Appendix E. Analytical Method

syngenta

Dicamba

Dicamba - Analytical Method GRM022.06A for the Determination of Dicamba and its Metabolite NOA414746 in Soil

Analytical Method

DATA REQUIREMENT(S): EPA OCSPP 850.6100 (2012)
EC SANCO/3029/99 rev 4 (2000)
EC SANCO/825/00 rev 8.1 (2010)

Appendix E: Analytical Method (continued)

Abbreviations and Symbols

Abbreviation	Definition
°C	degrees Celsius or Centigrade
CAS	Chemical Abstract Services
EPA	Environmental Protection Agency (U.S.)
EC	European Commission
EU	European Union
g	gram
GRM	Global Residue Method
HCl	Hydrochloric acid
HPLC	high performance liquid chromatography
i.d.	internal diameter
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
L	litre
LC-MS/MS	tandem liquid chromatography/mass spectrometry/mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
m	metre
MeCN	acetonitrile
MeOH	methanol
µg	microgram
µL	microlitre
µm	micrometre
mg	milligram
mL	millilitre
mm	millimetre
mmol	millimole
min	minute
mol	mole
ms	millisecond
MSDS	Material Safety Data Sheet
MS/MS	tandem mass spectrometry/mass spectrometry
mV	millivolt
MW	molecular weight
m/z	mass to charge ratio

Appendix E: Analytical Method (continued)

Abbreviation	Definition
N/A (or n/a)	not applicable
ND (or nd)	not detectable (below limit of detection)
ng	nanogram
No.	number
OES	Occupational Exposure Standards
OCSPP	Office of Chemical Safety and Pesticide Pollution
ppb	parts per billion or micrograms per kilogram or micrograms per litre
ppm	parts per million or milligrams per kilogram or milligrams per litre
pg	picogram
R (or r)	correlation coefficient
R^2 (or r^2)	coefficient of determination or square of correlation coefficient
RSD	relative standard deviation
Rt (or RT)	retention time
s (or sec)	second
SD	standard deviation
SPE	Solid Phase Extraction
UPW	ultra-pure water
V	volt
Vol (or vol)	volume

Appendix E: Analytical Method (continued)

1.0 INTRODUCTION

1.1 Scope of the Method

Analytical method GRM022.06A is suitable for the determination of dicamba and its metabolite NOA414746 (Figures 1 and 2) in soil. The limit of quantification (LOQ) of the method has been established at 0.0035 mg/kg (or 0.0035 ppm, 3.5 ppb).

This method satisfies US EPA guideline EPA OCSPP 850.6100 and EC Guidance Documents SANCO/3029/99 rev 4 and SANCO/825/00 rev 8.1.

1.2 Method Summary

10 g sub 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).

2.0 MATERIALS AND APPARATUS

2.1 Apparatus

The recommended equipment and apparatus are listed in Appendix 1. Equipment with equivalent performance specifications may be substituted.

2.2 Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used. Reagents of comparable purity may be substituted as long as acceptable performance is demonstrated. A list of reagents used in this method along with details of preparation of solutions is included in Appendix 2.

Appendix E: Analytical Method (continued)

2.3 Preparation of Analytical Standard Solutions

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

2.3.1 Stock Solutions

Prepare individual 100 µg/mL stock solutions for dicamba and NOA414746 by one of the following methods.

Weigh out accurately, using a five figure balance, sufficient dicamba and NOA414746 analytical standard into separate "Class A" amber volumetric flasks (100 mL size). Dilute to the mark with acetonitrile to give individual 100 µg/mL stock solutions of dicamba and NOA414746.

Alternatively, the appropriate volume of acetonitrile added to a known amount of standard material may be determined using the equation below. The standard concentration is corrected for its chemical purity.

$$V = \frac{W \times P}{C} \times 1000$$

P = Standard purity in decimal form (P(%)/100)
V = Volume of acetonitrile required
W = Weight, in mg, of the solid analytical standard
C = Desired concentration of the final solution, (µg/mL)
1000 = Unit conversion factor

In this case, the standard material is weighed directly into an appropriate storage vessel.

