Dicamba

Dicamba – Independent Laboratory Validation of Analytical Method GRM022.09A for the Determination of the Metabolite NOA414746 (DCSA) in Water

Final Report Amendment 1

TEST GUIDELINE(S): OECD ENV/JM/MONO(2007)17
EPA OCSPP 850.6100 (2012)
SANCO/3029/99 Rev. 4 (2000)
SANCO/825/00 Rev. 8.1 (2010)

2.0 INTRODUCTION

The analytical method GRM022.09A (Ref. 1) has been independently validated by Eurofins Agroscience Services Chem GmbH, Germany, for the determination of residues of NOA414746 in surface and drinking water.

Test Systems and Analytes Validated in this Study

Test System	Validated for Analyte
Surface and Drinking Water	NOA414746

This study was conducted to independently validate the analytical method GRM022.009A (Ref. 1), and specifically:

a) To establish that the method will produce recovery values which are within an acceptable range (i.e. mean recoveries between 70 % and 110 %, with a relative standard deviation within a run lower than or equal to 20 % of the LOQ level, and lower than or equal to 20 % of the 10xLOQ level, respectively), for each fortification level and overall for two fragment ions.

b) To establish that the limit of quantification (LOQ) of the analytical method is 0.05 µg/L each for NOA414746 in water.

c) To establish that residues in control samples and method blank are not present at levels above 30 % of the LOQ.

d) To investigate the relationship between instrument response and concentration over concentration ranges typical of those for which the method will be used.

e) To assess the suppression or enhancement of instrument response in the presence of matrix.

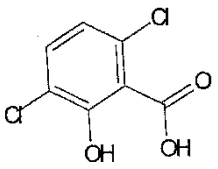
f) To assess and report the limit of detection (LOD).

This study was conducted in accordance with European guidelines SANCO/825/00 Rev. 8.1, SANCO/3029/99 Rev.4, US EPA guideline OCSPPS 850.6100 and OECD guidance document ENV/JM/MONO (2007) 17.

3.0 MATERIALS AND METHODS

3.1 Test Item and Reference Item

Common Name:	**NOA414746**
Compound Code Number:	CA3830
Chemical Name (IUPAC):	3,6-dichloro-2-hydroxy-benzoic acid
CAS-Registry-No.:	3401-80-7
Molecular Formula:	$C_7H_4Cl_2O_3$
Molecular Mass:	207.0 g/mol
Structural Formula:	



The reference item (analytical grade) and the certificate were supplied by the sponsor.

Name:	**NOA414746**
Lot Number:	BPS 988/1 (151642)
Purity:	99 %
Date of Certification:	29-Jun-2016
Storage Conditions (EAS Chem):	≤ -18 °C (in dark conditions)
Date of Expiry:	31-Jan-2020

3.2 Test Systems

Specimen Origin

Drinking water was taken from the local tap water supply. Characterisation data are shown in Table 1; and an analysis data sheet is archived within the analytical raw data file.

Untreated surface water was supplied by the test facility. Characterisation data are shown in Table 2. The source of surface water is given in the analysis data sheet which is archived within the analytical raw data file.

Specimen Preparation

Both water materials were shaken thoroughly to ensure sample homogeneity prior to analysis. To the surface water, an amount of 50 mg/L silt were added beforehand. Particulates were allowed to settle before taking aliquots for analysis.

3.3 Analytical Procedures

3.3.1 Sample Analysis

The samples were analysed for residues of NOA414746 using the analytical method, GRM022.09A (Ref. 1) detected by liquid chromatography with MS/MS detection.

The limit of quantification (LOQ) was 0.05 µg/L, with a limit of detection (LOD) estimated to be 0.015 µg/L (30 % of the LOQ). Full details of the methodology and the chromatography conditions used in this study are in Section 3.4.

3.3.2 Preparation and Stability of Analytical Standard Solutions

Analytical grade reference items of NOA414746 were used for preparing the fortification and external standard solutions.

The solvent standard solutions were prepared in acetonitrile/ultra-pure water (20/80, v/v). The matrix matched standard solutions were prepared using control matrix extracts. All standards were prepared over an appropriate range (0.075 ng/mL to 5.0 ng/mL).

Full details of the standard solutions are in Section 3.4.6.

3.3.3 Fortification

Recovery of NOA414746 was assessed by fortifying ten aliquots of the untreated matrix with the appropriate fortification solution (in acetonitrile) as detailed in the table below.

