Analytical method for fluoxastrobin (HEC-5725) and HEC 5725-deschlorophenyl and HEC 5725-oxazepine in water

Reports: ECM: EPA MRID No.: MRID 50428701 (Appendix 6, pp. 54-93). Ye, Y. 2017. An Analytical Method for the Determination of Residues of Fluoxastrobin (HEC-5725) and its Metabolites HEC 5725-deschlorophenyl and HEC 5725-oxazepine in Water Using LC/MS/MS. Report prepared, sponsored, and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 40 pages. Bayer Method No.: HE001-W17-01. Activity No.: RAHE0014. Final report issued September 6, 2017.

ILV: EPA MRID No.: MRID 50428701. Davidson, J.D. 2017. Independent Laboratory Validation of An Analytical Method for the Determination of Residues of Fluoxastrobin (HEC-5725) and its Metabolites HEC 5725-deschlorophenyl and HEC 5725-oxazepine in Water Using LC/MS/MS. Final Report. Report prepared by SynTech Research Laboratory Services, Inc., Stilwell, Kansas, sponsored, and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 93 pages. Study No.: RAHE0014 and 007SRUS17R0178. Activity No.: RAHE0014. Final report issued October 18, 2017.

Document No.: MRID 50428701

Guideline: 850.6100

Statements: ECM: The study was not conducted in compliance with Good Laboratory Practice (GLP) standards since the document was not the result of a study as defined by 40 CFR 160.3 (Appendix 6, p. 56 of MRID 50428701). Signed and dated Data Confidentiality and GLP statements were provided (Appendix 6, pp. 55-56). Quality Assurance and authenticity statements were not included. ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 50428701). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5). The statement of authenticity was not included.

Classification: This analytical method is classified as Supplemental. Percent recoveries were not reported in the ECM and only one sample was prepared for each analyte/fortification level. However, the ILV was able to appropriately quantify fluoxastrobin and its transformation products in water.

PC Code: 028869

EFED Final Reviewer:	Jessica L. O. Joyce, M.S. Physical Scientist	Signature: Date:	 04/29/2020
	Lisa Muto, Environmental Scientist	Signature: Date:	 02/18/2019
CDM/CSS-Dynamac JV Reviewers:	Mary Samuel, M.S., Environmental Scientist	Signature: Date:	 02/18/2019

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

Executive Summary

The analytical method, Bayer Method No. HE001-W17-01, is designed for the quantitative determination of fluoxastrobin (HEC-5725) and its metabolites HEC 5725-deschlorophenyl and HEC 5725-oxazepine in water at the stated LOQ of 0.05 ng/g (parts per billion). The LOQ is less than the lowest toxicological level of concern in water. The ILV and ECM used one uncharacterized and undescribed water matrix. The ILV validated the ECM in the second trial for all three analytes with insignificant modifications to the analytical equipment and parameters; however, HEC 5725-oxazepine samples passed in the second injection after alternating groupings of calibration standards and samples. The first method validation set had chromatography difficulties that resulted in failed integration parameters. All submitted ILV data pertaining to precision, repeatability, linearity, and specificity was acceptable at the LOQ and 10×LOQ; however, the reproducibility of the method could not be determined at LOQ and 10×LOQ because percent recoveries were not reported in the ECM. In the ECM, only one sample was prepared for each analyte/fortification level. The quantification linearity was not satisfactory for fluoxastrobin and HEC 5725-deschlorophenyl, but the confirmatory analysis was satisfactory. In the ECM, the LOD was not reported.

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						
Fluoxastrobin (HEC-5725)	MRID 50428701 (Appendix 6)[1]	MRID 50428701[2]	Supplemental	Water	06/09/2017	Bayer CropScience	LC/MS/MS	0.05 ng/g
HEC 5725-deschlorophenyl								
HEC 5725-oxazepine								

1 In the ECM, the test water was not described or characterized; water matrix source was not reported.

2 In the ILV, the test water was untreated water collected from the surrounding site of SynTech Research Laboratory Services, Inc. near Stilwell, Kansas (pp. 8, 13 of MRID 50428701). Water characterization was not provided, but it was retained in raw data. Water matrix source was not reported.

I. Principle of the Method

Water (50 ± 0.05 g) was fortified with mixed fortification solutions (0.02 or 1.0 µg/mL) in stoppered containers then mixed with 0.0625 mL of the 0.2 µg/mL internal standard (HEC 5725-dioxazin-D_4 and HEC 5725-deschlorophenyldioxazin-D_4; Appendix 6, pp. 60-62 of MRID 50428701). Aliquots were analyzed by LC/MS/MS.

