

SUMMARY OF RESULTS

An analytical methodology has been independently validated to quantitatively determine bixafen in surface water by high performance liquid chromatography system (HPLC) with tandem mass spectrometric detection (MS/MS) using multiple reaction monitoring (MRM) transitions. The method is described in “Analytical method 01073 for the determination of bixafen (BYF 00587) in drinking and surface water by HPLC-MS/MS”, R. Krebber and M. Braune, Bayer CropScience AG, Report no. MR-07/336.

The methodology was applied to the analysis of surface water samples and validated with 5 replicates at the limit of quantitation (LOQ, 0.05 ppb) and 10×LOQ (0.5 ppb). Mean recoveries for each fortification level were within the 70%–120% range for both MRM transitions (see Table 1). Although the first trial has met the guideline acceptance criteria, the recovery values were lower than expected, which most likely was due to a spiking problem. A second trial was then carried out and both analytical results are summarized in Table 1. No matrix effects were observed in this study and no method modifications are necessary, except an additional calibration standard at 0.01 ng/mL was added to cover 30% of LOQ for the second trial.

1. Introduction

1.1 Background

Bixafen is a pyrazole carboxamide fungicide provided by FMC Corp. Primera Analytical Solutions Corp. (hereafter referred to as PASC) has been contracted by FMC Corporation to conduct an independent laboratory validation (ILV) to demonstrate that the Bayer CropScience (BCS) AG analytical method reported in MR-07/336 (Reference 1) can be performed with acceptable recoveries for quantitative determination of bixafen in surface water by HPLC-MS/MS. The study protocol is provided in Appendix IV.

1.2 Purpose

This study is to validate the HPLC-MS/MS method for determination of bixafen in surface water. The results demonstrate that the analytical method is suitable for its intended use.

1.3 Scope

This report applies to the validation method for the analysis of bixafen in surface water, which was previously developed by Bayer CropScience AG and reported in MR-07/336, according to the study protocol PASC-PRT-1479.

2. References

- 2.1 R. Krebber and M. Braune, "Analytical method 01073 for the determination of bixafen (BYF 00587) in drinking and surface water by HPLC-MS/MS", Bayer CropScience AG, Report no. MR-07/336.
- 2.2 PASC-SOP-0402, Version No. 11: "Deviation Resolution"
- 2.3 EPA OCSPP 850.6100, "Ecological Effects Test Guidelines-Data Reporting for Environmental Chemistry Methods", EPA 712-C-001, January 2012
- 2.4 EPA OPP Pesticide Registration (PR) Notice 2011-3, Standard Format for Data Submitted Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Certain Provisions of the Federal Food, Drug, and Cosmetic Act (FFDCA), January 2012.

3. Materials and Equipment

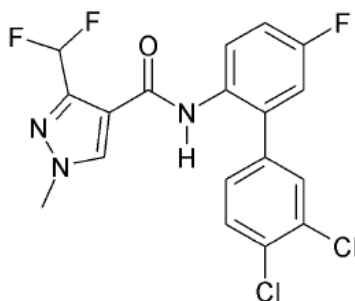
3.1 Chemicals and Reagents

Analytes

The reference standard for bixafen was provided by FMC, the certificate of analysis is provided in Appendix II.

Common Name:	Bixafen
Chemical Name (IUPAC):	N-(3',4'-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide
CAS Registry No.:	581809-46-3

Chemical Structure:



Empirical Formula:	C ₁₈ H ₁₂ Cl ₂ F ₃ N ₃ O
Molecular Mass:	414.21 g/mol
Supplier	FMC Agricultural Products
FMC Number	PL14-0108
Purity	99.7%
Expiration Date	April 2019

Sample Matrix

Surface water (PASC ID 170883) was provided by PASC. The surface water was collected from the Housatonic River in Kettletown State Park, CT. The GLP water characterization data is presented in Appendix II.

Chemicals

Chemical	Supplier	Catalog Number
Acetonitrile	PHARMCO-AAPER	30000HPLC
HPLC Water	PHARMCO-AAPER	23200HPLC
Methanol	J. T. Baker	9093-03
Milli-Q Water	Millipore Milli-Q Gradient A10	---
Ammonium Formate	Sigma-Aldrich	156264-100G
Formic Acid	EMD Millipore	FX0440-7

Reagent Solutions

Note: Solutions may be prepared in different volumes, as long as the components and proportions are not altered.

