

## Summary

This document describes the analytical method for the determination of residues of AE C638206 and its metabolites AE C657188 (PCA), and AE C653711 (BAM) in water.

### Principle of the method:

The parent compound and its both metabolites are extracted from water samples by mechanical shaking with a ethyl acetate acidified.

The analysis is carried out by LC/MS/MS and the quantification by external standardisation.

The limit of quantification (LOQ) of the method for each compound is 0.1 µg/L.

The method was successfully tested on samples from surface and tap water at the limit of quantification as well as ten times this limit.

The method was successfully tested at the limit of detection (LOD) at 0.05 µg/L only on tap water.

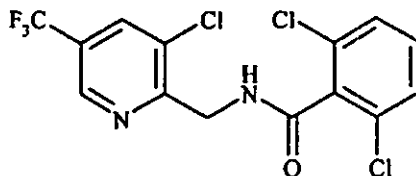
The method AR 307-03 was successfully validated according to the European requirements (96/46/EC of 16<sup>th</sup> July 1996):

- For each compound and at each level :
  - the mean recovery was between 70 % and 110 %
  - the repeatability, expressed as the relative standard deviation (RSD), was lower than 20 %
- For each compound, the overall relative standard deviation (RSD) was lower than 20 %.

## 1. GENERAL CHARACTERISTICS OF THE COMPOUNDS OF INTEREST

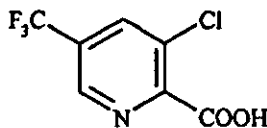
AE C638206 is a fungicide. Its characteristics are given below:

<i>Name or Code :</i>	AE C638206
<i>Chemical name (IUPAC) :</i>	2,6-dichloro-N-[(3-chloro-5-trifluoromethyl-2-pyridyl)methyl]benzamide
<i>Molecular formula :</i>	C <sub>14</sub> H <sub>8</sub> Cl <sub>3</sub> F <sub>3</sub> N <sub>2</sub> O
<i>Molecular weight :</i>	383.59
<i>Monoisotopic mass :</i>	381.97 with 3 <sup>35</sup> Cl
<i>Structure :</i>	



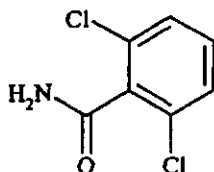
The both metabolites analysed in water are the following :

<i>Name or Code :</i>	AE C657188 (PCA)
<i>Chemical name (IUPAC) :</i>	3-chloro-5-(trifluoromethyl)pyridine-2-carboxylic acid
<i>Molecular formula :</i>	C <sub>7</sub> H <sub>3</sub> ClF <sub>3</sub> NO <sub>2</sub>
<i>Molecular weight :</i>	225.56
<i>Monoisotopic mass :</i>	224.98 with 1 <sup>35</sup> Cl
<i>Structure :</i>	



*Name or Code :* AE C653711 (BAM)  
*Chemical name (IUPAC) :* 2,6-dichlorobenzamide  
*Molecular formula :* C<sub>7</sub>H<sub>5</sub>Cl<sub>2</sub>NO  
*Molecular weight :* 190.0  
*Monoisotopic mass :* 188.98 with 2 <sup>35</sup>Cl

*Structure :*



## 2. ANALYTICAL METHOD PRINCIPLE

The analytical method allows the determination of residues of AE C638206 and its metabolites in water.

The water samples are extracted by shaking with ethyl acetate acidified.

The analysis is carried out by LC/MS/MS.

Quantification is carried out by external standardisation for the parent compound and its metabolites.

## 3. EQUIPMENT AND PRODUCTS

### 3.1. Apparatus

- \* Balance :  
  . accuracy ± 0.01 g (ex Mettler PM 2000)
- \* Sample Concentrator (ex Techne DB-3D)
- \* Diluter (ex Hamilton MicroLab 1000 )
- \* LC/MS/MS system (API 3000)
- \* Mechanical agitator (ex Heidolph REAX 2)

## 3.2. Supplies

### 3.2.1. Reagents and chemicals

Indicated grades of reagents must be respected.

