Analytical method for fluoxapiprolin (BCS-CS55621) in water

Reports: ECM: MRID 52041304. Krebber, R., and C. Sandau. 2019. Modification M007 of analytical method 01387 for the determination of fluoxapiprolin (BCS-CS55621) in drinking and surface water by HPLC-MS/MS. Method No.: Bayer AG Method 01387/M007 (p. 2). Study ID: P 684 157087. Activity ID: RATYN064. Report prepared, sponsored, and submitted by Bayer AG, Crop Science Division, Monheim am Rhein, Germany (pp. 1-2); 36 pages. Final report issued August 23, 2019.

ILV: MRID 52041305. Schmiedt, S. 2019. 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. Study ID: P 5402 G. Activity ID: RATYN073. Report prepared by EAG Laboratories GmbH, Ulm, Germany, and sponsored and submitted by Bayer AG, Crop Science Division, Monheim am Rhein, Germany (pp. 1-2); 34 pages. Final report issued November 11, 2019.

Document No.: MRIDs 52041304 & 52041305

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with German Good Laboratory Practices [GLP; German Chemical Law (ChemG)] and OECD GLP standards (p. 3; Appendix 7, pp. 35-36 of MRID 52041304). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5; Appendix 7, pp. 35-36). ILV: The study was conducted in accordance with German Good Laboratory Practices [GLP; German Chemical Law (ChemG)] and OECD, except for the characterization of the test system (p. 3; Appendix 6, p. 32 of MRID 52041305). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5; Appendix 6, p. 32).

Classification: This analytical method is classified as **unacceptable, but upgradeable**. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ. ECM MRID 52041304 method (Bayer AG Method 01387/M007) was Modification M007 of Analytical Method 01387. Analytical Method 01387 was not provided for review by PB&A/CSS Joint Venture personnel. Based on the method title, Analytical Method 01387 applied to drinking and surface water; however, the ECM MRID 52041304 and ILV MRID 52041305 validations justified the exclusion of drinking water as a test matrix since the drinking water LOQ was 0.1 µg/L. Only HPLC area counts were provided as recovery results in the ECM and ILV study reports; the reviewer calculated % recoveries based on the HPLC area count data and the linearity equations. No post-sampling storage data was provided for the ECM test water matrix which was sampled over four years prior to the experimental start date; the

	date of the characterization was not reported. The sample preparation for the method (Bayer AG Method 01387/M007) required some clarification. The time requirement for the method was not reported in the ILV or ECM.	
PC Code:	110304	
EFED Final Reviewer:	Richard Shamblen, Biologist	Signature: RICHARD SHAMBLEN Digitally signed by RICHARD SHAMBLEN Date: 2024.12.17 11:46:30 -05'00' Date: December 17, 2024
PB&A/CSS JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: Lisa Muto Date: 03/15/2023
	Summer Piantes, M.S., Environmental Scientist	Signature: Summer Piantes Date: 04/06/2023

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by PB&A/CSS Joint Venture personnel. The PB&A/CSS JV role does not include establishing Agency policies.

Executive Summary

This analytical method, Bayer AG Method 01387/M007 (Modification M007 of Analytical Method 01387), is designed for the quantitative determination of fluoxapiprolin (BCS-CS55621) at 0.0625 µg/L in surface and drinking water using LC/MS/MS. The LOQ is **less than** the lowest toxicological level of concern in water for fluoxapiprolin (BCS-CS55621). Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for fluoxapiprolin (BCS-CS55621) in surface water (0.0625 µg/L). Based on the method title, Analytical Method 01387 applied to drinking and surface water; however, the ECM MRID 52041304 and ILV MRID 52041305 validations justified the exclusion of drinking water as a test matrix since the drinking water (LOQ) was 0.1 µg/L.

The ILV and ECM validated the method using different characterized surface water matrices; one matrix per validation was used. A drinking water matrix was not included as a test matrix in the ECM and ILV.

The ILV validated Bayer AG Method 01387/M007 in the first trial for fluoxapiprolin (BCS-CS55621) in surface water at 0.0625 µg/L and 0.6250 µg/L with insignificant modifications to the sample fortification procedure and analytical parameters and equipment. No updated ECM is required based on ILV modifications. No communication occurred between the ILV personnel and the method developers or others familiar with the method. The method LOD was not reported in the ECM.