2.3.2 Fortification Solutions

Sample fortification solutions containing dicamba and NOA414746 should be prepared by serial dilution in acetonitrile down to 0.1 µg/mL.

Appendix E: Analytical Method (continued)

2.3.3 Preparation of Calibration Standards for LC-MS/MS

A significant matrix effect (suppression) of the instrument response for dicamba and NOA414746 has been observed in the soil types tested using the procedures described in Section 3 during method validation. Therefore, matrix-matched calibration standards should normally be used for quantification of all analytes.

For preparation of calibration standards, take additional 1 g (5 mL) aliquots (at least 5) of untreated soil extract at point 3.3 (d) and take through the analytical procedure to 3.4 (e). Combine the additional final control extracts into one composite sample to get a homogenous control matrix extract.

To prepare for example an LOQ equivalent matrix matched standard, add 17.5 µL of 0.1 mg/mL mixed dicamba and NOA414746 standard in acetonitrile to 0.5 mL of the composite control extract and adjust the final volume to 1 mL with the same composite control extract. Transfer an aliquot into a suitable autosampler vial for final determination by LC-MS/MS.

A calibration curve should be generated to quantify dicamba and NOA414746 residues. Standards over an appropriate concentration range should be prepared as described above, using the requisite volumes of dicamba and NOA414746 in acetonitrile.

Any matrix effects observed may be compensated for by use of matrix matched standards at the discretion of the study director, or by dilution of the final sample with 90/10 v/v ultra-pure water/acetonitrile where instrument sensitivity permits.

2.3.4 Standard Solution Storage and Expiration

All stock solutions should be stored between 2 and 8°C when not in use to prevent decomposition and/or concentration of the standard. Standard solutions should be allowed to equilibrate to room temperature prior to use.

An expiration date of 28 days for dicamba and NOA414746 in acetonitrile is recommended unless additional data are generated to support a longer expiration date.

2.4 Safety Precautions and Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate MSDS or a monograph such as 'Hazards in the Chemical Laboratory', edited by S G Luxon, The Chemical Society, London (Reference 2).

Appendix E: Analytical Method (continued)

Solvent and Reagent Hazards

	Acetonitrile	Potassium Hydroxide	Hydrochloric Acid	Diethyl Ether	Acetic Acid	Formic Acid
Harmful Vapour	✓	✓	✓	✓	✓	✓
Highly Flammable	✓	✗	✗	✓	✗	✗
Harmful by Skin Absorption	✓	✓	✓	✓	✓	✓
Irritant to respiratory system and eyes	✓	✓	✓	✓	✓	✓
Causes severe burns	✗	✓	✓	✗	✓	✓
OES Short Term (mg/m^3)	105	2	7	1500	37	19
OES Long Term (mg/m^3)	70	n/a	n/a	1200	25	9

n/a not known

Suitable personal protective equipment should be worn when handling chemicals and reagents. The appropriate MSDS should be consulted for each reagent and a local risk assessment should be carried out. In all cases avoid breathing vapour and contact with eyes and skin.

3.0 ANALYTICAL PROCEDURE

3.1 Sample Preparation

All samples should be prepared using an approved method of preparation to obtain a homogeneous sample prior to analysis.

3.2 Sample Fortification

In order to verify method performance and allow recovery corrections to be made (if appropriate), fortified control samples should be included with each sample set. To each pre-weighed control soil sample, add the appropriate amount of standard solution containing dicamba and NOA414746 in acetonitrile. Let each sample stand for at least five minutes after fortification to allow the spiking solution to soak into the matrix before proceeding with the extraction procedure. At least one untreated control and two fortified control samples should be analysed with each sample set.

3.3 Extraction

A summary of the method is included in flow-chart form in Appendix 4.

a) Weigh a representative amount of soil (10 g) into a round bottom flask (250 mL size).

