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Dicamba

Dicamba - Method GRM022.08A for the Determination of Dicamba from Air Sampling Tube and Filter Paper by LC-MS/MS

Analytical Method

DATA REQUIREMENT(S): EPA 850.6100

1.0 INTRODUCTION

1.1 Scope of the Method

Analytical Method GRM022.08A is suitable for the determination of Dicamba (Figure 1) from OVS XAD-2 sorbent (140/270 mg) air sampling tube (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).

The limit of quantitation (LOQ) of the method has been established at 1.0 ng/air sampling tube and 20 ng/filter paper.

This method satisfies US EPA 850.6100 guidelines.

1.2 Method Summary

Air sample tube contents and filter paper are 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 reconstituted to final volume with 0.1% formic acid ultra-pure water:methanol (95:5 v/v). Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

The limit of quantitation of the method is 1.0 ng/air sample type.

The limit of quantitation of the method is 20 ng/filter paper.

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.

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 appropriate PPE which includes chemical resistant gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

2.3.1 Stock Solutions

Prepare a 100 μg/mL stock solution for dicamba and dicamba internal standards by one of the following methods:

Weigh out accurately, using a five figure balance, sufficient Dicamba analytical standard into an amber "Class A" volumetric flask (100-mL). Dilute to the mark with methanol and mix well to give a 100 μg/mL stock solution of Dicamba. Standards should be prepared in amber bottles and stored under refrigeration.

Alternatively, the appropriate volume of solvent 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 methanol 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 should be prepared by serial dilution with methanol. It is recommended that the following solutions are prepared: 1.0 μg/mL, 0.1 μg/mL and 0.01 μg/mL for fortification purposes.

2.3.3 Preparation of Calibration Standards

No significant matrix effects, suppression or enhancement of the instrument response has been observed in the filter types tested using the procedures described in Section 3 during method development and non-matrix matched calibration standards should normally be used for quantitation using LC-MS/MS. $^{13}C_6$ Dicamba internal standard is recommended to offset any matrix related effect, if observed by additional to the final fraction.

A calibration curve should be generated to quantify dicamba. Standards over an appropriate concentration range should be prepared with a minimum of five levels using the recommended standard range 0.10 pg/µL – 80 pg/µL (5.0 pg to 4000 pg on column using a 50µL injection) in 0.1% formic acid ultra-pure water:methanol (95:5 v/v). Upper range levels are based on instrument sensitivity and may require adjustment due to detector saturation.

Dicamba internal standard concentration should be determined based on a mid-range calibration point. A 20 pg/µL concentration is recommended.

2.3.4 Standard Solution Storage and Expiration

All stock solutions should be stored in amber bottles and refrigerated (4°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 six months for dicamba 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 1).

Solvent and Reagent hazards

	Acetone	Methanol	Formic Acid
Harmful Vapor	✓	✓	✓
Highly Flammable	✓	✓	✖
Harmful by Skin Absorption	✓	✓	✓
Irritant to respiratory system and eyes	✓	✓	✓
Causes severe burns	✖	✖	✓
OES Short Term (mg/m^3)	3560	310	N./A
OES Long Term (mg/m^3)	1780	260	9

N/A not known

In all cases avoid breathing vapor. Avoid contact with eyes and skin.

3.0 ANALYTICAL PROCEDURE

A summary of the method is included in flow-chart form as shown in Appendix 4. In order to verify method performance and allow recovery corrections to be made (if appropriate), fortified control samples should be included in each sample set. At least one untreated control and two control samples fortified with known amounts of dicamba should be analyzed alongside each batch of samples to demonstrate acceptable performance of the method and allow recovery corrections to be made if desired.

3.1 Sample Preparation

All samples should be prepared/collected using an approved method of preparation to minimize any possible cross-contamination from sample to sample. Evaluation of potential compound break-through should be determined using the specific air sample type identified in the study. Tandem configuration may be required depending on the expected residue level.