All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity

were satisfactory for fluoxapiprolin (BCS-CS55621) in surface water at 0.0625 µg/L and 0.6250 µg/L. The LOQ was also reported as 0.05 µg/L in test water:acetonitrile (8:2, v:v) which corresponded to 0.0625 µg/L in the test water alone. Only HPLC area counts were provided as recovery results in the ECM and ILV study reports; the reviewer calculated % recoveries based on the HPLC area count data and the linearity equations. DER repeatability determinations were based on data available as % means (reviewer-calculated) and % RSDs (reviewer-calculated and study-reported).

No post-sampling storage data was provided for the ECM test water matrix which was sampled over four years prior to the experimental start date; the date of the characterization was not reported. The sample preparation for the method (Bayer AG Method 01387/M007) required some clarification. The time requirement for the method was not reported in the ILV or ECM.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Fluoxapiprolin (BCS-CS55621)	52041304[1]	52041305[2]		Water[3]	23/08/2019[3]	Bayer AG	LC/MS/MS	0.0625 µg/L[4]

1 In the ECM, the water matrix was surface (river) water (pH 7.5, 273 µS/cm conductivity, 4.6°dH water hardness, <2 mg/L total organic carbon, dissolved organic carbon not reported, and <5 mg/L filterable solids) from River Wupper, Leverkusen-Opladen, Germany (p. 12 of MRID 52041304). The laboratory which performed the characterization was not reported. The water matrix was sampled on January 13, 2015, which was over four years prior to the experimental start date (May 27, 2019; pp. 7, 12). No post-sampling storage data was provided for the test water matrix; the date of the characterization was not reported.

2 In the ILV, the water matrix was surface (river) water (pH 8.14, 511 µS/cm conductivity, 13.5°dH (2.42 mmol/L) water hardness, 3.6 mg/L total organic carbon; 2.8 mg/L dissolved organic carbon, and <0.1 mg/L filterable solids) from River Danube, Landeswasserversorgung, Langenau, Germany (p. 12; Appendices 7-8, pp. 33-34 of MRID 52041305). The water was characterized (non-GLP) by Institut Alpha, Ulm, Germany. The water matrix was sampled on June 27, 2019, which was *ca*. 2 months prior to the experimental start date (August 26, 2019; p. 6; Appendices 7-8, pp. 33-34).

3 The method (Bayer AG Method 01387/M007) was Modification M007 of Analytical Method 01387 (p. 1 of MRID 52041304). Analytical Method 01387 was not provided for review by PB&A/CSS Joint Venture personnel. Based on the method title, Analytical Method 01387 applied to drinking and surface water; however, drinking water was not included as a test matrix in the ECM or ILV validations for Modification M007 of Analytical Method 01387. The ECM and ILV reported that "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" (p. 12 of MRID 52041304; p. 12 of MRID 52041305).

4 LOQ was also reported as 0.05 µg/L in test water:acetonitrile (8:2, v:v) which corresponded to 0.0625 µg/L in the test water alone (pp. 10, 13, 17 of MRID 52041304; pp. 10, 14-15, 18-19 of MRID 52041305).

I. Principle of the Method

The method (Bayer AG Method 01387/M007) was Modification M007 of Analytical Method 01387 (p. 1 of MRID 52041304). Analytical Method 01387 was not provided for review by PB&A/CSS Joint Venture personnel. Based on the method title, Analytical Method 01387 applied to drinking and surface water; however, drinking water was not included as a test matrix in the ECM or ILV validations for Modification M007 of Analytical Method 01387. The ECM and ILV reported that "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" (p. 12 of MRID 52041304; p. 12 of MRID 52041305).

The fluoxapiprolin stock solution (A1; concentration 508.7 mg/L, corrected for purity) in water:acetonitrile (2:8, v:v) was diluted ten-fold with water:acetonitrile (2:8, v:v) then used to prepare the 5.01 mg/L fortification solution in test surface water:acetonitrile (8:2, v:v; A2; p. 13 of MRID 52041304). Test samples (20 mL) were prepared as test surface water:acetonitrile (8:2, v:v) mixtures which were fortified with fluoxapiprolin (BCS-CS55621) at 0.05 and 0.50 µg/L, which corresponded to 0.0625 and 0.625 µg/L, respectively, in test water alone, as necessary (pp. 10, 13, 17). The test samples were diluted with acetonitrile (25% of the water sample volume) then directly analyzed by HPLC/MS/MS or analyzed after further dilution with test surface water:acetonitrile (8:2, v:v).

