

FINAL REPORT

Study Title

**Independent Laboratory Validation of Syngenta Analytical Method GRM022.06A:
"Dicamba - Analytical Method GRM022.06A for the Determination of Dicamba and its
Metabolite NOA414746 in Soil"**

BASF/JRFA Study Code

BASF Study Number: 823033
JRFA Study Number: AU-2018-03

Guidelines

U.S. EPA Residue Chemistry Test Guidelines, OPPTS 850.6100 Environmental Chemistry Methods
and Associated ILV

1. INTRODUCTION

1.1 Scope of the Method

Syngenta Analytical Method No. GRM022.06A was developed to determine the residues of dicamba and NOA414746 in soil using LC-MS/MS and validated at CEMAS in Berkshire, United Kingdom (Reference 1). This report represents the validation by an independent laboratory, JRF America in Audubon, Pennsylvania.

The independent lab validation was conducted using fortification levels at the limit of quantitation (0.0035 mg/kg) and ten times of limit of quantitation (0.035 mg/kg) for soil matrix. For each fortification level, five replicates were analysed. Additionally, one reagent blank and two replicates of unfortified samples were examined.

1.2 Principle of the Method

10 g samples of soil are extracted by heating at reflux with 0.5 M potassium hydroxide solution. The extracts are allowed to cool to room temperature then centrifuged. An aliquot of the extract equivalent to 1 g is acidified and partitioned four times with diethyl ether. The combined diethyl ether fractions are evaporated to dryness and redissolved in 0.1 M hydrochloric acid. Samples are then taken through a solid phase extraction (SPE) procedure. Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method is 0.0035 mg/kg (0.0035 ppm, 3.5 ppb). The LOD was determined during the method validation and “for dicamba was estimated as 0.01 ng injected on column, equivalent to 0.6 µg/kg when using a 40 µL injection volume and based on the quantitation transition. The LOD for NOA414746 was estimated as 0.01 ng injected on column, equivalent to between 0.3 and 0.5 µg/kg when using a 40 µL injection volume and based on the quantitation transition” (Reference 1).

1.3 Specificity

To demonstrate the specificity of the analytical method, one additional confirmatory mass transition (dicamba m/z 220.8 → 37.0; NOA414746 m/z 204.8 → 124.9) was monitored simultaneous to the primary quantitation transition (dicamba m/z 218.9 → 35.1; NOA414746 m/z 204.8 → 160.7) for analysis of dicamba and NOA414746. The method was able to accurately determine residues of dicamba and NOA414746 and no interference was observed at the retention time of the analyte peaks.

Calibration standards, prepared in soil matrix extract, were used for the quantitation of both analytes. No sample dilutions were required.

2. REFERENCE SUBSTANCE AND SAMPLING HISTORY

2.1 Test Systems

The test system considered in this study was soil collected from Washington, Louisiana. A summary of soil characterization is presented in Table 8; the full Agvise characterization report can be found in Appendix C.

The control samples were obtained by JRFA. The soil samples were received on March 6, 2018. Upon arrival at the laboratory, the samples were opened, inspected, and checked against enclosed shipping forms. The test systems were received ambient and were stored in refrigeration at all times, unless necessary for laboratory analysis.

2.2 Test and Reference Substances

The standard substances were stored ambient until use. BASF has retained reserve samples of these chemicals, and has documentation specifying the location of the synthesis and characterization information available at BASF Crop Protection, Research Triangle Park, North Carolina.

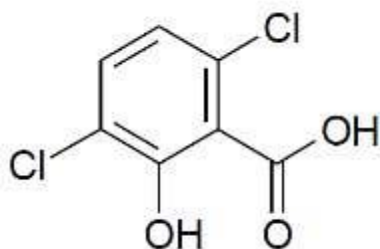
The dicamba (Batch No. L84-204) and NOA414746 (Batch No. 1221-67) reference substances were provided by the sponsor and received on February 28, 2018.

A summary of the reference substances is presented below.