Samples were analyzed for fluoxastrobin (HEC-5725), HEC 5725-deschlorophenyl, and HEC 5725-oxazepine using a Thermo TSQ Quantiva chromatograph/mass spectrometer (LC-MS/MS) equipped with electrospray ionization (ESI) interface in the positive ion, multiple reaction monitoring (MRM) mode (400°C; Appendix 6, pp. 59, 63-65 of MRID 50428701). The following LC conditions were used: Phenomenex Kinetex C18 column (2.1 mm x 100 mm, 1.7 µm; oven temperature 40°C), mobile phase of (A) 0.1% aqueous formic acid and (B) 0.1% methanol formic acid [mobile gradient phase of percent A:B (v:v) at 0.0-0.1 min. 95:5, 6.0-7.0 min. 5:95, 8.0-11.0 min. 95:5] and injection volume of 50 µL (adjusted as needed). Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 459.1→188 and *m/z* 459.1→427 for fluoxastrobin (HEC-5725), *m/z* 349.1→102.1 and *m/z* 349.1→317.1 for HEC 5725-deschlorophenyl, and *m/z* 409.1→169.1 and *m/z* 409.1→365 for HEC 5725-oxazepine. Expected retention times were *ca*. 6.2, 4.2, and 6.4 minutes for fluoxastrobin (HEC-5725), HEC 5725-deschlorophenyl, and HEC 5725-oxazepine, respectively.

The ILV performed the ECM method as written, except for insignificant modifications to the analytical equipment and parameters (p. 13; Appendix 3, pp. 49-50 of MRID 50428701). Samples were analyzed for fluoxastrobin (HEC-5725), HEC 5725-deschlorophenyl, and HEC 5725-oxazepine using an Ultimate 3000 XRS LPG-3400-XRS Serial # 32073 LC coupled with TSQ Quantiva Serial # TQH-Q1-0276 Mass Spectrometer (317°C). The LC/MS/MS parameters were the same as those of the ECM, except that injection volume was 60 µL. Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 459.2→188.1 and *m/z* 459.2→427.1 for fluoxastrobin (HEC-5725), *m/z* 349.2→102.1 and *m/z* 349.2→317.1 for HEC 5725-deschlorophenyl, and *m/z* 409.2→169.1 and *m/z* 409.2→365.1 for HEC 5725-oxazepine; these were similar to those of the ECM. Observed retention times were *ca*. 6.50, 4.38, and 6.66 minutes for fluoxastrobin (HEC-5725), HEC 5725-deschlorophenyl, and HEC 5725-oxazepine, respectively (Appendix 2, pp. 29-40).

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.05 ng/g for fluoxastrobin (HEC-5725), HEC 5725-deschlorophenyl and HEC 5725-oxazepine in water (pp. 15-16; Tables 7-8, p. 20; Appendix 6, p. 59 of MRID 50428701). In the ILV, the Limit of Detection (LOD) was calculated to be 0.0043-0.005 ng/g for fluoxastrobin (HEC-5725), 0.0062-0.0070 ng/g for HEC 5725-deschlorophenyl, and 0.0067-0.0096 ng/g for HEC 5725-oxazepine. In the ECM, the LOD was not reported.

II. Recovery Findings

ECM (MRID 50428701): The acceptability of recoveries could not be determined since percent recoveries were not reported. Only one sample was prepared for each analyte at fortification levels of 0.05 ng/g (LOQ) and 0.5 ng/g (10×LOQ; Appendix 6, pp. 76-87). Two ion pair transitions were monitored for fluoxastrobin (HEC-5725), HEC 5725-deschlorophenyl and HEC 5725-oxazepine using LC/MS/MS in positive mode. The quantification and confirmation ion data could not be compared. The test water was not described or characterized; water matrix source was not reported.

