Dicamba

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

Method Validation

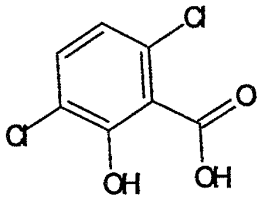
DATA REQUIREMENT(S): OCSPP 850.6100 (2012)
SANCO/3029/99 Rev. 4 (2000)
SANCO/825/00 Rev. 8.1 (2010)

3.0 MATERIALS AND METHODS

3.1 Test and Reference Items

3.1.1 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 Weight	: 207.0 g/mol
Chemical Structure	:



Name:	NOA414746
Supplier:	Syngenta
Batch:	BPS 988/1
Purity (%):	99
Expiration:	31 January 2020
Storage:	< 30 °C

3.2 Test System

The validation study was carried out using control samples sourced by CEMAS. Table 1 displays the characterisation results of the water samples. Characterisation was carried out under separate a GLP study (References 2 and 3). All control samples were stored in a coldroom set to maintain a specimen temperature of 2 – 8 °C.

Matrix	Source	Sample Reference	Preparation
Surface water	River Meon, UK	CCON/116/010	No specimen preparation required
Groundwater	Fawley Lodge, Henley-on-Thames, UK	CCON/116/005	
Seawater	Hayling Island, UK	CCON/116/011	

3.3 Analytical Procedures

3.3.1 Preparation of Analytical Standard Solutions

The standard solutions were prepared according to the procedures described in the draft analytical method GRM022.09A (Reference 1).

Two individual 400 µg/mL stock solutions (primary stock solutions) of NOA414746 were prepared in acetonitrile. See Appendix 2 for details.

3.3.2 Preparation of Fortification and Calibration Standard Solutions

Fortification solutions were prepared by serial dilution from one of the primary stock solutions (400 µg/mL). An intermediate 10 µg/mL solution was prepared by taking 0.5 mL of the 400 µg/mL stock solution into a volumetric flask (20 mL) and diluting with acetonitrile. The following solutions were then prepared by serial dilution in acetonitrile: 1 µg/mL, 0.1 µg/mL and 0.01 µg/mL.

Calibration solutions were prepared by serial dilution from the other primary stock solution (400 µg/mL). An intermediate 10 µg/mL solution was prepared by taking 0.5 mL of the 400 µg/mL stock solution into a volumetric flask (20 mL) and diluting with acetonitrile. The following solutions were then prepared by serial dilution in acetonitrile: 1 µg/mL, 0.1 µg/mL, 0.01 µg/mL and 0.001 µg/mL.

Calibration solutions for analytical determination by LC-MS/MS were prepared on the day of use from the 0.1 µg/mL, 0.01 µg/mL and 0.001 µg/mL calibration solutions over an appropriate range by dilution into acetonitrile/water (20/80, v/v).

The analytical standard solutions were stored at between 2 – 8 °C.

3.3.3 Sample Fortification

Recovery of NOA414746 in surface water, groundwater and seawater was assessed by fortifying 5 aliquots of each matrix with each analyte at the LOQ and 5 aliquots of each matrix at a higher level (10 × LOQ). The following table summarises the fortification levels.

Matrix	LOQ	Higher level (10 × LOQ)
Surface water	0.05 µg/L	0.5 µg/L
Groundwater	0.05 µg/L	0.5 µg/L
Seawater	0.05 µg/L	0.5 µg/L

In addition, two control samples and one reagent blank were analysed with each sample batch. The fortification levels are summarised in Table 2.

3.3.4 Sample Analysis

Samples were analysed according to the procedures described in analytical method GRM022.09A for the determination of residues of NOA414746 in water (see Appendix 2).

In summary, using analytical method GRM022.09A, 5 mL samples were extracted as per the following method:

5 mL samples were acidified with hydrochloric acid, cleaned-up and concentrated by SPE (Solid Phase Extraction), blown down to dryness and reconstituted in acetonitrile/water (20/80, v/v). The final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method was 0.05 µg/L for surface water, groundwater and seawater.

