

1.0 INTRODUCTION

1.1 Purpose of the Study

The purpose of the study is to demonstrate that the analysis of Pendimethalin in BASF DocID 2013/1037296 and 2013/1229814 can be performed with acceptable recoveries at an outside facility.

1.2 Summary of the Results

The independent laboratory validation of the BASF method was successfully completed in the first trial. Control and Recovery results are presented in **Table 11.1** and **Table 11.2**.

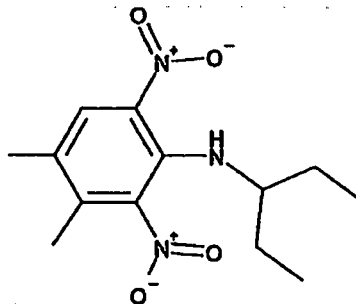
2.0 TEST AND REFERENCE SUBSTANCE AND SAMPLE HISTORY

2.1 Reference Materials

Pendimethalin (Reg. No. 900072) was used as both test and reference substance for the fortifications and the LC-MS/MS calibration. Concentrated (stock), fortification, and calibration standards were prepared according to the analytical method. Examples of standard solution preparations are presented in section, 2.2. A brief description of the reference standard used in this study is presented below.

Common Name:	Pendimethalin
BASF Code Name:	BAS 455 H
BASF Registry Number:	900072
CAS Number:	40487-42-1
Molecular Formula:	C ₁₃ H ₁₉ N ₃ O ₄
Molecular Weight:	281.3 g/mol
Batch Number:	AC12251-83
Purity:	99.2% ± 1.0%
Expiration Date:	January 1, 2016

Structural Formula:



The performance of the instrument was evaluated during the injection set. Standard solutions prepared for this study were stored under refrigerated conditions in refrigerator LETS#118 which had a temperature range of 2°C – 8°C during the course of the study. The COA for Pendimethalin is included in **Appendix B**.

2.2 Standard Solution

Stock Standard

Concentrated Standard Solutions (Solvent: Acetone)						
Lot Number	Analyte	Amount Weighed (mg)	Final Dilution Vol. (mL)	Final Conc. (mg/mL)	Stock Standard Solution ID No.	Prep. Date
AC12251-83	Pendimethalin	10.15	10.15	1.0	S03131401	03/13/14

Concentrated Standard Solutions (Solvent: Acetone)							
Parent ID No.	Analyte	Parent Conc. (µg/mL)	Aliquot Volume (µL)	Final Vol. (mL)	Final Conc. (µg/mL)	Stock Standard Solution ID No.	Prep. Date
S03131401	Pendimethalin	1000	100	10	10	S03131402	03/13/14

Fortification Solutions

Intermediate Standard and Fortification Standard Solutions (Solvent: Acetone)							
Parent ID No.	Analyte	Stock Conc. (µg/mL)	Aliquot Vol. (mL)	Dilution Vol. (mL)	Final Conc. (ng/mL)	Fort. Soln. ID	Prep. Date
S03131402	Pendimethalin	10	1	10	1000	F03181401	03/18/14
F03181401		1.0	1	10	100	F03181402	
F03181401		10	1	10	100	F03181403	
F03181401		1.0	0.2	10	20	F03181404	

Intermediate Standard and Fortification Standard Solutions (Solvent: Acetonitrile) cont.							
Parent ID No.	Analyte	Stock Conc. (µg/mL)	Aliquot Vol. (mL)	Dilution Vol. (mL)	Final Conc. (ng/mL)	Cali. Soln. ID	Prep. Date
F03181401	Pendimethalin	1.0	0.1	10	10	F03181405	03/18/14
F03181401		1.0	0.05	10	5	F03181406	
F03181404		0.02	1	10	2	F03181407	
F03181406		0.005	1	10	0.5	F03181408	

Example Calibration Solutions

Calibration Standard Solutions (Solvent: CA (Loamy Sand) Soil Control)							
Parent ID No.	Analyte	Stock Conc. (µg/mL)	Aliquot Vol. (mL)	Dilution Vol. (mL)	Final Conc. (ng/mL)	Fort. Soln. ID	Prep. Date
F03181403	Pendimethalin	100	0.05	1	5	C03311406	03/31/14
F03181404		20	0.05	1	1	C03311405	
F03181405		10	0.05	1	0.5	C03311404	
F03181406		5	0.05	1	0.25	C03311403	
F03181407		2	0.05	1	0.1	C03311402	
F03181408		0.5	0.05	1	0.025	C03311401	

2.3 Test System

Soil matrix was provided by BASF. Sandy and loamy sand soil matrices were sent from BASF Crop Protection, Inc. on March 10, 2014 and received by PASC on March 11, 2014. A second shipment of soil matrices were sent from BASF Crop Protection, Inc. on March 24, 2014 and received by PASC on March 25, 2014.

The extracted solutions were stored under refrigerated conditions in refrigerator LETS#118 which had a temperature range of 2°C – 8°C during the course of the study.

Soil Characterization reports are included in **Appendix C** for both matrices.

3.0 PROCEDURE - METHOD SYNOPSIS

Adaptation/Development and Validation of Residue Analytical Methods for the Determination of BAS 455 H (Pendimethalin) and Its Metabolites M455H001, Reg. No. 4061757 and Reg. No. 4982164 (P36) in Soil Reference 1

Report Amendment Reference 2

3.1 Summary of Analytical Procedure

3.1.1 Weighing and Fortification

Thirteen samples were prepared for each matrix (loamy soil (NJ) and sandy loam (CA)): One reagent blank, two control samples, five samples treated at the LOQ and five treated at 10 times the LOQ. For each, 10g of soil was weighed into a 50 mL PE centrifuge tube and spiked with the appropriate fortification solution using calibrated micropipette. The reagent blank received no soil.

