1.0 INTRODUCTION

1.1 Purpose of the Study

The purpose of the study was to demonstrate that BASF Analytical Method, "Determination of Dimethenamid-p and Its Metabolites M23, M27 and M31 in Water" for Dimethenamid-p only, could be performed successfully at an outside facility with no prior experience with the method.

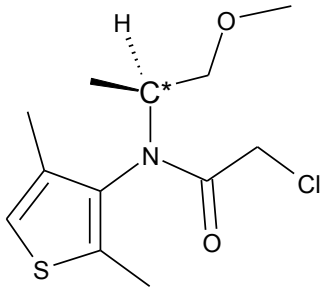
1.2 Summary of the Results

The independent laboratory validation of this BASF method was successfully completed. Detailed results for dimethenamid-p are presented in Tables 1 – 2 for drinking water. Certificate of analysis is presented in Appendix 1, and the detailed reports representing the analytical data are presented in Appendix 3.

2.0 REFERENCE SUBSTANCE AND SAMPLING HISTORY

2.1 Reference Materials

Reference substance dimethenamid-p was used for individual fortifications and LC-MS/MS calibration. Concentrated (stock), fortification, and calibration standards were prepared according to the analytical method. Example standard solution preparations are presented in Table 3. Standard solutions prepared for this study were stored at 4°C. A brief description of reference standards used in this study is presented below.

BASF Code Name:	BAS 656 H
Common Name:	Dimethenamid-p
Batch No.:	BEAU201204
BASF Registry Number:	363851
CAS Number:	163515-14-8
IUPAC Name:	(S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamide
Molecular Formula:	$C_{12}H_{18}ClNO_2S$
Molecular Weight:	275.8 g/mol
Purity:	96.4%
Expiration Date:	Jul 01, 2016
Chemical Structure:	

2.2 Test System

Drinking water matrices were sent from BASF Crop Protection on February 25, 2014 and received by Alliance Pharma on February 26, 2014. The water characterization report is presented in Appendix 2.

3.0 TECHNICAL PROCEDURE

BASF Analytical Method: "Determination of Dimethenamid-p and Its Metabolites M23, M27 and M31 in Water" for Dimethenamid-p Only.

3.1 Summary of Analytical Procedure

Drinking water containing 0.1% of formic acid was fortified with 0.02 mL of 1.5 ng/mL or with 0.02 mL of 15 ng/mL fortification solution to obtain the LOQ (0.03 µg/L) and higher level (0.30 µg/L), respectively. Then the samples were vortex and analyzed via LC-MS/MS.

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

Analyte	Transition (*m/z*)		Ionization Mode	Retention Time (min.)
	Primary	Secondary		
Dimethenamid-p	276.0 → 244.1	276.0 → 168.1	Positive	8.04

4.0 LIMITS OF QUANTITATION AND DETECTION

The LOQ and LOD for residues of dimethenamid-p in drinking water are 0.03 and 0.009 µg/L, respectively.

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 substances at multiple concentrations.

Individual standard curves were prepared for dimethenamid-p by injecting standard solutions at appropriate concentrations. Calibration standard concentrations for dimethenamid-p ranged from 0.009 –0.4 ng/mL (50 µL injected). Calibration standards were interspersed with sample injections. Analyst® 1.6 software created the standard curve based on linear regression using $1/x^2$ weighting. The regression functions were used to calculate the best-fit line by plotting the analyte found (ng/mL) 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 are presented in Figure 2.

Peak integration and quantitation were performed within Analyst® 1.6 software; using the calibration curve equation to determine the amount of analyte found (ng/mL) during sample analysis. Recovery results and additional sample concentrations were calculated for each

set of samples using Microsoft® Excel spreadsheet data reports, which are presented in Appendix 3.

For the validation recoveries, the exact sample weight was used in calculating the final residues (µg/L).

The following equations are used for residue and recovery calculations for dimethenamid-p in drinking water matrices: