syngenta

Dicamba

Dicamba - Residue Method GRM022.09A for the Determination of the Metabolite NOA414746 (DCSA) in Water

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)

1.0 INTRODUCTION

1.1 Scope of the Method

Analytical method GRM022.09A is suitable for the determination of NOA414746 (DCSA) (Figure 1) in water. The limit of quantification (LOQ) of the method has been established at 0.05 µg/L (or 0.05 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 Stereospecificity

NOA414746 is not a chiral molecule.

1.3 Method Summary

A 5 mL aliquot of the water sample is acidified with 50 µL concentrated hydrochloric acid. Aliquots are cleaned-up and concentrated by a solid phase extraction (SPE) procedure using reversed phase (Phenomenex Strata-X) cartridges. Samples are evaporated to dryness and redissolved in acetonitrile/ultra-pure water (20/80 v/v). Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

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 gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

2.3.1 Stock Solutions

Prepare individual 200 µg/mL stock solutions for NOA414746 by one of the following methods.

Note: the amount weighed out must be corrected for the purity of the analytical standard as indicated on the certificate of analysis and also any salt content, where the analytical standard is received as a salt e.g. Na^+, Cl^- etc.

Weigh out accurately, using a five figure balance, sufficient NOA414746 analytical standard into a "Class A" volumetric flask (50 mL size). Dilute to the mark with acetonitrile to give a 200 µg/mL stock solution of NOA414746.

Alternatively, the appropriate volume of acetonitrile to add to a known amount of standard material may be determined using the equation below. The standard concentration is corrected for its chemical purity and any salt content where the analytical standard is received as a salt e.g. Na^+, Cl^- etc.

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

P	=	Standard purity (including correction for salt content where the analytical standard is received as a salt e.g. Na^+, Cl^- etc.) 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 NOA414746 should be prepared by serial dilution in acetonitrile from the 200 µg/mL stock solutions previously prepared in acetonitrile. It is recommended that the following fortification solutions are prepared: 10 µg/mL, 1 µg/mL, 0.1 µg/mL and 0.01 µg/mL.

2.3.3 Preparation of Calibration Standards for LC-MS/MS

Significant matrix effects (suppression) of the instrument response for NOA414746 was observed in seawater using the procedures described in Section 3 during the method validation. It is recommended that matrix-matched calibration standards are used for quantification.

NOA414746 standards in acetonitrile at concentrations lower than 0.01 µg/mL required for the preparation of calibration standards are freshly prepared as necessary by serial dilution in acetonitrile from e.g. a 10 µg/mL standard solution in acetonitrile.

To prepare e.g. a 0.5 µg/L matrix-matched calibration standard, add 50 µL of a 0.01 µg/mL NOA414746 standard to 150 µL of acetonitrile and 800 µL of ultra-pure water in a vial containing evaporated control sample extract from stage 3.3 (f) and vortex mix.

A calibration curve may also be generated to quantify NOA414746 residues. Standards over an appropriate concentration range, such as 0.075 µg/L to 5 µg/L, should be prepared as described above.

2.3.4 Standard Solution Storage and Expiration

All stock solutions should be stored in a refrigerator or freezer 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 75 days for NOA414746 in acetonitrile is recommended (Reference 1) 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).

Solvent and Reagent hazards

	Acetonitrile	Hydrochloric acid	Acetic acid	Formic acid (98%)
Harmful Vapour	✓	✓	✓	✓
Highly Flammable	✓	×	×	×
Harmful by Skin Absorption	✓	✓	✓	✓
Irritant to respiratory system and eyes	✓	✓	✓	✓
Causes severe burns	×	✓	✓	✓
OES Short Term (mg/m^3)	105	N/A	37	N/A
OES Long Term (mg/m^3)	70	N/A	25	9

N/A – not available

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. Avoid contact with eyes and skin.

3.0 ANALYTICAL PROCEDURE

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

3.1 Sample Preparation

a) If water samples are received deep frozen they should be allowed to defrost completely at room temperature. Defrosted samples should be shaken thoroughly to ensure sample homogeneity prior to analysis.