The percentage recovery obtained for each sample was calculated and these results were used to assess the relative standard deviation (RSD) and limit of quantification of the analytical method.

3.3.5 Matrix Effects

The sample sets included appropriate matrix-matched standards for each matrix. The effect of the matrices on the LC-MS/MS response was assessed by preparing standards in the presence of each matrix and comparing the peak areas of each analyte against non-matrix standards (i.e. standard prepared in acetonitrile/water 20/80, v/v) at an equivalent concentration.

Percent matrix effects were calculated as follows:

$$((B/A)\times 100)-100$$

Where A is the non-matrix standard response and B is the matrix-matched standard response. The matrix effects were determined by triplicate injections of 0.5 µg/L calibration standards and the mean value used for the assessment of matrix effects. A difference of >20% is considered a significant matrix effect. The results are presented in Table 5.

3.3.6 Detector Linearity

Calibration standard solutions containing NOA414746 over a range of concentrations were analysed by LC-MS/MS, using the conditions specified in the method. The concentrations ranged from 0.075 to 5 µg/L (equivalent to 2.25 to 150 pg of analyte injected onto the column, based on a 30 µL injection). The detector response (peak area) for the LC-MS/MS was plotted against standard concentration. The lower margin of the linearity test was at least 30% of the LOQ and the upper margin was higher by at least 20% above the highest concentration in the final extract as required in SANCO guideline 825/00.

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

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

For NOA414746 for both the primary and confirmatory transitions, the baseline noise of control samples was measured at the appropriate retention time. The baseline noise was multiplied by 3 and then compared to the height of the LOQ equivalent standard of the calibration curve (0.25 µg/L standard) and the LOD estimated

The LOQ of the method is defined as the lowest analyte concentration in a sample at which the methodology has been successfully validated.

3.3.8 Storage Stability of Sample Extracts

The stability of NOA414746 was assessed by storing the final extracts in vials at a temperature of between 2 – 8 °C. The extracts were then re-analysed after 6 - 8 days of storage against freshly prepared calibration standards.

The extract stability comparisons (% Difference) were determined by:

$$((B/A)\times 100)-100$$

Where A is the original extracts mean recovery and B is the stored extracts mean recovery. Samples are said to be stable if there is less than 20% difference from the original analysis.

3.3.9 Storage Stability of Standards

The stability of NOA414746 in stock standard solutions prepared in acetonitrile was assessed by comparing dilutions of a freshly prepared standard solution (200 µg/mL) with dilutions of a stored standard solution (400 µg/mL) after 63 days storage, at between 2 – 8 °C. For analysis, 0.5 µg/L solutions in acetonitrile/water (20/80, v/v) were prepared from both the fresh and the stored standard solutions in acetonitrile. The results are presented in Table 15.

The stability of NOA414746 in working standard solutions was assessed by comparing a dilution of a freshly prepared standard solution with a dilution of a stored working standard solution after 75 days storage at between 2 – 8 °C. The stored 0.001 µg/mL working standard solution was diluted to 0.1 µg/L in acetonitrile/ultra-pure water (20/80, v/v) and compared with a freshly prepared 0.1 µg/L solution in acetonitrile/ultra-pure water.

The standard stability comparisons were determined by:

$$((B/A)\times 100)-100$$

Where A is the stored standard response and B is the freshly prepared standard response. Solutions are said to be stable when comparison of a new and old standard shows a difference of less than 20%.

4.0 RESULTS AND DISCUSSION

Draft method GRM022.09A "Dicamba - Residue Method GRM022.09A for the Determination of the Metabolite NOA414746 (DCSA) in Water" (Reference 1) was successfully validated.

4.1 Limit of Quantification (LOQ)

The LOQ of method GRM022.09A was established as 0.05 µg/L in surface water, groundwater and seawater for NOA414746.

4.2 Control Samples

Measured residues of NOA414746 were lower than 30% of the LOQ in all of the control and reagent blank samples used in this study. This demonstrates that no interferences were present at the retention time of the analyte in the test systems. This is in accordance with the level specified in SANCO guideline 825/00 rev. 8.1 and SANCO guideline 3029/99 rev. 4.