Samples were analyzed for fluoxapiprolin (BCS-CS55621) using an Agilent 1290 Binary Pump SL HPLC coupled to an AB Sciex API 5500 Triple Quadrupole Tandem Mass Spectrometer equipped with positive Turbo Ion Spray (ESI) ionization interface, multiple reaction monitoring (MRM) mode (pp. 14-15; Appendix 3, pp. 22-24 of MRID 52041304). The following LC conditions were used: Ascentis Express 2.7 µm C18 column (2.1 mm x 50 mm, 2.7 µm; column temperature not reported; oven temperature 80°C), mobile phase of (Bin Pump A) deionized water:formic acid (1000:0.120, v:v) + 10mM ammonium formate, (Bin Pump B) methanol:formic acid (1000:0.120, v:v) + 10mM ammonium formate, and (Iso Pump C) deionized water:methanol:formic acid (500:500:0.120, v:v:v), MS temperature 400°C, and injection volume of 50 µL. Expected approximate retention time was 2.6 minutes for fluoxapiprolin (BCS-CS55621). Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 650→610 and *m/z* 650→193 for fluoxapiprolin (BCS-CS55621). Matrix-match calibration standards were used (pp. 13, 17). The mobile phase gradient of the LC is shown below (p. 15).

Time (min.)	A (%)	B (%)	Into MS	Into Waste
0.0	95	5	Iso pump	Bin pump
0.1	95	5		
1.0			Bin pump	Iso pump
3.0	5	95		
4.0	5	95	Iso pump	Bin pump
4.1	95	5		
5.0	95	5		

The ILV performed the ECM method, Bayer AG Method 01387/M007, as written, except for the use of a pure acetonitrile stock solution for fortification of the calibration and test samples,

instead of a water:acetonitrile (2:8, v:v) solution or the matrix-matched standards, and insignificant modifications to the analytical parameters and equipment (pp. 13-16, 20; Appendix 3, p. 23 of MRID 52041305). Samples were analyzed for fluoxapiprolin (BCS-CS55621) using Agilent 1290 HPLC coupled to an Sciex 5500+ Triple Quadrupole Tandem Mass Spectrometer equipped with positive TurboIonSpray (ESI) interface, MRM mode. The LC conditions were the same as those of the ECM, except that oven temperature was 60°C, conversion from a tertiary to binary solvent system (eliminating the iso solvent C), and the addition of a pre-column (Phenomenex C18, 4 x 3 mm). Expected approximate retention time was 3.8 minutes for fluoxapiprolin (BCS-CS55621). Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 650→610 and *m/z* 650→193 for fluoxapiprolin (BCS-CS55621); the monitored ion transitions of the ILV were the same as those of the ECM. Matrix-match calibration standards were used (p. 15).

The Limit of Quantification (LOQ) in surface water in the ECM and ILV was reported as 0.0625 µg/L for fluoxapiprolin (BCS-CS55621; pp. 10, 13, 17 of MRID 52041304; pp. 10, 14-15, 18-19 of MRID 52041305). The Limit of Detection (LOD) in surface water for the method was reported as 0.015 µg/L in the ILV, but it was not reported in the ECM (Appendix 1, p. 21 of MRID 52041305). Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

II. Recovery Findings

ECM (MRID 52041304): Reviewer-calculated mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of fluoxapiprolin (BCS-CS55621) in one surface water matrix at the LOQ (0.0625 µg/L) and 10×LOQ (0.6250 µg/L; Table 8, p. 17; Table 10, p. 19; Appendix 5, p. 27; DER Excel Attachment). Results reported in the study report were presented as HPLC area counts for individual recoveries and mean recoveries and percent (%) for RSDs. Reviewer-calculated RSDs were the same as RSDs reported in the study report. Recovery results of the quantitative and confirmatory ion transitions were comparable. The water matrix was surface (river) water (pH 7.5, 273 µS/cm conductivity, 4.6°dH water hardness, <2 mg/L total organic carbon, dissolved organic carbon not reported, and <5 mg/L filterable solids) from River Wupper, Leverkusen-Opladen, Germany (p. 12 of MRID 52041304). The laboratory which performed the characterization was not reported. The water matrix was sampled on January 13, 2015, which was over four years prior to the experimental start date (May 27, 2019; pp. 7, 12). No post-sampling storage data was provided for the test water matrix; the date of the characterization was not reported.