Sample	Sample Volume [mL]	Fortification Solution	Fortification Solution Volume [µL]	Fortification Level [µg/L]
Drinking and surface water	5.0	Z1 (0.1 µg/mL)	25	0.50
		Z2 (0.01 µg/mL)	25	0.05

3.3.4 Calibration Information

Analytical grade of NOA414746 was used for preparing the external solvent and matrix matched standard solutions. Concentrations in the final extracts were calculated using the average response factor based on at least seven standard solutions injected in a sample set (evenly distributed over the analytical sequence), as well as the peak areas of the samples.

3.3.5 Example Calculations

Full details of calculations for fortification and quantification are in Sections 3.4.7.

3.3.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD 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 LOQ of the method is defined as the lowest analyte concentration in a sample at which the methodology has been successfully validated. A LOQ of 0.05 µg/L was confirmed for NOA414746 in surface and drinking water, with a limit of detection (LOD) estimated at 30 % of the LOQ to be 0.015 µg/L.

3.3.7 Detector Linearity

The linearity of the detector response was confirmed for water by injecting at least seven solvent standard solutions covering the working range of 0.075 ng/mL to 5.0 ng/mL. The lower margin of the linearity was below 30 % of the LOQ, and the upper margin was at least 20 % above the 10x LOQ concentration in the final extracts.

3.3.8 Matrix Effects

Each sample set included appropriate matrix matched standards, prepared in the presence of control matrix. For each matrix, the response of the analyte obtained from the matrix matched standard was compared against the response obtained from a solvent-based standard solution in acetonitrile/water (20/80, v/v) to allow calculation of any matrix effect (either suppression or enhancement of response).

Matrix effects (enhancement or suppression) of NOA414746 were assessed for each matrix type; these are deemed to be insignificant if ≤ 20 %.

3.4 Detailed Analytical Procedures

3.4.1 Apparatus and Equipment Used

- Ultrasonic bath Bandelin Sonorex RK 510 H, BANDELIN electronic GmbH & Co. KG, D-12207 Berlin, Germany, Art.No. 321
- Dilutor Hamilton Model ML530b, Hamilton Deutschland GmbH, D-82152 Martinsried, Germany, Art.No. ML530220
- Polypropylene centrifuge tubes with caps, 15 mL (e.g. Sarstedt, Art. No. 62.458.004)
- Vortex (e.g. Reax Top, HEIDOLPH Instruments, Schwabach, Germany)
- Volumetric pipettes (e.g. 1, 2 and 5 mL)
- Volumetric flasks (e.g. 10, 20 and 50 mL)
- Adjustable pipettes (reference 10-100 µL and reference 100-1000 µL, Eppendorf, Germany; Handy Step with 12.5 mL tip, Brand, Germany)
- Phenomenex Strata -X (100 mg, 3mL; Art. No. 8B-S100-EBJ)
- Common laboratory glassware
- LC-MS/MS detection system (see detection section)
- HPLC Autosampler vials, 1.8 mL

All glassware was rinsed with water (to remove detergents) and dried before use.

3.4.2 Reagents

- Distilled water, e.g. Braun Melsungen, Aqua ad iniectabilia, No. 536108
- Acetic acid, 100.0%, Merck, Art. No. 1000632511
- Hydrochloric acid, 37.0%, VWR, Art. No. 20252335
- Acetonitrile, HiPerSolv CHROMANORM for HPLC, VWR, > 99.9 %, Art.No. 20060.320
- Formic acid, 98-100 %, EMSURE ACS, Reag. Ph. Eur., analytical reagent, Merck, Art. No. 1002642500

3.4.3 Sample Extraction for Surface and Drinking Water

5.0 mL was measured into 15 mL polypropylene centrifuge tubes. Fortification was carried out at this step by adding an appropriate volume (25 µL) of fortification solution in acetonitrile. After the addition of 50 µL hydrochloric acid, the closed tubes were mixed well by vortex mixing.

3.4.4 Solid Phase Extraction

A Phenomenex Strata-X cartridge (size 100 mg, 3 mL) was taken for each water sample to be analysed, and placed on a suitable vacuum manifold. 3 mL of acetonitrile and 3 mL of ultra-pure water were added on to the cartridge and drawn through under vacuum to the level of the top frit at a rate of approximately 1 mL/min or percolate through under gravity. The cartridges were not allowed to run dry.

A sample (5 mL) was transferred to the cartridge and allowed to percolate through under gravity or low vacuum. The column eluate was discarded, and the polypropylene tube rinsed with 2 mL of ultra-pure water. The rinsing solution was added to the cartridge and the eluate was discarded again. The cartridge was dried under high vacuum for 10 min.

