

FINAL REPORT

Independent Laboratory Validation of BASF Analytical Method L0353/01:
“Method for the Determination of BAS 595 F (Triticonazole) and its Metabolites M595F001,
M595F002 and M595F014 in Soil by LC-MS/MS”

BASF Study Code

BASF Study Number: 808754
ADPEN Study Number: 17G0704

Guidelines

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

Sponsor

BASF Crop Protection
26 Davis Drive
P.O. Box 13528
Research Triangle Park, NC 27709-3528

1. INTRODUCTION

1.1 Scope of the Method

BASF Analytical Method No. L0353/01 was developed to determine the residues of BAS 595 F and its metabolites, M595F001, M595F002 and M595F014 in soil using LC-MS/MS at BASF SE Crop Protection in Limburgerhof, Germany (Reference 1). This report represents the validation by an independent laboratory, ADPEN Laboratories, Inc., in Jacksonville, FL.

The ILV was conducted using two fortification levels, the LOQ (0.002 mg/kg) and ten times the LOQ (0.02 mg/kg) for soil. Five replicates were analysed for each fortification level. 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 metabolites, M595F001, M595F002 and M595F014 are extracted from a 5 g soil samples by solid-liquid extraction using sonication and shaking with 0.1 M ammonium hydroxide and acetone. After each extraction, the suspension was centrifuged and decanted over cotton wool into a graduated cylinder. Then, an aliquot of the combined extracts was evaporated until the aqueous phase remained and subsequent redissolved in water. A sample clean-up by solid-phase extraction using a C18-SPE column was followed. The residues were eluted with acetonitrile/methanol (95/5, v/v) from the C18-SPE column. Afterwards, the liquid phase was evaporated to nearly dryness using a nitrogen evaporator and the remaining liquid phase was redissolved in water and acetonitrile. Samples known to exceed the highest calibration level were diluted an additional 10 times. The sample extracts were then analyzed by HPLC-MS/MS to determine the residues of BAS 595 F and its metabolites, M595F001, M595F002 and M595F014. The transitions for BAS 595 F at m/z 318→70 and at m/z 318→125 were monitored in positive mode for primary and confirmation quantification, respectively. The transitions for M595F001 at m/z 334→70 and at m/z 334→125 were monitored in positive mode for primary and confirmation quantification, respectively. The transitions for M595F002 at m/z 334→70 and at m/z 334→125 were monitored in positive mode for primary and confirmation quantification, respectively. The transitions for M595F014 at m/z 318→70 and at m/z 318→125 were monitored in positive mode for primary and confirmation quantification, respectively.

1.3 Specificity

To demonstrate the specificity of the analytical method, one additional mass transition for each BAS 595 F and its metabolites, M595F001, M595F002 and M595F014 was monitored simultaneous to the primary quantitation transition. The method was able to accurately determine residues of BAS 595 F and its metabolites, M595F001, M595F002 and M595F014 and no interference was observed at the retention time of the analyte peaks.

2. REFERENCE SUBSTANCE AND SAMPLING HISTORY

2.1 Test Systems

The test system in this study was soil.

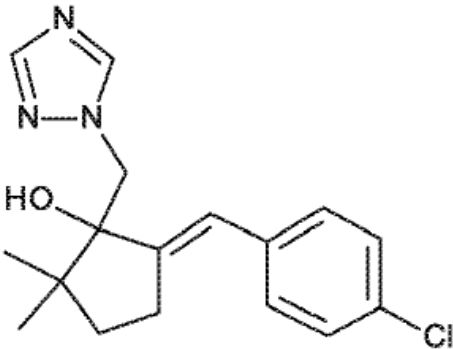
The control sample was provided by BASF. The soil sample was received on August 22, 2017. Upon arrival at the laboratory, the sample was opened, inspected, and checked against enclosed shipping forms. The test system was received frozen and was stored under frozen conditions at all times, unless necessary for laboratory analysis.

2.2 Test and Reference Substances

The standard substances were stored in a desiccator at room temperature until use. BASF has retained reserve samples of these chemicals, 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), M595F001 (Lot No. L67-148), M595F002 (Lot No. L85-198), and M595F014 (Lot No. BESS0578) reference substances were provided by the sponsor and received on July 19, 2017.

A summary of the reference substances is presented below.

