FINAL REPORT

Study Title

Independent Laboratory Validation of BASF Analytical Method D1612/01: "Method for the determination of BAS 595 F (Reg.No. 4378513) and Its Z-isomer (Reg.No. 5079359) in Surface and Drinking Water by LC-MS/MS"

BASF/JRFA Study Code

BASF Study Number: 808757
JRFA Study Number: AU-2016-30

Guidelines

EPA Residue Chemistry Test Guidelines, OPPTS 850.6100 Environmental Chemistry Methods and ILV; SANC0/825/00 rev 8.1, (Nov. 16, 2010)

1. INTRODUCTION

1.1 Scope of the Method

BASF Analytical Method No. D1612/01 was developed to determine the residues of BAS 595 F and its Z-isomer in aqueous matrices using LC-MS/MS and validated at BASF Crop Protection in Research Triangle Park, North Carolina (Reference 1). This report represents the validation by an independent laboratory, JRF America in Audubon, Pennsylvania.

The independent lab validation was conducted using fortification levels at the limit of quantitation (30 ng/L) and ten times of limit of quantitation (300 ng/L) for surface and drinking water matrices. For each fortification level and matrix, five replicates were analysed. Additionally, one reagent blank and two replicates of unfortified samples were examined.

1.2 Principle of the Method

The residues of BAS 595 F and its Z-isomer are determined by measuring 10 g (or 10 mL) of water and filtering samples with a 13 mm, 0.45µm PTFE syringe filter. Dilutions (if needed) are performed using a set volume of control sample for each matrix. Samples then are ready for HPLC-MS/MS or UPLC-MS/MS determination. The transitions for BAS 595 F and its Z-isomer at *m/z* 318.259 → 69.900 and at *m/z* 320.072 → 69.900 were monitored in positive mode for primary and confirmation quantitation, respectively.

1.3 Specificity

To demonstrate the specificity of the analytical method, one additional mass transition (*m/z* 320.072 → 69.900) was monitored simultaneous to the primary quantitation transition (*m/z* 318.259 → 69.900) for analysis of BAS 595 F and its Z-isomer. The method was able to accurately determine residues of BAS 595 F and its Z-isomer and no interference was observed at the retention time of the analyte peaks.

Calibration standards, prepared in both matrices, were used for the quantitation of both analytes. Any sample dilutions were made with control matrix.

2. REFERENCE SUBSTANCE AND SAMPLING HISTORY

2.1 Test Systems

The test systems considered in this study were surface and drinking waters.

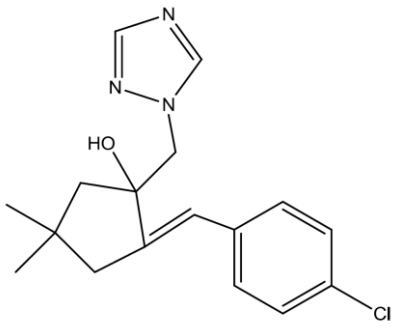
The control samples were provided by BASF. The water samples were received on November 15, 2016. Upon arrival at the laboratory, the samples were opened, inspected, and checked against enclosed shipping forms. The test systems were received frozen and were stored under frozen conditions at all times, unless necessary for laboratory analysis.

2.2 Test and Reference Substances

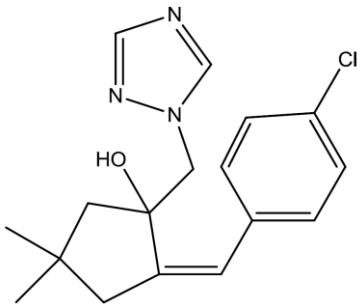
The standard substance was stored in a freezer (≤ -5°C) until use. BASF has retained a reserve sample of this chemical, and has documentation specifying the location of the synthesis and characterization information available at BASF Crop Protection, Research Triangle Park, North Carolina.

The BAS 595 F (Lot No. L85-136) and its Z-isomer (Lot No. BESS0578) reference substances were provided by the sponsor and received on November 16, 2016.

A summary of the reference substances is presented below.