3.2 Sample Fortification

To each pre-measured control water sample, add the appropriate amount of standard solution containing NOA414746 in acetonitrile. Mix the sample thoroughly by shaking. At least one untreated control and two fortified control samples should be analysed with each sample set.

a) Transfer 5 mL of the water sample to be analysed into a polypropylene centrifuge tube (15 mL size). Sample fortification, if required, is to be carried out at this time.

b) Acidify with 50 µL concentrated hydrochloric acid and vortex mix.

3.3 Solid Phase Extraction Procedure

a) Place a Phenomenex Strata -X solid phase extraction cartridge (100 mg, 3 mL size) on a suitable vacuum manifold (e.g. IST Vacmaster) for each water sample to be analysed. Add acetonitrile (3 mL) and allow to percolate through under gravity or draw through under vacuum at a rate of approximately 1 mL/min, discarding the eluate. Add ultra-pure water (3 mL) to the top of the cartridge and allow to percolate through under gravity or draw through under vacuum at the same rate, again discarding the eluate.

b) Transfer the sample aliquot from section 3.2 (b) onto the cartridge and allow to percolate through under gravity or low vacuum (approx. 200 mbar). Discard the eluate.

c) Rinse the empty tubes with ultra-pure water (2 mL), adding the rinse to the top of the cartridge and draw through under vacuum, discarding the eluate.

d) Dry the cartridge under high vacuum (≤500 mbar) for 10 minutes.

Note: Where achievable vacuums are less than specified or apparatus does not allow sufficient air flow through the cartridge, longer drying times may be required.

e) Place suitable collection tubes (e.g. glass vials or 15 mL polypropylene centrifuge tubes) under each port as required. Add 1% acetic acid in acetonitrile (v/v) (2 x 3

mL) to the top of the cartridge and allow to flow through under gravity or low vacuum (approx. 200 mbar) collecting the cartridge eluate.

f) Evaporate the samples to dryness under a stream of clean, dry air or nitrogen in a heating block with the temperature set to 40 ^{o}C (approximate time 1.5 hours for samples in glass vials).

g) Add acetonitrile/ultra-pure water (20/80 v/v, 1 mL) and vortex mix. Transfer the sample to an autosampler vial ready for final determination by LC-MS/MS. The final sample concentration is 5 mL/mL or 0.005 L/mL.

3.4 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, or preferably LC-MS 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 (> 0.5 µg/L) and samples with expected residues greater than 0.5 µg/L should be diluted so that the final analyte concentration does not exceed 5 ng/mL. It may also be useful to include blank injections of acetonitrile/ultra-pure water (10/90 v/v) after high level samples to clear any observed carry-over greater than 10% of the LOQ.

3.5 Time Required for Analysis

The methodology is normally performed with a batch of 20 samples. One person can complete the analysis of 20 samples in 1 day (8 hour working period).

3.6 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.

4.0 FINAL DETERMINATION

The method has been developed for use on an AB Sciex Triple Quad 5500 LC-MS/MS system. 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

Pump	:	Agilent 1200 series
Degasser	:	Agilent 1200 series
Column Oven	:	Agilent 1200 series
Detector	:	AB Sciex 5500 triple quadrupole mass spectrometer with Analyst™ software version 1.6.2
Autosampler	:	Agilent 1200 series
Gas Supply	:	Peak Scientific AB-3G generator

4.2 Chromatography Conditions for NOA414746

Column	:	Ace Ultracore Super C18 50 mm x 2.1 mm, 2.5 µm
Column Oven Temperature	:	30 °C
Injection volume	:	30 µL
Stop Time	:	6.0 min
Injection protocol	:	Analyse calibration standard after 3 to 4 sample injections
Mobile phase solvent 1	:	0.1% (v/v) formic acid in ultra-pure water
Mobile phase solvent 2	:	0.1% (v/v) formic acid in acetonitrile

Mobile Phase Composition

Time (min)	% Solvent 1	% Solvent 2	Flow (mL/min)
0	95	5	0.4
4.0	30	70	0.4
4.1	95	5	0.4
6.0	95	5	0.4

Valve Switching programme

Time (mins)	
0.0	To waste
2.5	To mass spectrometer
4.5	To waste

Notes: The column eluate is diverted to waste for the first 2.5 minute to prevent ionic material from the sample contaminating the mass spectrometer front plate. A secondary pump providing flow of mobile phase to the mass spectrometer when the column eluate is switched to waste has been found to be unnecessary.