10 mL glass test tubes were placed under the cartridge, and the analyte was eluted twice with 3 mL of 1 % acetic acid in acetonitrile. The eluate was allowed to flow through under low vacuum or gravity.

The collected eluates were evaporated to dryness under a stream of nitrogen in a heating block with the temperature set to 40 °C.

1.0 mL of acetonitrile/ultra-pure water (20/80, v/v) was added to the sample and vortex mixed. Subsequently, samples were transferred to auto sampler vials for final determination by LC-MS/MS.

3.4.5 Chromatography and Analysis

The final extracts were analysed for NOA414746 using an HPLC (Agilent Technologies) coupled to a PE-Sciex API 5500 tandem mass spectrometer with electrospray nebuliser. Typical HPLC and mass spectral operating conditions are summarized below.

LC-MS/MS Conditions:

HPLC-System:	Agilent 1200 Series			
Tandem Mass Spectrometer	API 5500 Mass Spectrometer			
Column:	ACE Ultracore Super C18, 50 x 2.1 mm, 2.5 µm particle size			
Column Temperature:	30 °C			
Injection Volume:	30 µL			
Mobile Phase Conditions:	A: Acetonitrile + 0.1% formic acid B: Water + 0.1 % formic acid			
	Time (min)	%A	%B	Flow (µL/min)
	0.0	5	95	400
	4.0	70	30	400
	4.1	5	95	400
	6.0	5	95	400
Retention Times (approx.):	NOA414746 ~ 3.8 min			
Valco Valve	To waste: 0 – 2.5 min; to MS: 2.5 – 4.5 min			

Mass Spectrometer Conditions:

MS System:	API 5500 Mass spectrometer, Applied Biosystems, Applera Deutschland GmbH				
Ionisation type:	Electrospray (ESI, TurboIon Spray)				
Polarity:	Negative ion mode				
Scan type:	MS/MS, Multiple Reaction Monitoring (MRM)				
Analyte Monitored	Ions Monitored	Declustering Potential (DP)	Collision Energy (CE)	Collision Cell Exit Potential (CXP)	Dwell Time
NOA414746	205 → 125*	-60 V	-26 eV	-20 V	0.10 s
	205 → 161**	-60 V	-16 eV	-20 V	0.10 s

* proposed for quantification, ** proposed for confirmation

3.4.6 Standard preparation

Preparation of stock solution No. 11808: 200 µg NOA414746/mL

10.10 mg of NOA414746 analytical reference standard (purity = 99 %, see section 3.1) was dissolved in acetonitrile and diluted to 50 mL in a volumetric, clear glass flask. The stock solution was stored at 1 °C to 10 °C in the dark and was allowed to equilibrate to room temperature prior to use.

Preparation of solvent-based standard solutions

Solvent working and standard solutions were prepared in clear glass vials in acetonitrile/ultra-pure water (20/80, v/v) using a dilutor, adjustable pipettes and volumetric pipettes as follows. All solutions were stored in clear glass vials at 1 °C to 10 °C in the dark. All solutions were allowed to equilibrate to room temperature prior to use.

Solution used	Volume used [mL]	Dilution volume [mL]	Concentration obtained [ng/mL]	Working / Standard Solution
Stock solution no. 11808	0.050	5.0	2000	DL1
DL1	0.25	10	50	DL2
DL1	0.125	10	25	DL3
DL2	1.0	5.0	10	DL4
DL2	0.10	1.0	5.0	L1
DL3	0.10	1.0	2.5	L2
DL4	0.10	1.0	1.0	L3
L1	0.10	1.0	0.50	L4
L2	0.10	1.0	0.25	L5
L3	0.125	1.0	0.125	L6
L4	0.15	1.0	0.075	L7

Preparation of matrix-matched standard solutions

Matrix-matched standard solutions were freshly prepared immediately prior to use in matrix extracts of untreated (control) samples using adjustable pipettes as follows.