BASF Code Name:	BAS 595 F
Common Name:	Triticonazole
Batch Number:	L85-136
BASF Registry Number:	4378513
CAS Number:	138182-18-0
IUPAC Name:	(RS)-(5E)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol
Molecular Formula:	$C_{17}H_{20}ClN_3O$
Molecular Weight:	317.8 g/mol
Purity:	99.4%
Expiration Date:	June 1, 2017
Chemical Structure:	

BASF Code Name: M595F014 (Z-isomer)
Batch Number: BESS0578
BASF Registry Number: 5079359
Molecular Formula: $C_{17}H_{20}ClN_3O$
Molecular Weight: 317.8 g/mol
Purity: 99.9%
Expiration Date: April 01, 2024
Structural Formula:



2.3 Test System

Water matrices were provided and homogenized by BASF. Surface and drinking waters were sent from BASF Crop Protection, Inc. on November 14, 2016 and received by JRF America on November 15, 2016.

3. ANALYTICAL METHOD

BASF Analytical Method D1612/01: "Method for the determination of BAS 595 F (Reg.No. 4378513) and Its Z-isomer (Reg.No. 5079359) in Surface and Drinking Water by LC-MS/MS" was used for the analysis of the samples.

The residues of BAS 595 F and its Z-isomer are determined by measuring 10 g (or 10 mL) of water and filtering samples with a 13 mm, 0.45µm PTFE syringe filter. Dilutions (if needed) are performed using a set volume of control sample for each matrix. Samples then are ready for HPLC-MS/MS or UPLC-MS/MS determination. Instrument parameters are described in Table 10.

The primary (quantitative) and secondary (confirmatory) transition ions monitored are presented below:

Analyte	Transition (*m/z*)		Polarity	RT (min)
	Primary	Secondary		
BAS 595 F	318.259 → 69.900	320.072 → 69.900	Positive	3.73
Z-Isomer				3.87

Several measures were taken to ensure the quality of the study results. The quality assurance unit at JRF America inspected the analytical procedures for compliance with Good Laboratory Practices that included adherence to the protocol. The dates inspected are detailed in the quality assurance unit statement. Study samples and test and reference items were maintained in a secured laboratory with limited access.

6. SUMMARY OF METHOD

Type of Method	LC-MS/MS
Test Systems	Surface, Drinking Water
Selected mass transitions (*m/z*)	**BAS 595 F/Z-Isomer** *m/z* 318.259 → 69.900* *m/z* 320.072 → 69.900 *Primary quantitation transition
Analytical Procedure	BASF Analytical Method D1612/01: "Method for the determination of BAS 595 F (Reg.No. 4378513) and Its Z-isomer (Reg.No. 5079359) in Surface and Drinking Water by LC-MS/MS" (Reference 1)
Confirmatory Technique	A secondary MRM transition was used for confirmation.
Method of Quantitation	The quantitation is based on the monitoring of one mass transition for BAS 595 F and its Z-isomer. Recovery data was reported for each mass transition considered.
LOD	6 ng/L
LOQ	30 ng/L (lowest fortification level)
Levels of Fortification	30 ng/L and 300 ng/L
Route of Administration	In this ILV, the test substances were applied as analytical standard solutions (in methanol) by volumetric pipette to ensure precise delivery of a small amount of the test substances.
Time Required	A set of 13 samples requires approximately 8 hours of work (calculation of the results included).
Justification of Ions	The ions used to conduct the ILV were determined in the validation (Reference 1) and are shown in Appendix D.

7. DISCUSSION

Recovery Findings

Specificity
Method D1612/01 determines residues of BAS 595 F and its Z-isomer in aqueous matrices. No interfering peaks were found at the retention time for BAS 595 F and its Z-isomer.

Limit of Quantitation and Limit of Detection
The LOQ was defined by the lowest fortification level successfully tested. The LOQ was found to be 30 ng/L for BAS 595 F and its Z-isomer in aqueous matrices. The LOD was set at 6 ng/L, which is 20% of the LOQ during ILV. The LOD is defined as the absolute amount of analyte injected into the LC-MS/MS when the lowest calibration standard was analyzed (0.006 ng/mL).

Repeatability
The overall relative standard deviation (RSD, %) for all fortification levels were below 20%.

It was demonstrated that the method D1612/01 fulfills the requirements with regards to specificity, repeatability, limit of quantitation, limit of detection, linearity and recoveries and is therefore applicable to correctly determine residues of BAS 595 F and its Z-isomer in aqueous matrices.

