

Analytical method for bixafen in water

Reports: ECM: EPA MRID No.: 49877182. Krebber, R., M. Braune. 2008. Analytical method 01073 for the determination of bixafen (BYF 00587) in drinking and surface water by HPLC-MS/MS. Report No.: MR-07/336. Method No. 01073. Laboratory Project ID: P 684 077020. Report prepared and sponsored by Bayer CropScience AG, Monheim am Rhein, Germany, and submitted by FMC Corporation, Ewing, New Jersey; 35 pages. Final report issued January 14, 2008.

ILV: EPA MRID No. 50470801. Xu, A. 2017. Independent Laboratory Validation for the Determination of Bixafen in Surface Water by HPLC-MS/MS. PASC Report No.: PASC-REP-1479; PASC Study No.: 058-2443. FMC Tracking No.: 2017AMT-BFN3924. Report prepared by Primera Analytical Solutions Corp. (PASC), Princeton, NJ, and sponsored and submitted by FMC Corporation, Ewing, New Jersey; 103 pages. Final report issued October 31, 2017.

Document No.: MRIDs 49877182 & 50470801

Guideline: 850.6100


Statements: ECM: The study was conducted in accordance with the OECD Principles of Good Laboratory Practice (GLP), as well as USEPA FIFRA (40 CFR Part 160), German and Japanese GLP standards (p. 3; Appendix 6, pp. 34-35 of MRID 49877182). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5; Appendix 6, pp. 34-35). A certification of authenticity was included with the QA statement (p. 5).

ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (p. 3 of MRID 50470801). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as Acceptable. Communication details between the ILV and ECM were not reported.

PC Code: 128400

EFED Final Reviewer: Mohammed A. Ruhman,
Senior Agronomist

Signature: 

Date: 08/09/2018

Lisa Muto, M.S.,
Environmental Scientist

Signature: 

Date: 3/9/17

CDM/CSS-Dynamac JV Reviewers:

Kathleen Ferguson, Ph.D.,
Environmental Scientist

Signature: 

Date: 3/9/17

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

This analytical method, Bayer Method 01073, is designed for the quantitative determination of bixafen (BYF 00587) in water at the LOQ of 0.05 µg/L using LC/MS/MS. At this time, the LOQ of Bayer Method 01073 is less than the lowest toxicological level of concern in water. The ECM and ILV used one characterized water matrix, surface water. Two ion transitions were monitored. Two trials of Bayer Method 01073 were performed by the ILV due to the fact that recovery results of the first trial were lower than expected (70-80%); the ILV validated the method in the first and second trial with insignificant modifications. Communication details between the ILV and ECM were not reported. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory. The LOD differed between the ECM and the ILV.

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						
Bixafen (BYF 00587)	49877182 (Method 01073)	50470801	08/09/2018	Water ^{1,2}	14/01/2008	FMC Corporation	LC/MS/MS	0.05 µg/L

1 In the ECM, surface water (pH 7.8, 3 mg/L dissolved organic carbon and total organic carbon, 10.7 °dH hardness), obtained from Rhine River, Leverkusen-Hitdorf, was used (p. 12 of MRID 49877182). The water characterization facility was not reported.

2 In the ILV, the surface water (PASC ID 170883; pH 8.3, 116 ppm total dissolved solids, 112 mg/L equivalent CaCO₃/L hardness), obtained from Housatonic River in Kettletown State Park, Connecticut, was used (p. 13; Appendix II, p. 83). The water was provided by PASC and characterized by Agvise Laboratories, Northwood, North Dakota.

I. Principle of the Method

Bayer Method 01073

Surface water samples (volume not reported) were fortified with bixafen in methanol for procedural recoveries (p. 14 of MRID 49877182). The samples were acidified (acidification solution and final desired pH not reported) and diluted with acetonitrile. The sample was analyzed by LC/MS/MS via direct injection or after appropriate dilution with surface water:acetonitrile:formic acid (800:200:0.1, v:v:v).