ILV (MRID 50428701): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD ≤20%) for analysis of fluoxastrobin (HEC-5725), HEC 5725-deschlorophenyl, and HEC 5725-oxazepine at fortification levels of 0.05 ng/g (LOQ) and 0.5 ng/g (10×LOQ) in one water matrix (Tables 1-6, p. 19). Two ion pair transitions were monitored for fluoxastrobin (HEC-5725), HEC 5725-deschlorophenyl and HEC 5725-oxazepine using LC/MS/MS in positive mode; the quantification and confirmation ion data was comparable. The test water was untreated water collected from the surrounding site of SynTech Research Laboratory Services, Inc. near Stilwell, Kansas (pp. 8, 13). Water characterization was not provided, but it was retained in raw data. Water matrix source was not reported. The ILV validated the ECM in the second trial for all three analytes with insignificant modifications to the analytical equipment and parameters; however, HEC 5725-oxazepine samples passed in the second injection after alternating groupings of calibration standards and samples (pp. 13, 16; Appendix 3, pp. 49-50). The first method validation set had chromatography difficulties that resulted in failed integration parameters.

Table 2. Initial Validation Method Recoveries for Fluoxastrobin (HEC-5725), HEC 5725-deschlorophenyl and HEC 5725-oxazepine in Water (ECM)

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Water[1]					
	Quantitation ion/Confirmation ion[2]					
Fluoxastrobin (HEC-5725)	0.05 (LOQ)	1	Not reported			
	0.5	1				
HEC 5725-deschlorophenyl	0.05 (LOQ)	1				
	0.5	1				
HEC 5725-oxazepine	0.05 (LOQ)	1				
	0.5	1				

Data (uncorrected recovery results; Appendix 6, p. 66) were obtained from Appendix 6, pp. 76-87 of MRID 50428701.
1 The water was not described or characterized; water matrix source was not reported.
2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 459.1→188 and *m/z* 459.1→427 for fluoxastrobin (HEC-5725), *m/z* 349.1→102.1 and *m/z* 349.1→317.1 for HEC 5725-deschlorophenyl, and *m/z* 409.1→169.1 and *m/z* 409.1→365 for HEC 5725-oxazepine.

Table 3. Independent Validation Method Recoveries for Fluoxastrobin (HEC-5725), HEC 5725-deschlorophenyl, and HEC 5725-oxazepine in Water

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Water[1]					
	Quantitation ion[2]					
Fluoxastrobin (HEC-5725)	0.05 (LOQ)	5	88-95	92	2.7	2.9
	0.5	5	95-100	97	2.2	2.3
HEC 5725-deschlorophenyl	0.05 (LOQ)	5	87-94	90	3.3	3.7
	0.5	5	92-99	97	3.0	3.1
HEC 5725-oxazepine	0.05 (LOQ)	5	86-90	89	5.1	5.7
	0.5	5	90-103	96	4.8	5.1
	Confirmation ion[2]					
Fluoxastrobin (HEC-5725)	0.05 (LOQ)	5	89-95	92	2.3	2.5
	0.5	5	95-100	98	2.2	2.3
HEC 5725-deschlorophenyl	0.05 (LOQ)	5	95-105	100	3.8	3.8
	0.5	5	94-103	98	3.3	3.4
HEC 5725-oxazepine	0.05 (LOQ)	5	87-95	92	3.6	3.9
	0.5	5	94-101	97	2.7	2.8

Data (uncorrected recovery results; p.14; Appendix 4, pp. 51-52) were obtained from Tables 1-6, p. 19 of MRID 50428701.
1 The water was untreated water collected from the surrounding site of SynTech Research Laboratory Services, Inc. near Stilwell, Kansas (pp. 8, 13). Water characterization was not provided, but it was retained in raw data. Water matrix source was not reported
2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 459.2→188.1 and *m/z* 459.2→427.1 for fluoxastrobin (HEC-5725), *m/z* 349.2→102.1 and *m/z* 349.2→317.1 for HEC 5725-deschlorophenyl, and *m/z* 409.2→169.1 and *m/z* 409.2→365.1 for HEC 5725-oxazepine; these were similar to those of the ECM.

III. Method Characteristics

In the ECM and ILV, the LOQ was 0.05 ng/g for fluoxastrobin (HEC-5725), HEC 5725-deschlorophenyl and HEC 5725-oxazepine in water (pp. 15-16; Tables 7-8, p. 20; Appendix 6, p. 59 of MRID 50428701). In the ILV, the LOQ was reported as the lowest level at which successful recoveries were run; no justification was provided in the ECM. No calculations were provided for the LOQ in the ECM or ILV. In the ILV, the LOD was calculated to be 0.0043-0.005 ng/g for fluoxastrobin (HEC-5725), 0.0062-0.0070 ng/g for HEC 5725-deschlorophenyl, and 0.0067-0.0096 ng/g for HEC 5725-oxazepine. The LOD was calculated for each analyte using the following equation:

$LOD = (t_{0.99} \times SD)$

Where, $t_{0.99}$ is the one-tailed t-statistic value of 3.747 at the 99% confidence level for n-1 replicates and SD is the standard deviation of the analyte recovery measurements at the target LOQ. In the ECM, the LOD was not reported.

