

1 INTRODUCTION

1.1 Scope of the Method

BASF Analytical Method L0257/01 (D1410) was developed to determine the residues of BAS 550 F (E) and BAS 550 F (Z) in drinking and surface water using LC-MS/MS at BASF Crop Protection, Research Triangle Park, N.C. The method was validated at BASF and independently validated at Eurofins Agrosience Services, Inc., 725 Cranbury Road, East Brunswick, NJ.

The independent lab validation was conducted using two fortification levels (0.05 and 0.5 µg/L) for surface and drinking water. For each fortification level and matrix, five replicates were analyzed. Additionally, two replicates of unfortified samples were examined.

1.2 Principle of the Method

Residues of BAS 550 F (E) and BAS 550 F (Z) were determined from water by direct injection analysis using LC-MS/MS in the positive ion mode.

In drinking and surface water, the method has a limit of quantitation of 0.05 µg/L for each analyte. The limit of detection for each analyte was set to 0.01 µg/L.

1.3 Specificity

BAS 550 F (E) and BAS 550 F (Z) were identified and quantified as individual analytes. The pair have mass transitions which are unique to the molecular entity, whereas the E and Z forms were differentiated chromatographically.

2 MATERIALS AND METHODS

2.1 Test systems

The following test systems were considered in this study:

Test System 1: Surface Water, Private pond, Durham, NC

Test System 2: Drinking (Tap) Water, BASF Research Triangle Park, NC

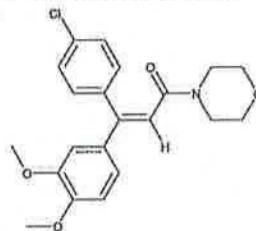
The description and characterization of the water used is given in the respective attached certificates (Appendix 10.3).

2.2 Test and Reference Substances

2.2.1 BAS 550 F (E-isomer)

BAS-Code	550 F
Common Name	<i>E</i> -Dimethomorph (<i>E</i>)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-acryloyl]morpholine
IUPAC Name	(<i>E</i>)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-acryloyl]morpholine
BASF Reg. No.	4110868
CAS-No.	113210-97-2
Molecular Formula	C ₂₁ H ₂₂ ClNO ₄
Molecular Weight	387.86 g/mol
Lot #	L83-214
Expiration Date	01 November 2015
Purity	99.3%

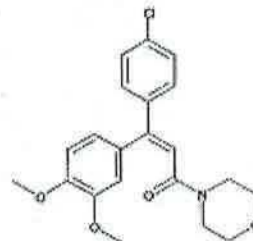
Chemical structure:



2.2.2 BAS 550 F (Z-isomer)

BAS-Code	550 F
Common Name	Z-isomer Dimethomorph (<i>Z</i>)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-acryloyl]morpholine
IUPAC Name	(<i>Z</i>)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-acryloyl]morpholine
BASF Reg. No.	4110869
CAS-No.	113210-98-3
Molecular Formula	C ₂₁ H ₂₂ ClNO ₄
Molecular Weight	387.86 g/mol
Lot #	L83-216
Expiration Date	01 November 2015
Purity	99.0%

Chemical structure:



2.3 Materials and Methods

2.3.1 Equipment

Equipment	Size, Description	Manufacturer
Balance, Top Loader	PM600	Mettler
Balance, Analytical	XP204	Mettler
Beakers	Various	PYREX Brand, VWR
Pipettes, volumetric	1 – 10 mL	Fisher Scientific – Class A
Pipettes, positive displacement	10-1000 μ L	Gilson
Pipettes, air displacement	10 – 10,000 μ L, various	Fisher and Eppendorf
Pipette, repeator	10 – 10,000 μ L, various tips	Eppendorf
Pipettes, Pasteur	Disposable, PP	Fisher
Volumetric Flasks	100, 50, 25, 10 and 5 mL	Various
Vortex Mixer	Genie 2	VWR
LC Vials	1.5 mL injection vials	Shimadzu
HPLC	Nexera UHPLC	Shimadzu
HPLC Column	Atlantis T3 (100 x 2.1mm, 3 μ), PN 186003718	Waters
Mass Spectrometer (MS)	API 6500	AB Sciex

2.3.2 Reagents

2.3.2.1 Chemicals

Chemical	Grade	Manufacturer/Supplier	Catalog No.
Methanol	HPLC Grade	Fisher	A452-4
Water	HPLC Grade	Fisher	W5-4
Formic Acid	Certified ACS	Sigma	695076

2.3.2.2 Solutions and Solvent Mixtures

Description	Composition and Preparation
HPLC Mobile Phase A	Water / formic acid (1000/1, v/v) Add 1 mL of concentrated formic acid into 1000 mL of water in a 1 L flask and mix well to ensure a homogeneous solution.
HPLC Mobile Phase B	Methanol / formic acid (1000/1, v/v) Add 1 mL of concentrated formic acid into 1000 mL of methanol in a 1 L flask and mix well to ensure a homogeneous solution.