8. MODIFICATIONS/CONCLUSIONS FROM ILV

This independent laboratory validation was successfully completed on the first trial at JRF America. Recovery results and statistical data demonstrate BASF Analytical Method D1612/01 can be performed successfully for quantitation of BAS 595 F (Reg.No. 4378513) and Its Z-isomer (Reg.No. 5079359) on surface and drinking water matrices.

The method is well-written and contains a fair amount of comments to guide the analyst through the procedure for the first time. Modifications performed to the analytical method are presented in Appendix A.

9. PROTOCOL, AMENDMENTS, AND DEVIATIONS

No amendments or deviations were noted during the ILV study.

Table 9 Example Standard Solutions Preparation and Dilution Data

Stock Standard Solutions

Standard ID#	Analyte	Parent Standard ID#	Adjusted Net Weight (mg)	Dilution Volume (mL)[1]	Final Conc. (µg/mL)	Prep. Date
JRFA-491/1-1	BAS 595 F	SAS-012-X	10.8	10.0	1080	11/17/2016
JRFA-491/1-2	Z-Isomer	SAS-012-Y	10.9	10.0	1090	11/17/2016

[1] Prepared in methanol

Fortification Standard Solutions

Standard ID#	Analyte	Parent Standard ID#	Parent Conc. (µg/mL)	Aliquot Volume (mL)	Dilution Volume (mL)[2]	Final Conc. (µg/mL)	Prep. Date
JRFA-491/1-3	BAS 595 F	JRFA-491/1-1	1080	0.0926	10.0	10.0	11/17/2016
	Z-Isomer	JRFA-491/1-2	1090	0.0917		10.0	
JRFA-491/1-4	Both	JRFA-491/1-3	10.0	2.50	25.0	1.00	
JRFA-491/1-5	Both	JRFA-491/1-4	1.00	10.0	100.0	0.100	
JRFA-491/1-6	Both	JRFA-491/1-5	0.100	1.00	10.0	0.010	

[2] Prepared in methanol

Matrix-matched Calibration Standard Solutions

Standard ID#	Analyte	Parent Standard ID#	Parent Conc. (ng/mL)	Aliquot Volume (mL)	Dilution Volume (mL)[3]	Final Conc. (ng/mL)	Prep. Date
JRFA-491/1-13	BAS 595 F, Z-Isomer	JRFA-491/1-5	100	3.00	50.0	6	11/18/2016
JRFA-491/1-14		JRFA-491/1-13	6	5.00	50.0	0.6	
JRFA-491/1-15		JRFA-491/1-13	6	1.25	50.0	0.15	
JRFA-491/1-16		JRFA-491/1-14	0.6	5.00	50.0	0.06	
JRFA-491/1-17		JRFA-491/1-15	0.15	10.00	50.0	0.03	
JRFA-491/1-18		JRFA-491/1-16	0.06	5.00	50.0	0.006	
JRFA-491/2-2		JRFA-491/1-13	6	1.25	25.0	0.3	11/23/2016

[3] Prepared in matrix – surface water (202206/CM16-016)

Table 10 Instrument Conditions and Parameters

UPLC Conditions			
Chromatographic System:	Agilent 1290 UPLC		
Column:	Waters Acquity HPLC HSS T3 2.1 x 100 mm; 1.8 µm (SN: 014332334156 40)		
Temperature:	50 °C		
Flow rate (µL/min)	600		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.00	85.0	15.0
	0.02	85.0	15.0
	0.40	60.0	40.0
	2.20	35.0	65.0
	3.80	10.0	90.0
	5.50	10.0	90.0
	6.50	85.0	15.0
	10.00	85.0	15.0
Mobile Phase A:	0.1% formic acid in water		
Mobile Phase B:	0.1% formic acid in methanol		
Injection Volume:	20 µL		

MS/MS Conditions						
Detection System:	AB SCIEX 6500 QTrap					
Ionization:	Turbo Spray					
Polarity:	Positive					
Curtain gas (CUR, psi):	35.0					
Temperature (TEM, ˚C):	600					
Collision gas setting (CAD):	High					
GS1 (psi):	80.0					
GS2 (psi):	70.0					
Entrance potential (EP, V):	10.0					
Scan type:	MRM					
MRM Conditions	Retention Time (min)	Transition (*m/z*)	DP (V)	CE (V)	CXP (V)	Dwell (msec)
BAS 595 F Reg. No. 4378513	3.73	318.259 → 69.900 (Quantitation, both analytes)	46.00	57.00	12.00	150
Z-Isomer Reg. No. 5079359	3.87	320.072 → 69.900 (Confirmation, both analytes)	71.00	53.00	6.00	150