Samples are analyzed using an Agilent 1100 HPLC coupled to an Ionics EP10+ upgrade of Applied Biosystems API 365 Mass Spectrometer (pp. 14-16; Appendices 1-2, pp. 23-26 of MRID 49877182). The following HPLC conditions were used: Phenomenex or equivalent Luna C18(2)-HST column (2 mm x 50 mm, 2.5 μ m; column temperature 60°C) with pre-column, mobile phase gradient of deionized water:methanol (900:100, v:v) + 10 mM ammonium formate + 120 μ L/L formic acid (A) and deionized water:methanol (100:900, v:v) + 10 mM ammonium formate + 120 μ L/L formic acid (B) [percent (A:B): 0-0.1 min. 70:30, 5.0-8.0 min. 5:95, 8.1 min. 70:30; flow rate 300 μ L/minute), TurboIonSpray in positive mode (TEM 380°C, or as needed for sensitivity), and MRM. Injection volume was 100 μ L (or as needed for sensitivity). Expected retention time for bixafen is *ca.* 5.5 minutes. Bixafen was identified using two ion transitions (primary and confirmatory, respectively): m/z 414 \rightarrow 394 and m/z 414 \rightarrow 266.

ILV

The ILV performed Bayer Method 01073 as written, except for insignificant modifications of the addition of a 0.01 ng/mL calibration standard and analytical instrumentation (pp. 13-14, 16-17 of MRID 50470801). Additionally, some important details of the method which were omitted from the ECM were reported, such as the sample volume (8.0 mL), volume of acetonitrile (2 mL), and the formic acid acidification (10 mL of 1:10 diluted formic acid solution was added). Samples were analyzed using a Shimadzu LC-10ADVP LC system and AB Sciex API 4000 Triple Quadrupole Mass Spectrometer. All other analytical parameters were the same as the ECM, other than minor adjustments allowed in the method. Expected retention time was *ca.* 5.94 minutes (Figure 2, p. 33).

In the ECM and ILV, the Limit of Quantification (LOQ) for bixafen in Bayer Method 01073 was reported as 0.05 μ g/L (pp. 10, 19 of MRID 49877182; pp. 10, 24 of MRID 50470801). The Limit of Detection (LOD) for bixafen was 0.02 μ g/L in the ECM and was 0.01 μ g/L in the ILV.

II. Recovery Findings

ECM (MRID 49877182): For Bayer Method 01073, mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of bixafen at the LOQ (0.05 μ g/L) and 10 \times LOQ (0.5 μ g/L) in one water matrix (Table 8, p. 18; Table 12, p. 20; DER Attachment 2). Two ion pair transitions were monitored; performance data (results) of the quantitation and confirmation ion analyses were comparable. The surface water (pH 7.8, 3 mg/L dissolved organic carbon and total organic carbon, 10.7 °dH hardness), obtained from Rhine River, Leverkusen-Hitdorf, was used in the study (p. 12). The water characterization facility was not reported.

ILV (MRID 50470801): For Bayer Method 01073, mean recoveries and RSDs were within guidelines for analysis of bixafen at the LOQ (0.05 μ g/L) and 10 \times LOQ (0.5 μ g/L) in one water matrix (Tables 13-16, pp. 26-29). Two ion pair transitions were monitored; performance data (results) of the quantitation and confirmation ion analyses were comparable. The surface water (PASC ID 170883; pH 8.3, 116 ppm total dissolved solids, 112 mg/L equivalent CaCO₃/L hardness), obtained from Housatonic River in Kettletown State Park, Connecticut, was used in

the study (p. 13; Appendix II, p. 83). The water was provided by PASC and characterized by Agvise Laboratories, Northwood, North Dakota. Two trials of Bayer Method 01073 were performed by the ILV due to the fact that recovery results of the first trial were lower than expected (70-80%); the ILV validated the method in the first and second trial with insignificant modifications of the addition of a 0.01 ng/mL calibration standard and analytical instrumentation (pp. 10, 13-14, 16-17, 24).