Under these conditions the retention time of NOA414746 is 3.1 minutes.

4.3 Mass Spectrometer Conditions for NOA414746

Interface	:	TurboIonSpray
Polarity	:	Negative
Curtain gas (CUR)	:	Nitrogen set at 35 (arbitrary units)
Temperature (TEM)	:	400 °C
Ionspray voltage	:	-3900 V
Collision gas setting (CAD)	:	Medium
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		**NOA414746 primary transition**	**NOA414746 confirmatory transition**
Q1 *m/z*	:	205	205
Q3 *m/z*	:	125	161
Dwell time	:	100 ms	100 ms
Resolution Q1	:	Unit	Unit
Resolution Q3	:	Unit	Unit
Declustering potential (DP)	:	-50 V	-50 V
Entrance potential (EP)	:	-10 V	-10 V
Collision energy (CE)	:	-28 V	-18 V
Collision cell exit potential (CXP)	:	-15 V	-10 V

Typical chromatograms are shown in the Figures Section.

4.4 Confirmatory Procedures for 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

NOA414746 residues may be calculated in µg/L 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 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.

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 (generally 1/x weighting 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 NOA414746 residues in the sample, expressed as µg/L, as follows

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

Where analyte found (µg/mL) is calculated from the standard calibration curve and sample conc. is the final sample concentration in L/mL, accounting for any concentration in the SPE step where used.

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

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \ (\mu\text{g/L})$$

5.2 Single-Point Calibration Procedure

NOA414746 residues may be calculated in µg/L 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 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 NOA414746.

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

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

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

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

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 (L/mL)

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

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} (\mu g/L)$$

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 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 110% and with a relative standard deviation of $\leq$20%.

Where 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 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 should be detergent washed and then rinsed with HPLC grade methanol, acetone or acetonitrile prior to use.

8.3 Derivatisation

Derivatisation is not required for the determination of residues of NOA414746 in water.

8.5 Limit of Quantification (LOQ)

The limit of quantification has been set at 0.05 µg/L (0.05 ppb).

8.6 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.

For surface water the LOD for the primary transition was determined 0.0025 µg/L, equivalent to 0.38 pg injected on column when using a 30 µL injection volume. For groundwater the LOD for the primary transition was determined 0.0008 µg/L, equivalent to 0.12 pg injected on column when using a 30 µL injection volume. For seawater the LOD for the primary transition was determined 0.0113µg/L, equivalent to 1.70 pg injected on column when using a 30 µL injection volume.

8.7 Matrix Effects

No significant matrix effects were observed in groundwater and surface water. Significant matrix effects were observed in seawater. Matrix-matched standards were used for quantification of all water types tested during the validation.

8.8 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.

Standard solutions containing NOA414746 at concentrations ranging from 0.075 to 5 µg/L (equivalent to 2.25 pg to 150 pg of NOA414746 injected onto the column

based on a 30 µL injection) were analysed by LC-MS/MS, using the conditions specified in the analytical method. The detector response for LC-MS/MS was plotted against standard concentration. The lowest concentration injected was at 30% of the LOQ of the method and the highest concentration was at least 20% above the highest analyte sample concentration in the final extracts.

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 NOA414746.

8.9 Extract Stability

Final water samples in acetonitrile/ultra-pure water (20/80, v/v) retained in vials and stored at a temperature of approximately 2 – 8ºC were suitable for NOA414746 residue analysis for storage periods of at least 6 days. Samples are assumed to be stable on storage when the reanalysis data are within ± 20% of the original analysis.

9.0 LIMITATIONS

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

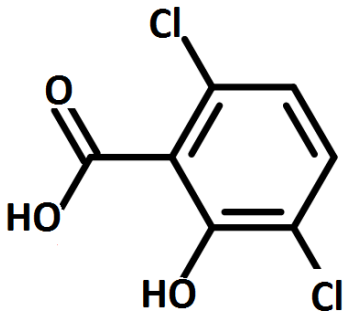
TABLE 1: Characterisation Data of Water Samples used for Method Validation.