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 air sample or filter, add the appropriate amount of standard solution (10-50µL recommended) containing dicamba in methanol. Let each sample stand for at least five minutes after fortification to allow the spiking solvent to penetrate before proceeding with the extraction procedure. At least one untreated control and two fortified control samples should be analyzed with each sample set.

3.3 Extraction

1. Air Sampler: Remove all contents (PTFE ring, filter, sorbent, puf…etc.) of air sample by pushing forward from small opening to large opening with a glass/metal rod or suitable implement directly into appropriately size vessel (15 mL or 50 mL polypropylene tube). To avoid contamination from the exterior surface of the air sampler, the glass tube should not be included in the extraction.

   Filter Paper: It is recommended filter papers to be shipped and extracted in original collection tube (50 mL polypropylene tube). Filter paper samples should be located in the lower half of the tube to provide the most effective agitation.

2. Air Sampler: Extract dicamba residue from sample using 10 mL to 40 mL of acidified acetone (1% formic acid) depending on sample type.

   Filter Paper: Extract dicamba residue from sample using 40 mL of acidified methanol (1% formic acid).

3. Shake samples (air sampler and filter paper) for 30 minutes using a platform, orbital or other (GENO Grinder/FastPrep) that visibly agitates samples without maceration or causing very fine particulates from dusting. Sonicate sample for 10 minutes using a water level equal to or slightly above sample. Allow samples to settle prior to transferring aliquot. Centrifugation may be required if cloudiness or fine particulates are observed (5 minutes at 5000 rpm).

4. Transfer sample extract aliquot (1 mL to 20.0 mL dependent upon LOQ requirement and sensitivity of mass spectrometer) to a polypropylene tube and evaporate to near dryness at 50°C under a gentle stream of nitrogen or air (≤ 100 µL).

   For internal standard calibration - dd 50 µL of 0.20 ug/mL $^{13}C_6$ dicamba to each sample type including blanks, UTC, unknowns, QC, and calibrators, reconstitute sample to 0.5 mL final volume using 0.1% formic acid ultra-pure water:methanol (95:5 v/v). Vortex well, then transfer to low volume autosampler vial. If sensitivity permits, further dilution can be performed.

5. Analyze by LC-MS/MS (ESI-Negative Ionization Mode).

3.4 Time Required for Analysis

The methodology is normally performed with a batch of 15 samples per set. One skilled analyst can complete the analysis of 1 to 2 sets in 1 day (8 hour working period).

3.5 Method Stopping Points

The analytical procedure can be stopped at various points for overnight and weekends 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.

3.4 Problems and Modifications

If matrix effect is present, the use of internal standard is recommended.

Proper lab techniques should be implemented to prevent possible contamination of samples, labware and instrumentation.

4.0 FINAL DETERMINATION

4.1 Instrument Description (LC-MS/MS)

UPLC System	:	Acquity UPLC
Detector	:	Sciex API 5500QTRAP

4.2 Chromatography Conditions

Column	:	Phenomenex Kinetex Phenyl-Hexyl 2.1 x 100mm, 2.6µ
Alternate Column	:	Ace 3 C18 3.0 x 50mm, 3.0µ
Column Oven Temperature	:	50°C
Injection volume	:	10 µL to 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

Mobile Phase Composition

Time (min)	0.1% Formic Acid in Water	0.1% Formic Acid in MeOH	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

Divert to Waste: 0-3 minutes
Divert To MS: 3-5 minutes
Divert to Waste: 5-10 minutes
Under these conditions the retention time of dicamba is 3.9 minutes.