Reagent	Preparation
Surface Water:Acetonitrile:Formic Acid (800:200:0.1, v/v/v)	400 mL Surface Water 100 mL Acetonitrile 0.05 mL Formic Acid
HPLC Water:Acetonitrile:Formic Acid (800:200:0.1, v/v/v)	400 mL HPLC Water 100 mL Acetonitrile 0.05 mL Formic Acid

3.2 Equipment

Equipment	Supplier/Model
Analytical Balance	Mettler Toledo XS105
Laboratory Class A Glassware (beakers, flasks, volumetric flasks, glass pipettes)	Various
Liquid Chromatograph	Shimadzu LC-10ADVP liquid chromatograph CTO-10ASVP Column Oven SIL-HTC Auto Sampler
Mass Spectrometer	AB Sciex API 4000

4. Instrument Conditions/Parameters

4.1 Chromatographic Conditions

Column:	Luna 2.5 μ C18(2)-HST, 50 \times 2 mm, 100 \AA Part No. 00B-4446-B0	
Injection Volume:	50 μ L	
Column Flow Rate:	0.30 mL/min	
Oven Temperature	60 $^{\circ}$ C	
Mobile Phase		
A:	DI water/methanol, 900/100, v/v + 10 mM Ammonium formate + 120 μ L/L formic acid	
B:	DI water/methanol, 100/900, v/v + 10 mM Ammonium formate + 120 μ L/L formic acid	
Time Table		
Time (min)	A (% , v/v)	B (% , v/v)
0.0	70	30
0.1	70	30
5.0	5	95
8.0	5	95
8.1	70	30
12.0	Stop time	

4.2 Mass Spectrometer Method Properties

General MS Acquisition Parameters for Analytes	
Ionization:	Turbo IonSpray (ESI)
Gas Temperature:	380 $^{\circ}$ C
Gas supply (GS 1):	45 (arbitrary units)
Gas supply (GS 2)	50 (arbitrary units)
Curtain gas (CUR):	15 (arbitrary units)
Collisionally activated dissociation (CAD) gas:	7 (arbitrary units)
Entrance potential (EP):	10 V
Declustering potential (DP):	20 V
IonSpray voltage:	5500 V
Cell exit potential (CXP):	15 V
Polarity:	positive
Scan Type:	MRM

4.3 Mass Transitions and Voltages

Analyte Group	Precursor Ion Q1 Mass (m/z)	Product Ion Q3 Mass (m/z)	Dwell Time (msec)	Collision Energy (V)
Bixafen Quantitation	414	394	250	21
Bixafen Confirmation	414	266	250	33

5. Validation Procedures

The method validation was conducted from October 04, 2017 to October 10, 2017, at PASC in Princeton, New Jersey. The standard stock solution, the fortification standard solutions, and calibration standard solutions were prepared on October 04, 2017. Surface water samples were fortified with bixafen and diluted on October 04, 2017.

The analytical set was first run on October 06, 2017. The acceptance criteria for recovery and % RSD were met for both transitions. However, the percent recoveries of the fortified samples were lower than expected, which most likely due to a spiking problem. A second trial was then carried out.

The second sequence was injected on October 10, 2017, using the freshly-prepared fortification samples and an additional calibration standard at 0.01 ng/mL to cover 30% of LOQ. The criteria for recovery and % RSD were met for both transitions with satisfactory percent recovery for the fortified samples.

5.1 Stock and Fortification Standard Solutions Preparation

Stock Standard Solution Preparation

The stock standard solution was prepared by weighing a defined amount of bixafen into a Class A volumetric flask and making up to volume with acetonitrile. Weight was corrected for purity. The stock standard solution was stored in freezer when not in use. The preparation of the stock standard solution is summarized in Table 2.

Table 2. Preparation of the Stock Standard Solution

Analyte	Purity	Weight (mg)	Final Volume (mL)	Final Concentration (mg/L)
Bixafen	99.7%	10.740	100	107.1

Fortification Standard Solutions Preparation

Fortification solutions were prepared from the stock solution by serial dilution with acetonitrile. The preparation of the fortification solution is summarized in Table 3.

Table 3. Preparation of the Fortification Solutions

No.	Concentration of Source Solution (ng/mL)	Volume of Source Solution (mL)	Final Volume (mL)	Final Concentration (ng/mL)
F1	1.071×10^5	0.934	100	1000
F2	1000	1.00	10	100
F3	1000	1.00	100	10.0

5.2 Sample Preparation

Fortification

To each of twelve 15 mL centrifugation tubes, 8.0 mL of surface water were transferred and fortified with the fortification solution as outlined in Table 4.

Table 4. Surface Water Sample Fortification

Trial No.	Sample Type	Number of Replicates	Fortification Solution Concentration ($\mu\text{g/mL}$)	Fortification Volume (mL)	Fortification Level (ppb)
1	Reagent Blank	1	N/A	0	0
	Control	2	N/A	0	0
	LOQ	5	10	0.040	0.05
	10 \times LOQ	5	100	0.040	0.5
2	Reagent Blank	1	N/A	0	0
	Control	2	N/A	0	0
	LOQ	5	10	0.040	0.05
	10 \times LOQ	5	10	0.400	0.5

Sample Dilution

To the above fortified samples, control samples, and reagent blank (i.e., deionized water), 2 mL of acetonitrile was added to each tube and vortexed. Ten mL of diluted (1:10) formic acid solution was then added to each tube and the mixtures were subject to vortex again.

Fortified samples were stored in freezer when not in use.

