1.0 EXECUTIVE SUMMARY

Draft analytical method GRM030.04A has been validated for the determination of residues of NOA449280 and metabolites SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 in two different soil types.

Control samples of prepared soil matrices were fortified with NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 and extracted according to the analytical procedures in GRM030.04A and analysed by high performance liquid chromatography using triple quadrupole mass spectrometric detection (LC-MS/MS).

A reagent blank sample, control samples in duplicate and fortified samples in quintuplet at the limit of quantification (LOQ, 0.001 mg/kg) and in quintuplet at ten times the LOQ (0.01 mg/kg) were all analysed for each matrix.

On all matrices tested, residues in the reagent blank and control samples were found to be lower than 30% of the LOQ. Acceptable mean recoveries between 70% and 110% with a relative standard deviation lower than 20% were found for:

- NOA449280 primary transition *m/z* 398.0→175.0 and confirmatory transition *m/z* 398.0→137.1
- SYN503780 primary transition *m/z* 278.0→202.0 and confirmatory transition *m/z* 278.0→145.8
- CSCD656832 primary transition *m/z* 205.9→142.0 and confirmatory transition *m/z* 205.9→162.0
- CSCD642512 primary transition *m/z* 398.0→340.0 and confirmatory transition *m/z* 398.0→137.1
- CSCC163768 primary transition *m/z* 233.9→146.0 and confirmatory transition *m/z* 233.9→126.0
- CSAA806573 primary transition *m/z* 219.9→156.0 and confirmatory transition *m/z* 219.9→176.0

No matrix effect higher than 10 % was observed for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 for either soil type. It was therefore appropriate to use non matrix-matched standards for calibration and quantification.

The response of the LC-MS/MS was shown to be linear for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 for each transition over a concentration range of 0.0002 to 0.01 µg/mL (0.0002 to 0.0075 µg/mL for NOA449280).

The stability of each compound in the final extracts when stored at approximately 4ºC (between 0 and 9°C) for 12 days was assessed. Residues were found to be stable when stored under these conditions.

These results demonstrate the stability of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 in final extracts when stored between 0 and 9°C.

The repeatability and specificity of the method have been demonstrated and GRM030.04A has been validated successfully for the determination of residues of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 in soil matrices at the LOQ of 0.001 mg/kg. The method has been validated according to the EU guidelines SANCO/3029/99 Rev. 4 and SANCO/825/00 Rev. 7. The method validation also complies with US EPA guidelines OPPTS 850.7100 and OPPTS 860.1340.

2.0 INTRODUCTION

Draft analytical method GRM030.04A has been validated by Eurofins|ADME Bioanalyses laboratories for the determination of residues of NOA449280 and its metabolites SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 in two soil matrices at the LOQ of 0.001 mg/kg using commercially available instrumentation (Reference 1). This study was conducted to validate the analytical method.

This study was conducted in compliance with guidelines SANCO/3029/99 Rev. 4 and SANCO/825/00 Rev. 7. The method validation also complies with US EPA guidelines OPPTS 850.7100 and OPPTS 860.1340.

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 ≤ 20%), for each fortification level and overall.

b) To establish that the limit of quantification (LOQ) of the analytical method is 0.001 mg/kg for NOA449280 and metabolites in soil matrices.

c) To establish that residues of NOA449280 and its metabolites SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 in control samples are not present at levels above 30% of the LOQ.

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

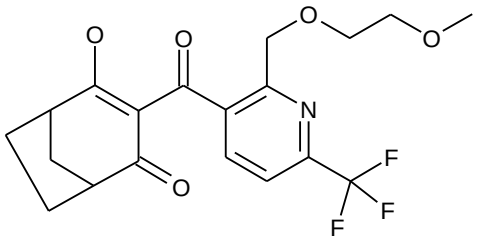
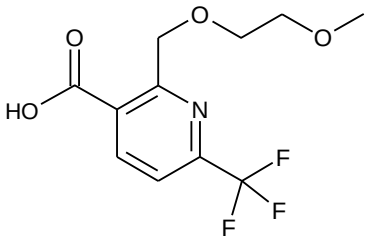
e) To assess suppression or enhancement of instrument response to the analytes in the presence of matrix.