Matrix	Source	pH	Silt Content (% w/w)	Dissolved Organic Carbon (DOC) (mg/L)	Total Hardness As $CaCO_3$ (mg/L)
Surface water	River Meon, Meonstoke, Hampshire, UK	8.0	100	3.36	275
Groundwater	Fawley Lodge, Henley-on-Thames, UK	7.8	100	3.21	300
Seawater	Hayling Island, UK	7.9	<1	8.80	6606

CHEMICAL STRUCTURES

FIGURE 1: NOA414746

Alternative compound code number	**:**	-
IUPAC Name	**:**	3,6 -dichloro-2-hydroxy-benzoic acid
Molecular Formula	:	$C_7H_4Cl_2O_3$
Molecular Weight	**:**	207.0 g/mol



APPENDIX 1 APPARATUS

Recommended Suppliers

Equipment	Description	Supplier
Laboratory supplies	General glassware, centrifuge tubes, autosampler vials and caps	Thermo Fisher
SPE cartridges	Phenomenex Strata -X (100 mg, 3 mL)(Part Number: 8B-S100-EBJ)	Phenomenex
LC-MS/MS system	AB Sciex QTRAP 5500 equipped with a TurboIonSpray source	Applied Biosystems
HPLC system	Agilent 1260/1290 series	Agilent
HPLC column	Ace Ultracore Super C18 50 mm x 2 mm i.d., 2.5 µm particle size (Part Number: CORE-25A-0502U)	Hichrom
Nitrogen generator	Peak Scientific ABN2ZA	Peak Scientific

APPENDIX 2 REAGENTS

Recommended Suppliers

Reagent	Description	Supplier
Ultra-pure water	HPLC grade	Fisher Scientific
Acetonitrile	HPLC grade	Fisher Scientific
Formic acid	Analytical grade	Fisher Scientific
Hydrochloric acid	Laboratory grade	Fisher Scientific
Acetic acid	Analytical grade	Sigma Aldrich
NOA414746 analytical standard	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) 1% acetic acid in acetonitrile – add 1 mL of concentrated acetic acid to 100 mL of acetonitrile in a suitable container. Stopper the vessel and shake to mix thoroughly.

b) Acetonitrile/ultra-pure water (20/80 v/v) – mix 80 mL HPLC or LC-MS grade water and 20 mL acetonitrile in a suitable container and shake to mix thoroughly.

c) 0.1% formic acid in ultra-pure water – add 1 mL of concentrated formic acid to 1000 mL of HPLC or LC-MS grade water in suitable container and shake to mix thoroughly.

d) 0.1% formic acid in acetonitrile – add 1 mL of concentrated formic acid to 1000 mL of acetonitrile in a suitable container and shake to mix thoroughly.

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 NOA414746

Note: NOA414746 has 2 chlorine atoms present. Chlorine has two isotopic forms ^{35}Cl and ^{37}Cl and therefore the mass spectrum may show a two chlorine isotope pattern ratio of 100:64:10. These isotopic masses based on the chlorine isotopes can be used as required to achieve analytical sensitivity and specificity. In this method ^{35}Cl has been used exclusively.

Infuse standard solutions of NOA414746 (0.1 to 1.0 µg/mL) in mobile phase (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* 205 for NOA414746 in negative ionisation mode.

Using the Analyst software quantitative optimisation routine, tune the instrument for 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 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 NOA414746, in negative, the deprotonated/protonated 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* 125 and *m/z* 161) are then selected and used for quantitative analysis.

Daughter Ion *m/z*	Structure
125	$[M - H - CO_2 - HCl]^-$
161	$[M - H - CO_2]^-$

APPENDIX 4 METHOD FLOW CHART

5 mL aliquot of water

↓

Acidify with 50 µL concentrated hydrochloric acid

↓

Mix thoroughly

↓

Clean-up and concentrate by SPE procedure

↓

Evaporate to dryness and redissolve in acetonitrile/ultra-pure water (20/80 v/v, 1 mL).
Vortex mix thoroughly

↓

Transfer to a suitable autosampler vial

↓

Analysis by LC-MS/MS