Table 2. Initial Validation Method Recoveries for Bixafen in Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Surface Water						
Quantitation ion						
Bixafen	0.05	10	95-101	98	2	1.7
	0.5	10	102-106	104	2	1.7
Confirmation ion						
Bixafen	0.05	10	96-102	100	2	2.0
	0.5	10	98-105	102	2	2.1

Data (uncorrected recovery results; pp. 16-17) were obtained from Table 8, p. 18 and Table 12, p. 20 of MRID 49877182 and DER Attachment 2.

- 1 The surface water (pH 7.8, 3 mg/L dissolved organic carbon and total organic carbon, 10.7 °dH hardness), obtained from Rhine River, Leverkusen-Hitdorf, was used in the study (p. 12). The water characterization facility was not reported.
- 2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 414→394 and m/z 414→266.
- 3 Standard deviations were reviewer-calculated based on data provided in the study report since the study author did not report these values (see DER Attachment 2). Rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for Bixafen in Water¹

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Surface Water – Trial 1						
Quantitation ion						
Bixafen	0.05	5	57.8-89.1	73.3	11.4	15
	0.5	5	67.9-84.4	76.1	6.8	8.9
Confirmation ion						
Bixafen	0.05	5	63.3-90.4	74.9	10.1	13
	0.5	5	69.0-86.1	76.7	7.1	9.3
Surface Water – Trial 2						
Quantitation ion						
Bixafen	0.05	5	80.7-94.1	86.6	4.8	5.5
	0.5	5	81.6-108	99.4	10	10
Confirmation ion						
Bixafen	0.05	5	80.9-100	87.9	7	8.1
	0.5	5	81.2-109	99.8	11	11

Data (uncorrected recovery results) were obtained from Tables 13-16, pp. 26-29 of MRID 50470801.

- 1 The surface water (PASC ID 170883; pH 8.3, 116 ppm total dissolved solids, 112 mg/L equivalent CaCO₃/L hardness), obtained from Housatonic River in Kettletown State Park, Connecticut, was used in the study (p. 13; Appendix II, p. 83). The water was provided by PASC and characterized by Agvise Laboratories, Northwood, North Dakota.
- 2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 414→394 and m/z 414→266.
- 3 Standard deviations were reviewer-calculated based on data provided in the study report since the study author did not report these values (see DER Attachment 2). Rules of significant figures were followed.

III. Method Characteristics

In the ECM and ILV, the LOQ for bixafen in Bayer Method 01073 was 0.05 µg/L (pp. 10, 19 of MRID 49877182; pp. 10, 24 of MRID 50470801). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which yielded a mean recovery of 70-110% and relative standard deviation of ≤20%. No justifications of the LOQ were provided in the ILV. The LOD for bixafen was 0.02 µg/L in the ECM and was 0.01 µg/L in the ILV. The LOQ was defined as 20% of the LOQ in the ILV; no justifications of the LOD were provided in the ECM.

Table 4. Method Characteristics for Bixafen in Water

		Bixafen (BYF 00587)	
Limit of Quantitation (LOQ)	ECM	0.05 µg/L	
	ILV		
Limit of Detection (LOD)	ECM	0.02 µg/L	
	ILV	0.01 µg/L (20% of the LOQ)	
Linearity (calibration curve r^2 and concentration range)	ECM		$r^2 = 0.9994$ (Q) $r^2 = 0.9997$ (C) [0.04-10 µg/L]
	ILV ¹	Trial 1	$r^2 = 0.9982$ (Q) $r^2 = 0.9986$ (C) [0.02-10.0 µg/L]
		Trial 2	$r^2 = 0.9954$ (Q) $r^2 = 0.9960$ (C) [0.01-10.0 µg/L]
Repeatable	ECM ²		Yes at LOQ and 10×LOQ.
	ILV ^{3,4}		Yes at LOQ and 10×LOQ (Trials 1 and 2).
Reproducible		Yes at LOQ and 10×LOQ.	
Specific	ECM		Yes, matrix interferences were <7% of the LOQ (based on peak area).
	ILV		Yes, matrix interferences were <10% of the LOQ (based on peak comparison). Some minor baseline noise was observed at the LOQ.

