1. INTRODUCTION

1.1 Scope of the Method

In Smithers Viscient Study No. 986.6265, an analytical method was developed to determine residues of BAS 315 I and its metabolites (Compound E, Compound UK, Compound R, Compound C and Compound F) in water matrices using LC-MS/MS at Smithers Viscient in Wareham, Massachusetts. This method was independently validated at ADPEN Laboratories, Inc. during this ILV study.

1.2 Principle of the Method

No sample extraction or clean-up was required for the determination of BAS 315 I and Compounds E and UK. An aliquot (5 mL) of control water was fortified and the entire sample diluted with 5 mL of methanol. The resultant solution (10 mL of 50/50 methanol/matrix (v/v)) was submitted for LC-MS/MS analysis.

Solid-phase extraction clean-up was required for the determination of Compound R. An aliquot of control water (25 mL aliquot for LOQ fortification level or 5 mL aliquot for 10x LOQ fortification level) was fortified and then acidified with formic acid prior to clean-up with a Waters Oasis MCS SPE column. Residues were concentrated with evaporation and then reconstituted (with aid of vortex) to a final volume of 5 mL with 20/80 caustic methanol/water (v/v). The resultant solution (5 mL of caustic methanol/water (20/80, v/v) was submitted for LC-MS/MS analysis.

No sample extraction or clean-up was required for the determination of Compounds C and F. An aliquot (8 mL) of control water was fortified and the entire sample diluted with 2 mL of methanol. The resultant solution (10 mL of 20/80 methanol/matrix (v/v)) was submitted for LC-MS/MS analysis.

2. REFERENCE SUBSTANCE AND SAMPLING HISTORY

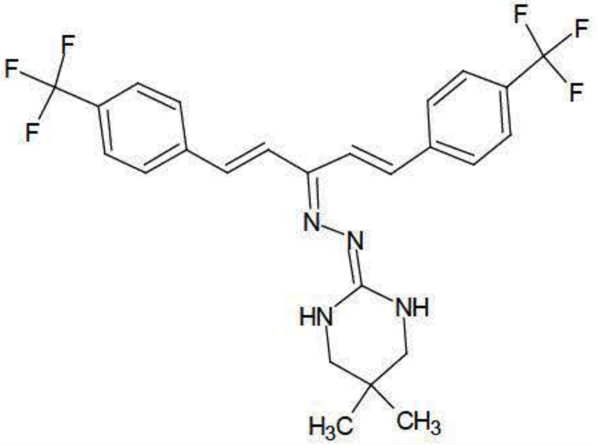
2.1 Test Systems

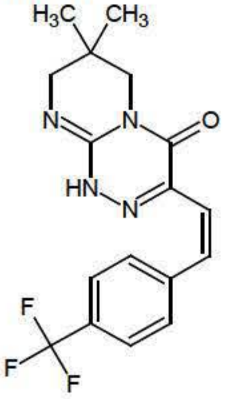
The test systems used in this study were groundwater and surface water.

The control groundwater and surface water samples used in this study were provided by Smithers Viscient, and were received at ADPEN Laboratories, Inc. on July 11, 2018. Upon arrival at the laboratory, the samples were inspected and were assigned unique numbers through ADPEN’s Laboratory Information Management System (LIMS). The samples were received ambient and in good condition, then stored in frozen (c.a. -20 ºC) until needed for analyses. The characterization reports for the control samples are maintained by Smithers Viscient.

2.2 Test and Reference Substances

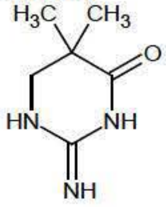
The following reference standard substances were provided by the Sponsor and were stored frozen (< -5 °C) upon receipt at the testing facility. Characterization and stability data for the substances is maintained by the Sponsor, and a reserve sample of these standards is retained at BASF, Research Triangle Park, North Carolina. Detailed information regarding the test substances, including the certificates of analysis are presented in Appendix A. A brief description of each analyte follows:

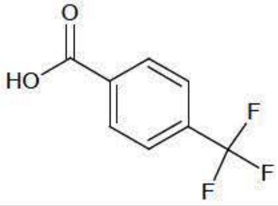
Code No.	BAS 315 I
Chemical Name (IUPAC):	5,5-dimethylperhydropyrimidin-2-one 4-trifluoromethyl-alpha-(4-trifluoromethylstyryl)cinnamylidenehydrazone
CAS Registry No.:	67485-29-4
BASF Reg. No.:	4111109
Molecular Formula:	$C_{25}H_{24}F_6N_4$
Molecular Weight:	494.5 g/mol
Batch No.:	L83-26
Purity:	99.5%
Expiration:	October 1, 2020
Storage:	Frozen
Molecular Structure:	

