syngenta

Dicamba

Independent Laboratory Validation of Analytical Method (GRM022.08A) for the Determination of Dicamba from Air Sampling Tube and Filter Paper by LC-MS/MS

ILV Final Report

DATA REQUIREMENT(S): EPA 850.6100

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2.0 INTRODUCTION

Described in this report is the independent laboratory validation (ILV) of Syngenta analytical method GRM022.08A, entitled "Independent Laboratory Validation of Analytical Method (GRM022.08A) for the Determination of Dicamba in Air Sampling Tube and Filter Paper by LC-MS/MS."

This study was designed to satisfy harmonized guideline requirements described in EPA OCSPP 850.6100 (Data Reporting for Environmental Chemistry Methods). This study was conducted in compliance with EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 (3).

BRIEF SUMMARY OF METHOD

Dicamba trapped in air sampling tubes and filter paper was extracted by acidified organic solvent and then the diluted extraction solution was analyzed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The LOQ of the method is 0.001 µg/air sample tube and 0.02 µg/filter paper.

For analysis of Dicamba, 10 µL of 0.1 µg/mL (LOQ) and 10 µL of 1.0 µg/mL (10XLOQ) were spiked into the OVS tube samples. For filter paper samples, 20 µL of 1.0 µg/mL (LOQ) and 20 µL of 10 µg/mL (10XLOQ) were spiked. The samples were extracted using a 30 minute shake with acidified (1%formic acid) organic solvent followed by a 10 minute sonication. An aliquot of sample is evaporated to near dryness then to final volume with 5% methanol and 0.1% formic acid aqueous solution, vortexed well and transferred to autosampler vials for analysis by LC-MS/MS.

3.0 MATERIALS AND METHODS

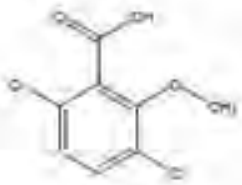
3.1 Test/Reference Substance

The test reference substance was obtained from Syngenta Crop Protection, LLC. The following test reference substance was used:

CHEMICAL STRUCTURES

FIGURE 1 Dicamba

Compound Code Number	:	SAN837
Common NAme	:	Dicamba
CAS Number	:	1918-00-9
IUPAC Name	:	3,6-dichloro-2-methoxybenzoic acid
Molecular Formula		$C_8H_6Cl_2O_3$
Molecular Mass	:	221



3.2 Test System

The test system evaluated for this ILV was OVS XAD-2 sorbent air sampling tubes, PUF sorbent air sampling tubes and filter paper. These matrices were chosen because they are the representative of the matrices the method was designed for. The control samples used in this study were provided by Syngenta Crop Protection, LLC.

3.3 Equipment and Reagents

The equipment and reagents used for this study were selected and prepared as outlined in the method. Identical or equivalent equipment and materials may have been used, as permitted by the method.

3.3.1 Equipment

Equipment	Description	Supplier
General lab glassware	General lab glassware	Thermoscientific
General lab plastic ware	General lab plastic ware	Thermoscientific
Autosampler vials	Snap cap, 2 mL size	Thermoscientific
LC-MS/MS system Including HPLC and autosampler units	AB Sciex 6500 triple quadrupole mass spectrometer with Analyst software version 1.6.2	AB Sciex&Waters
HPLC column	Phenomenex Kinetex Phenyl-Hexyl 2.1x100mm, 2.6µ	ACE
Balance	Mettler Toledo	Mettler Toledo

3.3.2 Reagents

Reagent	Description	Supplier
Water	HPLC grade	Pharmco-Aaper
Formic acid	ACS grade	Sigma Aldrich
Acetonitrile	HPLC grade	Pharmco-Aaper
Methanol	HPLC grade	J.T. Baker
Acetone	HPLC grade	Pharmco-Aaper
Dicamba	GLP certified	Syngenta Crop Protection, LLC P.O Box 8300 Greensboro, NC 27419-8300

3.3.3 Preparation of Reagents

a) Acidified Acetone: Prepared by mixing 5 mL formic acid in 500 mL acetone.
b) Acidified Methanol: Prepared by mixing 10 mL formic acid in 1000 mL Methanol.
c) 5% methanol and 0.1% formic acid aqueous solution; prepared by mixing 25mL HPLC grade methanol with 475mL Milli-Q water and added 0.5mL Formic acid.
d) “Mobile Phase A”; 0.1% Formic acid in water prepared by mixing 4000 mL of Milli-Q water and 4.0 mL of Formic acid.
e) “Mobile Phase B”; 0.1% Formic acid in methanol prepared by mixing 4000 mL of methanol and 4.0 mL of Formic acid.

3.4.3 Calibration Standards

A calibration curve was generated to quantify Dicamba. Standards over an appropriate concentration range were prepared with seven levels using the standard range 0.20 ng/mL – 20 ng/mL in ultra-pure water: methanol (95:5 v/v) with 0.1% formic acid.