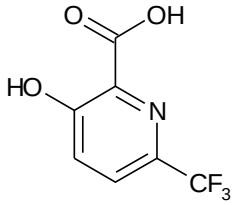
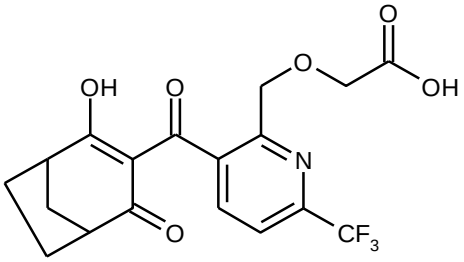
f) To assess the stability of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 stored at approximately 4°C (between 0 and 9°C) in the final soil extracts.

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

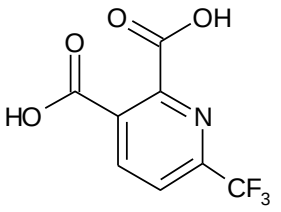
3.0 MATERIALS AND METHODS

3.1 Test and Reference substances

Common name:	**NOA449280**
Chemical name (IUPAC):	4-Hydroxy-3-[2-(2-methoxy-ethoxymethyl)-6-trifluoromethyl-pyridine-3-carbonyl]-bicyclo[3.2.1]oct-3-en-2-one
CAS-Registry-No.:	[*352010-68-5*]
Structural formula:	
Molecular formula:	$C_{19}H_{20}F_3NO_5$
Molecular mass:	399.4 g/mol
Batch:	AMS 1144/1
Purity:	99.9 %
Storage conditions	20°C ± 4°C (at Eurofins|ADME Bioanalyses)
Date of expiry:	31 May 2011
Common name:	**SYN503780**
Chemical name (IUPAC):	2-(2-Methoxy-ethoxymethyl)-6-trifluoromethyl-nicotinic acid
CAS-Registry-No.:	[*380355-55-5*]
Structural formula:	
Molecular formula:	$C_{11}H_{12}F_3NO_4$
Molecular mass:	279.2 g/mol
Batch:	KI 6386/18
Purity:	100 %
Storage conditions	between 0 and 9°C (at Eurofins|ADME Bioanalyses)
Date of expiry:	31 Mar 2012

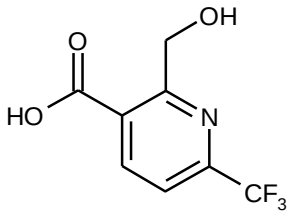
Common name:	**CSCD656832**
Alternative Code Number	SYN545680
Chemical name (IUPAC):	3-Hydroxy-6-trifuoromethyl-pyridine-2-carboxylic acid
CAS-Registry-No.:	not listed
Structural formula:	
Molecular formula:	$C_7H_4F_3NO_3$
Molecular mass:	207.1 g/mol
Batch:	MES 109/1
Purity:	100%
Storage conditions	between 0 and 9°C
Date of expiry:	30 April 2010
Common name:	**CSCD642512**
Alternative Code Number	SYN545859
Chemical name (IUPAC):	[3-(2-hydroxy-4-oxo-bicyclo[3.2.1]oct-2-ene-3-carbonyl)-6-trifluoromethyl- pyridine-2-yl-methyloxy]-acetic acid
CAS-Registry-No.:	not listed
Structural formula:	
Molecular formula:	$C_{18}H_{16}F_3NO_6$
Molecular mass:	399.3 g/mol
Batch:	MES 128/2
Purity:	96%
Storage conditions	between 0 and 9°C
Date of expiry:	28 February 2011
Common name:	**CSCC163768**
Alternative Code Number	SYN504810
Chemical name (IUPAC):	6-Trifluromethyl-pyridine-2,3-dicarboxylic acid
CAS-Registry-No.:	not listed

Structural formula:



Molecular formula:	$C_8H_4F_3NO_4$
Molecular mass:	235.1g/mol
Batch:	MES 135/1
Purity:	98%
Storage conditions	between 0 and 9°C
Date of expiry:	31 December 2010
Common name:	**CSAA806573**
Chemical name (IUPAC):	2-(Hydroxymethyl)-6-trifuoromethyl-nicotinic acid
CAS-Registry-No.:	not listed

Structural formula:



Molecular formula:	$C_8H_6F_3NO_3$
Molecular mass:	221.1 g/mol
Batch:	MES 132/1
Purity:	93%
Storage conditions	between 0 and 9°C
Date of expiry:	30 November 2010

The certified reference items of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 were supplied by Syngenta Crop Protection Münchwilen AG, 4333 Münchwilen, Switzerland.