- \* Acetone for organic trace analysis (SupraSolv) (Merck 1.00012)
- \* Acetonitrile for organic trace analysis (SupraSolv) (Merck 1.00017)
- \* Ethyl acetate (Pestipur) (SDS 00.22216)
- \* Formic acid for analysis (Prolabo 20.318.264)
- \* Water for HPLC (Millipore : Milli Q system)

### 3.2.2. Analytical supplies

- \* Inorganic membrane filter Anotop 25 (0.2  $\mu\text{m}$ ) (Whatman 6809-2024)
- \* Disposable Polystyrene pipettes (20mL) (Prolabo 07.903.052)
- \* Glass pipettes (10mL) (OSI A.1247030)
- \* Disposable glass bottles (60 mL) with caps (SDL 2414 and CB28)
- \* Disposable glass evaporation tubes with caps (Merck 11125.01 et 11163.01)
- \* Pipette controller (Digital Powerpette Jencons) (Bioblock A94826)
- \* Pipette controller for spiking (Multipette Eppendorf) (Bioblock 4780)
- \* HPLC columns :

Uptisphere HDO C18 ( UP3 HDO#15QS) for parent compound and BAM :

length :	150	mm
Internal diameter:	2.0	mm
particle size :	3	$\mu\text{m}$

Thermo Hypersil Betabasic-18 for PCA

length :	100	mm
Internal diameter:	2.0	mm
particle size :	3	$\mu\text{m}$

### 3.3. Reagent and chemical solutions preparation

**Dilution mixture used for diluting samples and for preparation of standards (calibration curve) : Water - acetonitrile – formic acid (95: 5:0.1:volume).**

Using a measuring cylinder, prepare the mixture of solvents and add 1 mL of formic acid (for 1L of the mixture).

### 3.4. Preparation of standard solutions

*- Characteristics of analytical standards.*

Compound	Code Number	Batch Number	Origin	Purity g/kg
AE C638206	AE C638206 00 1B99 0002	R001737	ACS	993
AE C657188 (PCA)	AE C657188 00 1B97 0001	RAW244055/1	ACS	972
AE C653711 (BAM)	AE C653711 00 1B97 0001	8808018	ACS	970

#### 3.4.1. Preparation of standard solutions

*- Stock solutions (1000 mg/L)*

Into a 100 mL amber screw-cap flask, weigh accurately between 20 and 50 mg of analytical standard. Using a burette, add a volume of acetonitrile to obtain a stock solution of exactly 1000 mg/L. Mix thoroughly until completely dissolved using a magnetic stirrer. Two separate stock solutions must be prepared.

*- Verification of the preparation of the stock solutions*

Dilute each stock solution in water - acetonitrile - formic acid (95:5:0.1:volume) to obtain a 100 µg/L concentration, analyse the solutions obtained and compare the response, as follows :

$$\left( \frac{\text{solution1} - \text{solution2}}{(\text{solution1} + \text{solution2}) / 2} \right) \times 100\%$$

The results were found to be between ± 10 % (internal acceptable range of variation).

**- Mixture solution (100 mg/L)**

Pipette 10 mL of each stock solution using a class «A<sup>+</sup>» pipette. Pour into a class «A» 100 mL volumetric flask. Adjust volume with acetonitrile, cap and mix by shaking.

**- Fortifying solution (1 mg/L)**

By serial dilutions of the above mixture solution (100 mg/L), prepare the following solution used to fortify control samples : 1 mg/L with acetonitrile.

Note the mixture solution is also used as fortifying solution.

**- Intermediate standard solution (1 mg/L)**

By serial dilutions of the above mixture solution (100 mg/L), prepare the following standard solution used to prepare standards for calibration curve : 1 mg/L with acetonitrile.

**Nota Bene :**

All these solutions must be stored in amber glass bottles at  $\leq 8^{\circ}\text{C}$  when not in use.