4.3 Mass Spectrometer Conditions (LC-MS/MS)

Interface	:	ESI
Ionization mode	:	Negative
Curtain gas (CUR)	:	Nitrogen set at 20 (arbitrary units)
Source temperature (TEM)	:	500 °C
Ionspray Voltage (IS)	:	-2000
Collision gas setting (CAD)	:	Nitrogen set at Medium (arbitrary units)
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

MRM Conditions		**Dicamba Primary**	**Dicamba Confirmatory**	**Dicamba IS**
Q1 *m/z*	:	219	221	225
Q3 *m/z*	:	175	177	181
Dwell time	:	50 ms	50 ms	50 ms
Resolution Q1	:	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit
Declustering potential (DP)	:	-60 V	-60 V	-60 V
Entrance potential (EP)	:	-10 V	-10 V	-10 V
Collision energy (CE)	:	-10 V	-10 V	-10 V
Collision cell exit potential (CXP)	:	-10 V	-10 V	-10 V

Representative chromatograms are shown in the Figures Section.

4.4 Confirmatory Procedures for Dicamba

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 residues may be calculated in µg/sample for each sample as follows:

a) Prepare standard solutions over a concentration range appropriate to the expected residues in the samples (for example, 30% LOQ to 20 x LOQ). An appropriate number of different concentrations within this range should be prepared (at least five levels). And combined with an internal standard to normalize data.

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to respective target ions. Quality Control standard solutions should be interspersed throughout the analysis to monitor any matrix effects.

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

d) The linear regression 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 (slope) 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 coefficient of determination, $\mathbf{r^2}$, and correlation coefficient, **r**, for the regression.

Re-arrangement for *x* gives

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

e) Calculate residues of interest in a sample, expressed as µg/sample, as follows:

$$\text{Residue (ug/sample)} = \frac{\text{Analyte found } (\mu\text{g/mL})}{\text{Sample Vol. (mL)}}$$

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

f) Determine the recovery by first subtracting the residue found in the control sample, if any, from the residue found in the recovery sample. Calculate the recovery as a percentage (%) by the equation:

$$Recovery\ (\%) = \frac{(Residue\ in\ Recovery\ Sample) - (Residue\ in\ Control)}{Amount\ Fortified} \times 100\%$$

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

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

5.2 Single Point Calibration Procedure

Dicamba residues may be calculated in µg/sample 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 at an appropriate concentration operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak areas obtained for dicamba.

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

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

d) Calculate the dicamba residues in the sample, expressed as µg/sample using a mean standard response from each of the injections bracketing the sample as follows:

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

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 volume (mL)

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

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

6.0 CONTROL AND RECOVERY SAMPLES

Control samples should be analyzed 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 analyzed with each batch of samples. Control samples from the same matrix are recommended to monitor any instrumental matrix effects present.

At least two recovery samples (control samples accurately fortified with known amounts of dicamba), including one at the method LOQ and one at the expected residue level, should also be analyzed alongside each set of samples. The fortification levels should be appropriate to the residue levels expected in the sample.

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

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

7.0 SPECIFICITY

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

7.1 Matrix

LC-MS/MS is a highly specific detection technique. Interferences arising from the matrices tested have 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 should be detergent washed and then rinsed with HPLC grade methanol, acetone or acetonitrile prior to use.

8.0 METHOD VALIDATION

8.2 Limit of Quantitation (LOQ)

The limit of quantitation 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 quantitation, the response for an analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time.

The limit of quantitation of the method has been established at 1.0 ng/air sample tube and 20 ng/filter.

8.3 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 was determined to be 0.10 pg/µL, equivalent to 5 pg on column when using a 50 µL injection volume by LC-MS/MS.

8.4 Matrix Effects

No significant matrix effects were observed in the air sample type tested. Non Matrix-matched standards should generally be used for quantitation. Internal standard may be used to compensate for matrix effect or sample-to-sample variability.

8.5 Detector Linearity

For accurate quantitation 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 was tested in the range from 5 pg to 4000 pg injected on column (equivalent to 0.10 pg/µL to 80 pg/µL standards when using a 50 µL injection volume) and was found to be linear. This is equivalent to 10% LOQ - >120% of the validated levels.