The certificates of analysis are under the responsibility of the supplier. Samples of the reference items will be retained at the test facility as long as their quality can be maintained.

3.2 Test system

The validation study was carried out using control samples from two Syngenta soil dissipation studies (The physico-chemical characteristics are reported in Table 1). The samples were homogenised by standard Eurofins|ADME Bioanalyses preparation procedures.

3.3 Preparation and stability of calibration and quantification analytical standard solutions

The preparation of standard solutions is detailed in Appendix 1.

3.4 Fortification levels

The preparation of fortification solutions is detailed in Appendix 1. Fortification levels are summarised in below.

Matrix	NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768, and CSAA806573 Fortification Level (mg/kg)	Minimum Number of Replicates
Soil	Reagent blank	1
	Control	2
	0.001	5
	0.010	5

3.5 Analytical procedures

3.5.1 Limit of detection (LOD) and limit of quantification (LOQ)

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 four times background noise. Note that the LOD may vary between runs and from instrument to instrument.

The LOD was estimated for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 for both transitions. The results are presented in Tables 2 – 7 and summarised in Section 4.0.

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 0.001 mg/kg was

confirmed for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 in soil.

3.5.2 Sample analysis

Samples were analysed according to the procedures described in draft analytical method GRM030.04A. The method is detailed in Appendix 1.

In summary, soil samples were heated at reflux in 50/50 v/v acetonitrile/1 M NH_4OH for 1 hour. After cooling to room temperature, extracts were centrifuged and then aliquots evaporated to remove the acetonitrile. Aliquots were then diluted with ultra pure water and acidified to < pH 2 prior to clean-up and concentration with a solid phase extraction (SPE) procedure using Phenomenex Strata-X cartridges. Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method is 0.001 mg/kg for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573.

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

3.5.3 Detector linearity

Standard solutions containing NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 at concentrations ranging from 0.0002 to 0.01 µg/mL (equivalent to 10 to 500 pg of analyte injected on to the column, based on a 50 µL injection, which was mainly used) 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 50% of the LOQ of the method. The highest concentration level injected was equivalent to a residue level of 0.025 mg/kg or 25×LOQ.

3.5.4 Storage stability of sample extracts

The stability of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 was assessed by storing the final extracts of representative soil matrix (sandy loam) in 90/10 v/v ultra pure water/acetonitrile refrigerated at approximately 4°C (between 0 and 9°C). The soil matrices samples were then re-analysed against freshly prepared calibration standards after 12 days of storage.

3.5.5 Matrix effects

Each sample set included an appropriate matrix-matched standard, prepared in soil matrices. For each matrix, the response obtained from the matrix-matched standard was

compared against the response obtained from the standard in 90/10 v/v ultra pure water/acetonitrile to allow calculation of any matrix effect (either suppression or enhancement of response). No significant matrix effects were observed in either soil type and non-matrix standards were used for quantification.

APPENDIX 1 Method GRM030.04A

1.0 METHOD SUMMARY

10 g soil samples are heated at reflux in 50/50 v/v acetonitrile/1 M NH_4OH for 1 hour. After cooling to room temperature, extracts are centrifuged and then aliquots evaporated to remove the acetonitrile. Aliquots are diluted with ultra pure water and acidified to < pH 2 prior to clean-up and concentration with a solid phase extraction (SPE) procedure using Phenomenex Strata-X cartridges. Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method is 0.001 mg/kg for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573.

2.0 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.

2.1 Stock solutions

Prepare individual 200 µg/mL stock solutions for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 by one of the following methods.