Appendix E: Analytical Method (continued)

b) Add 0.5 M potassium hydroxide (50 mL) and place the round bottom flask in a heating mantle. Attach a suitable water cooled condenser and heat the sample at reflux for 45 minutes. Allow the sample to cool to room temperature and remove the flask from the heating mantle.

c) Carefully transfer the contents of the flask into a clean, disposable centrifuge bottle (250 mL size) and centrifuge at a speed which visibly separates the solid material from the extract (e.g. 4000 rpm for 5 minutes). Decant the supernatant extract into a clean centrifuge tube (50 mL size).

d) Transfer an aliquot (5 mL, equivalent to 1 g soil) into a plastic, screw cap centrifuge tube (15 mL size). Add concentrated hydrochloric acid (0.5 mL) and vortex mix for a few seconds. Check that the pH is at least pH 1 using suitable indicator paper. A precipitation of the humic and fulvic acids is normally observed and should be left in the sample tube.

e) Add diethyl ether (2 mL), cap the tube securely, and shake the sample vigorously for about 15 seconds, venting the sample tube periodically to release the pressure.

f) Centrifuge the sample at a speed which separates the two phases (e.g. 4000 rpm for 5 minutes). Care should be taken not to transfer any of the precipitate present at the interface of the aqueous and organic phases as this may adversely affect the performance of the SPE clean-up in the next stage.

g) Repeat steps 3.3 (e) and 3.3 (f) three more times, combining the upper diethyl ether fractions.

h) Place the combined diethyl ether fractions in a heating block set at 30 °C and evaporate the sample just to dryness under a stream of air or nitrogen.

Note that dicamba and NOA414746 are volatile compounds and low recovery may be observed should the sample be left at dryness for extended periods.

i) Add 0.1 M hydrochloric acid (1 mL) to the sample and ultrasonicate briefly to mix thoroughly. Proceed to the SPE clean-up described in Section 3.4

3.4 Solid Phase Extraction Procedure.

a) Take one Phenomenex Strata-X cartridge (60 mg, 3 mL size) for each sample to be analysed and place on a suitable vacuum manifold (e.g. IST Vacmaster). Add acetonitrile (3 mL) and allow to percolate through under gravity or draw through under vacuum to the level of the top frit at a rate of approximately 1 mL/min, discarding the column eluate. Do not allow the cartridges to become dry. Add ultra-pure water (3 mL) to the top of each cartridge and allow to percolate through under gravity or draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluate. Do not allow the cartridges to become dry.

Appendix E: Analytical Method (continued)

b) Add the sample from 3.3 (i) in 0.1 M hydrochloric acid to the top of the SPE cartridge and allow to percolate through the cartridge under gravity. Discard the cartridge eluate.

c) Rinse the empty sample tube with 2 x 1 mL ultra-pure water, adding the rinses to the top of the cartridge as before. Discard the cartridge eluates.

d) Wash the cartridge with ultra-pure water (2 x 2 mL), discarding the cartridge eluates.

e) Dry the cartridges under high vacuum (e.g. -0.5 bar) for approximately 10 minutes, depending on the vacuum achievable on the SPE manifold.

f) Elute the cartridges with 1% acetic acid in acetonitrile (2 x 3.5 mL). Collect the cartridge eluate in a clean disposable glass test tube and evaporate the combined eluates just to dryness by placing the sample in a heating block set at 40 °C under a stream of air or nitrogen.

Note that dicamba and NOA414746 are volatile compounds and low recovery may be observed should the sample be left at dryness for extended periods.

g) Add 90/10 v/v ultra-pure water/acetonitrile (2 mL) and ultrasonicate briefly to mix thoroughly. Transfer samples in to suitable autosampler vials for analysis by LC-MS/MS using matrix matched standards. The final sample concentration is 0.5 g/mL.

3.5 Experimental Precautions

a) The SPE procedure has been developed using cartridges from the stated manufacturer. Similar cartridges from other manufacturers may be used. In all cases however, it is strongly recommended that the elution profile of the chosen batch of cartridges is checked prior to commencing analysis to assess any variation in manufacturers' products and between batches.

b) Bottled HPLC grade ultra-pure water is used to prepare the LC mobile phase, which produces a lower background noise in the MS/MS chromatograms than water taken from a laboratory water purification system.

c) To prevent contamination of the instrument and to minimise possible carry-over issues, it is recommended that high level recoveries (>0.1 mg/kg) and samples with expected residues greater than 0.1 mg/kg should be diluted so that the final analyte concentration does not exceed 0.005 µg/mL. It may also be useful to include blank injections of acetonitrile/ultra-pure water (50/50 v/v) after high level samples to clear any observed carry-over greater than 10% of the LOQ.