The following scheme was used:

Sample Type	Sample Weight	Concentration of Spiking Solution [µg/mL]	Volume of Spiking Solution [µL]	Level of Fortification [ppb]
Reagent Blank	-	-	-	0.00
Control	10 g	-	-	0.00
Fortification (LOQ)	10 g	0.1	100	1.00*
Fortification (10× LOQ)	10 g	1.0	100	10.00

* Limit of quantification

3.1.2 Extraction of Sample Material

Each sample was extracted three separate times, with different extraction solutions. For the first extraction, 20 mL of an extraction solution consisting of 2% HCl in MeOH

was added to the samples. Samples were shaken at approximately 225 RPM for 30 minutes, then sonicated for 10 minutes, and then centrifuged for 5 minutes at 4000 RPM. The samples were decanted into separate 100 mL graduated cylinders. The second extraction used a solution of Methanol: Water (7:3, v/v), and the third extracted used a Methanol: Water (1:1, v/v) for the extraction solution. All three extractions for each sample were collected into the same graduated cylinder.

3.1.3 Preparation for Measurement

The combined samples extracts were brought up to a volume of 60 mL using Methanol: Water (7:3, v/v). Samples were shaken, then transferred to HPLC vials for analysis. The expected final concentration of Pendimethalin is presented in the table below.

Sample Type	Pendimethalin Concentration (ng/mL)
Control	< 20%LOQ
Fortification (LOQ)	0.1667
Fortification (10× LOQ)	1.667

Residue results were determined by LC-MS/MS. All the detailed instrument parameters are listed in Table 11.3.

The LC-MS/MS analysis was conducted as shown in the following tables.

Transition Monitored	Pendimethalin
Primary Quantification	m/z 282→212
Confirmatory Quantification	m/z 282→194

4.0 LIMIT OF QUANTITATION AND DETECTION

The method limit of quantitation (LOQ) for residues per analyte in soil was 1 ppb and the method limit of detection (LOD) was set at 0.3 ppb (30% of LOQ).

5.0 CALIBRATION, CALCULATIONS, AND STATISTICS

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

A standard curve was generated by injecting standard solutions at a range of concentrations bracketing the LOQ and 10x LOQ levels. Calibration standard concentrations for Pendimethalin

ranged from 0.025 – 5.0 ng/mL. Each calibration standard was injected in duplicate. Analyst® 1.4.2 software created the standard curve based on linear regression using 1/x. 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. A calibration curve for Pendimethalin in CA (Loamy sand) soil is presented in Figures 12.1. Representative chromatograms of calibration standards for Pendimethalin in CA (Loamy sand) soil are presented in Figure 12.2. The performance of the instrument was evaluated during each injection set.

Peak integration and quantitation were performed within Analyst® 1.4.2 software using the calibration curve equation to determine the amount of analyte found (ng/mL) during sample analysis. Recovery results were calculated for each set of samples using Microsoft® Office Excel spreadsheets which are presented in Appendix A.

Calculation of results is based on area measurements. The recoveries and residues of Pendimethalin in ppb were calculated using the following formulas:

$$\text{Residue [mg/kg]} = \frac{C_{\text{end}} \times DF \times V_{\text{ex}}}{1000 \times W}$$

C_{end} = Concentration of analyte in final sample volume [ng/mL]
DF = Dilution Factor
W = Weight of the sample extracted [g]
 V_{ex} = Final extract volume (mL)
1000 = Factor remaining after all unit conversions

The recoveries of spiked compounds were calculated using the following equation:

$$\text{Recovery \%} = \frac{\text{Residue in fortified Sample} \times 100}{\text{Amount of analyte fortified}}$$

As an example, calculations to obtain the residue level and recovery results of Pendimethalin (LOQ-1, m/z 282→212) in NJ (Loam) soil are shown below:

$$\text{Residue [mg/kg]} = \frac{0.162 \text{ ng/mL} \times 1 \times 60 \text{ mL}}{1000 \times 10.01 \text{ g}}$$

$$\begin{aligned} \text{Residue [mg/kg]} &= 0.00097103 \\ \text{Residue [ppb]} &= 0.97103 \end{aligned}$$

$$\text{Recovery \%} = \frac{0.97103 \text{ ppb} \times 100}{0.999 \text{ ppb}}$$

$$\text{Recovery \%} = 97.2\%$$

Percent relative standard deviation (% RSD) calculations were performed using Microsoft® Office Excel results and were rounded only for reporting purposes. No calculations were made with rounded numbers.

9.0 CONCLUSIONS

This independent laboratory validation was successfully completed at PASC. Recovery results and statistical data demonstrate that the analysis of Pendimethalin in DocID 2013/1037296 and 2013/1229814 can be performed successfully down to a level of 1 ppb 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.

10.0 REFERENCES

- 1) Nicole Heinz. Adaptation/Development and Validation of Residue Analytical Methods for the Determination of BAS 455 H (Pendimethalin) and Its Metabolites M455H001, Reg. No. 4061757 and reg. No. 4982164 (P36) in Soil. BASF DocID 2013/1037296(April 18, 2013).
- 2) Thomas Class. Report Amendment for Adaptation/Development and Validation of Residue Analytical Methods for the Determination of BAS 455 H (Pendimethalin) and Its Metabolites M455H001, Reg. No. 4061757 and reg. No. 4982164 (P36) in Soil. BASF DocID 2013/1229814(August 8, 2013)