2.3.2.3 Standard Solutions

Stock Solutions

Individual 1 mg/mL stock solutions were prepared by weighing approximately 10 mg of each analyte into a 22 mL amber vial, correcting for purity, and adding the proportional volume of methanol to make a 1.00 mg/mL solution. Two stock solutions of each isomer were prepared and compared for equivalence.

ID	Purity	Weight (mg)	Volume CH ₃ OH (mL)	Concentration (mg/mL)
BAS 550 F (E)-1	99.3 %	11.5	11.4	1.00
BAS 550 F (E)-2	99.3 %	11.0	10.9	1.00
BAS 550 F (Z)-1	99.0 %	12.4	12.3	1.00
BAS 550 F (Z)-2	99.0 %	10.5	10.4	1.00

Fortification Solutions

Fortifications Solution Preparation

Mixed Isomer QC Fortification Solutions were diluted volumetrically with methanol according to the table below and mixed to ensure a complete homogeneous solution.

Initial Concentration (µg/mL)	Aliquot Volume (µL)	Total (Final) Volume (mL)	Final Concentration (Each Isomer)	Final Solution ID
1000 (Stock)	100 (each Stock)	10	10 µg/mL	FS_H
10 (FS_H)	100	10	0.1 µg/mL	FS_MH
0.1 (FS_MH)	2,500	5	50 ng/mL	FS_ML
0.1 (FS_MH)	250	5	5 ng/mL	FS_L

Calibration Standard Solutions

Intermediate Calibration Standard Solutions Preparation

Mixed Isomer Calibration Standard Fortification Solutions were diluted volumetrically with methanol according to the table below and mixed to ensure a homogeneous solution.

Initial Concentration (µg/mL)	Aliquot Volume (µL)	Total (Final) Volume (mL)	Final Concentration (Each Isomer)	Final Solution ID
1000 (Stock)	100 (each Stock)	10	10 µg/mL	CFS_H
10 (CFS_H)	100	10	0.1 µg/mL	CFS_MH
0.1 (CFS_MH)	2,500	5	50 ng/mL	CFS_ML
0.1 (CFS_MH)	250	5	5 ng/mL	CFS_L

Calibration Standard Solutions Preparation

Mixed Isomer Calibration Standard Solutions for LC-MS/MS analysis were prepared using the Calibration Standard Fortification Solutions and diluting volumetrically with water according to the table below. Each dilution was mixed to ensure a homogeneous solution.

Source	Initial Concentration (ng/mL)	Aliquot Volume (µL)	Total Volume (mL)	Final Concentration (ng/mL)	Standard ID
CFS_ML	50	100	5	1.00	STD_7
STD_7	1	2,500	7.5	0.333	STD_6
CFS_L	5	250	5	0.250	STD_5
CFS_L	5	100	5	0.100	STD_4
CFS_L	5	50	5	0.0500	STD_3
STD_5	0.25	500	5	0.0250	STD_2
STD_4	0.1	500	5	0.0100	STD_1

3. Analytical Procedure

3.1 Recovery QC Fortification

Twelve samples were prepared with each matrix (surface and drinking water): 2 control samples, 5 samples treated at the LOQ, and 5 treated at 10 times the LOQ. For each, 1.0 mL of water was pipetted into an autosampler vial, fortified volumetrically according to the table below, and mixed to ensure a homogeneous solution.

Sample Type	Sample Volume	Spiking Solution Concentration (ID)	Spiking Solution Volume (µL)	Fortification Level (ppb [ng/mL])
Control	1 mL	-	-	0
Fortification (LOQ)	1 mL	5 ng/mL (FS_L)	10	0.0500*
Fortification (10×LOQ)	1 mL	50 ng/mL (FS_ML)	10	0.500

* Limit of quantitation

3.2 Preparation for Measurement

Standards, recovery QCs, and controls were injected as prepared above onto the LC-MS/MS system.

4 LC-MS/MS Instrumentation and Conditions

BAS 550 F (E) and BAS 550 F (Z) – Primary & Confirmatory Transitions

Component	Parameter	
Chromatographic System	Shimadzu Nexera UHPLC	
Analytical-column	Atlantis T3 (100 x 2.1mm, 3µ), PN 186003718 (Waters)	
Column Temperature	45 °C	
Injection Volume	15 µL	
Mobile Phase A	Water / formic acid (1000/1, v/v)	
Mobile Phase B	Methanol / formic acid (1000/1, v/v)	
Flow Rate	600 µL/min	
LC Gradient	Time (min)	% Mobile Phase B
	0.0	34
	0.25	34
	2.0	70
	5.75	99
	6.4	99
	6.5	34
7.0	34	
Detection System	AB Sciex 6500 Mass Spectrometer	
Ionization	Positive TurbolonSpray™	

Ionization Temperature	550 °C	
Analyte	MS Transitions (m/z)	Approximate Retention Time (min)
BAS 550 F (E)	388 → 301* 388 → 165	3.21
BAS 550 F (Z)	388 → 301* 388 → 165	3.33

* Primary quantitation transition. Either MS transition could be used for quantitation if interference is observed at the same retention time

4.1 Calibration Procedures

The calibration curve was obtained by direct injection of calibration standards containing known amounts of BAS 550 F (E) and BAS 550 F (Z) in the range of 0.01 ng/mL to 1.0 ng/mL. LC-MS/MS quantitation was achieved using a standard curve plotted from chromatographic peak area measurements versus several concentrations of standards. Linear standard calibration functions were used for evaluation. Mean, standard deviation, and percent relative standard deviation calculations were performed on the data generated at each fortification level. A correlation coefficient (r) was determined from the linearity data and an acceptable quantitation should achieve an r² of 0.98 or higher.

4.2 Calculation of Residues and Recoveries

Calculation of results was based on area measurements of each integrated peak. Each analyte's concentration was calculated relative to the linear calibration curve generated with each set according to **Equation I**. Results were calculated by AB Sciex Analyst® Software version 1.6.2 (validated system) and reported to 3 significant figures.

$$I. \quad \text{Concentration [ng/mL]} = \frac{\text{Response} - \text{Intercept}}{\text{Slope}} = C_A$$

The recoveries of spiked compounds are calculated according to **Equation II**:

$$II. \quad \text{Recovery \%} = \frac{\text{Conc. of Fortified Sample (ng/mL)} - \text{Conc. of Control (ng/mL)}}{\text{Conc. of Sample as Prepared}} \times 100$$

Example: Example: BAS 550 F (E), 388 → 301; Tap Water TAP_LOQ_1 fortified at 0.05 µg/mL:

The following values were used in this calculation:

Response of fortified sample	9.10e4
Response of control sample	0
Slope:	1.83e6
Intercept:	-2.3e3

I. **Concentration (ng/mL)** = $(91000 + 2300) / 1,830,000 = 0.0520 \text{ ng/mL}$

II. **Recovery %** = $\frac{0.0510 \text{ ng / mL}}{0.0500 \text{ ng / mL}} = 102.0\%$

Note: Manually calculated results may differ as a result of rounding.

5.2 Summary of Method

Type of method: LC-MS/MS

Test systems: Drinking (Tap) Water
Surface (Pond) Water

Analytes and selected mass transitions (*m/z*):

BAS 550 F (E) 388 → 301*
388 → 165

BAS 550 F (Z) 388 → 301*
388 → 165

* Primary quantification transition

Analytical procedure: L0257/01 (D1410)

Confirmatory technique: Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary.

Limit of detection: 0.01 µg/mL for BAS 550 F (E) and BAS 550 F (Z)

Limit of quantification (LOQ): 0.05 µg/L (lowest fortification level), corresponding to a concentration of 0.05 ng/mL in the final extract.

Levels of fortification: 0.05 µg/L and 0.5 µg/L of each analyte

Time required: A set of 13 samples requires about 4 hours of work

9. REFERENCES

- [1] BASF Method Number L0257/01 (D1410): Method for the Determination of the Geometric Isomers of Dimethomorph (BAS 550 F; Reg.No. 4110868, 4110869) in Water at LOQ of 0.05 ppb using LC/MS/MS, 23 October 2014.