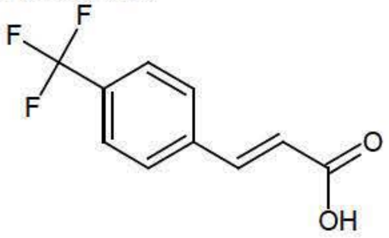
Code No.	(Compound E)
Chemical Name (IUPAC):	7,7-dimethyl-3-{(E)-2-[4-(trifluoromethyl)phenyl]ethenyl}-1,6,7,8-tetrahydro-4H-pyrimido[2,1-c][1,2,4]triazin-4-one
CAS Registry No.:	N/A
BASF Reg. No.:	4435553
Molecular Formula:	$C_{17}H_{17}F_3N_4O$
Molecular Weight:	350.3 g/mol
Batch No.:	L83-278
Purity:	99.4%
Expiration:	February 1, 2019
Storage:	Frozen
Molecular Structure:	

The following reference standard substances were obtained from a commercial source and were stored frozen (< -5 °C) upon receipt at the testing facility. A purity statement accompanied the shipment of these materials to the testing facility. Detailed information regarding the test substances, including the certificates of analysis (if available) are presented in Appendix A. A brief description of each analyte follows:

Code No.	Compound UK
Chemical Name (IUPAC):	N/A
CAS Registry No.:	N/A
Commercial Source:	Ricerca Bioscience
Molecular Formula:	N/A
Molecular Weight:	N/A
Batch No.:	N/A
Purity:	93.0%
Expiration:	N/A
Storage:	Frozen
Molecular Structure:	N/A

Code No.	Hydramethylnon Metabolite R (Compound R)
Chemical Name (IUPAC):	N/A
CAS Registry No.:	N/A
Commercial Source:	Concord Biosciences
Molecular Formula:	$C_6H_{11}N_3O$
Molecular Weight:	141.2 g/mol
Batch No.:	55658-28-35
Purity:	95.98%
Expiration:	December 20, 2019
Storage:	Frozen
Molecular Structure:	

Code No.	Hydramethylnon Metabolite C (Compound C)
Chemical Name (IUPAC):	N/A
CAS Registry No.:	N/A
Commercial Source:	Concord Biosciences
Molecular Formula:	$C_8H_5F_3O_2$
Molecular Weight:	190.1 g/mol
Batch No.:	MKBW7296V
Purity:	97.68%
Expiration:	December 20, 2019
Storage:	Frozen, dark
Molecular Structure:	

Code No.	Hydramethylnon Metabolite F (Compound F)
Chemical Name (IUPAC):	N/A
CAS Registry No.:	N/A
Commercial Source:	Concord Biosciences
Molecular Formula:	$C_{10}H_7F_3O_2$
Molecular Weight:	216.2 g/mol
Batch No.:	1402309V
Purity:	100%
Expiration:	December 20, 2019
Storage:	Frozen, dark
Molecular Structure:	

3. ANALYTICAL METHOD

Smithers Viscient Study 986.6265 “Validation of the Analytical Method for the Determination of BAS 315 I and Metabolites in Groundwater and Surface Water ” (**Reference 1**) was used for the analysis of the samples. The validation report is presented in Appendix D.

Final determination of BAS 315 I and its metabolites was conducted using the following transition ions.

Analyte	Polarity	Quantitation (*m/z*)	Confirmation (*m/z*)
BAS 315 I	Positive	495→323	495→368
Compound E	Positive	351→331	351→275
Compound UK	Positive	511→323	511→314
Compound R	Positive	142→70	142→72
Compound C	Negative	189→145	235→189
Compound F	Negative	215→171	261→215

3.1 Method Selectivity

The method selectivity was evaluated with the collection of mass spectra (product ion scans) to justify the selection of ion transitions used for LC-MS/MS determination. Single-analyte stock solutions were prepared according to the analytical method, diluted as necessary and infused directly into the MS-MS detector to confirm the quantitation and confirmation ion transitions for BAS 315 I and each metabolite. A total of 6 product ion scans were collected to confirm method selectivity. In addition, method selectivity was also evaluated by screening unfortified test system samples for the quantitation and confirmation ion transitions listed above.

