eurofins | agroscience services

Title

Independent Laboratory Validation of Modification M007 of analytical method 01387 for the determination of fluoxapiprolin (BCS-CS55621) in drinking and surface water by HPLC-MS/MS

Test Items

Fluoxapiprolin (BCS-CS55621)

Data Requirements/Guidelines

- 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.
- Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010
- 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 rev. 4, 11/07/00
- US EPA OCSPP 850.6100

Completion Date

2019-11-11
(yyyy-mm-dd)

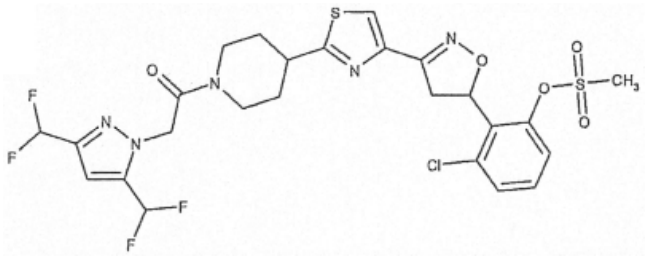
2 Introduction and Objective

The objective of the study was to validate independently the analytical method 01387/M007 for the determination of concentrations of fluoxapiprolin in surface water by HPLC-MS/MS.

3 Compounds

3.1 Reference Items

The purity of the reference item was certified prior to its use. Copies of "Certificates of Analyses" for the reference item are kept in the study raw data.
The reference item was stored as specified in the certificate. The chemical structures, nomenclature, purity, and expiration dates of the reference item is presented below.

Test / Reference Item 1			
Common name	Fluoxapiprolin	Synonym(s)	BCS-CS55621
Chemical name	2-{3-[2-(1-{[3,5-bis(difluoromethyl)-1H-pyrazol-1yl-]acetyl}piperidin-4-yl)1,3-thiazol-4-yl]4,5-dihydro-1,2-oxazol-5-yl}-3-chlorophenyl methanesulfonate		
Chemical structure			
Certificate No.	AZ 21323	Empirical formula	$C_{25}H_{24}ClF_4N_5O_5S_2$
Molecular weight (average)	650.07 g/mol	CAS number	1360819-11-9
Supplier	Bayer AG	Purity analysed	98.2 % (w/w)
Mol ID	25996	Batch / Lot number	NLL 9039-13-1
Date of analysis	12 Jan 2017	Expiry date	12 Jan 2020
Date of receipt at test facility	10 Mai 2019	Storage conditions at test facility	5 ± 5 °C (target)

4 Experimental Section

4.1 Test System

The analytical method was validated for surface water. A validation for drinking water was not necessary because the limit of quantitation for surface water is equal or below the drinking water limit of 0.1 µg/L.
For independent laboratory validation of method 01387/M007 surface water from the river Danube sampled at Landeswasserversorgung Langenau was used. Characteristics of the test system are listed in Table 3. Details are given in Appendix 7. Data were generated non-GLP.

Table 3: Characteristics of the Surface Water From River Danube, Sampled on 2019-06-27 in Langenau (Germany)

Parameter Danube water	Value
Total organic carbon (TOC)	3.6 mg/L
Dissolved organic carbon (DOC)	2.8 mg/L
Conductivity	511 µS/cm
pH	8.14
Water hardness	13.5°dH
corresponds to	2.42 mmol/L
Filterable solids	<0.1 mg/L
Magnesium	12.4 mg/L
Calcium	76.4 mg/L

4.2 Safety

The German guidelines for laboratories of the Employees' Liability Insurance Association, e.g. Working Safely in Laboratories [5] or comparable guidelines in other countries should be observed.

The following chemicals were used, which are classified by the hazardous material regulations. The classification is based on the Globally Harmonised System (GHS).

Substance	Signal word	Hazard statements
Methanol	Danger	H225 Highly flammable liquid and vapour H301 Toxic if swallowed H311 Toxic in contact with skin H331 Toxic if inhaled H370 Causes damage to organs
Acetonitrile	Danger	H225 Highly flammable liquid and vapour H302 Harmful if swallowed H312 Harmful in contact with skin H319 Causes serious eye irritation H332 Harmful if inhaled
Formic acid	Danger	H226 Flammable liquid and vapour H314 Causes severe skin burns and eye damage
Ammonium formate	Warning	H315 Causes skin irritation H319 Causes serious eye irritation H335 May cause respiratory irritation
Fluoxapiprolin (BCS-CS55621)		Caution – not fully tested yet

The pertinent safety instructions must be observed when working with all compounds mentioned in this method.