4.6 Limit of Detection (LOD)

The limit of detection (LOD) was calculated for NOA414746 for both primary and confirmatory transitions in surface water, groundwater and seawater and was found to be equivalent to less than 30% of the LOQ (0.015 µg/L) when using a 30 µL injection volume.

4.7 Storage Stability of Sample Extracts

Sample extracts from fortified water samples were re-analysed after at least 6 days of storage between 2 – 8 °C. The results show that NOA414746 is stable in fortified surface water, groundwater and seawater samples for at least 6 – 8 days when stored in vials at between 2 – 8 °C. Results are presented in Tables 12 to 14.

4.8 Storage Stability of Standards

NOA414746 has been shown to be stable in stock standard solutions prepared in acetonitrile when stored between 2 – 8 °C for a period of at least 63 days.

NOA414746 has been shown to be stable in working standard solutions prepared in acetonitrile when stored between 2 – 8 °C for a period of at least 75 days.

4.9 Specificity

Since two characteristic MS/MS mass transitions were used to monitor NOA414746 the method achieves a high level of specificity and no further confirmation on a different detector was necessary.

.

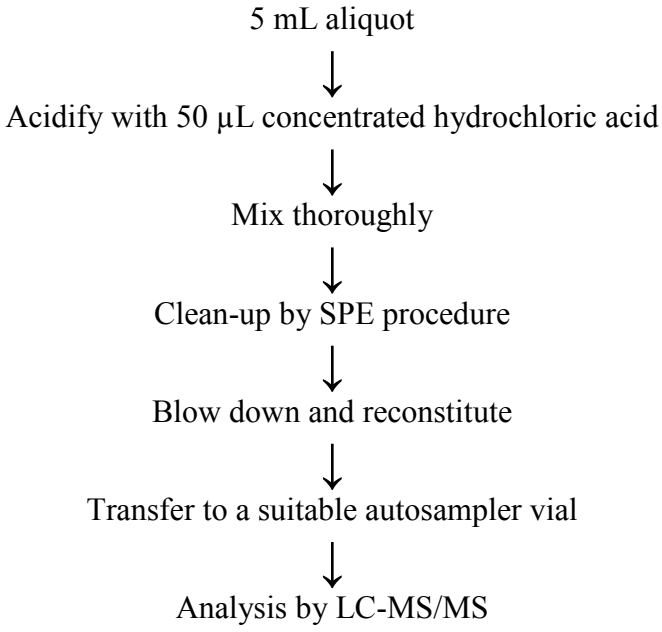
TABLE 1 Characterisation Data of Control Water Samples used for Method Validation

Matrix	Surface Water	Groundwater	Seawater
Source	River Meon, Meonstoke, Hampshire, UK	Fawley Lodge, Henley-on-Thames, UK	Hayling Island, UK
Sample Reference	CCON/116/010	CCON/116/005	CCON/116/011
pH	8.0	7.8	7.9
Total Hardness as $CaCO_3$ (mg/L)	275	300	6606
Silt Content (mg/L)	100	100	<1
Dissolved Organic Carbon (DOC) (mg/L)	3.36	3.21	8.80

TABLE 2 Fortification Levels

Matrix	**Reference Item**	**Untreated Replicates**	**Replicates at LOQ Fortification Level**	**Replicates at 10 × LOQ Fortification Level**
Surface water	NOA414746	2	5 at 0.05 µg/L	5 at 0.5 µg/L
Groundwater	NOA414746	2	5 at 0.05 µg/L	5 at 0.5 µg/L
Seawater	NOA414746	2	5 at 0.05 µg/L	5 at 0.5 µg/L

APPENDIX 1 Method Flow Chart



APPENDIX 2 Analytical Method Description (Excerpt from GRM022.09A)

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.

APPENDIX 2 Analytical Method Description (Continued)

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.

APPENDIX 2 Analytical Method Description (Continued)

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 °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.

APPENDIX 2 Analytical Method Description (Continued)

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

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

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.

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

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).