Example of Calibration Standard Solution Preparation

Intermediate Calibration Solution			Calibration Solution					
Intermediate Calibration Solution ID	**Compound**	**Conc. (ng/mL)**	**Aliquot Taken (mL)**	**50ng/mL Internal Standard added(mL)**	**Diluted To (mL)**	**Solvent**	**Conc. (ng/mL)**	**Calibration Solution ID**
F20170124-6	Dicamba	200	1.0	1.0	10	Water:methanol (95:5, v/v) with 0.1% formic acid	20	C20170124-1
F20170124-6	Dicamba	200	0.5	1.0	10		10	C20170124-2
C20170124-1	Dicamba	20	2.5	1.0	10		5.0	C20170124-3
C20170124-1	Dicamba	20	1.0	1.0	10		2.0	C20170124-4
C20170124-1	Dicamba	20	0.5	1.0	10		1.0	C20170124-5
C20170124-4	Dicamba	2.0	2.5	1.0	10		0.5	C20170124-6
C20170124-4	Dicamba	2.0	1.0	1.0	10		0.2	C20170124-7

3.4.4 Preparation of Internal Standard Solution.

Prepared a 100 µg/mL stock solution for Dicamba internal standard following method preparation instructions:

2mg of Dicamba internal standard was transferred into an amber “Class A” volumetric flask (20-mL). Diluted to the mark with methanol and mixed well to give a 100 µg/mL stock solution concentration.

The stock solution was further diluted with water: methanol 95:5(v/v) with 0.1% formic acid to yield a 50ng/mL of internal standard solution.

3.5 Analytical Procedures and Modifications

3.5.1 Sample Fortification

In the validation, 10 µL or 20 µL of fortification solution containing Dicamba in methanol was added to air sampling tube or filter paper samples respectively. Each sample was left to stand for five minutes after fortification to allow the spiking solvent to penetrate/evaporate before proceeding with the extraction procedure. One reagent blank, two control samples, five control samples fortified at LOQ and five control samples fortified at 10xLOQ were included in the sample set.

Sample Type	Concentration of Spiking Solution [µg/mL]	Volume of Spiking Solution [µL]	Level of Fortification [µg/tube or paper]	Inject Conc.
Control	Methanol	50	0	0
Fortification (LOQ) Tube	0.1	10 (Tube)	0.001	0.2 ng/mL (if aliquot 1 mL to dry)
Fortification (10×LOQ) Tube	1.0	10 (Tube)	0.01	2.0 ng/mL (if aliquot 1 mL to dry)
Fortification (LOQ) Filter paper	1.0	20 (Paper)	0.02	1 ng/mL (if aliquot 1 mL to dry)
Fortification (10×LOQ) Filter paper	10	20 (paper)	0.2	10 ng/mL (if aliquot 1 mL to dry)

Extraction

1. Remove all contents (media, filter, foam plug, plastic holder…etc.) of air sample by pushing forward from small opening to large opening with a glass rod or suitable implement directly into appropriately size vessel (50 mL polypropylene tube). To avoid contamination from the exterior surface of the air sampler, the glass OVS tube should not be included in the extraction.
2. Extract RS16509 residue from the sample contents using 10 mL of acidified acetone (1% formic acid).
   Filter paper: Extract RS16509 residue from the sample contents using 40 mL of acidified methanol (1% formic acid).
3. Shake samples for 30 minutes and then sonicate for 10 minutes.
4. Transfer 1ml of filter paper extract or 2 mL of air sampling tube extract to a polypropylene tube and evaporate to near dryness. (<100 µL)
5. Reconstitute sample to 0.5 mL final volume using ultra-pure water: acetonitrile (95:5 v/v) with 0.1% formic acid, vortex well.
6. Analyze by LC-MS/MS (ESI Negative Mode).

Instrumentation

3.5.2 LC-MS/MS Instrument Description for Analysis of Dicamba

HPLC System:	Shimadzu system
MS Detector:	AB Sciex 6500 with Analyst^{TM} software version 1.6.2

Chromatography Conditions

Column	:	Phenomenex Kinetex Phenyl-Hexyl 2.1x100mm, 2.6µ
Column Oven Temperature	:	50°C
Injection volume	:	50 µL
Stop Time	:	10 minutes
Injection protocol	:	Analyze calibration standard after 5 sample injections
Mobile phase	:	Solvent 1 = 0.1% Formic Acid in Optima Water Solvent 2 = 0.1% Formic Acid in Optima Methanol

Isocratic/Gradient Flow:

Time (min)	0.1% Formic Acid in Water	0.1% Formic Acid in Methanol	Flow rate, mL/min
0.0	90	10	0.3
1.0	90	10	0.3
2.0	50	50	0.3
4.9	50	50	0.3
5.0	20	80	0.3
7.0	20	80	0.3
7.1	90	10	0.6
10.0	90	10	0.6

Under these conditions the retention time of Dicamba is 4.5 minutes.