5.3 Matrix-Matched Calibration Standards Preparation

Serial Dilution

The calibration standard solutions were prepared by serially diluting the fortification solutions with a mixture of surface water/acetonitrile/formic acid or deionized water/acetonitrile/formic acid (800/200/0.1, v/v/v), according to Table 5.

Table 5. Preparation of the Calibration Standards

No.	Concentration of Source Solution (ng/mL)	Volume of Source Solution (mL)	Final Volume (mL)	Diluent	Final Concentration (ng/mL)
C1	1000	0.10	10	*	10.0
C2	1000	0.10	20	*	5.0
C3	1000	0.10	100	*	1.0
C4	1000	0.050	100	*	0.50
C5	10.0	0.10	10	*	0.10
C6	10.0	0.10	20	*	0.050
C7	10.0	0.10	25	*	0.040
C8	10.0	0.10	50	*	0.020
C9	10.0	0.050	50	*	0.010
C10	1000	0.050	100	**	0.50

* surface water/acetonitrile/formic acid 800/200/0.1 (v/v/v)

** deionized water/acetonitrile/formic acid 800/200/0.1 (v/v/v)

5.4 Modifications to the Original Method

The following modification was applied to the methods:

An additional calibration standard at 0.01 ng/mL was added to cover 30% of LOQ for the second trial.

5.5 Injection Sequence

Five replicate samples at two fortification levels were used to evaluate the method efficiency. Calibration standards were injected within the analysis set to ensure detector linearity and stable response.

The validation set contained at least one reagent blank, two unfortified matrix controls, five matrix control samples fortified at LOQ (0.05 ppb) and five matrix control samples fortified at 10×LOQ (0.5 ppb). The injection sequences for the two valid sample runs are outlined in Tables 6 and 7.

Table 6. Injection Sequence for First Trial

Injection Sequence	Sample Type
1	Solvent
2	Solvent
3	Solvent
4	Standard C8 (0.02 ng/mL)
5	Standard C7 (0.04 ng/mL)
6	Standard C6 (0.05 ng/mL)
7	Standard C5 (0.10 ng/mL)
8	Standard C4 (0.50 ng/mL)
9	Standard C3 (1.0 ng/mL)
10	Standard C2 (5.0 ng/mL)
11	Standard C1 (10.0 ng/mL)
12	Solvent
13	Standard C8 (0.02 ng/mL)
14	Standard C7 (0.04 ng/mL)
15	Solvent
16	reagent blank
17	control 1
18	control 2
19	Standard C6 (0.05 ng/mL)
20	Standard C5 (0.10 ng/mL)
21	LOQ-1
22	LOQ-2
23	LOQ-3
24	LOQ-4
25	LOQ-5
26	Standard C4 (0.50 ng/mL)
27	Standard C3 (1.0 ng/mL)
28	10×LOQ-1
29	10×LOQ-2
30	10×LOQ-3
31	10×LOQ-4
32	10×LOQ-5
33	Standard C2 (5.0 ng/mL)
34	Standard C1 (10.0 ng/mL)
35	Solvent
36	Solvent
37	Standard C10 (0.50 ng/mL)
38	Standard C10 (0.50 ng/mL)
39	Standard C10 (0.50 ng/mL)
40	Standard C4 (0.50 ng/mL)
41	Standard C4 (0.50 ng/mL)
42	Standard C4 (0.50 ng/mL)
43	Solvent

Table 7. Injection Sequence for Second Trial

Injection Sequence	Sample Type
1	Solvent
2	Solvent
3	Solvent
4	Standard C9 (0.01 ng/mL)
5	Standard C8 (0.02 ng/mL)
6	Standard C7 (0.04 ng/mL)
7	Standard C6 (0.05 ng/mL)
8	Standard C5 (0.10 ng/mL)
9	Standard C4 (0.50 ng/mL)
10	Standard C3 (1.0 ng/mL)
11	Standard C2 (5.0 ng/mL)
12	Standard C1 (10.0 ng/mL)
13	Solvent
14	Standard C9 (0.01 ng/mL)
15	Standard C8 (0.02 ng/mL)
16	Standard C7 (0.04 ng/mL)
17	Solvent
18	reagent blank
19	control 1
20	control 2
21	Standard C6 (0.05 ng/mL)
22	Standard C5 (0.10 ng/mL)
23	LOQ-1
24	LOQ-2
25	LOQ-3
26	LOQ-4
27	LOQ-5
28	Standard C4 (0.50 ng/mL)
29	Standard C3 (1.0 ng/mL)
30	10×LOQ-1
31	10×LOQ-2
32	10×LOQ-3
33	10×LOQ-4
34	10×LOQ-5
35	Standard C2 (5.0 ng/mL)
36	Standard C1 (10.0 ng/mL)
37	Solvent
38	Solvent
39	Standard C10 (0.50 ng/mL)
40	Standard C10 (0.50 ng/mL)
41	Standard C10 (0.50 ng/mL)
42	Standard C4 (0.50 ng/mL)
43	Standard C4 (0.50 ng/mL)
44	Standard C4 (0.50 ng/mL)
45	Solvent