Weigh out accurately, using a five figure balance, sufficient NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 analytical standards and carefully transfer into separate “Class A” volumetric flasks (50 mL). Dilute to the mark with acetonitrile to give 200 µg/mL stock solutions of NOA449280, SYN503780, CSCD656832, CSCD642512 and CSAA806573.

Note: CSCC163768 is not soluble in pure acetonitrile. CSCC163768 should be dissolved in 80/20 v/v acetonitrile/ultra pure water.

2.2 Fortification solutions

Sample fortification solutions should be prepared in ultra pure water from the primary stock solution in “Class A” volumetric flasks. It is recommended that, as a minimum, the following solutions are prepared by serial dilution: 10 µg/mL, 1.0 µg/mL and 0.1 µg/mL. Mixed NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573

standards may be prepared if desired. The preparation of LC-MS/MS calibration standards is discussed in Section 3.8.

Note: it is recommended that fresh dilutions of the fortification solutions are prepared on a daily basis from the 10 µg/mL standard using ultra pure water.

An expiration date of six months for stock solutions and 10 µg/mL fortification solution is recommended unless additional data are generated to support a longer expiration date.

3.0 ANALYTICAL PROCEDURE

The method is summarized in flow chart form in Appendix 8.

3.1 Modifications and potential problems

a) Bottled HPLC grade water is used to prepare aqueous mobile phase as this gives a reduced MS/MS background signal when compared to water from a laboratory water purification system.

b) To prevent contamination of the instrument and to minimise possible carry-over issues, it is recommended that high level recoveries (>0.1 mg/kg) and samples with expected residues greater than 0.1 mg/kg should be diluted so that the final analyte concentration does not exceed 0.005 µg/mL. It may also be useful to include blank injections of acetonitrile/ultra pure water (50:50 v/v) after high level samples to clear any observed carry-over greater than 10% of the LOQ.

3.2 Sample preparation

All samples should be prepared using an approved method of preparation to obtain a homogeneous sample prior to analysis.

3.3 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 pre-weighed control soil sample, add the appropriate amount of standard solution containing NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 in ultra pure water. Let each sample stand for at least five minutes after fortification to allow the spiking solution to soak into the matrix before proceeding with the extraction procedure. At least one untreated control and two fortified control samples should be analysed with each sample set.

3.4 Extraction

a) Weigh a representative amount of soil (10 g) into a round bottom flask (100 mL size). Fortify samples as required at this point. Add 50/50 v/v acetonitrile/1 M NH_4OH (50 mL) and record the weight of the flask and contents on a suitable balance. This allows correction for any loss of solvent due to evaporation during reflux.

b) Place the flasks in a suitable electric heating mantle and securely attach a water cooled reflux condenser to each flask. Heat at reflux for 1 hour then allow the samples to cool to room temperature with the condensers still attached.

c) Carefully remove the condensers and flasks from the heating mantle and check the weight of the flask and contents. Any losses due to evaporation should be corrected for by addition of ultra pure water. Swirl the flask and contents to mix thoroughly.

d) Decant the sample into a clean plastic centrifuge tube (50 mL size) and centrifuge at a speed that visibly separates the supernatant from the soil e.g. 4000 rpm for 5 minutes. The sample concentration is 0.2 g/mL.

e) Transfer aliquots (2 mL, equivalent to 0.4 g) into graduated plastic centrifuge tubes (e.g. 15 mL size) and place under a stream of nitrogen in a sample concentrator set at 40^{o}C. Evaporate the samples to 1 mL ± 0.1 mL then adjust the final volume to 4 mL with ultra pure water. Ultrasonicate the samples briefly to mix the sample thoroughly.

f) Add concentrated formic acid (200 µL) to each sample. Cap the tubes and shake gently to ensure thorough mixing. Check that the pH is < 2 using suitable indicator paper. It is important to ensure that the sample is sufficiently acidic so that the carboxylic acids are fully protonated. In the ionised form at higher pH, the metabolites will not be retained on the SPE cartridge, resulting in low recovery.