**- Standard solutions used for calibration**

By serial dilutions of the intermediate standard solution (1 mg/L), prepare extemporaneously the following standards 0.2, 0.5, 1, 5, 10, 25  $\mu\text{g/L}$  in water - acetonitrile - formic acid (95:5:0.1:volume).

### 3.4.2. Stability of standard solutions

The stock solutions (1000 mg/L), the mixture solution (100 mg/L) and the fortifying solution or intermediate standard solution (1 mg/L) all prepared in acetonitrile and stored at  $\leq 8^{\circ}\text{C}$ , were found to be stable at least for 5 months.

### 3.5. Samples

The method was validated with tap water and surface water (Saône river water samples collected in Lyon - France).

Some characteristics of surface water are recorded in the following table (\*):

<b>date of collect</b>	<b>pH at 27.2 °C</b> (NF T 90-008)	<b>Ca<sup>++</sup> (mg/L)</b>  potentiometric detection	<b>clay particles (mg/L)</b> (NF EN 872)	<b>total organic carbon(TOC) (mg/L)</b> (NF EN 1484)	<b>conductivity (µS/cm)</b> (NF EN ISO 27 888)
07/07/2003	7.4	60	15	3.40	595

These characteristics were determined by :

**Cemagref**  
Division qualité des eaux et prévention des pollutions  
Groupement de Lyon  
3 bis quai Chauveau  
69336 Lyon cedex 09

(\*): The characteristics mentioned in this table were not generated under GLP standards.)



## 4. SAMPLE PREPARATION PROCEDURE

### 4.1. Extraction procedure

- Accurately, transfer  $W = 20.0$  mL of sample into a 60 mL glass bottle.
- Add about 25  $\mu$ L formic acid
- For recoveries, fortify the sample with the appropriate fortifying standard solution.
- Add  $V_{EA} = 20$  mL of ethyl acetate
- Extract for 5 min by mechanical agitation at room temperature.
- After the separation of the phases, transfer an aliquot  $V_A = 10$  mL of the organic phase in a glass evaporation tube
- Evaporate to dryness at around 40 °C under nitrogen gas.
- Add  $V_F = 2$  mL of the mix water-acetonitrile-formic acid (95:5:0.1:volume) and sonicate the final extract.
- The extract is diluted by the same mix of solvent as above (if it is necessary) and transfer into a vial for analysis by LC/MS/MS.

### 4.2. Stability of the final extracts

During the validation, all the final extracts were analysed within 24 hours following their preparation.

The compounds AE C638206, BAM, and PCA in surface and tap water final extracts were found to be stable on the Peltier rack autosampler at around 10 °C for a least for 3 days (see study n°01-23).

**4.3. Schematic analytical procedure for the sample preparation**

20 mL of water + about 25  $\mu$ L formic acid



**Extraction with ethyl acetate**



Agitation



**Sampling of 10 mL ethyl acetate phase**



Evaporation



**LC/MS/MS Analysis**

## Extraction with ethyl acetate

*20.0 mL of water acidified with about 25  $\mu$ L  
formic acid (around 25  $\mu$ L)*

*+*

*20 mL ethyl acetate*



*Manual agitation (around 30 seconds)  
at ambient temperature*



*Sampling 10 mL of ethyl acetate phase (upper layer)*



*Evaporation to dryness at around 40 °C under N<sub>2</sub> stream*



*Add 2 mL of acetonitrile-water-formic acid  
( 5/95/0.1 : volume)*



*Sonication*



**FINAL EXTRACT**

## 5. INSTRUMENTAL ANALYSIS

### 5.1. LC/MS/MS operating conditions

The final extracts were analysed by LC/MS/MS system. The quantitative determination was carried out by external standardisation for AE C638206, AE C653711 (BAM) and AE C657188 (PCA) .

