1.0 INTRODUCTION

1.1 Purpose of the Study

The objective of this independent laboratory validation (ILV) study was to demonstrate that BASF Analytical Method D1501/01 (L0273/01), “Method for the Determination of the Enantiomers of BAS 555 F (Reg. No. 5836046, Reg. No. 4677200, Reg. No. 5836047, and Reg. No. 5836048) in Surface and Drinking Water by LC-MS/MS”, could be performed successfully at an outside facility with no prior experience with the method.

1.2 Summary of the Results

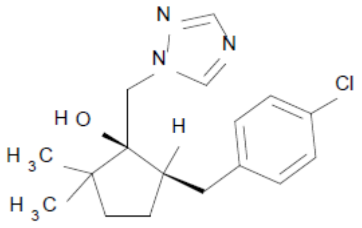
The independent laboratory validation of the BASF method was successfully completed in the first trial.

2.0 REFERENCE SUBSTANCE AND SAMPLING HISTORY

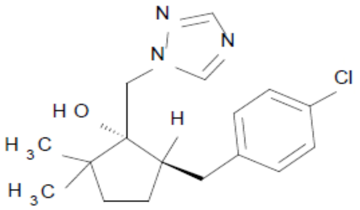
2.1 Reference Materials

Reference substances, BAS 555 F (cis-metconazole) BASF registration number 4079468 and BAS 555 F (trans-metconazole) BASF registration number 4079654 were used for individual fortifications and for LC-MS/MS calibration. Concentrated (stock), fortification, and calibration standards were prepared according to the analytical method. The certificates of analysis for all reference substances are presented in Appendix A. Standard solutions prepared for this study were stored in the refrigerator E-109, which operated at an average temperature of 4 °C. A brief description of the reference standard used in this study is presented below.

Common Name:	Cis-Metconazole
IUPAC Name:	Cis-1-(1H-1,2,4-triazole-1-ylmethyl)-2,2-dimethyl-5-(4-chlorobenzyl)cyclopentanol
BASF Reg. Number:	44079468
CAS Number:	115850-27-6
Molecular Formula:	$C_{17}H_{22}ClN_3O$
Molecular Weight (g/mol):	319.8
Batch Number:	AC8879-136A
Purity:	99.3%
Storage:	Freezer
Expiration Date:	May 1, 2016
Structural Formula:	



Common Name: Trans-Metconazole
IUPAC Name: Trans-1-(1H-1,2,4-triazole-1-ylmethyl)-2,2-dimethyl-5-(4-chlorobenzyl)cyclopentanol
BASF Reg. Number: 4079654
CAS Number: 115850-28-7
Molecular Formula: $C_{17}H_{22}ClN_3O$
Molecular Weight (g/mol): 319.8
Batch Number: AC9339-122[a]
Purity: 99.1%
Storage: Freezer
Expiration Date: June 01, 2026
Structural Formula:



2.2 Test System

Two control water samples (surface and drinking) were sent from BASF Crop Protection, Inc. on November 10, 2014 and received by ADPEN Laboratories, Inc. on November 11, 2014. Upon receipt, the samples were stored in refrigerator E-122, which operated at an average temperature of 5.6 °C during the course of the validation study.

The Laboratory Information Management System (LIMS) provided unique laboratory analysis codes (surface = 150317004-001 and drinking = 150317004-002) for each control sample, which is cross-referenced on detailed analytical data reports to the sample identification number.

3.0 TECHNICAL PROCEDURE

BASF Analytical Method D1501/01 (L0273/01), “Method for the Determination of the Enantiomers of BAS 555 F (Reg. No. 5836046, Reg. No. 4677200, Reg. No. 5836047, and Reg. No. 5836048) in Surface and Drinking Water by LC-MS/MS” was used for the analysis of water samples.

3.1 Preparation of Solutions

All solutions used for the conduct of the ILV were prepared as described in the analytical method. Below are the solutions and solvent mixtures used for this study and their composition:

Description	Code	Composition
HPLC mobile phase A	**LC1**	0.1% Formic Acid in Water Add 1000 mL of water and 1 mL of concentrated formic acid into a, e.g., 1L Erlenmeyer flask and mix well to ensure a completely homogeneous solution.
HPLC mobile phase B	**LC2**	0.1% Formic Acid in Methanol Add 1000 mL of methanol and 1 mL of concentrated formic acid into a, e.g., 1L Erlenmeyer flask and mix well to ensure a completely homogeneous solution.

3.2 Preparation of Standard Solutions

All stock, fortification, tuning and calibration standard solutions were stored refrigerated in amber bottles at approximately 4 °C and were brought to room temperature prior to use. Example preparations of standard solutions are presented in Table 18.