BASF Code Name:	BAS 183 H
Common Name:	Dicamba
Batch Number:	L84-204
BASF Registry Number:	196095
CAS Number:	1918-00-9
IUPAC Name:	3, 6-dichloro-2-methoxy-benzoic acid
Molecular Formula:	$C_8H_6Cl_2O_3$
Molecular Weight:	221.0 g/mol
Purity:	99.9%
GLP Recertified Date:	August 16, 2017
Expiration Date:	September 1, 2022
Chemical Structure:	



BASF Code Name:	NOA414746
Common Name:	DCSA
Batch Number:	1221-67
BASF Registry Number:	395220
CAS Number:	3401-80-7
IUPAC Name:	3, 6-dichloro-2-hydroxybenzoic acid
Molecular Formula:	C ₇ H ₄ Cl ₂ O ₃
Molecular Weight:	207.0 g/mol
Purity:	100%
GLP Recertified Date:	October 2, 2009
Expiration Date:	April 01, 2019
Structural Formula:	



3. ANALYTICAL METHOD

Syngenta “Dicamba - Analytical Method GRM022.06A for the Determination of Dicamba and its Metabolite NOA414746 in Soil” was used for the analysis of the samples.

The residues of dicamba and NOA414746 are determined by the following: 10 g samples of soil are extracted by heating at reflux with 0.5 M potassium hydroxide solution. The extracts are allowed to cool to room temperature then centrifuged. An aliquot of the extract equivalent to 1 g is acidified and partitioned four times with diethyl ether. The combined diethyl ether fractions are evaporated to dryness and redissolved in 0.1 M hydrochloric acid. Samples are then taken through a solid phase extraction (SPE) procedure. Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method is 0.0035 mg/kg (0.0035 ppm, 3.5 ppb). The LOD was determined during the method validation and “for dicamba was estimated as 0.01 ng injected on column, equivalent to 0.6 µg/kg when using a 40 µL injection volume and based on the quantitation transition. The LOD for NOA414746 was estimated as 0.01 ng injected on column, equivalent to between 0.3 and 0.5 µg/kg when using a 40 µL injection volume and based on the quantitation transition” (Reference 1). These LOD values (0.6 µg/kg for dicamba and 0.3-0.5 µg/kg for NOA414746) were calculated by taking three times the estimated baseline noise of one control sample and dividing it by the intensity of the lowest standard (0.0005 µg/mL) injected. Calculations are shown in Table 3 and 4 of the method validation report (Reference 2).

Instrument parameters are described in Table 6.

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

Analyte	Transitions (m/z)		Polarity	Retention Time (min)
	<u>Quantitation</u>	<u>Confirmatory</u>		
Dicamba	218.9→35.1	220.8→37.0	Negative	~2.36
NOA414746	204.8→160.7	204.8→124.9		~3.49

5. STATISTICS AND DATA INTEGRITY

Statistical treatment of the data was simple descriptive statistics. These included determinations of averages, standard deviation and/or relative standard deviation (RSD) for the procedural recoveries. Also performed were area counts and calculation of the calibration curve and correlation coefficient (r) by linear regression of the instrument responses for the reference standards. The statistical calculations throughout this report were performed and reported using an automated computer spreadsheet (Microsoft Excel®) and were rounded for presentation purposes. Slight differences may be noted in hand calculations using the recoveries presented in the tables. These are due to rounding and have no effect on the scientific conclusions presented in this report. The detailed analytical data may be consulted for confirmation of the calculated results.

Several measures were taken to ensure the quality of the study results. The quality assurance unit at JRF America inspected the analytical procedures for compliance with Good Laboratory Practices that included adherence to the protocol. The dates inspected are detailed in the quality assurance unit statement. Study samples and test and reference items were maintained in a secured laboratory with limited access.

6. SUMMARY OF METHOD

Type of Method	LC-MS/MS
Test System	Soil
Selected mass transitions (<i>m/z</i>)	Dicamba <i>m/z</i> 218.9 → 35.1* <i>m/z</i> 220.8 → 37.0 NOA414746 <i>m/z</i> 204.8 → 160.7* <i>m/z</i> 204.8 → 124.9 *Primary quantitation transition
Analytical Procedure	“Dicamba - Analytical Method GRM022.06A for the Determination of Dicamba and its Metabolite NOA414746 in Soil” (Reference 1)
Confirmatory Technique	A secondary MRM transition was used for confirmation.
Method of Quantitation	The quantitation is based on the monitoring of one mass transition for dicamba and NOA414746. Recovery data was reported for each mass transition considered.
LOQ	0.0035 mg/kg (lowest fortification level)

Levels of Fortification	0.0035 and 0.035 mg/kg
Route of Administration	In this ILV, the test substances were applied as analytical standard solutions (in acetonitrile) by volumetric pipette to ensure precise delivery of a small amount of the test substances.
Time Required	A set of 13 samples required approximately 14 hours of work (calculation of the results included).
Justification of Ions	The ions used to conduct the ILV were determined in the validation (Reference 1) and are shown in Appendix C. The chosen ions were switched due to instrument optimization and stability of the respected ions.