Instrument :	Perkin Elmer API 3000 LC/MS/MS System PE Sciex TurboIon Spray Interface. Hewlett Packard Series 1100 HPLC Pump with column selecteur and Valco valve. Auxiliary HPLC Pump Hewlett Packard series 1100
Ionisation and MS Mode :	TIS (TurboIon Spray) Positive and Negative ion mode MS/MS with multiple reaction monitoring (MRM).
Column :	ambient temperature
Positive mode :	Uptisphere HDO C18, 150 x 2 mm, 3µm particle size
Negative mode :	Thermo Hypersil Betabasic-18, 100 x 2mm, 3µm particle size
Mobile phase flow rate :	0.300 or 0.200 mL/min no split
Mobile phase composition :	
Positive mode :	start gradient : 50 % acetonitrile - 50 % water - 0.1% HCOOH
Negative mode :	isocratique : 10 % acetonitrile - 90 % water
Injection volume :	50 or 100 µL
Retention times :	about 2.5 min for BAM about 7.7 min for AE C638206  and, about 4.3 min for PCA

**All parameters, used in the method, are given in appendices 9 and 10 as a guidance; they were established using the apparatus, the analytical column described above, as well as the samples.**

## 5.2. Calibration

The calibration was carried out by injecting only once each standard solution at 6 levels during each sample set. Standards should be interspersed with samples to compensate for any minor change in instrument response.

The standard solutions bracketed the working range of the final extracts.

The calibration curves were obtained by linear regression weighting  $1/x$  (peak area expressed in counts, versus the concentration expressed in  $\mu\text{g/L}$  with least squares method) and plotted for each compound (See Appendix 2).

The final concentration in  $\mu\text{g/L}$  was determined as follows :

$$C_{\text{final extract}} (\mu\text{g/L}) = \frac{(\text{Peak area (counts)} - b)}{a}$$

The corresponding model to determine the concentration in final extracts was calculated using the Analyst Software (Version 1.3) where :

a : slope coefficient of the calibration curve

b : intercept of the calibration curve

## 5.3. Dilution factor

The dilution factor ( $f_D$ ) is defined as follows :

$$f_D = \frac{\text{Volume after dilution (mL)}}{\text{pipetted volume (mL)}}$$

For sample final extracts fortified at ten times of the limit of quantification, a dilution was not necessary and  $f_D = 1$ .

## 5.4. Calculation method

Each final extract was injected only once using the same conditions as previously described for the standard solutions.

Then, the concentration in  $\mu\text{g/L}$  of the compound was calculated, using the following formula :

$$C_{\text{sample}} (\mu\text{g/L}) = \frac{V_{EA} \times V_f}{W \times V_a} \times C_{\text{final extract}} (\mu\text{g/L})$$

where :

W : volume of the sample (20 mL)

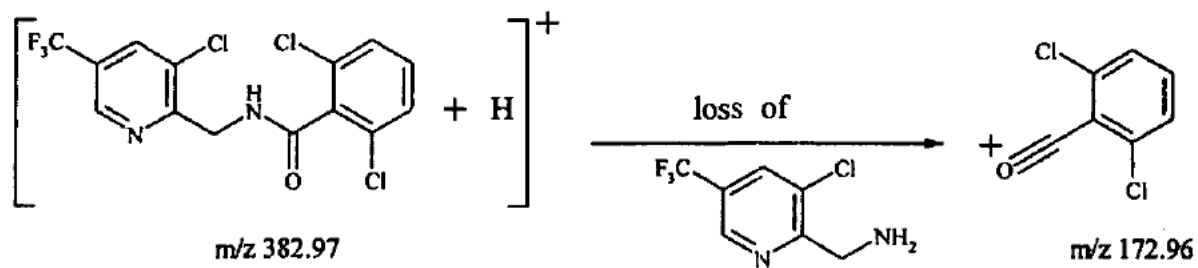
**V<sub>f</sub>** : volume of the final extract (2 mL)  
**V<sub>a</sub>** : volume of the aliquot (10 mL)  
**V<sub>EA</sub>** : volume of ethyl acetate (20 mL)

For each sample, the results are expressed in  $\mu\text{g/L}$  of parent compound and the two metabolites by dividing  $C_{\text{sample}}$  ( $\mu\text{g/L}$ ) by 1000.