3.5 Solid Phase Extraction (SPE) Procedure

a) Take one Phenomenex Strata-X SPE cartridge (60 mg, 3 mL) for each sample to be analysed and place on a suitable vacuum manifold (e.g. IST Vacmaster). Add methanol (2 mL) and allow to percolate through each cartridge under gravity or draw through under vacuum to the level of the top frit at a rate of approximately 1 mL/min, discarding the column eluate. Do not allow the cartridges to become dry. Add ultra pure water (2 mL) to the top of each cartridge and allow to percolate through under gravity or draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluate. Do not allow the cartridges to become dry.

b) Load the samples from Section 3.4 (f) onto the SPE cartridges and allow to percolate through under gravity or under low vacuum, at a rate of approximately 1 - 2 mL/min, to the level of the top frit. Do not allow cartridges to become dry. NOA449280,

SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 are retained on the SPE cartridges.

c) On completion of loading, wash the empty sample tubes with ultra pure water containing 2% v/v formic acid (2 mL) and add the rinse to the column reservoir. Allow to percolate through under gravity or draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluate. Do not allow the cartridges to become dry.

d) Wash SPE cartridges with a further 2% v/v formic acid in ultra pure water. Allow to percolate through under gravity or draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluate.

e) Briefly apply a high vacuum for approximately 5 - 10 seconds to remove excess water from the cartridges but do not dry for extended periods. Extended periods of drying the cartridge may result in low recovery of NOA449280 especially.

f) Place suitable collection tubes (e.g. 10 mL glass test tubes) under each port, as required, in the manifold rack. Add 95/5 v/v methanol/formic acid (2 mL) onto the cartridges and allow the mixture to enter the cartridges for 5 minutes, then elute under gravity or draw through under low vacuum at a rate of approximately 1-2 mL/min to the level of the top frit collecting the column eluate.

g) Elute with further 3 mL of 95/5 v/v methanol/formic acid under gravity or draw through under low vacuum at a rate of approximately 1-2 mL/min to the level of the top frit collecting the column eluate. Apply a high vacuum for approximately 5 – 10 seconds to collect the excess solvent from the SPE cartridges. NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 are eluted in this step.

h) Evaporate the samples to dryness under a stream of nitrogen in a sample concentrator with the temperature set to 40 ºC. This should take approximately 20-30 minutes. Although there is no information to suggest that losses occur if samples are left at dryness for extended periods it is recommended that samples are removed immediately the solvent has evaporated.

i) Add acetonitrile (100 µL) to the dried residue and ultrasonicate carefully. Add ultra pure water (900 µL and again ultrasonicate thoroughly to ensure the sample is completely dissolved and thoroughly mixed.

j) Transfer the sample to a suitable autosampler vial ready for final determination by LC-MS/MS. The final sample concentration is 0.4 g/mL.

3.6 Preparation of calibration standards for LC-MS/MS

Samples should be quantified against non-matrix calibration standards where possible. Any significant matrix effects observed may be compensated for using matrix matched standards, at the discretion of the study director. Alternatively, further sample dilution may be used to reduce matrix effects where instrument sensitivity allows.

For example, to prepare e.g., an LOQ equivalent non-matrix calibration standard (0.0004 μg/mL) of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573, transfer 90/10 v/v ultra pure water/acetonitrile (approximately 9 mL) into a 10 mL volumetric flask and add 400 μL of a 0.1 μg/mL mixed NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 standard in ultra pure water. Adjust to the 10 mL mark with 90/10 v/v ultra pure water/acetonitrile. Stopper the flask securely and shake to mix thoroughly. Dilute 10-fold with 90/10 v/v ultra pure water/acetonitrile, mixing throughly. Transfer an aliquot of the standard into a suitable autosampler vial for analysis by LC-MS/MS.

A calibration curve may also be generated to quantify NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 residues. Standards over an appropriate concentration range should be prepared as described above, using the requisite volumes of NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573 standards in ultra pure water.

4.0 FINAL DETERMINATION

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. The method has been developed for use on an Applied Biosystems API4000 LC-MS/MS.

