

**Dicamba**

**Dicamba - Independent Laboratory Validation of Analytical Method GRM022.02A  
for the Determination of Residues of Dicamba (SAN837) in Water**

**Method Validation**

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

**VOLUME 1 OF 1 OF STUDY**

**PAGE 1 OF 47**

## **1.0 EXECUTIVE SUMMARY**

### **1.1 Study Design**

This study validates the method GRM022.02A to quantitatively determine residues of dicamba (SAN837) in water. The analytes are extracted by means of solid phase extraction, derivatised and determined with gas chromatography (GC) coupled to mass spectrometric detection (MS). Primary and two confirmatory ions were recorded and evaluated for all samples and all analytes.

## **2.0 INTRODUCTION**

This method was based on the method (GRM022.02A) provided by the sponsor and was kept as close to the original method as possible. A GC-MS device was used, with similar column material and settings.

Analysis was performed according to the Syngenta method GRM022.02A. In summary, water samples were prepared by solid phase extraction followed by derivatization and finally measured by GC-MS. Primary and two confirmatory ions were recorded and evaluated for all samples. The limit of quantification of the method was 0.05 µg/L.

### 3.0 MATERIALS AND METHODS

#### 3.1 Test Item

Code Number	SAN837 (Dicamba)
Chemical name (IUPAC)	3,6-dichloro-o-anisic acid
Molecular formula	C <sub>8</sub> H <sub>6</sub> Cl <sub>2</sub> O <sub>3</sub>
Molecular mass	221.0

The test item used for this validation study was the following:

Test Item	Batch	Purity (%)	Valid until:	Storage Conditions
SAN837 (Dicamba)	AMS 163/3	99.9 w/w	End of January 2018	< 30°C

The certificate of analysis has been provided by the sponsor. The remaining test item will be stored at < 30°C until expiry at which point it will be discarded. The structure is shown in Figure 1.

#### 3.2 Test System

The method was validated for drinking water. Drinking water was taken from the institute's kitchen tap. The water originates from a spring of the water board Eslohe/Schmallenberg, in the surrounding of the institute. Details of the water characterisation are given in Table 1.

#### 3.3 Preparation of Analytical Standard Solutions

0.858 g/L stock solution of dicamba (SAN837) was prepared in acetone. Further serial dilutions in acetonitrile were made to prepare final concentrations of 5.0 mg/L and 125.0 µg/L. Using these solutions, calibration standards in acetonitrile in the range from 0.625 µg/L to 50.0 µg/L (7 levels) were prepared.

#### 3.4 Fortification Levels

Fortification samples were prepared at 0.05 µg/L (LOQ) and at 0.50 µg/L.

For each fortification level, five recovery samples were prepared. In addition, two control samples were prepared for each sample batch. Reagent blanks do not apply. The fortification levels are summarized in Table 2.

The fortified samples at 0.05 µg/L were prepared by taking 50 µL from the calibration solution at 50 µg/L and adding them to 50 mL tapwater containing 200 µL concentrated hydrochloric acid.

The fortified samples at 0.50 µg/L were prepared by taking 50 µL from the calibration solution at 500 µg/L and adding them to 50 mL tapwater containing 200 µL concentrated hydrochloric acid.

### 3.5 Sample Analysis

The untreated controls and fortified samples were analysed according to the procedures specified in analytical method GRM022.02A. The GC-MS conditions and method details are summarized in Appendix 1.

Calibration standard solutions containing dicamba (SAN837) at concentrations ranging from 0.625 µg/L to 50.0 µg/L were injected along with the samples. The detector responses (peak areas) for GC-MS was plotted against standard concentrations injected to generate calibration curves using analyst software. The lowest concentration injected was at 50% of the LOQ of the method. The highest concentration injected was equivalent to 40×LOQ.

### 3.6 Modifications to the method and potential problems

Analytical Method GRM022.02A was followed as written with modifications to the method as follows:

- GC system: Varian 450-GC
- Mass spectrometer: Varian 320-MS
- Software: Varian MS Workstation Ver.6.9.3
- Column: Restex Rxi-5Sil MS (20 m x 0.18 mm ID; 0.18 µm film)
- Carrier gas and head pressure: Helium at 1.0 mL min<sup>-1</sup> constant flow
- Injection volume: 1 µL
- Injector temperature: 275°C
- Injection mode: Splitless
- Inlet: Varian Deactivated Double Gooseneck Splitless 4 mm
- Transferline temperature: 280°C
- Temperature programme: 100°C (hold for 1 minute), 20°C min<sup>-1</sup> to 300°C (hold for 1 minute)
- Mode and reagent gas: Negative CI with methane

All other parameters were followed as written. Only solvents and chemicals with the required purity and quality were used.