Mass Spectrometer Conditions

Interface	:	ESI
Ionization mode	:	Negative
Curtain gas (CUR)	:	Nitrogen set at 30 (arbitrary units)
Source temperature (TEM)	:	300 °C
Ionspray Voltage (IS)	:	-4500
Collision gas setting (CAD)	:	Nitrogen set at 8 (arbitrary units)
Gas 1 (GS1)	:	Air set at 60 (arbitrary units)
Gas 2 (GS2)	:	Air set at 50 (arbitrary units)
Interface heater (ihe)	:	On
Scan type	:	MRM

		Dicamba Primary	Dicamba Confirmatory	Internal Standard
MRM Conditions				
Q1 *m/z*	:	219	221	225
Q3 *m/z*	:	175	177	181
Dwell time	:	100 ms	100 ms	100 ms
Resolution Q1	:	High	High	High
Resolution Q3	:	High	High	High
Declustering potential (DP)	:	-60 V	-60 V	-60 V
Entrance potential (EP)	:	-3 V	-3 V	-3 V
Collision energy (CE)	:	-10 V	-10 V	-10 V
Collision cell exit potential (CXP)	:	-10 V	-10 V	-10 V

Note: Instrument parameters were modified as AB Sciex 6500 was used in place of API 5500.

Data Acquisition
The peak integration and peak area count quantitation were performed by AB Sciex Analyst® (Version 1.6.2). A best-fit linear regression equation with 1/x weighting was selected in conjunction with the analyte response in each sample to calculate the concentration of analyte. The correlation coefficient (r) for the calibration curves for each analytical set was greater than 0.99. Recovery results were computed for each sample.

A statistical treatment of the data includes the calculation of averages, standard deviations, and relative standard deviations. Mean percent recoveries, standard deviations, and relative standard deviations were calculated using a current Microsoft Office Excel package.

4.0 RESULTS AND DISCUSSION

4.1 Method Establishment/Pre-Validation Evaluation

Initially, the mass spectrometer was optimized by infusing analyte standards to determine the optimum instrument operation parameters. Instrument parameters were established using the AB Sciex 6500, the retention times of the analyte, instrument detection limits, and response linearity were established by injecting a series of calibration reference standards. Prior to analysis of validation samples, a reagent blank and untreated control samples were analyzed to confirm the absence of contamination.

4.3 Potential Interferences

No potential interferences were observed.

4.4 Communications

Communication with the Sponsor Monitor occurred for the following:

1. Clarification/approval of the protocol and method,
2. Acquisition of analytical standard and control sample.

The communication emails were included in the raw data.

4.5 Time Required for Analysis

A single analyst completed a set of 13 samples in one working day with LC-MS/MS and analyses performed overnight.

4.6 Deviations

One deviation was written to address the preparation of standard solutions in clear volumetric flasks.

4.7 Circumstances Affecting Data

No circumstances occurred during this validation that affected the quality or integrity of the data.

4.8 Matrix Effects

Iostopitc labeled internal standard was used to compensate matrix effect for all matrices.

5.0 CONCLUSIONS

Syngenta Analytical Method GRM022.08A for the determination of Dicamba from OVS XAD-2 sorbent (140 mg/270 mg) air sampling tubes (SKC Inc. Cat No. 226-30-16), PUF sorbent (76-mm plug) air sampling tube (SKC Inc. Cat No. 226-92) and Filter Paper (Whatman™ Qualitative Grade Plain Circles and Sheets-Grade 3, 15 cm diameter) was successfully independently validated by PASC.

The LOQ of the method was established at 0.001µg/tube and 0.02µg/filter paper by acceptable recoveries and precision. This method was demonstrated to be suitable for the determination of Dicamba in OVS XAD-2 sorbent air sampling tubes, PUF sorbent air sampling tube and filter paper samples.

TABLE 1 Samples Fortification Levels

Soil Type	Fortification Level	Number of Recovery Samples
OVS XAD-2 sorbent air sampling tube	Control	2
	LOQ (0.001 μg/tube)	5
	10×LOQ (0.01 μg/tube)	5
PUF sorbent air sampling tube	Control	2
	LOQ (0.001 μg/tube)	5
	10×LOQ (0.01 μg/tube)	5
Filter paper	Control	2
	LOQ (0.02 μg/tube)	5
	10×LOQ (0.2 μg/tube)	5

FIGURE 1 Dicamba Chemical Structure

Compound Code Number	**:**	SAN837
Common Name	**:**	Dicamba
CAS Number	**:**	1918-00-9
IUPAC Name	**:**	3.6-dichloro-2-methoxybenzoic acid
Molecular Formula	:	$C_8 H_6 Cl_2 O_3$
Molecular Weight	**:**	221