4.1 Instrument description

Pump	:	PE200 quaternary or Shimadzu LC20AD
Column Oven	:	PE200 or Shimadzu CTO20AC column switching valve
Detector	:	Applied Biosystems API 4000 triple quadrupole mass spectrometer with Analyst™ software version 1.4.2
Gas Supply	:	Peak Scientific NM20ZA gas station

4.2 Chromatography conditions for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573

Column	:	Chromolith Performance RP18 2 μm 100 x 3.0 mm i.d.
Column Oven Temperature	:	40°C
Injection volume	:	50 μL (10 μL for stability of extracts)
Stop Time	:	10 minutes
Injection protocol	:	Analyse calibration standard after 3 to 4 sample injections
Mobile phase	:	Solvent 1 = methanol Solvent 2 = 0.2% acetic acid in ultra pure water

Mobile phase composition

Time (min)	% Solvent 1	% Solvent 2	Flow (mL/min)
0.0	10	90	0.75
2.0	80	20	0.75
5.5	80	20	0.75
5.6	10	90	0.75
10.0	10	90	0.75

Under these conditions the approximate retention times are given in the table below.

Analyte	Retention time (min)
CSCC163768	3.4 – 3.7
CSAA806573	4.3 – 4.4
CSCD656832	4.7 – 5.1
SYN503780	4.7 – 4.9
CSCD642512	4.9 – 5.1
NOA449280	5.2 – 5.3

Notes: The column eluate is diverted to waste for the first 0.8 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.

4.3 Mass spectrometer conditions for NOA449280, SYN503780, CSCD656832, CSCD642512, CSCC163768 and CSAA806573

Interface	:	TurboIonSpray			
Polarity	:	negative			
Curtain gas (CUR)	:	Nitrogen set at 17 (arbitrary units)			
Temperature (TEM)	:	550 °C			
Ionspray voltage	:	-4500 V			
Collision gas setting (CAD)	:	Nitrogen set at 6 (arbitrary units)			
Gas 1 (GS1)	:	Air set at 60 (arbitrary units)			
Gas 2 (GS2)	:	Air set at 60 (arbitrary units)			
Interface heater (ihe)	:	On			
Scan type	:	MRM			
MRM Conditions		**NOA449280 Primary Transition**	**NOA449280 Confirmatory Transition**	**SYN503780 Primary Transition**	**SYN503780 Confirmatory Transition**
Q1 *m/z*	:	398.0	398.0	278.0	278.0
Q3 *m/z*	:	175.0	137.1	202.0	145.8
Dwell time	:	50 ms	50 ms	50 ms	50 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	-75 V	-75 V	-40 V	-40 V
Entrance potential (EP)	:	-10 V	-10 V	-10 V	-10 V
Collision energy (CE)	:	-38 V	-54 V	-18 V	-26 V
Collision cell exit potential (CXP)	:	-9 V	-8 V	-15 V	-10 V

MRM Conditions		**CSCD656832 Primary Transition**	**CSCD656832 Confirmatory Transition**	**CSCD642512 Primary Transition**	**CSCD642512 Confirmatory Transition**
Q1 *m/z*	:	205.9	205.9	398.0	398.0
Q3 *m/z*	:	142.0	162.0	340.0	137.1
Dwell time	:	50 ms	50 ms	50 ms	50 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	-45 V	-45 V	-75V	-75 V
Entrance potential (EP)	:	-10 V	-10 V	-10 V	-10 V
Collision energy (CE)	:	-31 V	-23 V	-35 V	-54V
Collision cell exit potential (CXP)	:	-9 V	-9 V	-10 V	-8 V

MRM Conditions		**CSCC163768 Primary Transition**	**CSCC163768 Confirmatory Transition**	**CSAA806573 Primary Transition**	**CSAA806573 Confirmatory Transition**
Q1 m/z	:	233.9	233.9	219.9	219.9
Q3 m/z	:	146.0	126.0	156.0	176.0
Dwell time	:	50 ms	50 ms	50 ms	50 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	-35 V	-35 V	-30 V	-30 V
Entrance potential (EP)	:	-10 V	-10 V	-10 V	-10 V
Collision energy (CE)	:	-22 V	-32 V	-25 V	-15 V
Collision cell exit potential (CXP)	:	-10 V	-9 V	-12 V	-12 V

Typical chromatograms are shown in Appendix 2.