#### **4.0 RESULTS AND DISCUSSION**

The limit of quantification (LOQ) of the analytical method was confirmed at 0.05 µg/L for dicamba (SAN837) in water.

#### **5.0 CONCLUSIONS**

Analytical method GRM022.02A has been successfully validated for determination of residues of dicamba (SAN837) in water by this laboratory according EC guidance documents SANCO/3029/99 Rev. 4 [3] and SANCO/825/00 Rev. 8.1 [4].

Two control samples and fortified samples in quintuplet at the LOQ (0.05 µg/L) and in quintuplet at 10 times the LOQ (0.5 µg/L) were analysed in the study.

No interferences (<50% LOQ) were found at or near the retention times of dicamba (SAN837) in untreated controls for the primary ion and both confirmatory ions recorded.

**TABLE 1: Drinking Water Characteristics**

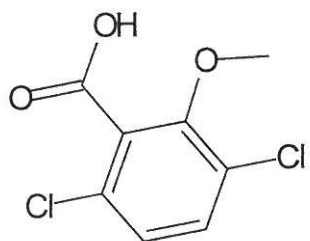
	<b>TOC (mg/L)</b>	<b>Hardness (°dH)</b>	<b>pH</b>	<b>silt content (mg/L)</b>
according to internal SOP	V3-034/02	V7-520/05	G3-022/02	V7-402/01
Drinking water	0.5519	6.34	6.89	n/a

°dH: degrees German hardness

**TABLE 2: Fortification Levels**

<b>Matrix</b>	<b>Fortification Level (µg/L)</b>	<b>Minimum Number of Replicates</b>
Drinking water	Control	2
	0.05	5
	0.50	5

**FIGURE 1: Structure of Dicamba (SAN837)**



## APPENDICES SECTION



## APPENDIX 1 Analytical Method Description

### 1. PREPARATION AND USE OF THE STANDARD SOLUTIONS

The stock solutions were stored in a refrigerator when not in use. The stock, fortification and calibration solutions used for this study were stored in a refrigerator or a freezer when not in use (according to the Syngenta method GRM022.02A). The solutions were stable during the performance of the study. The following tables show the preparation of stock solutions, calibration solutions and fortification solutions of the test item Dicamba (SAN837).

#### 1.1. Preparation of Stock solutions and calibration standards

##### **solution prepared in acetone**

<b>Stock solution I</b>	Weight (mg)	Volume (mL)	Purity (%)	Conc. (g/L)
Dicamba	17.180	20	99.9	<b>0.858</b>

##### **solution prepared in acetonitrile**

<b>Stock solution II</b>	Stock I (µL)	Volume (mL)	Conc. (mg/L)
Dicamba	117	20	5.0

##### **solution prepared in acetonitrile**

<b>Stock solution III</b>	Stock II (µL)	Volume (mL)	Conc. (µg/L)
Dicamba	500	20	125.0

##### **solution prepared in acetonitrile**

##### **Calibration standards**

	Stock III (µL)	Stock II (µL)	Volume (mL)	Conc. (µg/L)
Kal 1	50	-	10	0.625
Kal 2	100	-	10	1.250
Kal 3	200	-	10	2.500
Kal 4	400	-	10	5.000
Kal 5	800	-	10	10.000
Kal 6	-	40	10	20.000
Kal 7	-	100	10	50.000

The concentrations of the stock solutions were different to the concentrations of the stock solutions mentioned in Syngenta method GRM022.02A.

## APPENDIX 1 Analytical Method Description

### 1.2. Preparation of Fortification samples

#### solution prepared in acetone

Stock solution I	Weight (mg)	Volume (mL)	Purity (%)	Conc. (g/L)
Dicamba	17.180	20	99.9	<b>0.858</b>

#### solution prepared in acetone

Stock solution II	Stock I (µL)	Volume (mL)	Conc. (mg/L)
Dicamba	117	20	5.0

#### solution prepared in acetone

	Stock II (µL)	Volume (mL)	Conx. (µg/L)
Spike solution 10x-LOQ	1000	10	500.0
Spike solution LOQ	100	10	50.0

#### solution prepared in drinking water

Fortification	Spike sol. 10x-LOQ (µL)	Spike sol. LOQ (µL)	Volume (mL)	Conc. (µg/L)
10x-LOQ	50	-	50	0.5
LOQ	-	50	50	0.05

## **APPENDIX 1      Analytical Method Description**

### **2. ANALYTICAL SUPPLIES AND APPARATUS**

According to availability and laboratory equipment, analytical supplies from other suppliers and apparatus of different design may be used.