BAS Code	BAS 595 F	
Common Name	Triticonazole	
Chemical Name	(RS)-(5E)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	
BASF Reg. No.	4378513	
CAS No.	138182-18-0	
Lot No.	L85-136	
Purity (Certificate)	98.5%	
Expiry	March 01, 2018	
Molecular Formula	C ₁₇ H ₂₀ ClN ₃ O	
Molecular Weight	317.8 g/mol	

Common Name	M595F001	
Chemical Name	(1RS,2E,3SR)-2-(4-chlorobenzylidene)-5,5-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-1,3-cyclopentane-1,3-diol	
BASF Reg. No.	5079285	
CAS No.	None	
Lot No.	L67-148	
Purity (Certificate)	99.3%	
Expiry	July 01, 2020	
Molecular Formula	C ₁₇ H ₂₀ ClN ₃ O ₂	
Molecular Weight	333.8 g/mol	

Common Name	M595F002	
Chemical Name	(1RS,2E,3RS)-2-(4-chlorobenzylidene)-5,5-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-1,3-cyclopentane-1,3-diol	
BASF Reg. No.	5059144	
CAS No.	None	
Lot No.	L85-198	
Purity (Certificate)	72.5%	
Expiry	July 01, 2018	
Molecular Formula	C ₁₇ H ₂₀ ClN ₃ O ₂	
Molecular Weight	333.8 g/mol	

Common Name	M595F014	
Chemical Name	(1RS,5Z)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	
BASF Reg. No.	5079359	
CAS No.	None	
Lot No.	BESS0578	
Purity (Certificate)	99.9%	
Expiry	April 01, 2024	
Molecular Formula	C ₁₇ H ₂₀ ClN ₃ O	
Molecular Weight	317.8 g/mol	

2.3 Test System

The control soil samples were provided and homogenized by BASF, and were identified as sample numbers R1405910005 and R1405910006. Sample number R1405910006 (PA.NY.T.CHAR 18-24.A.) was used for the validation. The soil was sent from BASF Crop Protection, Inc., on August 21, 2017 and received by ADPEN Laboratories, Inc. on August 22, 2017.

The control sample was logged in to ADPEN's laboratory information management system (LIMS) and given a unique lab code (170823001-002).

3. ANALYTICAL METHOD

BASF Analytical Method L0353/01 "Method for the Determination of BAS 595 F (Triticonazole) and its Metabolites M595F001, M595F002 and M595F014 in Soil by LC-MS/MS" was used for the analysis of the samples.

The residues of BAS 595 F and its metabolites, M595F001, M595F002 and M595F014 are extracted from a 5 g soil sample by solid-liquid extraction using sonication and shaking with 0.1 M ammonium hydroxide and acetone. After each extraction, the suspension was centrifuged and decanted over cotton wool into a graduated cylinder. Then, an aliquot of the combined extracts was evaporated until the aqueous phase remained and subsequent redissolved in water. A sample clean-up by solid-phase extraction using a C18-SPE column was followed. The residues were eluted with acetonitrile/methanol (95/5, v/v) from the C18-SPE column. Afterwards, the liquid phase was evaporated to nearly dryness using a nitrogen evaporator and the remaining liquid phase was redissolved in water and acetonitrile. Samples known to exceed the highest calibration level were diluted an additional 10 times. The sample extracts were then analyzed by HPLC-MS/MS. Instrument parameters are described in Table 10.

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

Analyte	Transition (<i>m/z</i>)		Polarity	RT (min)
	Primary	Secondary		
BAS 595 F	318→70	318→125	Positive	5.9
M595F001	334→70	334→125		2.5
M595F002	334→70	334→125		3.2
M595F014	318→70	318→125		6.2

STATISTICS AND DATA INTEGRITY

Statistical treatment of the data included simple descriptive statistics, such as determinations of averages, standard deviation, and/or relative standard deviation (RSD) for the procedural recoveries and area counts and calculation of the calibration curve and coefficient of determination (r^2) by linear regression of the instrument responses for the reference standards. The statistical calculations throughout this report were performed using Microsoft® Excel® and were rounded for presentation purposes. Slight differences may be noted in hand calculations using the recoveries presented in the tables. These are due to rounding and have no effect on the scientific conclusions presented in this report. The detailed analytical data may be consulted for confirmation of the calculated results.

Several measures were taken to ensure the quality of the study results. The quality assurance unit at ADPEN 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.