3.2.1 Stock Standard Solutions

Stock standard solutions were prepared as per method in 5-mL volumetric flasks, The standards were dissolved, using sonication and/or vortexing and then diluted to the mark. For the cis- and trans-BAS 555 F isomers a separate stock solution containing 2 mg/mL of each individual diastereomeric form was prepared in methanol.

3.2.2 Fortification Standard Solutions

Fortification solutions were prepared at 100 and 10 ng/mL using the scheme shown in the method. Mixed standard solution for fortifications were prepared by combining individual stock solutions of cis- and trans-BAS 555 F in volumetric flasks. The two volumetric flasks containing the fortification standards were diluted volumetrically with methanol as described in the method and complete homogeneous solutions were accomplished by sonication and/or vortexing.

3.2.3 Calibration Standard Solutions

Calibration standard solutions for LC-MS/MS analysis were prepared using stock or intermediate solutions described above and in the method by diluting them as noted below. Calibration standard solutions were prepared as follows:

Preparation of Calibration Standards for cis- and trans- BAS 555 F mixed standard

Initial solution (ng/mL)	Aliquot Volume (mL)	Diluted with HPLC water to a final volume of (mL)	Concentration (ng/mL)
10	2.5	25	1.0
10	1.25		0.5
10	0.625		0.25
10	0.25		0.1
1.0	1.25		0.05
1.0	0.625		0.025
1.0	0.25		0.01
0.5	0.25		0.005

3.3 Analysis by Direct Injection

Bulk drinking and surface water control samples were allowed to equilibrate to room temperature and inverted several times to ensure homogeneity. A 10-mL aliquot was transferred to a culture tube. Recovery samples were fortified at the LOQ and 10x LOQ, as described in the lab benchsheet, and vortexed thoroughly. A portion of the sample was added to an HPLC vial and analyzed by UPLC-MS/MS.

3.4 LC-MS/MS Instrumentation and Conditions

The LC-MS/MS instrumentation and conditions including the primary and confirmatory mass transitions (m/z) monitored during this validation study are presented in Table 19.

4.0 LIMITS OF QUANTITATION AND DETECTION

The limit of quantitation of both cis- and trans-BAS 555 F is 25 ng/L (ppt) per isomer in all matrices. The limit of detection is 20% of LOQ, equivalent to 5 ng/L per isomer.

5.0 CALIBRATION, CALCULATIONS, AND STATISTICS

Quantitation of residues in all samples was achieved using an external calibration curve calculated by linear regression of instrument responses for the reference substance at multiple concentrations.

A standard curve was prepared for each BAS 555 F enantiomer (RR, RS, SR, and SS) by injecting standard solutions at appropriate concentrations. Calibration standard concentrations ranged from 0.005 to 1.0 ng/mL. A calibration standard was interspersed with sample injections. ABSciex Analyst® software (version 1.6.2) created the standard curve based on linear regression using 1/x weighting. The regression functions were used to calculate the best-fit line by plotting the analyte found (ng) on the x-axis versus the detector's peak response (peak area) on the y-axis. Typical calibration curves are presented in Figure 1. Representative chromatograms of calibration standards for all mass transitions (m/z) are presented in Figures 2 through 9.

Peak integration and quantitation were performed within Analyst®; using the calibration curve equation to determine the amount of analyte found (ng) during sample analysis. Recovery results and additional sample concentrations were calculated for each set of samples within the LIMS and reported in Microsoft® Excel spreadsheet as detailed analytical data reports, which are presented in Appendix B.

The following equations are used for residue and recovery calculations for water samples:

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

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

b) $\text{Amount of sample injected (mL)} = \frac{\text{injection size (mL)}}{\text{final volume (mL)} \times \text{DF}} \times \text{sample amount (mL)}$

c) $\text{Residue found (ng/mL)} = \frac{\text{Amount found (ng)}}{\text{Amount of sample injected (mL)}}$

Residue found (ng/L) = ng/mL × 1000

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

As an example, calculations to obtain M555000-

a) Calibration curve: y = (2.62e+007)x + 4.34e+003

Solving for x:

b) $\text{Amount of sample injected (mL)} = \frac{0.1\ \text{mL}}{10\ \text{mL}} \times 10\ \text{mL} = 0.10\ \text{mL}$

c) $\text{Residue found (ng/mL)} = \frac{0.002239\ \text{ng}}{0.10\ \text{mL}} = 0.02239\ \text{ng/mL}$

Residue found (ng/L) = ng/mL found x 1000

d) $\text{Recovery (\%)} = \frac{0.02239\ \text{ng/L} - 0.00000\ \text{ng/L}}{25\ \text{ng/L}} \times 100 = 90\%$

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 and LIMS software. Results were rounded only for reporting purposes. No calculations were made with rounded numbers.

Table 1 Flow Diagram of the Technical Procedure