#### **2.1. Apparatus and material**

- GC-MS system: see APPENDIX 1, chapter 4.1
- Analytical balance (Mettler)
- Cabinet dryer (up to 60°C)
- Fridge for reference standards
- Fridge for samples
- Graduated flask, volumens 10 mL, 20 mL
- Graduated cylinder, 50 mL
- Pipettes, 10 µL, 25 µL, 50 µL, 100 µL, 1000 µL, 5000 µL
- 15 mL polypropylene (PP) centrifuge tubes with screw caps
- 50 mL polypropylene (PP) centrifuge tubes with screw caps
- Extraction cartridges IST ISOLUTE 100 mg/10 mL

#### **2.2. Reagents**

- Acetonitrile:                      Chem Solute, LC-MS, 99.95 %
- Hydrochloric acid:              > 37.00 %, Merck
- Acetone:                            > 99.80 %, J.T: Baker
- N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA):  
   > 97.00 %, Sigma-Aldrich
- Drinking Water:                  Tap water, from the institute's canteen at Fraunhofer IME. The water originates from a spring of the surrounding of the institute as part of the local water board of Schmallingenberg/Eslohe, Germany.

### **3. ANALYTICAL PROCEDURE**

Analysis was performed according to the Syngenta method GRM022.02A. The method in short: Water samples were prepared by solid phase extraction, derivatised and measured by GC-MS. Primary and confirmatory mass transitions were recorded and evaluated for all samples. The limit of quantification of the method was 0.05 µg/L.

## APPENDIX 1 Analytical Method Description

### 4. PARAMETERS FOR CHROMATOGRAPHIC ANALYSIS

#### 4.1. Operating conditions

The following GC-MS parameters were used during the study. They may be adapted if alternative equipment is used.

- GC-System: Varian 450-GC
- Detector: Varian 320-MS
- Software: Varian MS Workstation Ver.6.9.3
- Column: Restex Rxi-5Sil MS (20m x 0.18mm ID; 0.18µm film)
- Temperature programme: 100°C (hold for 1 minute), 20°C min<sup>-1</sup> to 300°C (hold for 1 minute)
- Retention time Dicamba: 6.97 minutes
- Injection volume: 1 µL
- Injector temperature: 275°C
- Flow: Helium at 1.0 mL/min constant flow
- Inlet: Varian Deactivated Double Gooseneck Splitless 4mm
- Injection mode: Splitless
- Ionisation mode: Negative CI
- Scan Type: SIM (Selective Ion Monitoring)
- Calibration range: 0.625 µg/L – 50.0 µg/L

**APPENDIX 1      Analytical Method Description**

<b>Analyte</b>	<b>Primary Ion (<i>m/z</i>)</b>	<b>Confirmatory ion 1 (<i>m/z</i>)</b>	<b>Confirmatory Ion 2 (<i>m/z</i>)</b>
Dicamba (SAN837)	184	185	186

## Calibration

Calibration standards were injected before each series of test sample analyses. The determination coefficient  $R^2$  was found to be higher than 0.999 in all cases.

### 4.2. Result calculation

The chromatographic system was calibrated using separate calibration curves for each analyte as external standards. Linear calibration curves were calculated using the method of least squares with  $1/x$  weighting:

$$Y = A \times C + B$$

Y = detector response (as peak area) for each analyte.

A = slope of the linear least squares fit of the calibration curve

C = concentration determined from standard curve ( $\mu\text{g/L}$ )

B = Y-intercept of the linear least squares fit of the calibration curve.

The concentration determined from standard curve is:  $C = (Y-B)/A$

Due to SPE extraction a concentration factor of 25 is applied. So the concentration in the test sample is the concentration determined from standard curve divided by 25.

Procedural recovery data from fortified samples are calculated via the following equation:

$$\text{Recovery (\%)} = A / S \times 100$$

where:

A = concentration of analyte found in test sample ( $\mu\text{g/L}$ ).

S = concentration of analyte added to test sample ( $\mu\text{g/L}$ ).