Table 11 Equipment, Reagents and Mobile Phases

Equipment	Size, Description	Manufacturer	Catalog No.
Analytical Balance	AT 200	Mettler Toledo	----
Flasks, Volumetric	Various sizes	Various	----
Pipettes	Various volumes	Eppendorf	----
Syringes	10 mL, Slip Tip	BD	301604
Bottle, Amber glass	Qorpak , 2 oz and 4 oz Boston Round, Amber with Teflon®-lined screw cap	VWR Scientific Products	----
Plastic Centrifuge Tubes	15 mL	Globe Scientific	6264
Mixer	BenchMark, vortex	BenchMark Scientific, Inc.	13112194
Syringe Filters	PTFE, 0.45 µm	Agilent	5190-5688
HPLC vials	2 mL	Agilent Technologies	5182-0716
HPLC vial caps	PTFE/red silicone septa	Agilent Technologies	5182-0717

Chemical	Grade	Manufacturer/Supplier	Lot No.
Water	LC-MS	EMD	56112
Methanol	LC-MS	EMD	56070
Formic Acid	LC-MS	Fluka	BCBR2425V

Description	Composition
HPLC mobile phase A	0.1% Formic Acid (FA) in Water 1.00 mL FA + ~999 mL water in 1L volumetric flask
HPLC mobile phase B	0.1% FA in Methanol (MeOH) 1.00 mL FA + ~999 mL MeOH in 1L volumetric flask

Figure 19 Residue Calculations

Peak integration and quantitation were performed within Analyst® 1.6.2 software; using the calibration curve equation to determine the amount of analyte found (ng) during sample analysis.

The following equations are used for residue and recovery calculations for BAS 595 F and Z-isomer in matrices of surface and drinking waters.

a) Calibration curve: $y = mx + b$ Solving for x: $x = \frac{y-b}{m}$

Where, m = slope
b = y-intercept
x = Amount found (ng/mL)
y = Peak area

b) Residue found (ng/L) = $\frac{\text{Amount found (ng/mL) x dilution factor x 1000 mL}}{\text{1 L}}$

c) Recovery (%) = $\frac{\text{Residue in sample (ng/L) - Residue in control sample (ng/L)}}{\text{Amount fortified (ng/L)}} \times 100$

As an example, calculations to obtain BAS 595 F (primary transition) recovery results using sample “UTC + LOQ E” from surface water (Figure 11) are shown below:

a) Calibration curve: $y = 7906476x + 75418$ (Figure 1)

Solving for x: $x = \frac{303722 - 75418}{7906476} = 0.0289$ ng/mL

c) Residue found (ng/L) = $\frac{0.0289 \text{ ng/mL x 1 x 1000 mL}}{\text{1 L}} = 28.9$ ng/L

d) Recovery (%) = $\frac{28.9 \text{ ng/L} - 0.0 \text{ ng/L}}{30.0 \text{ ng/L}} \times 100 = 96.3\%$

Statistical treatment of the data included calculation of means, standard deviations (SD), and percent relative standard deviations (%RSD). These calculations were performed using Microsoft® Excel. Results were rounded only for reporting purposes. No calculations were made with rounded numbers.

APPENDICES SECTION

Appendix A. Modifications for BASF Analytical Method D1612/01

The following modifications were performed to the technical procedure:

1. Column: A Waters Acquity HPLC HSS T3 column with the following dimensions and particle size was used: 2.1 x 100 mm, 1.8 µm. This is different from the column listed in the method (2.1 x 150 mm, 2.5 µm).

2. Mobile Phase Gradient: The following gradient was used for both matrices.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.00	85.0	15.0
0.02	85.0	15.0
0.40	60.0	40.0
2.20	35.0	65.0
3.80	10.0	90.0
5.50	10.0	90.0
6.50	85.0	15.0
10.00	85.0	15.0

This varies from the gradient listed in the method.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.00	85	15
0.02	85	15
0.63	60	40
3.35	35	65
4.10	5	95
5.45	5	95
5.50	85	15
6.00	85	15