5. SUMMARY OF METHOD

Summaries of the method parameters and characteristics are provided in Table 10.

Summary of Method

Type of Method	LC-MS/MS
Test System	Soil
Selected mass transitions (m/z)	BAS 595 F m/z 318→ m/z 70* m/z 318→ m/z 125 M595F001 m/z 334→ m/z 70* m/z 334→ m/z 125 M595F002 m/z 334→ m/z 70* m/z 334→ m/z 125 M595F014 m/z 318→ m/z 70* m/z 318→ m/z 125 *Primary quantification transition
Analytical Procedure	BASF Analytical Method L0353/01 “ Method for the Determination of BAS 595 F (Triticonazole) and its Metabolites M595F001, M595F002 and M595F014 in Soil by LC-MS/MS ”

Confirmatory Technique	A secondary MRM transition was used for confirmation.
Method of Quantitation	The quantitation is based on the monitoring of two mass transitions for BAS 595 F and its metabolites, M595F001, M595F002 and M595F014. Recovery data were reported for each mass transition considered.
LOD	0.0004 mg/kg
LOQ	0.002 mg/kg (lowest fortification level)
Levels of Fortification	0.002 mg/kg and 0.02 mg/kg
Time Required	A set of 13 samples requires approximately 8 hours of work (calculation of the results included).

6. DISCUSSION

Standard Stability

Stock and fortification solutions of BAS 595 F and its metabolites, M595F001, M595F002 and M595F014 were prepared in acetonitrile. Calibration standard solutions were prepared by serial dilution of the intermediate standard solutions using water/acetonitrile (80:20, v:v). During the course of this study, the test/reference substance solutions were stored under refrigerated conditions and all solutions were used within the demonstrated time period of stability.

All analytes have been shown to be stable in stock and fortification solutions prepared in acetonitrile for at least 22 days when stored under refrigeration (Reference 1). Each analyte has been shown to be stable in calibration standard solutions prepared by serial dilution of the intermediate standard solutions with water/acetonitrile (80:20, v:v) and held under refrigeration for at least 22 days for all analytes (Reference 2).

Extract Stability

Extract stability was established in the validation study. The initial extracts stored under refrigeration and HPLC final volume held under refrigeration indicated that each analyte is stable in soil for at least 5 days (Reference 1).

Specificity

Method L0353/01 determines residues of BAS 595 F and its metabolites, M595F001, M595F002 and M595F014 in soil. No interfering peaks were found at the retention time for any of the analytes.

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

The LOQ of the method was set at 0.002 mg/kg in soil for BAS 595 F and its metabolites, M595F001, M595F002 and M595F014. The LOQ is also defined as the lowest fortification level for each analyte. The LOD for BAS 595 F and its metabolites, M595F001, M595F002 and M595F014 in soil was set at 0.0004 mg/kg, which was 20% of the defined LOQ. The LOD for each analyte was shown to be detectable as the absolute amount of analyte injected (0.007 ng on-column) with acceptable signal to noise ratio (S/N > 3:1).

Repeatability

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

It was demonstrated that the method L0353/01 fulfills the requirements with regards to specificity, repeatability, LOQ, LOD, linearity, and recoveries and is therefore applicable to correctly determine residues of BAS 595 F and its metabolites, M595F001, M595F002 and M595F014 in soil.

7. RECOMMENDATIONS/CONCLUSIONS FROM ILV

This ILV was successfully completed in the first trial at ADPEN. Recovery results and statistical data demonstrate BASF Analytical Method L0353/01 can be performed successfully for quantitation of BAS 595 F (Reg. No. 4378513) and Its Metabolites M595F001 (Reg. No. 5079285), M595F002 (Reg. No. 5059144), and M595F014 (Reg. No. 5079359) in soil.

The method is well-written and contains a fair amount of comments to guide the analyst through the procedure for the first time. Recommendations for improvement of the analytical method are presented in Appendix A and it is recommended that they be incorporated into the method.