4.3 Materials

4.3.1 Apparatus and Reagents

For apparatus and reagents please see Appendix 2.

4.3.2 Stock Solutions

The stock solution was prepared by weighing a defined amount of the reference item into a volumetric flask and making up to volume with acetonitrile.

Table 4: Preparation Scheme of Reference Item Stock Solutions

Reference Item	Mass [mg]	Volume [mL]	Solvent	Final Concentration* [mg/L]
fluoxapiprolin	5.09	10	acetonitrile	500

*: Concentration is corrected for purity

The stock solution was stored in a refrigerator in the dark until use.

4.3.3 Spiking Solutions

Fortification solutions were prepared by appropriate dilution in acetonitrile of the stock solution, e.g. as follows:

Table 5: Preparation Scheme of Fortification Solutions

Analyte	Final Conc. Required [µg/L]	Final Volume [mL]	Standard Solution Used		Solvent
			Vol. [mL]	Concentration of used Solution [µg/L]	
fluoxapiprolin	5000	10	0.10	500000 (stock)	acetonitrile
	50	10	0.10	5000	
	10	10	2.0	50	

The fortification solutions were stored in a refrigerator in the dark when not in use.

4.3.4 Standard Solutions in Solvent

Two intermediate standard solutions were prepared for the preparation of matrix-matched standards.

Table 6: Preparation Scheme of Intermediate Standard Solutions in Solvent

Compound			Standard Solution		Solvent
Analyte	Final Conc. Required [µg/L]	Final Volume [mL]	Vol. [mL]	Concentration of used Solution [mg/L]	
fluoxapiprolin	5000	10	0.1	500 (stock)	water/acetonitrile (8/2, v/v)
	50	10	0.1	5.0	

The intermediate calibration solutions were stored in a refrigerator in the dark when not in use.

4.3.5 Matrix Matched Standards

Matrix-matched standards were prepared by adding intermediate solution in solvent to surface water/acetonitrile (8/2, v/v).

Table 7: Preparation Scheme for Standard Solutions (Secondary Standards)

Compound			Standard Solution		Solvent
Analyte	Final Conc. Required [µg/L]	Final Volume [mL]	Vol. [mL]	Concentration of used Solution [µg/L]	
fluoxapiprolin	5.0	10	1.0	50	surface water/acetonitrile (8/2, v/v)
	1.0	10	2.0	5.0	
	0.50	10	1.0	5.0	
	0.10	10	1.0	1.0	
	0.050	10	1.0	0.50	
	0.015	10	1.5	0.10	

The matrix-matched standard solutions were stored in a refrigerator in the dark when not in use.

4.4 Sample Preparation

The water samples were added with acetonitrile (25% of the water sample volume) and directly injected into the HPLC.

4.5 Instrumental Analysis

4.5.1 Principle of Measurement

An aliquot of the sample solution was injected into the high performance liquid chromatograph and subjected to reversed phase chromatography coupled with tandem mass spectrometry (MS/MS) with electrospray ionisation. The MS/MS instrument was operated in the Multiple Reaction Monitoring mode (MRM). The pseudo molecular ions of the analytes ($[M+H]^+$, $[M-H]^-$ or any adducts) were selected by the first quadrupole. These precursor ions were impulsed with nitrogen in the collision cell (second quadrupole) and the resulting fragment ions (product ions) were separated according to their m/z ratio in the third quadrupole. Two of these product ions per analyte were selected: one product ion (MRM-transition) serving for quantitation and the second for confirmation.

4.5.2 Chromatography

Instrument: Agilent 1290 HPLC System (vacuum solvent degasser, binary HPLC pump, Multisampler, Multicoulumn Thermostate)

Column: Supelco Ascentis Express C18, 2.7 µm, 50 x 2.1 mm.

Injection Volume: 50 µL

Oven temperature: 60°C

Mobile Phase: Pump A: Water + 0.012 % formic acid + 10 mM ammonium formate

Pump B: Methanol + 0.012 % formic acid + 10 mM ammonium formate

Time [min]	A [%, v/v]	B [%, v/v]
0.0	95	5
0.1	95	5
3.0	5	95
4.0	5	95
4.1	95	5
5.0	95	5

Flow (Column): 0.5 mL/min

Retention times: fluoxapiprolin approx. 3.8 min

4.5.3 Detection

The detection by MS/MS was performed on a triple-quadrupole tandem mass spectrometer, equipped with a Turbo Ion Spray (ESI) interface operated in the positive ion mode and multiple reaction monitoring (MRM).