Table 4. Method Characteristics for Fluoxastrobin (HEC-5725), HEC 5725-deschlorophenyl and HEC 5725-oxazepine in Water

Parameter		Fluoxastrobin (HEC-5725)	HEC 5725-deschlorophenyl	HEC 5725-oxazepine
Limit of Quantitation (LOQ)	ECM	0.05 ng/g		
	ILV			
Limit of Detection (LOD)	ECM	**Not reported**		
	ILV	0.0043-0.005 ng/g	0.0062-0.0070 ng/g	0.0067-0.0096 ng/g
Linearity (calibration curve r^2 and concentration range)	ECM	r^2 = **0.9943** (Q) r^2 = 0.9970 (C)	r^2 = **0.9925** (Q) r^2 = 0.9978 (C)	r^2 = 0.9969 (Q) r^2 = 0.9968 (C)
	ILV	r^2 = 0.9998 (Q) r^2 = 0.9996 (C)	r^2 = 0.9989 (Q) r^2 = 0.9980 (C)	r^2 = 0.9993 (Q) r^2 = 0.9991 (C)
	Range	0.03-1.00 ng/g		
Repeatable	ECM[1]	**Could not be determined** at LOQ and 10×LOQ. **No recovery data reported**; n = **1**. (uncharacterized water used)		
	ILV[2,3]	Yes at LOQ and 10×LOQ. (uncharacterized water used)		
Reproducible		**Could not be determined** at LOQ and 10×LOQ. Only one set of performance data submitted.		
Specific	ECM	Yes, only minor baseline noise was observed at the LOQ, which was more significant for HEC 5725-deschloropehnyl. **No** representative chromatograms of the control samples were provided.		
	ILV	Yes, no matrix interferences were observed, but some minor baseline noise was observed in the control.		

Data were obtained from Appendix 6, p. 59 (ECM LOQ/LOD); Appendix 6, pp. 70-75 (ECM calibration curve); Appendix 6, pp. 76-87 (ECM chromatograms); pp. 15-16; Tables 7-8, p. 20 (ILV LOQ/LOD); Tables 1-6, p. 19 (ILV recovery results); Appendix 1, pp. 22-27 (ILV calibration curve); Appendix 2, pp. 41-48 (ILV chromatograms) of MRID 50428701. Q = quantitation ion; C = confirmation ion. All results reported for Q and C ions unless specified otherwise.

1 In the ECM, the test water was not described or characterized; water matrix source was not reported.

2 In the ILV, the test water was untreated water collected from the surrounding site of SynTech Research Laboratory Services, Inc. near Stilwell, Kansas (pp. 8, 13 of MRID 50428701). Water characterization was not provided, but it was retained in raw data. Water matrix source was not reported.

3 The ILV validated the ECM in the second trial for all three analytes with insignificant modifications to the analytical equipment and parameters; however, HEC 5725-oxazepine samples passed in the second injection after alternating groupings of calibration standards and samples (pp. 13, 16; Appendix 3, pp. 49-50 of MRID 50428701). The first method validation set had chromatography difficulties that resulted in failed integration parameters.

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. The reproducibility of the method could not be determined at LOQ and 10×LOQ because percent recoveries were not reported in the ECM. OCSPP Guideline 850.6100 states that two sets of performance data should be submitted, one for the initial or other internal validation and one for the ILV.

2. The ECM contained the following additional deficiencies:

   In the ECM, only one sample was prepared for each analyte at fortification levels of 0.05 ng/g (LOQ) and 0.5 ng/g (10×LOQ; Appendix 6, pp. 76-87 of MRID 50428701). OCSPP Guideline 850.6100 states that a minimally complete sample set includes a reagent blank, two matrix blanks, five samples spiked at the LOQ, and five samples spiked at 10× LOQ for each matrix.

   ECM linearity was not satisfactory for the quantitation ion analysis of fluoxastrobin (r^2 = 0.9943) and HEC 5725-deschlorophenyl (r^2 = 0.9925) in water, however it was satisfactory in the confirmatory ion analysis (Appendix 6, pp. 70-75 of MRID 50428701). Linearity is satisfactory when $r^2 \geq 0.995$.

   In the ECM, no representative chromatograms of the control samples were provided. The level of matrix interferences in the controls could not be assessed.