Table 5 Example Standard Solutions Preparation and Dilution Data

Stock Standard Solutions

Standard ID#	Analyte	Parent Standard ID#	Adjusted Net Weight (g)	Dilution Volume (mL) ¹	Final Conc. (µg/mL)	Prep. Date
JRFA-545/1-1	Dicamba	SAS-012-Z	0.0121	100.00	121	3/6/18
JRFA-545/1-2	NOA414746	SAS-012-AA	0.0101	100.00	101	3/6/18

¹ Prepared in acetonitrile

Fortification Standard Solutions

Standard ID#	Analyte	Parent Standard ID#	Parent Conc. (µg/mL)	Aliquot Volume (mL)	Dilution Volume (mL) ²	Final Conc. (µg/mL)	Prep. Date
JRFA-545/1-3	Dicamba	JRFA-545/1-1	121	0.21	25.00	1.00	3/6/18
	NOA414746	JRFA-545/1-2	101	0.25		1.00	
JRFA-545/4-2	Both	JRFA-545/1-3	1.00	2.50	25.0	0.10	3/14/18

² Prepared in acetonitrile

Matrix-matched Calibration Standard Solutions

Standard ID#	Analyte	Parent Standard ID#	Parent Conc. (µg/mL)	Aliquot Volume (mL)	Dilution Volume (mL) ³	Final Conc. (µg/mL)	Prep. Date
JRFA-545/9-1	Both	JRFA-545/4-2	0.10	0.50	2.00	0.025	4/10/18
JRFA-545/9-2		JRFA-545/9-1	0.025	0.80	2.00	0.010	
JRFA-545/9-3		JRFA-545/9-2	0.010	1.00	2.00	0.005	
JRFA-545/9-4		JRFA-545/9-3	0.005	1.00	2.00	0.0025	
JRFA-545/9-5		JRFA-545/9-4	0.0025	0.80	2.00	0.001	
JRFA-545/9-6		JRFA-545/9-5	0.001	1.00	2.00	0.0005	

³ Prepared in matrix extract– JRFA-545/8-8

Table 6 Instrument Conditions and Parameters

UPLC Conditions			
Chromatographic System:	Shimadzu UFLC XR (L20224912063)		
Column:	Waters XSelect CSH 2.5 µm, 3.0x50 mm (01433724915805)		
Temperature:	30 °C		
Flow rate (mL/min)	0.750		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.00	95	5
	2.0	30	70
	4.0	30	70
	4.1	95	5
	6.0	95	5
Mobile Phase A:	0.1% formic acid in water		
Mobile Phase B:	Acetonitrile		
Injection Volume:	40 µL		

MS/MS Conditions						
Detection System:	AB SCIEX 4000 API MS (V29931111)					
Ionization:	Turbo Spray					
Polarity:	Negative					
Curtain gas (CUR, psi):	30.00					
Temperature (TEM, °C):	500.00					
Collision gas setting (CAD):	4.00					
GS1 (psi):	70.00					
GS2 (psi):	50.00					
Entrance potential (EP, V):	-10.0					
IQ1	10.00					
ST	18.00					
Scan type:	MRM					
MRM Conditions	Retention Time (min)	Transition (m/z)	DP (V)	CE (V)	CXP (V)	Dwell (msec)
Dicamba Reg. No. 196095	~2.36	218.9 → 35.1 (Quantitation)	-50.00	-30.00	-3.00	100.00
Dicamba Reg. No. 196095	~2.36	220.8 → 37.0 (Confirmatory)	-45.00	-32.00	-3.00	100.00
NOA414746 Reg. No. 395220	~3.49	204.8 → 160.7 (Quantitation)	-45.00	-18.00	-9.00	100.00
NOA414746 Reg. No. 395220	~3.49	204.8 → 124.9 (Confirmatory)	-45.00	-30.00	-7.00	100.00

