14**	N	Y	ILV to adapted method/New data requirements	TF- BCS-CHA
KCA 4.2 /19	Konrad, St.; Neuland, M.	2011	Validation of analytical BCS method 01316 for the determination of residues of fenoxaprop-P-ethyl and its metabolites fenoxaprop-P and AE F054014 (using LC-MS/MS in animal tissues: liver, kidney, muscle, milk, egg, fat) Currenta GmbH & Co. OHG, Leverkusen, Germany TF- BCS-CHA, Report No.: P603117507, Edition Number: M-404091-01-1 Method Report No.: 2011/0007/1 Date: 2011-03-22 GLP/GEP: yes, unpublished	N	Y	Replacement of unacceptable method with new one according to current standards	TF- BCS-CHA
KCA 4.2 /20	Lakaschus, S.; Amann, S.	2011	Independent method validation of BCS method 01316 for the determination of residues of fenoxaprop-p-ethyl and its metabolites in animal tissues TF- BCS-CHA, Report No.: 01316, Edition Number: M-411523-01-1 Method Report No.: S11-01374 Date: 2011-06-30 GLP/GEP: yes, unpublished	N	Y	ILV to replacement method	TF- BCS-CHA

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KCA 4.2 /21	Braune, M.	2013	Analytical method 01357 for the determination of fenoxaprop-p-ethyl and its metabolite fenoxaprop-p in drinking and surface water by HPLC-MS/MS TF- BCS-CHA, Report No.: MR-12/103, Edition Number: M-445048-01-1 Date: 2013-01-21 GLP/GEP: no, unpublished	N	N		TF- BCS-CHA
KCA 4.2 /22	Thies, S.	2015	Independent laboratory validation of the BCS analytical method 01357 for the determination of fenoxaprop-P-ethyl and its metabolite fenoxaprop-P in surface water by HPLC-MS/MS Currenta GmbH & Co. OHG, Leverkusen, Germany Bayer CropScience, Report No.: M-533252-01-1, Edition Number: M-533252-01-1 Date: 2015-09-04 GLP/GEP: yes, unpublished	N	Y	ILV to adapted method/New data requirements	Bayer CropScience
KCA 4.2 /23	Burton, D.	2016	Fenoxaprop-P-ethyl and fenoxaprop-P: Validation of methodology for the determination of residues as the benzoxazolone metabolite in foodstuff of animal origin Envigo CRS Limited, Eye, Suffolk, United Kingdom TF- BCS-CHA, Report No.: ENK0020, Edition Number: M-552660-01-1 Date: 2016-03-30 GLP/GEP: yes, unpublished **...also filed: KCA 4.1.2 /15**	N	Y	Analytical method used in new hen feeding study	TF- BCS-CHA