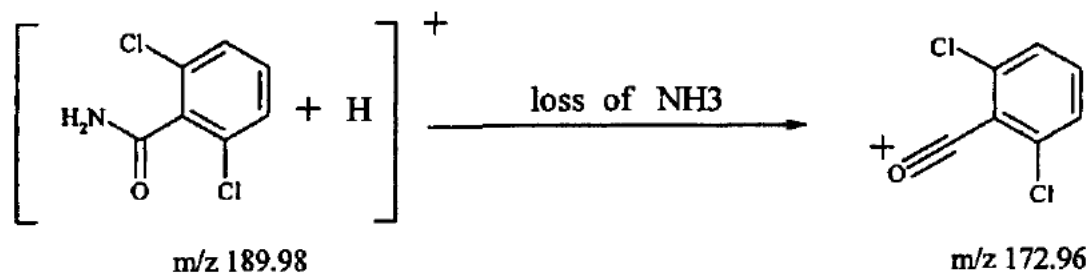
## Appendix 8

### LC/MS/MS fragmentation

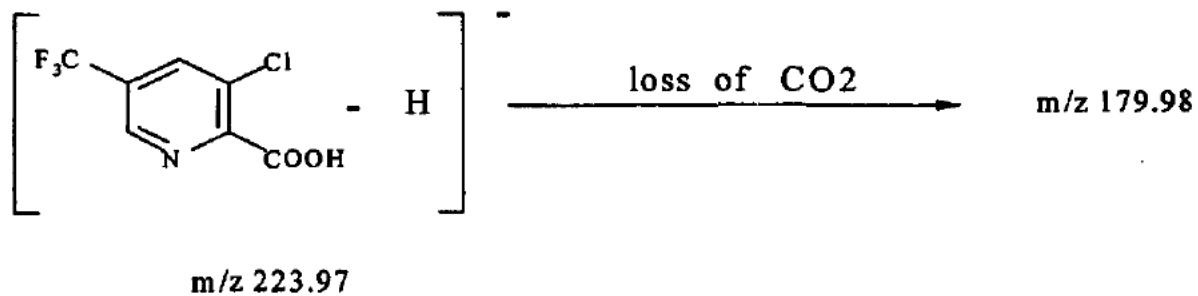
#### AE C638206



#### BAM



#### PCA



## Appendix 9

### Details of parameters used for positive LC/MS/MS mode Run for BAM and AE C638206

Comment: 26Mai03-206 eau-Pression basse-POS  
Synchronization Mode: LC Sync  
Auto-Equilibration: Off  
Acquisition Duration: 9min0sec  
Number Of Scans: 893  
Periods In File: 2  
Acquisition Module: Acquisition Method  
Software version Analyst 1.3.1

^^startperiodPeriod 1:

-----  
Scans in Period: 347  
Relative Start Time: 0.00 msec  
Experiments in Period: 1

^^startexperimentPeriod 1 Experiment 1:

-----  
Scan Type: MRM (MRM)  
Polarity: Positive  
Scan Mode: N/A  
Ion Source: Turbo Spray  
Resolution Q1: UNIT  
Resolution Q3: LOW  
Intensity Thres.: 0.00 cps  
Settling Time: 0.0000 msec  
MR Pause: 5.0000 msec  
MCA: No  
Step Size: 0.00 amu

@Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param	Start	Stop
190.10	173.00	600.00			

Parameter Table(Period 1 Experiment 1):

NEB:	8.00
CUR:	8.00
CAD:	9.00
IS:	5500.00
TEM:	475.00
DP:	41.00
FP:	180.00
EP:	10.00
CE:	25.00
CXP:	12.00

^^endexperiment^^endperiod

^^startperiodPeriod 2:

-----  
Scans in Period: 546  
Relative Start Time: 3.50 min  
Experiments in Period: 1

^^startexperimentPeriod 2 Experiment 1:

-----  
Scan Type: MRM (MRM)  
Polarity: Positive

BCS-D-ROCS/CRLD /0315132

Study n° 03-27 : Method AR 307-03



## Appendix 9 (continuation)

### Details of parameters used for positive LC/MS/MS mode Run for BAM and AE C638206

Scan Mode: N/A  
Ion Source: Turbo Spray  
Resolution Q1: UNIT  
Resolution Q3: UNIT  
Intensity Thres.: 0.00 cps  
Settling Time: 0.0000 msec  
MR Pause: 5.0000 msec  
MCA: No  
Step Size: 0.00 amu

@Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param	Start	Stop
383.00	173.00	600.00			
Parameter Table(Period 2 Experiment 1):					
NEB:		8.00			
CUR:		8.00			
CAD:		9.00			
IS:		5500.00			
TEM:		475.00			
DP		51.00			
FP		275.00			
EP		10.00			
CE		31.00			
CXP		10.00			

^^endexperiment^^endperiod

^^starteditorAgilent 1100 LC Pump Method Properties  
Pump Model: Agilent 1100 LC Binary Pump  
Minimum Pressure (psi): 0.0  
Maximum Pressure (psi): 5801.0  
Dead Volume (µl): 40.0  
Maximum Flow Ramp (ml/min<sup>2</sup>): 100.0  
Maximum Pressure Ramp (psi/sec): 290.0

Step Table:	@Step	Total Time(min)	Flow Rate(µl/min)	A (%)	B (%)
	0	0.00	300	50.0	50.0
	1	3.00	300	40.0	60.0
	2	5.00	300	40.0	60.0
	3	5.20	300	50.0	50.0
	4	9.00	300	50.0	50.0

Left Compressibility: 75.0  
Right Compressibility: 75.0  
Left Dead Volume (µl): 40.0  
Right Dead Volume (µl): 40.0  
Left Stroke Volume (µl): -1.0  
Right Stroke Volume (µl): -1.0  
Left Solvent: A2  
Right Solvent: B1

^^endeditor

^^starteditorAgilent 1100 Autosampler Properties  
Autosampler Model: Agilent 1100 Thermo Autosampler  
Syringe Size (µl): 100

BCS-D-ROCS/CRLD/0315132

Study n° 03-27 : Method AR 307-03

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## Appendix 9 (continuation)

### Details of parameters used for positive LC/MS/MS mode Run for BAM and AE C638206

Injection Volume (µl): 50.00  
Draw Speed (µl/min): 300.0  
Eject Speed (µl/min): 750.0  
Needle Level (mm): 0.00  
Temperature Control Enabled  
Setpoint (4 - 40 C): 10  
Wash is not used

Use Custom Injector Program No

^^endeditor

^^starteditorAgilent 1100 LC Pump Method Properties

Pump Model: Agilent 1100 LC Quaternary Pump

Minimum Pressure (psi): 0.0

Maximum Pressure (psi): 5801.0

Compressibility: 100.0

Dead Volume (µl): 40.0

Stroke Volume (µl): -1.0

Maximum Flow Ramp (ml/min<sup>2</sup>): 100.0

Maximum Pressure Ramp (psi/sec): 290.0

Step Table:

@Step (%)	Total Time (min)	Flow Rate (µl/min)	A (%)	B (%)	C (%)	D
0	0.00	300	0.0	50.0	50.0	0.0
	open open open open					
1	9.00	300	0.0	50.0	50.0	0.0
	open open open open					