Appendix E: Analytical Method (continued)

d) Dicamba and NOA414746 are volatile compounds and low recovery may be observed should the samples be left at dryness for extended periods.

3.6 Time Required for Analysis

The methodology is normally performed with a batch of 12 samples. One person can complete the analysis of 12 samples in 1 day (8 hour working period).

3.7 Method Stopping Points

The analytical procedure can be stopped at various points for overnight and weekend breaks unless otherwise specified in the analytical procedure. Acceptable method recoveries will validate any work flow interruptions. Samples should be stored refrigerated in sealed containers where the analysis cannot be completed in a single day.

Appendix E: Analytical Method (continued)

4.0 FINAL DETERMINATION

The method has been developed for use on an Applied Biosystems AB Sciex 5500. The following instrumentation and conditions have been found to be suitable for this analysis. Other instrumentation can also be used, though optimisation may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum use.

4.1 Instrument Description

HPLC system (binary pump, column oven and degasser)	:	Agilent technologies 1200
Detector	:	AB Sciex 5500 Q Trap
Autosampler	:	Agilent 1200 - high performance autosampler SL
Gas Supply	:	Peak Scientific

4.2 Chromatography Conditions for Dicamba and NOA414746

Column	:	Waters XSelect CSH™ C18 2.5 µm 3.0 x 50 mm
Column Oven Temperature	:	30 °C
Injection volume	:	40 mL
Stop Time	:	6.0 minutes
Injection protocol	:	Analyse calibration standard after 3 to 4 sample injections
Mobile phase	:	Solvent 1: acetonitrile Solvent 2: 0.1% formic acid in ultra-pure water

Mobile Phase Composition

Time (mins)	% solvent 1	% solvent 2	Flow rate (mL/min)
0.0	5	95	750
2.0	70	30	750
4.0	70	30	750
4.1	5	95	750
6.0	5	95	750

Under these conditions, the retention time for dicamba is approximately 2.7 minutes and NOA414746 is 3.8 minutes.

Appendix E: Analytical Method (continued)

Valco Valve Diverter Programme

Time	Position
0.0	Waste
2.5	Mass spectrometer
3.0	Waste
3.5	Mass spectrometer
4.6	Waste

4.3 Mass Spectrometer Conditions for Dicamba and NOA414746

Interface	:	TurboIonSpray
Polarity	:	Negative
Curtain gas (CUR)	:	Nitrogen set at 35 (arbitrary units)
Temperature (TEM)	:	400°C
Ionspray voltage	:	-4200V
Collision gas setting (CAD)	:	Nitrogen set at Low
Gas 1 (GS1)	:	Air set at 50 (arbitrary units)
Gas 2 (GS2)	:	Air set at 50 (arbitrary units)
Interface heater (ihe)	:	On
Scan type	:	MRM

Appendix E: Analytical Method (continued)

MRM Conditions		Dicamba primary transition	Dicamba confirmatory transition	NOA414746 primary transition	NOA414746 confirmatory transition
Q1 *m/z*	:	219.0	221.0	204.6	204.6
Q3 *m/z*	:	35	37	125	161
Dwell time	:	100 ms	100 ms	100 ms	100 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	-55 V	-55 V	-55 V	-55 V
Entrance potential (EP)	:	-10 V	-10 V	-10 V	-10 V
Collision energy (CE)	:	-38 V	-38 V	-30 V	-15 V
Collision cell exit potential (CXP)	:	-6 V	-6 V	-15 V	-14 V

Typical chromatograms are shown in the Figures Section.

4.4 Confirmatory Procedures for Dicamba and NOA414746

Final determination by LC-MS/MS with two transitions is considered to be highly specific; hence no further confirmatory conditions are included.