ILV (MRID 52041305): Reviewer-calculated mean recoveries and RSDs met requirements for the quantitation ion transition analysis of fluoxapiprolin (BCS-CS55621) in one surface water matrix at the LOQ (0.0625 µg/L) and 10×LOQ (0.6250 µg/L; Tables 10-11, pp. 18-19; and Appendix 4, pp. 24-25; DER Excel Attachment). Results reported in the study report were presented as HPLC area counts for individual recoveries and mean recoveries and percent (%) for RSDs. Reviewer-calculated RSDs were the same as RSDs reported in the study report. Recovery results of the quantitative and confirmatory ion transitions were comparable. The water matrix was surface (river) water (pH 8.14, 511 µS/cm conductivity, 13.5°dH (2.42 mmol/L)

water hardness, 3.6 mg/L total organic carbon; 2.8 mg/L dissolved organic carbon, and <0.1 mg/L filterable solids) from River Danube, Landeswasserversorgung, Langenau, Germany (p. 12; Appendices 7-8, pp. 33-34 of MRID 52041305). The water was characterized (non-GLP) by Institut Alpha, Ulm, Germany. The water matrix was sampled on June 27, 2019, which was *ca*. 2 months prior to the experimental start date (August 26, 2019; p. 6; Appendices 7-8, pp. 33-34).

The method, Bayer AG Method 01387/M007, was validated by the ILV in the first trial for fluoxapiprolin (BCS-CS55621) in surface water at 0.0625 µg/L and 0.6250 µg/L with insignificant modifications to the sample fortification procedure and analytical parameters and equipment (pp. 19-20 of MRID 52041305). No updated ECM is required based on ILV modifications.

ECM & ILV (MRIDs 52041304 & 52041305): The method (Bayer AG Method 01387/M007) was Modification M007 of Analytical Method 01387 (p. 1 of MRID 52041304). Analytical Method 01387 was not provided for review by PB&A/CSS Joint Venture personnel. Based on the method title, Analytical Method 01387 applied to drinking and surface water; however, drinking water was not included as a test matrix in the ECM or ILV validations for Modification M007 of Analytical Method 01387. The ECM and ILV reported that "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" (p. 12 of MRID 52041304; p. 12 of MRID 52041305).

The LOQ was also reported as 0.05 µg/L in test water:acetonitrile (8:2, v:v) which corresponded to 0.0625 µg/L in the test water alone (pp. 10, 13, 17 of MRID 52041304; pp. 10, 14-15, 18-19 of MRID 52041305).

Table 2. Initial Validation Method Recoveries for Fluoxapiprolin (BCS-CS55621) in Surface Water[1,2,3,4]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range	Mean Recovery[5]	Standard Deviation[6]	Relative Standard Deviation[7]
Surface (River) Water						
Quantitation ion transition						
Fluoxapiprolin (BCS-CS55621)	0.0625 (LOQ)	5[8]	62198-64999 ct*	63084 ct	--	1.4%
			99.1-103.6%	100.6%	1.4%	1.4%
	0.625	5	612962-630344 ct	622778 ct	--	0.9%
			98.4-101.2%	100.0%	0.9%	0.9%
Confirmation ion transition						
Fluoxapiprolin (BCS-CS55621)	0.0625 (LOQ)	5	22677-24041 ct	23289 ct	--	1.6%
			96.5-102.4%	99.2%	1.6%	1.6%
	0.625	5	226606-234633 ct	230454 ct	--	1.2%
			97.2-100.6%	98.8%	1.2%	1.2%

Data (uncorrected results, p. 16) were obtained from Table 8, p. 17; Table 10, p. 19; and Appendix 5, p. 27 of MRID 52041304. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

* ct = HPLC area counts.

1 Only HPLC area counts were provided as recovery results in the study report. The reviewer calculated % recoveries based on the HPLC area count data and the linearity equations (See DER Excel Attachment) based on the calculation of concentration equations reported on p. 16 of the study MRID 52041304. The reviewer also calculated means, standard deviations, and relative standard deviations for all sample sets.