^^endeditor

^^starteditorValco Valve Method Properties

Valco Valve Diverter

	Total Time (min)	Position
1	0.0	WASTE
2	1.3	MS/MS
3	9.0	WASTE

## Appendix 9 (continuation)

### Details of parameters used for positive LC/MS/MS mode Run for BAM and AE C638206

Gas pressure		Temperature	
N2	3 bar	Peltier rack	10 °C
Air gas 1	5 bar	Column	ambient
Air gas 2	6 bar		
Air Exhaust gas	3 bar		
<b>Gas 2 Manifold Setting</b>		<b>Valco valve</b>	<b>Column selector</b>
Heated Nebulizer gas	5 bar	C2-0000EP VIC1	C5-0006EMTD
Turbo IonSpray gas	8000 mL/min	Ten ports	6 positions
gas 2 valve	partially opened	2 positions	
<b>Pumps</b>		<b>Source</b>	<b>TurboIonSpray</b>
Binary pump HP1100	2750 psi	Device n°	1164
Flow	0.3 mL/min	Horizontal Position	+ 6
A2 = H2O + 0.1 % HCOOH	50 %	Lateral Position	- 5
B1 = ACN	50 %		
Linear gradient		<b>Mass Spectrometer</b>	
		Device n°	1194
Quaternary pump HP1100	233 psi	<b>Column</b>	
Flow	0.3 mL/min	Precolumn	SMPREAR2
A	-	Column	SMAR 2-1
B = ACN	50 %	Uptisphere HDO C18 150 x 2.0 mm 3µm	
C = H2O + 0.1 % HCOOH	50 %		
D	-		
Gradient mode			

All parameters are given here as a guidance;  
they were established using the apparatus, the analytical column, as well as the samples.

## Appendix 10

### Details of parameters used for negative LC/MS/MS mode Run for PCA

Comment: 26Mai03-Betabasic Isocratic 90-10  
Synchronization Mode: LC Sync  
Auto-Equilibration: Off  
Acquisition Duration: 10min0sec  
Number Of Scans: 997  
Periods In File: 1  
Acquisition Module: Acquisition Method  
Software version: Analyst 1.3.1

^^startperiodPeriod 1:

-----  
Scans in Period: 997  
Relative Start Time: 0.00 msec  
Experiments in Period: 1

^^startexperimentPeriod 1 Experiment 1:

-----  
Scan Type: MRM (MRM)  
Polarity: Negative  
Scan Mode: N/A  
Ion Source: Turbo Spray  
Resolution Q1: UNIT  
Resolution Q3: UNIT  
Intensity Thres.: 0.00 cps  
Settling Time: 0.0000 msec  
MR Pause: 2.0000 msec  
MCA: No  
Step Size: 0.00 amu

@Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param	Start	Stop
224.00	180.00	600.00			

Parameter Table(Period 1 Experiment 1):

NEB:	8.00
CUR:	8.00
IS:	-5500.00
TEM:	475.00
CAD:	9.00
DP -	31.00
FP	-170.00
EP	-10.00
CE	-14.00
CXP	-8.00

^^endexperiment^^endperiod

^^starteditorAgilent 1100 LC Pump Method Properties

Pump Model: Agilent 1100 LC Binary Pump  
Minimum Pressure (psi): 0.0  
Maximum Pressure (psi): 5200.0  
Dead Volume (µl): 40.0  
Maximum Flow Ramp (ml/min<sup>2</sup>): 100.0

## Appendix 10 (continuation)

### Details of parameters used for negative LC/MS/MS mode Run for PCA

Maximum Pressure Ramp (psi/sec): 290.0

Step Table:

@Step	Total Time(min)	Flow Rate(µl/min)	A (%)	B (%)
0	0.00	200	90.0	10.0
1	10.00	200	90.0	10.0

Left Compressibility: 75.0  
Right Compressibility: 75.0  
Left Dead Volume (µl): 40.0  
Right Dead Volume (µl): 40.0  
Left Stroke Volume (µl): -1.0  
Right Stroke Volume (µl): -1.0  
Left Solvent: A1  
Right Solvent: B1