Matrix	Working / Calibration Solution used*	Volume used [µL]	Dilution (End) Volume [µL]	Concentration obtained [ng/mL]	Matrix-matched Calibration Solution
Drinking Water	DL2	25	250	5.0	M11
	DL3	25	250	2.5	M12
	DL4	25	250	1.0	M13
	L1	25	250	0.50	M14
	L2	25	250	0.25	M15
	L3	30	400	0.075	M16
Surface Water	DL2	25	250	5.0	M21
	DL3	25	250	2.5	M22
	DL4	25	250	1.0	M23
	L1	25	250	0.50	M24
	L2	25	250	0.25	M25
	L3	30	400	0.075	M26

* in acetonitrile/water (20/80, v/v)

Preparation of fortification solutions

Fortification solutions were prepared in clear glass vials in acetonitrile using a volumetric pipette and volumetric flasks. All solutions were stored at 1 °C to 10 °C in the dark. All solutions were allowed to equilibrate to room temperature prior to use.

Solution used	Volume used [mL]	Dilution volume [mL]	Concentration obtained [µg/mL]	Working / Fortification Solution
Stock solution no. 11808 *	0.050	2.5	4.0	DZ1
DZ1	0.060	2.4	0.10	Z1
Z1	0.25	2.5	0.01	Z2

* in acetonitrile

3.4.7 Calculations

The evaluation of the results was based on the average response factor which was calculated from the calibration standards. Two ion mass transitions were evaluated for each analyte.

The residues (R) in µg/L were calculated according to the following equation:

$$R = \frac{A_A \times V_{End} \times DF}{AvF \times V}$$

where:

R: Residue of the analyte in µg/L

A_A: Peak area of the analyte in final solution in counts

AvF: Average response factor: average of peak area / standard conc. (ng/mL) calculated from the calibration standards in each sequence.

The average response factor was calculated as follows:

$$AvF = \frac{(A_{St1} / C_{St1} + A_{St2} / C_{St2} + ... A_{StN} / C_{StN})}{N}$$

C_{St}: Concentration of analyte in external standard solution, in ng/mL

A_{St}: Peak area of analyte in external standard solution, in counts

N: Number of external standard solutions

V: Volume of sample = 5.0 mL

V_{End}: Final volume = 1.0 mL

DF: Dilution factor (for no dilution = 1)

Percent recovery from fortified specimen was calculated using the following expressions:

$$\text{Recovery (\%)} = \frac{(R_{\text{fortified}})}{F} \times 100$$

where:

$R_{fortified}$: Residues of fortified specimen, in µg/L

F: Fortification, in µg/L

3.4.8 Calculation example

For a 0.05 µg/L fortified drinking water specimen (205/125, quantification), the concentration of NOA414746 found was calculated as follows:

$$R = \frac{8910 \times 1.0 \times 1}{4.4575 \times 10^4 \times 5.0} = 0.0400 \text{ µg/L (rounded)}$$

The percent recovery found was calculated as follows:

$$\text{Recovery (\%)} = \frac{0.0400}{0.0500} \times 100\% = \underline{\underline{80\ \%}}$$

4.0 RESULTS AND DISCUSSION

The applicability of the analytical method GRM022.09A (Ref. 1) for the analysis of the residues of NOA414746 was tested as follows:

Test Systems and Analytes Validated in this Study

Test systems	Validated for Analyte
Drinking and surface water	NOA414746

4.1 Limit of Quantification / Limit of Detection

The limit of quantification (LOQ) was 0.05 µg/L with a limit of detection (LOD) estimated to be 0.015 µg/L (30 % of the LOQ).

4.2 Control samples

No residues of NOA414746 were detected in any of the control specimens indicating that no interferences were present at the retention time of the analytes in the test systems. This is in accordance with the level specified in SANCO Guideline 825/00 rev. 8.1 (16/11/2010), which demands a blank level of less than 30% of the LOQ.

TABLE 1 Characterisation Data of Drinking Water used for Validation

Matrix	Source	pH	Turbidity (NTU *)	Total Organic Carbon (TOC) (mg/L)	Total Hardness as $CaCO_3$ (mg/L)
Drinking water	Local tap water supply: Groundwater-works Süderelbmarsch, Hamburg, Germany	8.1	0.23	1.1	119.3

* NTU = Nephelometric Turbidity Units

A corresponding analysis data sheet with mean values for 2016 is archived with the analytical raw data.

TABLE 2 Characterisation Data of Surface Water used for Validation

Matrix	Source	pH	Silt content * (mg/L)	Dissolved Organic Carbon (DOC) (mg/L)	Total Hardness as $CaCO_3$ (mg/L)
Surface water	Surface water (raw water), River Alster, Hamburg, Germany	7.8	50	5.9	185.1

* added manually to surface water matrix

The corresponding analysis data sheet is archived with the analytical raw data.

APPENDIX 1 Flowchart of the Method