5.0 CALCULATION OF RESULTS

5.1 Multi-Point Calibration Procedure

Dicamba and NOA414746 residues may be calculated in mg/kg for each sample as follows.

a) Prepare standard solutions over a concentration range appropriate to the expected residues in the samples (30% LOQ to at least 20% above the highest fortified level as a minimum). An appropriate number of different concentrations within this range should be prepared (at least five).

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to dicamba and NOA414746. Calibration standard solutions should be interspersed throughout the analysis, after a maximum of four injections of sample solutions.

c) Generate calibration curve parameters using an appropriate regression package.

Appendix E: Analytical Method (continued)

d) The following equation can be rearranged and used to calculate residues as follows:

$$y = mx + c$$

Where y is the instrument response value, x is the standard concentration, m is the gradient of the line of best fit ("X-variable 1" in MS Excel) and c is the intercept value. An example of this equation generated using the experimental values of m and c should be included in the raw data, as should the "R-Squared" value for the regression.

Re-arrangement for x gives

$$x = \frac{y - c}{m}$$

e) Calculate the dicamba and NOA414746 residues in the sample, expressed as mg/kg, as follows

$$\text{Residue (mg/kg)} = \frac{\text{Analyte found } (\mu\text{g/mL})}{\text{Sample conc. (g/mL)}}$$

Where analyte found (µg/mL) is calculated from the standard calibration curve and sample conc. is the final sample concentration in g/mL.

If residues need to be corrected for average percentage recovery e.g. for storage stability studies, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \text{ (mg/kg)}$$

5.2 Single-Point Calibration Procedure

Dicamba and NOA414746 residues may be calculated in mg/kg for each sample using a mean standard response from each of the injections bracketing the sample as follows.

a) Make repeated injections of a standard containing dicamba and NOA414746 at an appropriate concentration into the LC-MS/MS operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak areas obtained for Dicamba and NOA414746.

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to dicamba and NOA414746.

c) Re-inject the standard solution after a maximum of four injections of sample solutions.

Appendix E: Analytical Method (continued)

d) Calculate the dicamba and NOA414746 residues in the sample, expressed as mg/kg using a mean standard response from each of the injections bracketing the sample as follows.

$$\text{Residue (mg/kg)} = \frac{\text{PK area (SA)}}{\text{PK area (STD)}} \times \frac{\text{Standard Conc.}}{\text{Sample Conc.}}$$

PK area (SA) = Peak response for sample
PK area (STD) = Average peak response for bracketing standards
Standard Conc. = Concentration of standard (µg/mL)
Sample Conc. = Sample concentration (g/mL)

If residues need to be corrected for average percentage recovery e.g. for storage stability studies, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \text{ (mg/kg)}$$

Although single point calibration may be used to quantify residues it is recommended that a calibration curve is generated with each analytical run to demonstrate the linearity of instrument response (Reference 3).

6.0 CONTROL AND RECOVERY SAMPLES

Control samples should be analysed with each set of samples to verify that the sample used to prepare recovery samples is free from contamination. A minimum of one control should be analysed with each batch of samples.

At least two recovery samples (control samples accurately fortified with known amounts of dicamba and NOA414746 in acetonitrile) should also be analysed alongside each set of samples. Provided the recovery values are acceptable they may be used to correct any residues found. The fortification levels should be appropriate to the residue levels expected.

Recovery efficiency is generally considered acceptable when the mean values are between 70% and 120% and with a relative standard deviation of ≤20%.

Where the method is used for monitoring purposes, control and recovery samples are not required where suitable control samples are not available.

Appendix E: Analytical Method (continued)

7.0 SPECIFICITY

It is recommended that reagent blank samples be included in a sample set if contamination is suspected.

7.1 Matrix

LC-MS/MS is a highly specific detection technique. Interference arising from the matrices tested has not been observed.

7.2 Reagent and Solvent Interference

Using high purity solvents and reagents no interference has been found.

7.3 Labware Interference

This method uses mainly disposable labware. All reusable glassware (round bottom flasks and condensers) should be detergent washed and then rinsed with HPLC grade methanol, acetone or acetonitrile prior to use.