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

8.6 Final Extract Stability

Final extracts in 0.1% formic acid ultra-pure water:methanol (95:5 v/v) retained in vials and stored at a temperature of approximately 4ºC were suitable for dicamba residue analysis, for storage periods of up to 7 days.

9.0 LIMITATIONS

The method has been tested on representative sorbents including OVS XAD-2 sorbent (140/270 mg) air sampling tube (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).

10.0 CONCLUSIONS

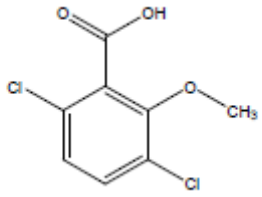
This procedure has been demonstrated to be a reliable and accurate procedure for the determination of dicamba from OVS XAD-2 sorbent, PUF sorbent and Filter Paper. Only commercially available laboratory equipment and reagents are required. The analysis of 1 to 2 sets (25+ samples) can be completed by one person in 1 day (8 working hour period). Untreated and fortified samples should be analyzed with each set of samples to demonstrate absence of any interference and adequate recovery, if possible. The limit of quantitation of the method is 1.0 ng/air sampling tube and 20 ng/filter.

This method satisfies US EPA 850.6100 guidelines.

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



APPENDIX 1 Apparatus

Recommended Suppliers

Equipment	Description	Supplier
General lab glassware	General lab glassware	ThermoFisher
General lab plastic-ware	General lab plastic-ware	ThermoFisher
OVS XAD-2 Tube	SKC Cat No. 226-30-16	SKC Inc.
PUF Sampler	SKC Inc. Cat No. 226-92	SKC Inc.
Whatman Filter Paper	Grade 3, 15 cm Cat No. 09-820D	ThermoFisher
LC Column	Kinetex Phenyl-Hexyl 2.1 x100 mm 2.6 µm (00D-4495-AN)	Phenomenex
LC Column	Ace 3 C18 3.0 x 50 mm, 3.0 µm (111-0503)	ACE

APPENDIX 2 Reagents

Recommended Suppliers

Reagent	Description	Supplier
Solvents	HPLC grade	www.thermoscientific.com
Dicamba analytical standards	GLP certified	Syngenta Crop Protection, LLC

Preparation of Reagents

a) Acidified Acetone (1% Formic Acid): 10 mL Reagent Grade Formic Acid to 990 mL ACS Grade acetone.

b) Acidified Methanol (1% Formic Acid): 10 mL Reagent Grade Formic Acid to 990 mL ACS Grade methanol.

c) Ultra-pure water: acetonitrile (95:5 v/v): 950 mL of ultra-pure water added to 50 mL ACS Grade Methanol.

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

Note: Dicamba has 2 chlorine atoms present both with isotopic forms ^{35}Cl and ^{37}Cl with a mass spectrum for ions at M (^{35}Cl x2), 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 Dicamba standard (0.1 to 1.0 µg/mL) in methanol (see section 4) 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 for Dicamba in negative ionization mode.

Using the Analyst software quantitative optimisation routine, tune the instrument for Dicamba, 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 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 ionization mode, the deprotonated molecular ion generated in the ion source (*m/z* 219 and ^{37}C Isotope 221) is selected and subjected to further fragmentation by collisional activation. The two most sensitive daughter ions (*m/z* 175 and ^{37}Cl isotope *m/z* 177) are then selected and used for quantitative analysis.

APPENDIX 4 Method Flow Chart

Extract contents of sample in a poly tube
↓
Add acidified acetone (1% formic acid) for air sampler
↓
Add acidified methanol (1% formic acid) for filter paper
↓
Shake sample for 30 minutes
↓
Sonicate sample for 10 minutes
↓
Transfer aliquot for concentration
↓
Evaporate to near dryness
↓
Add Internal Standard
↓
Reconstitute to final volume using ultra-pure water:methanol (95:5 v/v) and vortex
↓
Analyze by LC-MS/MS