2 In the ECM, the water matrix was surface (river) water (pH 7.5, 273 µS/cm conductivity, 4.6°dH water hardness, <2 mg/L total organic carbon, dissolved organic carbon not reported, and <5 mg/L filterable solids) from River Wupper, Leverkusen-Opladen, Germany (p. 12 of MRID 52041304). The laboratory which performed the characterization was not reported. The water matrix was sampled on January 13, 2015, which was over four years prior to the experimental start date (May 27, 2019; pp. 7, 12). No post-sampling storage data was provided for the test water matrix; the date of the characterization was not reported.

3 Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 650→610 and *m/z* 650→193 for fluoxapiprolin (BCS-CS55621).

4 The LOQ was also reported as 0.05 µg/L in test water:acetonitrile (8:2, v:v) which corresponded to 0.0625 µg/L in the test water alone (pp. 10, 13, 17 of MRID 52041304). Target concentrations were 0.0501 µg/L and 0.501 µg/L (p. 13 of MRID 52041304).

5 Reported in the study as HPLC area counts (Table 8, p. 17; Table 10, p. 19 of MRID 52041304); reviewer-calculated as % based on individual calculated % recoveries (See DER Excel Attachment).

6 Reviewer-calculated as % based on individual calculated % recoveries (See DER Excel Attachment). Standard deviations were not calculated/reported in the study.

7 Reported in the study as %; reviewer-calculated as % based on individual calculated % recoveries (See DER Excel Attachment).

8 Duplicate aliquots were taken for each sample analysis. All results were included in calculations and ranges.

Table 3. Independent Validation Method Recoveries for Fluoxapiprolin (BCS-CS55621) in Surface Water[1,2,3,4]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range	Mean Recovery[5]	Standard Deviation[6]	Relative Standard Deviation[7]
Surface (River) Water						
Quantitation ion transition						
Fluoxapiprolin (BCS-CS55621)	0.0625 (LOQ)	5[8]	27600-30500 ct*	29000 ct	--	2.8%
			106.1-117.0%	111.2%	3.1%	2.8%
	0.625	5	278000-289000 ct	282000 ct	--	1.2%
			105.3-109.4%	106.9%	1.3%	1.2%
Confirmation ion transition						
Fluoxapiprolin (BCS-CS55621)	0.0625 (LOQ)	5	8050-9010 ct	8480 ct	--	4.1%
			105.7-118.2%	111.3%	4.5%	4.1%
	0.625	5	78800-84100 ct	81600 ct	--	2.1%
			102.5-109.3%	106.0%	2.2%	2.1%

Data (uncorrected results, p. 17) were obtained from Tables 10-11, pp. 18-19; and Appendix 4, pp. 24-25 of MRID 52041305. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

* ct = HPLC area counts.

1 Only HPLC area counts were provided as recovery results in the study report. The reviewer calculated % recoveries based on the HPLC area count data and the linearity equations (See DER Excel Attachment), based on the calculation of concentration equations reported on p. 17 of the study MRID 52041305. The reviewer also calculated means, standard deviations, and relative standard deviations for all sample sets.

2 In the ILV, the water matrix was surface (river) water (pH 8.14, 511 µS/cm conductivity, 13.5°dH (2.42 mmol/L) water hardness, 3.6 mg/L total organic carbon; 2.8 mg/L dissolved organic carbon, and <0.1 mg/L filterable solids) from River Danube, Landeswasserversorgung, Langenau, Germany (p. 12; Appendices 7-8, pp. 33-34 of MRID 52041305). The water was characterized (non-GLP) by Institut Alpha, Ulm, Germany. The water matrix was sampled on June 27, 2019, which was *ca*. 2 months prior to the experimental start date (August 26, 2019; p. 6; Appendices 7-8, pp. 33-34).

3 Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 650→610 and *m/z* 650→193 for fluoxapiprolin (BCS-CS55621).

4 The LOQ was also reported as 0.05 µg/L in test water:acetonitrile (8:2, v:v) which corresponded to 0.0625 µg/L in the test water alone (pp. 10, 14-15, 18-19 of MRID 52041305).

5 Reported in the study as HPLC area counts (Tables 10-11, pp. 18-19 of MRID 52041305); reviewer-calculated as % based on individual calculated % recoveries (See DER Excel Attachment).

6 Reviewer-calculated as % based on individual calculated % recoveries (See DER Excel Attachment). Standard deviations were not calculated/reported in the study.

7 Reported in the study as %; reviewer-calculated as % based on individual calculated % recoveries (See DER Excel Attachment).