Detector: Triple Quadrupole Tandem Mass Spectrometer, Sciex 5500+, Windows 10, Analyst 1.7.1 software versions

Interface: Turbo Ion Spray (ESI)

Scan Type: MRM (Multiple Reaction Monitoring)

Table 8: MS/MS Parameters for the Determination of Fluoxapiprolin

	Precursor Ion Q1 Mass (amu)	Product Ion Q3 Mass (amu)	Dwell Time (msec)	Collision Energy (eV)	Polarity
fluoxapiprolin quantitation	650	610	100	29	pos
fluoxapiprolin confirmation	650	193	100	47	pos

See Appendix 4 for more details.

4.6 Calculation

No recovery calculation was made for the direct injection of the sample for the independent method validation.

The example calculation displayed below can be used for the calculation of the concentration of the analyte in the sample.

4.6.1 Calculation of Concentrations

The measured concentration is calculated by comparison of the analyte response to a standard calibration curve (1/x weighted).

The following equation is used for calculation in case of a linear calibration curve of type y = mx + b:

$$C_S = \frac{A_{(S)}\text{Sample} - \text{Intercept (b)}}{\text{Slope (a)}} \times \text{Dilution factor}$$

C_S = concentration of the analyte in the sample
$A_{(S)}$Sample = peak area of the analyte in the sample solution
Intercept = point where the calibration curve crosses the y-axis
Slope = slope of the calibration curve

The calculation of concentrations is also possible by comparison of the peak areas of the samples with the peak areas of the external standard solutions.

The concentration in water samples can be calculated according to the following equation:

$$\text{Conc}_{\text{Sample}}\,[\mu g/L] = \frac{\text{Peak Area}_{\text{Sample}} \times \text{Conc}_{\text{Standard}}\,[\mu g/L] \times \text{Dilution Factor}}{\text{Peak Area}_{\text{Standard}}}$$

$Conc_{Standard}$ = concentration of the analyte in the standard solution
$Conc_{Sample}$ = concentration of the analyte in the sample solution

4.6.2 Calculation of the Repeatability (Precision)

The repeatability or precision of the method is defined as the dispersion of the validation results and is expressed as the relative standard deviation (RSD).

At each fortification level, the relative standard deviation was calculated as follows:

$$\text{Relative Standard Deviation [\%]} = \frac{\text{Standard Deviation}}{\text{Mean of Peak area}} \text{ X } 100\%$$

5 Results and Discussion

5.1 Selectivity

The high selectivity of the method resulted from the HPLC separation in combination with MS/MS detection. Two MRM transitions were monitored for the analyte. No signals/peaks interfering with the detection of the analytes were observed in solutions of untreated control specimens.

5.2 Linearity

The correlation between the injected amount of substance and the detector response was linear (1/x weighted) for standard solutions in surface water ranging from 0.015 µg/L to 5.0 µg/L (corresponding to 0.0188 µg/L to 6.25 µg/L in test water) for fluoxapiprolin. The correlation coefficient was ≥ 0.99 for both MRM transitions (see Appendix 4).

5.3 Untreated Control Samples

Two untreated control samples were examined. The concentration was below 30% of the LOQ of 0.0625 µg/L for fluoxapiprolin (BCS-CS55621) (see Appendix 5).

Table 9: Apparent Concentrations in Control Samples (LOQ = 0.0625 µg/L)

Control Material		**Fluoxapiprolin Concentration [µg/L]**
Surface water	Sampled at Landeswasserversorgung Langenau on 2019-06-27	< 0.3 x LOQ

5.5 Matrix Effects

Determination of matrix effects was not part of this study; it was determined within the original validation study.

5.6 Storage Stability

The stability of extracts was not part of this study; it was determined within the original validation study.

5.8 Conclusion

The method was successfully validated independently for the determination of fluoxapiprolin in drinking and surface water from the tested LOQ of 0.0625 µg/L according to the guidance document SANCO/825/00, rev. 8.1 and SANCO/3029/99 rev.4.

Primary validation and independent laboratory validation were carried out at different locations, by different study personnel, and using different instrumentation and stocks of chemicals. No communication with the method developers or others familiar with the method was necessary to carry out the analysis and the first attempt made resulted in the validation data reported here.

5.9 Minor modifications of the method

Following minor modifications of the method were done:

The stock solution was prepared in pure acetonitrile instead of acetonitrile/water (8/2, v/v). There is no impact on the outcome of the independent method validation. The stability of the analyte in acetonitrile has been shown for 165 days under refrigerated conditions [6].

The fortified samples were prepared using a slightly different procedure.
In the original method validation the analyte was fortified to an acetonitrile/water (2/8, v/v) mixture using matrix-matched calibration solutions.
In this independent method validation study fortification solutions containing the analyte were prepared in acetonitrile. These fortification solutions were used to fortify the analyte to surface water samples, which were diluted with acetonitrile afterwards to obtain the same fortification levels and solvent mixtures as used in the original method validation.