8.0 METHOD VALIDATION

8.1 Extractability

Dicamba and NOA414746 have been shown to be efficiently extracted in a radiolabelled metabolism study using the conditions described in Section 3 of the analytical procedure (References 4 and 5).

8.3 Limit of Quantification (LOQ)

The limit of quantification of the method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-120% with a relative standard deviation of ≤ 20% has been obtained. Generally, for accurate quantification, the response for an analyte peak should be no lower than three times the mean amplitude of the background noise in an untreated sample at the corresponding retention time.

The limit of quantification has been set at 0.0035 mg/kg (0.0035 ppm, 3.5 ppb).

Appendix E: Analytical Method (continued)

8.4 Limit of Detection (LOD)

The limit of detection of the method is defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as three times background noise. Note that the LOD may vary between runs and from instrument to instrument.

The LOD 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.

8.5 Matrix Effects

A comparison of the response obtained from the matrix-matched standards against the response obtained from the standards in 90/10 v/v ultra-pure water/acetonitrile revealed that matrix effects (suppression (-)) on the instrument response caused by the soil matrices were considered to be significant (>20%) for dicamba and NOA414746. Matrix-matched calibration standards should generally be used for quantification of all analytes in this method. A summary of the matrix effects data is presented in Table 6.

8.6 Detector Linearity

For accurate quantification of residue concentrations, analyses should be carried out within the linear range of the detector. For multi-point calibration, detector range and linearity will be demonstrated within each sample set.

The linearity of the LC-MS/MS detector response for dicamba and NOA414746 was tested in the range from 0.02 ng to 1.0 ng injected on column (equivalent to 0.0005 µg/mL to 0.025 µg/mL standards when using a 40 µL injection volume) and was found to be linear when using linear regression.

If a residue beyond the tested concentration range is expected, dilute the sample appropriately to bring it within the tested linear range prior to quantification.

Inject standards in the range 30% LOQ – 20% above highest concentration required and plot the peak area against the standard concentration, using Microsoft Excel for both primary and confirmatory transitions for dicamba and NOA414746.

Appendix E: Analytical Method (continued)

8.7 Extract Stability

Final soil extracts in 90/10 v/v ultra-pure water/acetonitrile retained in vials and stored at a temperature of approximately 4°C were suitable for dicamba and NOA414746 residue analysis for storage periods of at least 7 days. .

9.0 LIMITATIONS

The method has been tested on representative soil types. It can reasonably be assumed that the method can be applied to other soil types not tested in this method provided successful recovery tests at the relevant levels validate the suitability of the method.

10.0 CONCLUSIONS

This procedure has been demonstrated to be a reliable and accurate procedure for the determination of dicamba and NOA414746 residues in soil. Only commercially available laboratory equipment and reagents are required. The analysis of 12 soil samples for dicamba and NOA414746 can be completed by one person in 1 day (8 working hour period). Untreated and fortified samples should be analysed with each set of samples to demonstrate absence of any interference and adequate recovery, if possible. The limit of quantification of the method is 0.0035 mg/kg (0.0035 ppm, 3.5 ppb).

This method satisfies US EPA guideline EPA OCSPP 850.6100 and EC Guidance Documents SANCO/3029/99 rev 4 and SANCO/825/00 rev 8.1.

Appendix E: Analytical Method (continued)

TABLE 1: Characterisation Data for Soil Samples used in Method Validation

Matrix	Source	Sample Reference	pH (Water)	pH (0.01M CaCl2)	Organic Matter % w/w	Organic Carbon % w/w	CEC meq/ 100g	Textural Class
Soil (Gartenacker)	Syngenta	CCON/033/002	7.7	7.2	3.5	2.0	11.1	Loam
Soil (18 Acres)	Syngenta	CCON/034/003	6.4	5.8	4.0	2.3	16.8	Sandy Clay Loam

Appendix E: Analytical Method (continued)

CHEMICAL STRUCTURES

FIGURE 1: Dicamba

Compound Code Number	:	SAN 837
CAS Number	:	1918-00-9
IUPAC Name	:	3,6-Dichloro-2-methoxybenzoic acid
Molecular Formula	:	$C_8H_6Cl_2O_3$
Molecular Mass	:	221 g/mol