8 Duplicate aliquots were taken for each sample analysis. All results were included in calculations and ranges.

III. Method Characteristics

The LOQ surface water in the ECM and ILV was reported as 0.0625 µg/L for fluoxapiprolin (BCS-CS55621; pp. 10, 13, 17 of MRID 52041304; pp. 10, 14-15, 18-19 of MRID 52041305). The LOQ was also reported as 0.05 µg/L in test water:acetonitrile (8:2, v:v) which corresponded to 0.0625 µg/L in the test water alone. No justifications or calculations for the LOQ were reported in the ECM or ILV. The LOD in surface water for the method was reported as 0.015 µg/L in the ILV, but it was not reported in the ECM (Appendix 1, p. 21 of MRID 52041305).

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

Table 4. Method Characteristics

		Fluoxapiprolin (BCS-CS55621)
Limit of Quantitation (LOQ)*	ECM	0.0625 µg/L (test water) 0.05 µg/L [test water:acetonitrile (8:2, v:v)]
	ILV	
Limit of Detection (LOD)	ECM	**Not reported**
	ILV	0.015 µg/L (test water)
Linearity (calibration curve r and concentration range)	ECM	r = 0.9999372 (Q) r = 0.9998641 (C)
	ILV	r = 1.0000 (Q) r = 1.0000 (C)
	Range	0.015-5.0 µg/L
Repeatable	ECM[1,2,3]	Yes for LOQ (0.0625 µg/L) and 10×LOQ (0.6250 µg/L) in one characterized water matrix (surface).
	ILV[1,2,4,5]	Yes for LOQ (0.0625 µg/L) and 10×LOQ (0.6250 µg/L) in one characterized water matrix (surface).
Reproducible		Yes for 0.0625 µg/L (LLMV)* and 0.6250 µg/L in surface water.[6]
Specific	ECM	Yes, no matrix interferences were observed at the RT of fluoxapiprolin (BCS-CS55621). Some minor baseline noise was observed in the LOD and LOQ C chromatograms.
	ILV	Yes, matrix interferences were <1% of the LOQ (based on peak area) at the RT of fluoxapiprolin (BCS-CS55621). A minor contaminant (RT 0.35 min.) was noted in the LOD and LOQ Q chromatograms.

Data were obtained from pp. 10, 13, 17 (LOQ/LOD); Table 8, p. 17; Table 10, p. 19 (recovery data); Appendix 5, p. 27 (calibration curves); Appendix 6, pp. 30-34 (chromatograms) of MRID 52041304; pp. 10, 14-15, 18-19; Appendix 1, p. 21 (LOQ/LOD); Tables 10-11, pp. 18-19 (recovery data); Appendix 4, pp. 24-25 (calibration curves); Appendix 5, pp. 27-31 (chromatograms) of MRID 52041305; DER Excel Attachment. Q = quantitative ion transition; C = confirmatory ion transition.

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 Only HPLC area counts were provided as recovery results in the ECM and ILV study reports. The reviewer calculated % recoveries based on the HPLC area count data and the linearity equations (See DER Excel Attachment) based on the calculation of concentration equations reported on p. 16 of the study MRID 52041304 and p. 17 of the study MRID 52041305. The reviewer also calculated means, standard deviations, and relative standard deviations for all sample sets.

2 DER repeatability determinations were based on data available as %: 1) reviewer-calculated means as % based on individual calculated % recoveries (See DER Excel Attachment) and 2) reviewer-calculated and study-reported RSDs as % (reviewer-calculated and study-reported RSDs were the same for each sample set; See DER Excel Attachment).

3 In the ECM, the water matrix was surface (river) water (pH 7.5, 273 µS/cm conductivity, 4.6°dH water hardness, <2 mg/L total organic carbon, dissolved organic carbon not reported, and <5 mg/L filterable solids) from River Wupper, Leverkusen-Opladen, Germany (p. 12 of MRID 52041304). The laboratory which performed the characterization was not reported. The water matrix was sampled on January 13, 2015, which was over four years prior to the experimental start date (May 27, 2019; pp. 7, 12). No post-sampling storage data was provided for the test water matrix; the date of the characterization was not reported.