^^endeditor

^^starteditorAgilent 1100 LC Pump Method Properties

Pump Model: Agilent 1100 LC Binary Pump

Minimum Pressure (psi): 0.0  
Maximum Pressure (psi): 5200.0  
Dead Volume (µl): 40.0  
Maximum Flow Ramp (ml/min<sup>2</sup>): 100.0  
Maximum Pressure Ramp (psi/sec): 290.0

Step Table:

@Step	Total Time(min)	Flow Rate(µl/min)	A (%)	B (%)
0	0.00	200	90.0	10.0
1	10.00	200	90.0	10.0

Left Compressibility: 75.0  
Right Compressibility: 75.0  
Left Dead Volume (µl): 40.0  
Right Dead Volume (µl): 40.0  
Left Stroke Volume (µl): -1.0  
Right Stroke Volume (µl): -1.0  
Left Solvent: A1  
Right Solvent: B1

^^endeditor

^^starteditorAgilent 1100 Autosampler Properties

Autosampler Model: Agilent 1100 Thermo Autosampler

Syringe Size (µl): 100  
Injection Volume (µl): 100.00  
Draw Speed (µl/min): 700.0  
Eject Speed (µl/min): 700.0  
Needle Level (mm): 0.00  
Temperature Control Enabled  
Setpoint (4 - 40 C): 10  
Wash Vial Number: 100  
Wash Rack Number: 1

Use Custom Injector Program No

## Appendix 10 (continuation)

### Details of parameters used for negative LC/MS/MS mode Run for PCA

^^endeditor  
^^starteditorAgilent 1100 LC Pump Method Properties  
Pump Model: Agilent 1100 LC Quaternary Pump  
Minimum Pressure (psi): 0.0  
Maximum Pressure (psi): 5200.0  
Compressibility: 100.0  
Dead Volume (µl): 40.0  
Stroke Volume (µl): -1.0  
Maximum Flow Ramp (ml/min<sup>2</sup>): 100.0  
Maximum Pressure Ramp (psi/sec): 290.0

Step Table:

@Step (%)	Total Time (min)	Flow Rate (µl/min)	A (%)	B (%)	C (%)	D
	TE#1 TE#2 TE#3 TE#4					
0	0.00 open open open open	200	90.0	10.0	0.0	0.0
1	10.00 open open open open	200	90.0	10.0	0.0	0.0

^^endeditor  
^^starteditorValco Valve Method Properties  
Valco Valve Diverter

	Total Time (min)	Position
1	0.0	WASTE
2	3.0	MS/MS
3	10.0	WASTE

## Appendix 10 (continuation)

### Details of parameters used for negative LC/MS/MS mode Run for PCA

Gas pressure		Temperature	
N2	3 bar	Peltier rack	10 °C
Air gas 1	5 bar	Column	ambient
Air gas 2	6 bar		
Air Exhaust gas	3 bar		
<b>Gas 2 Manifold Setting</b>		<b>Valco valve</b>	<b>Column selector</b>
Heated Nebulizer gas	5 bar	C2-0000EP V1C1	C5-0006EMTD
Turbo IonSpray gas	8000 mL/min	Ten ports	6 positions
gas 2 valve	partially opened	2 positions	
<b>Pumps</b>		<b>Source</b>	<b>TurboIonSpray</b>
Binary pump HP1100	1956psi	Device n°	1164
Flow	0.2 mL/min	Horizontal Position	+ 6
A1 = H2O	95 %	Lateral Position	- 5
B1 = ACN	5 %		
Isocratic mode		<b>Mass Spectrometer</b>	
		Device n°	1194
Quaternary pump HP1100	148 psi		
Flow	0.2 mL/min	<b>Column</b>	
A = H2O	90 %	Precolumn	SMPREAR2
B = ACN	10 %	Column	SMAR 35-1
C	-	Thermo Hypersil Betabasic-18 100 x 2.0mm 3µ	
D	-		
Isocratic mode			

All parameters are given here as a guidance;  
they were established using the apparatus, the analytical column, as well as the samples.