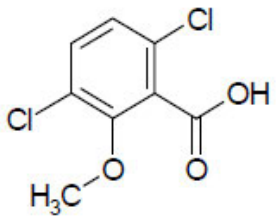
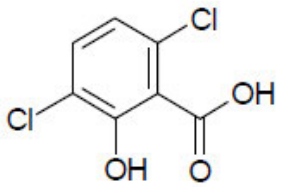


FIGURE 2: NOA414746

Compound code number	:	NOA414746
CAS Number	:	3401-80-7
IUPAC Name	:	3,6-dichloro-2-hydroxy-benzoic acid
Molecular Formula	:	$C_7H_4Cl_2O_3$
Molecular Mass	:	207.0 g/mol



Appendix E: Analytical Method (continued)

APPENDIX 1 APPARATUS

Recommended Suppliers

Equipment	Description	Supplier
General glassware	General glassware	www.thermofisher.com/global/en/home.asp
Plastic centrifuge bottles	Polypropylene, 250 mL size	www.thermofisher.com/global/en/home.asp
Centrifuge	e.g. Hettich Rotanta 460	www.hettichlab.com
Plastic centrifuge tubes	Polypropylene, 50 mL and 15 mL size	www.thermofisher.com/global/en/home.asp
Plastic pipettes	Disposable, 3 mL size	www.thermofisher.com/global/en/home.asp
SPE cartridges	Phenomenex Strata-X 33 µm 60 mg, 3 mL size Part no. 8B-S100-UBJ	www.phenomenex.com
SPE vacuum manifold	To hold up to 20 cartridges	www.phenomenex.com
Ultrasonic bath		www.thermofisher.com/global/en/home.asp
Autosampler vials	2 mL size	www.agilent.com
LC-MS/MS system	AB Sciex 5500 equipped with a TurboIonSpray source	www.AppliedBiosytems.com
HPLC system	Agilent 1200 equipped with binary pump, autosampler and column oven.	www.agilent.com
Autosampler	CTC Analytics HTC Pal	www.ctc.ch
HPLC column	Waters XSelect CSH™ 2.5 µm 3.0 x 50 mm. Part No. 186006105	www.waters.com
Nitrogen generator	Peak Scientific AB-3G gas station	www.peakscientific.com

Appendix E: Analytical Method (continued)

APPENDIX 2 REAGENTS

Recommended Suppliers

Reagent	Description	Supplier
Ultra-pure water	HPLC grade	www.thermofisher.com/global/en/home.asp
Acetonitrile	HPLC grade	www.thermofisher.com/global/en/home.asp
Potassium hydroxide	Analytical grade	www.thermofisher.com/global/en/home.asp
Conc. HCl (36% w/v)	Analytical grade	www.thermofisher.com/global/en/home.asp
Diethyl ether	Analytical grade	www.thermofisher.com/global/en/home.asp
Acetic acid	Analytical grade	www.thermofisher.com/global/en/home.asp
Formic acid (98%)	Analytical grade	www.thermofisher.com/global/en/home.asp
Dicamba and NOA414746analytical standards	GLP certified	GLP Testing Facility, Syngenta, CH-4333, Munchweilen, Switzerland or Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, NC 27419-8300.

Preparation of Reagents

a) 0.5 M potassium hydroxide
Add 28 g of potassium hydroxide to ultra-pure water in a 1 L flask and mix to dissolve the pellets. Adjust to the 1 L mark with ultra-pure water. Stopper the flask securely and shake to mix thoroughly.

b) 0.1 M hydrochloric acid
Carefully add 8.5 mL of concentrated hydrochloric acid to ultra-pure water in a 1 L flask. Adjust to the 1 L mark with ultra-pure water. Stopper the flask securely and shake to mix thoroughly.

c) 1% v/v acetic acid in acetonitrile
Add 1 mL glacial acetic to acetonitrile in 100 mL volumetric flask. Add acetonitrile to the 100 mL mark. Stopper the flask securely and shake to mix thoroughly.

d) 0.1% formic acid in ultra-pure water
Add 1 mL concentrated formic acid to ultra-pure water in a 1 L volumetric flask. Adjust to the 1 L mark with ultra-pure water. Stopper the flask securely and shake to mix thoroughly.

e) 90/10 v/v ultra-pure water/acetonitrile
Add 10 mL acetonitrile to 90 mL ultra-pure water in a 100 mL volumetric flask. Stopper the flask securely and shake to mix thoroughly.