Data were obtained from pp. 10, 19; Table 8, p. 18 and Table 12, p. 20 (recovery results); Appendix 4, p. 29 (calibration curves); Appendix 5, pp. 31-33 (chromatograms) of MRID 49877182; pp. 10, 24; Tables 13-16, pp. 26-29 (recovery results); Tables 8-11, pp. 20-23 (calibration data); Figures 3-5, pp. 41-53 and Figures 9-11, pp. 66-78 (chromatograms) of MRID 50470801; DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition.

1 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (Figures 41-44, pp. 50-51 of MRID 50470801; see DER Attachment 2).

2 In the ECM, surface water (pH 7.8, 3 mg/L dissolved organic carbon and total organic carbon, 10.7 °dH hardness), obtained from Rhine River, Leverkusen-Hitdorf, was used (p. 12 of MRID 49877182). The water characterization facility was not reported.

3 In the ILV, the surface water (PASC ID 170883; pH 8.3, 116 ppm total dissolved solids, 112 mg/L equivalent CaCO₃/L hardness), obtained from Housatonic River in Kettletown State Park, Connecticut, was used (p. 13; Appendix II, p. 83). The water was provided by PASC and characterized by Agvise Laboratories, Northwood, North Dakota.

4 Two trials of Bayer Method 01073 were performed by the ILV due to the fact that recovery results of the first trial were lower than expected (70-80%); the ILV validated the method in the first and second trial with insignificant modifications of the addition of a 0.01 ng/mL calibration standard and analytical instrumentation (pp. 10, 13-14, 16-17, 24 of MRID 50470801).

IV. Method Deficiencies and Reviewer's Comments

1. No communication details between the ILV and ECM were reported. The reviewer noted that no personnel of the ECM were reported in the ILV signatures, other than the Submitter (p. 2 of MRID 49877182; pp. 2-5 of MRID 50470801).
2. The ECM reported that the validation was not necessary for drinking water because the LOQ for surface water is below the drinking water limit of 0.1 µg/L (p. 12 of MRID 49877182). The reviewer did not find a reference for the LOQ for drinking water. Also,

the reviewer concluded that this method could be proposed to be applied to other water matrices, such as drinking water, but certainty cannot be stated until a validated method validation is submitted with a drinking water matrix.

3. The estimations of the LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 10, 19 of MRID 49877182; pp. 10, 24 of MRID 50470801). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which yielded a mean recovery of 70-110% and relative standard deviation of $\leq 20\%$. No justifications of the LOQ were provided in the ILV. The LOD for the method differed between the ECM and the ILV. The LOQ was defined as 20% of the LOD in the ILV; no justifications of the LOD were provided in the ECM. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.
4. No reagent blank was included in the ECM.
5. In the ECM, the matrix effects were determined to be insignificant (p. 24; Table 12, p. 24 of MRID 49877182).
6. In the ECM, samples of bixafen in surface water (acidification was not specified) were found to be stable for up to 7 days at $\leq -18^{\circ}\text{C}$ (pp. 10, 19, 22 of MRID 49877182). The standard solutions were reported to be stable for at least 10 months under refrigeration.
7. The time requirement for the method was not reported in the ILV or ECM.

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**Bixafen (BYF 00587)**

IUPAC Name: N-(3',4'-Dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide

CAS Name: Not reported

CAS Number: 581809-46-3

SMILES String: Not found