Table 7 Equipment, Reagents and Mobile Phases

Equipment	Size, Description	Manufacturer	Catalog No.
Analytical Balance	AT 200	Mettler Toledo	----
Top loading Balance	PC 2000	Mettler Toledo	----
Flasks, Volumetric	Various sizes	Various	----
Pipettes	Various volumes	Eppendorf	----
Bottle, Amber glass	Qorpak , 2 oz and 4 oz Boston Round, Amber with Teflon®-lined screw cap	VWR Scientific Products	----
Plastic Centrifuge Tubes	250 mL	Thermo Scientific	332189-0008
	50 mL	UCT	RFV0050CT-D
	15 mL	Globe Scientific	1224M62 (Thomas)
Mixer	BenchMark, vortex	BenchMark Scientific, Inc.	13112194
HPLC vials	2 mL	Agilent Technologies	5182-0716
HPLC vial caps	PTFE/red silicone septa	Agilent Technologies	5182-0717
SPE cartridges	StrataX, polymeric reversed phase	Phenomenex	8B-5100-UBJ
Sonicator	FS30	Fischer	----
Centrifuge	Allegra 6R	Beckman Coulter	----
TurboVap LV	5x10 slots	Zymark	43750/29
Heating Mantle	6 mantles	Electrothermal Engineering	EMEA5-0500/CEX1 EME6-0500/CE

Chemical	Grade	Manufacturer/Supplier	Lot No.
Water	LC-MS	EMD	57297, 58004
Acetonitrile	LC-MS	EMD	57335, 58010
Hydrochloric Acid	ACS	JT Baker	0000107033
Acetic Acid	Glacial	JT Baker	0000079267
Formic Acid	LC-MS	Honeywell-Fluka	G350S
Potassium Hydroxide	ACS	EMD	171195203
Diethyl Ether	HPLC	Alfa Aesar	A26Y022

Description	Composition
HPLC mobile phase A	0.1% Formic Acid (FA) in Water 1.00 mL FA + ~999 mL LC-MS water in 1L volumetric flask
HPLC mobile phase B	100% LC-MS Acetonitrile

Table 8 Soil Characterization

Soil 203730	
Percent Sand	21
Percent Silt	57
Percent Clay	22
USDA Textural Class (hydrometer method)	Silt Loam
Cation Exchange Capacity	13.1 meq/100 g
Percent Moisture at 1/3 bar	26.2
Percent Organic Carbon –Walkley Black	1.1
Percent Organic Matter –Walkley Black	1.8
pH in 1:1 soil:water ratio	6.4

Figure 27 Residue Calculations

Peak integration and quantitation were performed within Analyst® 1.6.2 software; using the calibration curve equation to determine the amount of analyte found during sample analysis. Recovery results and additional sample concentrations were calculated for each set of samples and reported in Microsoft® Excel spreadsheets,

The following equations are used for residue and recovery calculations for dicamba and NOA414746 in soil.

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

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

b) Residue found ($\mu\text{g/g}$) = $\frac{\text{Amount found (ng/mL)} \times \text{Extraction Volume (mL)} \times \text{Final Volume (mL)}}{\text{Aliquot Volume (mL)} \times \text{Sample Weight (g)} \times 1000 \text{ ng}/\mu\text{g}}$

c) Recovery (%) = $\frac{\text{Residue found } (\mu\text{g/g}) - \text{Residue in control sample } (\mu\text{g/g})}{\text{Amount fortified } (\mu\text{g/g})} \times 100\%$

As an example, calculations to obtain dicamba (primary transition) recovery results using sample "LOQ R3" from soil (Figure 10) are shown below:

a) Calibration curve: $y = 2954.82230x + -454.032544$ (Figure 1)

Solving for x: $x = \frac{4414.547 + 454.032544}{2954.82230} = 1.648 \text{ ng/mL}$

c) Residue found ($\mu\text{g/g}$) = $\frac{1.648 \text{ ng/mL} \times 50 \text{ mL} \times 2 \text{ mL}}{5 \text{ mL} \times 9.97 \text{ g} \times 1000 \text{ ng}/\mu\text{g}} = 0.00331 \mu\text{g/g}$

d) Recovery (%) = $\frac{0.00331 \mu\text{g/g} - 0.0 \mu\text{g/g}}{0.0035 \mu\text{g/g}} \times 100\% = 94.4\%$

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