Appendix E: Analytical Method (continued)

APPENDIX 3 LC-MS/MS TUNING PROCEDURE

Calibration of Instrument

The instrument must be mass calibrated on a regular basis using polypropylene glycol (PPG) solutions according to the manufacturer's instructions. Calibrate both mass resolving quadrupoles (Q1 and Q3).

Tuning Instrument for Dicamba and NOA414746

Note: Dicamba and NOA414746 both have 2 chlorine atoms present. Chlorine has two isotopic forms ^{35}Cl and ^{37}Cl and therefore the mass spectrum may show ions at M (^{35}Cl x 2), M+2 (^{35}Cl + ^{37}Cl) and M+4 (^{37}Cl x2) at a ratio of 9:6:1, respectively. On the instrument, these ion masses based on the chlorine isotopes can be used as required to achieve analytical sensitivity and specificity.

Infuse standard solutions of dicamba and NOA414746 (0.1 µg/mL) in acetonitrile directly into the mass spectrometer interface at a rate at of approximately 10-20 µL/min. Roughly adjust interface parameters (sprayer position, spray, heater/auxiliary gas flows, as well as voltages of spray, orifice, and focusing ring) for a sufficiently high parent ion signal at *m/z* 219 and *m/z* 221 for dicamba and *m/z* 205 for NOA414746 in negative ionisation mode.

Using the Analyst software quantitative optimisation routine, tune the instrument for dicamba and NOA414746, ensuring that the correct ion is selected. If desired, manual tuning of the ion optics and collision energy can be carried out to ensure maximum sensitivity.

Finally, connect the LC-pump via the autosampler directly to the MS/MS instrument. Perform repetitive flow injection of a dicamba and NOA414746 standard using mobile phase at the flow rate to be used. Tune the interface parameters (sprayer position, spray and heater gas flows, spray, orifice, and focusing ring voltages) and the collision gas flow for maximum sensitivity.

For dicamba, in negative ionisation mode, the deprotonated molecular ion generated in the ion source (*m/z* 219) is selected and subjected to further fragmentation by collisional activation. The most sensitive and specific daughter ion (*m/z* 35) corresponding to ^{35}Cl is then selected and used for quantitative analysis. Similarly, the confirmatory daughter ion for parent ion (*m/z* 221) is ^{37}Cl (*m/z* 37) and is selected for confirmatory analysis.

For NOA414746, in negative ionisation mode, the deprotonated molecular ion generated in the ion source (*m/z* 205) is selected and subjected to further fragmentation by collisional activation. The two most sensitive daughter ions (*m/z* 161 and *m/z* 125) are then selected and used for quantitative analysis.

The fragment *m/z* 161 corresponds to loss of CO_2 and the fragment *m/z* 125 corresponds to subsequent loss of HCl.

Appendix E: Analytical Method (continued)

APPENDIX 4 METHOD FLOW CHART

Weigh 10 g soil into a 250 mL round bottom flask

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Add 0.5 M KOH (50 mL) and heat at reflux for 45 minutes

↓

Allow to cool to room temperature

↓

Partition a 5 mL (1 g) aliquot with 4 x 2 mL Et_2O

↓

Evaporate the Et_2O just to dryness and redissolve sample in 0.1 M HCl

↓

Strata-X SPE procedure

↓

Elute dicamba and NOA405873 with 1% AcOH in MeCN

↓

Evaporate to dryness and redissolve in 90/10 v/v ultra-pure water/acetonitrile (2 mL) with ultrasonication

↓

Analyse by LC-MS/MS using matrix matched standards