4 In the ILV, the water matrix was surface (river) water (pH 8.14, 511 µS/cm conductivity, 13.5°dH (2.42 mmol/L) water hardness, 3.6 mg/L total organic carbon; 2.8 mg/L dissolved organic carbon, and <0.1 mg/L filterable solids) from River Danube, Landeswasserversorgung, Langenau, Germany (p. 12; Appendices 7-8, pp. 33-34 of MRID 52041305). The water was characterized (non-GLP) by Institut Alpha, Ulm, Germany. The water matrix

was sampled on June 27, 2019, which was *ca*. 2 months prior to the experimental start date (August 26, 2019; p. 6; Appendices 7-8, pp. 33-34).

5 The ILV validated Bayer AG Method 01387/M007 in the first trial for fluoxapiprolin (BCS-CS55621) in surface water at 0.0625 µg/L and 0.6250 µg/L with insignificant modifications to the sample fortification procedure and analytical parameters and equipment (pp. 19-20 of MRID 52041305). No updated ECM is required based on ILV modifications.

6 The method (Bayer AG Method 01387/M007) was Modification M007 of Analytical Method 01387 (p. 1 of MRID 52041304). Analytical Method 01387 was not provided for review by PB&A/CSS Joint Venture personnel. Based on the method title, Analytical Method 01387 applied to drinking and surface water; however, drinking water was not included as a test matrix in the ECM or ILV validations for Modification M007 of Analytical Method 01387. The ECM and ILV reported that "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" (p. 12 of MRID 52041304; p. 12 of MRID 52041305).

IV. Method Deficiencies and Reviewer's Comments

1. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ (pp. 10, 13, 17 of MRID 52041304; pp. 10, 14-15, 18-19 of MRID 52041305). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for fluoxapiprolin (BCS-CS55621) in water (0.0625 µg/L).

   The LOQ was also reported as 0.05 µg/L in test water:acetonitrile (8:2, v:v) which corresponded to 0.0625 µg/L in the test water alone (pp. 10, 13, 17 of MRID 52041304; pp. 10, 14-15, 18-19 of MRID 52041305).

2. The ECM MRID 52041304 method (Bayer AG Method 01387/M007) was Modification M007 of Analytical Method 01387 (p. 1 of MRID 52041304). Analytical Method 01387 was not provided for review by PB&A/CSS Joint Venture personnel. Based on the method title, Analytical Method 01387 applied to drinking and surface water; however, drinking water was not included as a test matrix in the ECM or ILV validations for Modification M007 of Analytical Method 01387. The ECM and ILV reported that "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" (p. 12 of MRID 52041304; p. 12 of MRID 52041305).

   To fully assess the justification of the exclusion of drinking water in Bayer AG Method 01387/M007, Analytical Method 01387 would need to be provided for review by PB&A/CSS Joint Venture personnel.

3. Only HPLC area counts were provided as recovery results in the ECM and ILV study reports (Table 8, p. 17; Table 10, p. 19 of MRID 52041304; Tables 10-11, pp. 18-19 of MRID 52041305). The reviewer calculated % recoveries based on the HPLC area count data and the linearity equations (See DER Excel Attachment), based on the calculation of

concentration equations reported on p. 16 of the study MRID 52041304 and p. 17 of the study MRID 52041305. The reviewer also calculated means, standard deviations, and relative standard deviations for all sample sets.

DER repeatability determinations were based on data available as %: 1) reviewer-calculated means as % based on individual calculated % recoveries (See DER Excel Attachment) and 2) reviewer-calculated and study-reported RSDs as % (reviewer-calculated and study-reported RSDs were the same for each sample set; See DER Excel Attachment).

4. The ECM surface water matrix was sampled on January 13, 2015, which was over four years prior to the experimental start date (May 27, 2019; pp. 7, 12 of MRID 52041304). No post-sampling storage data was provided for the test water matrix; the date of the characterization was not reported.

5. The reviewer believed that the sample preparation for the method (Bayer AG Method 01387/M007) required some clarification (p. 13 of MRID 52041304). It was reported in the ECM and ILV that "water samples are added with acetonitrile (25% of the **water sample** volume) and directly injected into the HPLC" (p. 13 of MRID 52041304; p. 15 of MRID 52041305). Since the test sample was prepared as test surface water:acetonitrile (8:2, v:v) mixtures, the reviewer believed that some clarification about the volume of the acetonitrile should be reported. The reviewer also noted that this detail may have been reported in Analytical Method 01387, which was not provided for review by PB&A/CSS Joint Venture personnel.