Besides the minor modifications described above, no addition or modification to the original method [1] other than optimization of instrumental parameters was done.

Appendix 1:
Method characteristics

Table 12: Summary Parameters for the Analytical Method Used for the Quantitation of Fluoxapiprolin Residues in Surface Water

Method ID	**01387/M007**
Analytes	fluoxapiprolin (BCS-CS55621)
Extraction solvent/technique	None – direct sample injection
Clean-up strategies	None – direct sample injection
Instrument/Detector/Column	Agilent 1290 HPLC System (Vacuum Solvent Degasser, Binary HPLC Pump, Multisampler, Multicolumn Thermostat) Sciex 5500+ Triple Quadrupole Ascentis Express C18 2.7 µm, 50mm x 2.1 mm id
Standardization method	Linear regression with 1/x weighting
Storage stability	not assessed in this study
Retention time	Fluoxapiprolin: approx. 3.8 min

Table 13: Characteristics for the Analytical Method Used for the Quantitation of Fluoxapiprolin Residues in Surface Water

Analyte	fluoxapiprolin (BCS-CS55621)
Limit of detection (LOD)	0.015 µg/L in surface water
Limit of quantitation (LOQ)	Surface water: Fluoxapiprolin: 0.0625 µg/L
Precision	The relative standard deviation for the peak area counts is ≤ 4.1% for both MRM transitions.
Reliability of the Method/ [ILV]	ILV was performed successfully.
Linearity	The method/detector response was linear (correlation coefficient r = 1.0000 for both MRM transitions)
Specificity	The control chromatograms generally have no peaks above the chromatographic background and the spiked sample chromatograms contain only the analyte peak of interest. Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.

Appendix 2:
Apparatus and Reagents

Apparatus

- Balance, XS205DU, Mettler Toledo GmbH
- Agilent 1290 HPLC system (Vacuum Solvent Degasser, Binary HPLC Pump, Multisampler, Multicolumn Thermostat).
- Sciex 5500+ Triplequadrupole mass spectrometerwith Turbo IonSpray ESI source. Analyst 1.7.1 Instrument control and data acquisition software.
- Reversed phase chromatography column,
  Supelco Ascentis Express C18, 2.7 µm, 50 x 2.1 mm (Purchase No.: 53822-U)
  with pre-column Phenomenex C_{18}, 4 x 3 mm (Purchase No.: AJ0-4287)
- Small instruments, e.g. autosampler vials
- Transonic bath USC 300 TH, VWR international bvba

Reagents

- Formic acid, 98-100% Sigma Aldrich
- Acetonitrile, HPLC grade, VWR
- Methanol, LC-MS grade, Merck
- Water, LC-MS grade, Merck
- Millipore water, EAG Laboratories GmbH
- Ammonium formate, ≥ 99.0%, Sigma Aldrich

Appendix 3:
Detailed Summary of Chromatographic and Mass Spectrometric Conditions

LC/MS System	Agilent 1290 HPLC System (Vacuum Solvent Degasser, Binary HPLC Pump, Multisampler, Multicolumn Thermostat) Sciex 5500+ Triple Quadrupole mass spectrometer with Turbo IonSpray ESI source. Analyst 1.7.1 Instrument control and data acquisition software.					
LC Column	Ascentis Express C18, 2.7 µm, 50 x 2.1 mm with pre-column Phenomenex C18, 4x3 mm					
Injection volume	50 µL					
Column oven temperature	60°C					
Mobile Phase	A – Water + 0.012 % formic acid + 10 mM ammonium formate B – Methanol + 0.012 % formic acid + 10 mM ammonium formate					
Flow Rate:	500 µL/min					
Gradient:	Time, min. 0.0 0.1 3.0 4.00 4.10 5.00	A, % 95 95 5 5 95 95	B, % 5 5 95 95 5 5			
Retention time	Fluoxapiprolin: approx. 3.8 min					
Interface:	TurboIon Spray					
Polarity:	Positive					
GS1	50		GS2		60	
Curtain Gas (CUR):	40		Collision Gas (CAD):		9	
Temperature (TEM):	400 °C		Ion Spray Voltage (IS)		4500 V	
Entrance Potential (EP)	10 V					
Compound	Ion, m/z		Dwell time	DP	CE	CXP
	Q1	Q3	ms	V		
Fluoxapiprolin (Quantitation)	650	610	100	201	29	32
Fluoxapiprolin (Confirmation)	650	193	100	201	47	16