3.2 Influence of Matrix Effects on Analysis

Matrix effects were evaluated during the conduct of this study. To assess the potential matrix effect of each analyte in each test system, matrix-matched standards and non-matrix-matched standards were prepared according to the analytical method and analyzed (in triplicate) on the LC-MS/MS. Matrix effects found to be ≥ 20 % were considered significant and justified the use of matrix-matched calibration standards for method validation trials. Matrix effects found to be less than 20% were considered insignificant and did not require the use of matrix-matched standards for method validation trials.

3.3 Validation of Method

For validation of the method to determine BAS 315 I and Compounds E and UK, untreated samples of groundwater and surface water were fortified with BAS 315 I, Compound E and Compound UK then analyzed according to the established method guidelines. For validation of the method to determine Compound R, untreated samples of groundwater and surface water were fortified with Compound R then analyzed according to the established method guidelines. For validation of the method to determine Compounds C and F, untreated samples of groundwater and surface water were fortified with Compound C and Compound F then analyzed according to the established method guidelines. To test the repeatability of the method, the analytical sets for each method/test system consisted of a reagent blank, two unfortified control samples, five replicates fortified at the method LOQ (0.10 µg/L) and five replicates fortified at a higher level, corresponding to 10x the method LOQ (1.0 µg/L).

3.4 Extract Stability

Stability of the final volume solutions was evaluated to determine extract stability. Final volume solutions were stored (refrigerated, in autosampler vials) for a period of at least 8 days. After an appropriate storage interval, the retained vials were re-analyzed against freshly-prepared calibration solutions to assess analyte recovery after extract storage. Acceptable mean recoveries were used to confirm extract stability after storage.

7. RECOMMENDATIONS/CONCLUSIONS FROM ILV

The independent laboratory validation of the method validated in Smithers Viscient Study No. 986.6265 was successfully completed for all analytes in groundwater and surface water in the first trial. Upon completion of the independent laboratory validation, the following recommendations were noted:

1. *Matrix Effects*: Matrix effects were assessed for each transition (quantitation and confirmation) in each test system (groundwater and surface water) according to Sections 2.8.4-2.8.6, and all matrix effects were found to be insignificant (< 20%). However, when solvent-based calibration standards were used to quantify BAS 315 I transitions in surface water, the average recoveries at the LOQ fortification level

were found to be approximately 20-30% below the average recoveries at the 10x LOQ fortification level. When the samples prepared at the LOQ fortification level were re-analyzed using matrix-matched calibration standards, the average recoveries were within the acceptable range of 70-120% . The matrix effects of BAS 315 I should be evaluated prior to the analysis of additional test systems, and the use of matrix-matched calibration standards should be considered when matrix effects are found to be below the 20% threshold.

2. *Fortification Volumes*: The fortification volumes range from 1-10% of the sample volumes. It is recommended that fortification volumes should consistently be less than or equal to 1% of the sample volume.

3. *Compound R/ Sample Volume*: For Compound R, the method instructs to use 25 mL sample volume for samples fortified at the LOQ fortification level and 5 mL sample volume for samples fortified at the 10x LOQ fortification level. It is recommended that the sample processing for Compound R (Section 2.10.2) be re-developed to use a consistent sample volume before the method is used to analyze water samples containing unknown levels of Compound R.

4. *Chromatography*: The chromatography observed during the ILV identified a mixture of cis-trans isomers for BAS 315 I, Compound E and Compound UK. However, the chromatography represented in the validation report only shows multiple isomers for Compound E. It is recommended that some discussion be added to the method to address the chromatographic discrepancies which may arise during the analysis of compounds containing multiple isomers.

5. *Extract Storage Stability*: The ILV study did not confirm the stability of BAS 315 I (both transitions; groundwater), Compound E (both transitions; groundwater and quantitation transition; surface water) or Compound UK (both transitions; groundwater and surface water) after 11 days of refrigerated storage in final volume solutions. Instead, acceptable recovery of fortified samples within 1 day of extraction was used to suggest extract stability for at least 1 day. Additional stability analyses are required to determine > 1 day extract stability of these analytes in groundwater and surface water.

8. PROTOCOL, AMENDMENTS, AND DEVIATIONS

A single protocol amendment was issued during the course of the ILV study. Protocol amendment #1 defined the limit of detection (LOD) for the study as 0.03 µg/L, clarified that 6 product ion scans were collected to confirm method selectivity and removed standard stability from the experimental design of the study.

No protocol deviations were documented during the course of the ILV study.