8. PROTOCOL, AMENDMENTS, AND DEVIATIONS

No amendment or deviations were documented during this validation 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) ^a	Final Conc. (ng/μL)	Prep. Date
C8742	BAS 595 F	P5719	9.998	10	999.8	8/9/2017
C8743	M595F001	P5720	10.38	10	1038	
C8744	M595F002	P5721	7.460	10	746	
C8745	M595F014	P5722	10.69	10	1069	

^a Prepared in acetonitrile

Fortification Standard Solutions

Standard ID#	Analyte	Parent Standard ID#	Parent Conc. (ng/μL)	Aliquot Volume (mL)	Dilution Volume (mL) ^b	Final Conc. (ng/μL)	Prep. Date
I9421	BAS 595 F	C8742	999.8	1.0	10	100	8/10/2017
	M595F001	C8743	1038	0.963			
	M595F002	C8744	746	1.34			
	M595F014	C8745	1069	0.935			
W14296-1	4 - mix	I9421	100	1.0	10	10	8/10/2017
W14296-2		W14296-1	10			1.0	
W14296-3		W14296-2	1			0.1	

^b Prepared in water:acetonitrile (80:20, v/v)

Table 10 Instrument Conditions and Parameters

UPLC Conditions			
Chromatographic System:	Agilent 1290 UPLC		
Column:	Phenomenex Luna Phenyl Hexyl; 5 µm, 4.6 x 100 mm		
Guard Column:	Phenomenex SecurityGuard C18; 4 x 3.0 mm		
Temperature:	25 °C		
Flow rate (L/min):	1.0		
Isocratic Elution:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.00	30	70
	10.0	30	70
Mobile Phase A:	Water / formic acid (1000/2, v/v)		
Mobile Phase B:	Methanol / formic acid (1000/2, v/v)		
Injection Volume:	35 µL		

MS/MS Conditions						
Detection System:	Ab Sciex 5500 Triple Quad Mass Spectrometer					
Ionization:	Turbo Spray (ESI)					
Polarity:	Positive					
Gas Temp:	550 °C					
Nebulizer:	35 psi					
Capillary:	5000 V					
MRM Conditions	Transition (m/z)	Dwell (msec)	DP	CE	CXP	Retention Time (min)
BAS 595 F (Reg. No. 4378513)	318→70	200	106	53	8	5.9
	318→125			51	6	
M595F001 (Reg. No. 5079285)	334→70	200	85	54	9	2.5
	334→125			54	11	
M595F002 (Reg. No. 5059144)	334→70	200	85	54	9	3.2
	334→125			54	11	
M595F014 (Reg. No. 5079359)	318→70	200	106	53	8	6.3
	318→125			51	6	

Figure 21 Residue Calculations for Soil

Peak integration and quantitation were performed within AB Sciex Analyst[®] 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 its metabolites, M595F001, M595F002 and M595F014 in soil.

- a) Concentration in the final volume (ng) = $\frac{\text{Response} - \text{Intercept}}{\text{Slope}}$
- b) Residue found (mg/kg) = $\frac{\text{Final Volume (mL)} \times \text{Dilution Factor} \times \text{Concentration (ng)}}{\text{Sample Weight (g)} \times \text{Injection Volume (mL)} \times 1000}$
- c) Recovery (%) = $\frac{\text{Residue in sample (mg/kg)} - \text{Residue in control sample (mg/kg)}}{\text{Amount fortified (mg/kg)}} \times 100$

As an example, calculations to obtain BAS 595 F (primary transition) recovery results using 17082802-Recovery1-1 from data set QC-17082802 are shown below:

- a) Calibration curve: $y = (4.10024e+6)x + 4093.5$

Solving for x: $x = \frac{111836 + 4093.5}{4.10024e+6} = 0.02628 \text{ ng}$

- b) Residue found (mg/kg) = $\frac{10 \text{ mL} \times 1 \times 0.02628 \text{ ng}}{5.00 \text{ g} \times 0.035 \times 1000} = 0.00150 \text{ mg/kg}$

- c) Recovery (%) = $\frac{0.00150 \text{ mg/kg} - 0.00 \text{ mg/kg}}{0.002 \text{ mg/kg}} \times 100 = 75.1\%$

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.

Appendix A. Recommendations for BASF Analytical Method L0353/01

The following recommendations should be incorporated into the technical procedure:

1. A different method of filtration might be considered rather than cotton wool to facilitate a cleaner extract.
2. After rotary evaporation, the tapered flasks are rinsed with 0.4 mL of water, which may be insufficient to transfer all residues. A larger rinse volume would allow proper transfer while the SPE column would still accommodate the larger sample load volume.
3. After nitrogen evaporation, when adjusting the final volume, reconstitution is typically done in the vessel used for evaporation followed by sonication and vortexing to dissolve any residues.