6. The time requirement for the method was not reported in the ILV or ECM.

7. The ILV reported that no communication with the method developers or others familiar with the method occurred (p. 19 of MRID 52041305).

8. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 10, 13, 17 of MRID 52041304; pp. 10, 14-15, 18-19 of MRID 52041305). No justifications or calculations for the LOQ were reported in the ECM or ILV. The LOD in surface water for the method was not reported in the ECM. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

   Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

9. In the ECM, the storage stability of the fluoxapiprolin (BCS-CS55621) in surface water at 0.5 µg/L was determined to be up to 8 days under freezer storage (≤-18°C; p. 18; Table 9, p. 18 of MRID 52041304). The solutions (20 mL) were mixed with acetonitrile (5 mL) prior to analysis. Storage stability was not studied in the ILV but cited as having been studied in the "original laboratory validation" (p. 19 of MRID 52041305). However, the

ILV cited that fluoxapiprolin (BCS-CS55621) in acetonitrile has been shown to be stable for up to 165 days under refrigeration (temperature not reported; Bayer Analytical Method 01554; p. 20 of MRID 52041305).

10. Matrix effects were studied in the ECM and determined to not affect MS/MS detection of fluoxapiprolin (BCS-CS55621); however, matrix-match calibration standards were used in the ECM and ILV (pp. 13, 18 of MRID 52041304). Matrix effects were not studied in the ILV but cited as having been studied in the "original laboratory validation" (p. 19 of MRID 52041305).

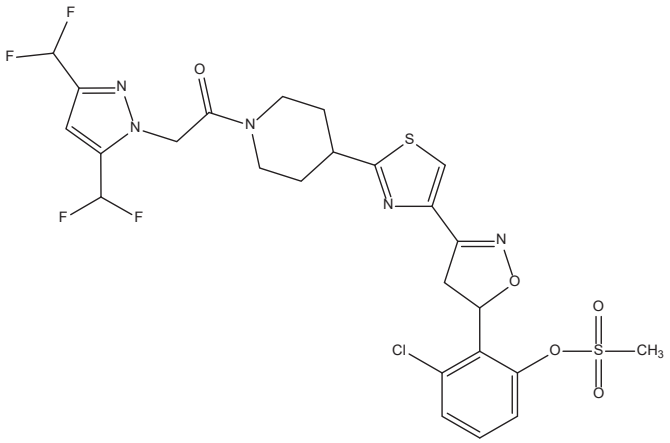
V. References

U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 344-347, and Revision 2; 2015 and 2016.

DER ATTACHMENT 1. Fluoxapiprolin and Its Environmental Transformation Products. [A]

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)	Final %AR (study length)
PARENT						
Fluoxapiprolin (BCS-CS55621)	**IUPAC:** 2-{(5RS)-3-[2-(1-{[3,5-bis(difluoromethyl)-1H-pyrazol-1-yl]acetyl}-4-piperidyl)thiazol-4-yl]-4,5-dihydroisoxazol-5-yl}-3-chlorophenyl methanesulfonate **CAS:** 2-[3,5-bis(difluoromethyl)-1H-pyrazol-1-yl]-1-[4-[4-[5-[2-chloro-6-[(methylsulfonyl)oxy]phenyl]-4,5-dihydro-3-isoxazolyl]-2-thiazolyl]-1-piperidinyl]ethanone **CAS No.:** 1360819-11-9 **Formula:** $C_{25}H_{24}ClF_4N_5O_5S_2$ **MW:** 650.1g/mol **SMILES:** FC(F)C1=NN(CC(N2CCC(C3=NC(C4=NOC(C5=C(Cl)C=CC=C5OS(C)(=O)=O)C4)=CS3)CC2)=O)C(C(F)F)=C1		850.6100 ECM Soil	52041304	PRT	PRT
			850.6100 ILV Soil	52041305		
MAJOR (>10%) TRANSFORMATION PRODUCTS						
No major transformation products were identified.						
MINOR (<10%) TRANSFORMATION PRODUCTS						
No minor transformation products were identified.						
REFERENCE COMPOUNDS NOT IDENTIFIED						
All compounds used as reference compounds were identified.						

[A] AR means “applied radioactivity”. MW means “molecular weight”. PRT means “parent”. ECM means "environmental chemical methods". ILV means "independent laboratory validation".