3. The ECM and ILV test water matrices were not described or characterized; the water matrix sources were not reported (pp. 8, 13 of MRID 50428701). The ILV water characterization was reportedly retained in raw data.

4. The communications between the ECM and ILV were reportedly limited to email exchange regarding study progress and trial results (p. 16; Appendix 5, p. 53 of MRID 50428701). Detailed communication records were not provided, but they were retained in the raw data.

5. 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. 15-16; Tables 7-8, p. 20; Appendix 6, p. 59 of MRID 50428701). In the ILV, the LOQ was reported as the lowest level at which successful recoveries were run; no justification was provided in the ECM. No calculations were provided for the LOQ in the ECM or ILV. In the ECM, the LOD was estimated based on three times the baseline noise; the calculations were not provided. In the ILV, the LOD was calculated for each analyte using the following equation: LOD = ($t_{0.99}$ x SD), where, $t_{0.99}$ is the one-tailed t-statistic value of 3.747 at the 99% confidence level for n-1 replicates and SD is the standard deviation of the analyte recovery measurements at the target LOQ. In the ECM, the LOD was not reported. Detection limits should not be based on arbitrary values.

6. The reviewer noted that the ECM noted that the internal standard. HEC 5725-dioxazin-d4, consists of a pair of E/Z isomers, and the separated Z isomer by chromatography was not integrated (Appendix 6, pp. 76-77 of MRID 50428701).

7. In the ILV, the time required to complete the extraction of one set of 13 samples (one reagent blank, two matrix controls and ten fortified samples) was reported as 2-3 hours, followed by 6 hours for LC/MS/MS analysis (p. 16 of MRID 50428701).

V. References

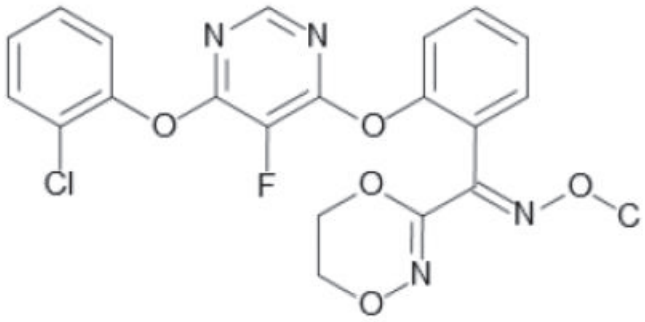
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

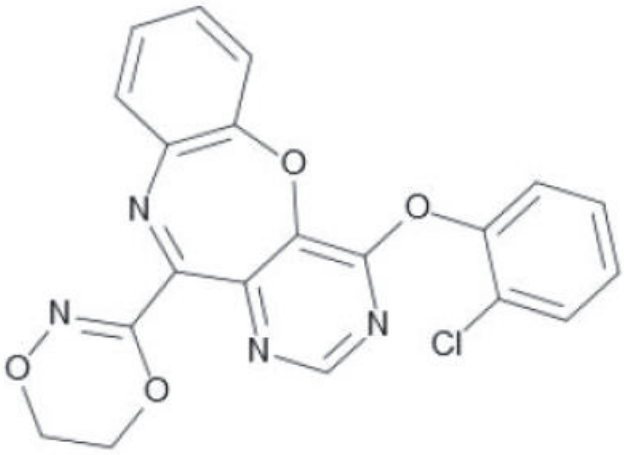
Fluoxastrobin (HEC-5725)

IUPAC Name: Not reported
CAS Name: (1E)-[2-[[6-(2-Chlorophenoxy)-5-fluoro-4-pyrimidinyl]oxy]phenyl](5,6-dihydro-1,4,2-dioxazin-3-yl)methanone O-methyloxime
CAS Number: 361377-29-9
SMILES String: Not found



HEC 5725-oxazepine

IUPAC Name: Not reported
CAS Name: 6-[2-[(5,6-Dihydro-1,4,2-dioxazin-3-yl)(methoxyimino)methyl]phenoxy]-5-fluoro-4(1*H*)-pyrimidinone
CAS Number: Not reported
SMILES String: Not found



HEC 5725-deschlorophenyl

IUPAC Name:	Not reported
CAS Name:	4-(2-Chlorophenyl)-11-(5,6-dihydro-1,4,2-dioxazin-3-yl)pyrimido[5,4-*b*]benzoxazepine
CAS Number:	Not reported
SMILES String:	Not found