

Summary

This document describes the analytical method for the determination of residues of AE C638206 and its metabolites AE C657188 (PCA), AE C653711 (BAM), and RPA 427967 (hydroxy) in soil.

Principle of the method:

the parent compound and its three metabolites are extracted from soil samples by mechanical shaking with a mixture of water and acetonitrile 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 0.005 mg/kg

The method was successfully tested on samples from a European site (Senas, France) at the limit of quantification as well as one hundred times this limit.

Note : a few changes have been done only in the positive LC/MS/MS analysis for the modified method (while keeping the same extraction procedure).

The method AR 265-01 was successfully validated according to the European requirements (96/46/EC of 16th 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:

Name or Code : AE C638206

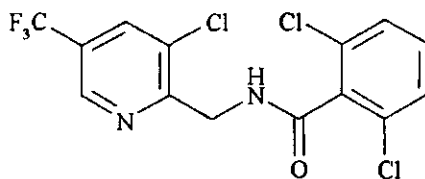
Chemical name (IUPAC) : 2,6-dichloro-N-[(3-chloro-5-trifluoromethyl-2-pyridyl)methyl]benzamide

Molecular formula : $C_{14}H_8Cl_3F_3N_2O$

Molecular weight : 383.59

Monoisotopic mass : 381.97 with 3 ^{35}Cl

Structure :



The three metabolites analysed in soil are the following :

Name or Code : AE C657188 (PCA)

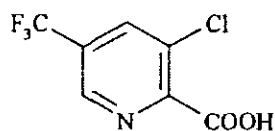
Chemical name (IUPAC) : 3-chloro-5-(trifluoromethyl)pyridine-2-carboxylic acid

Molecular formula : $C_7H_3ClF_3NO_2$

Molecular weight : 225.56

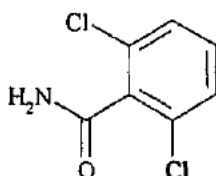
Monoisotopic mass : 224.98 with 1 ^{35}Cl

Structure :



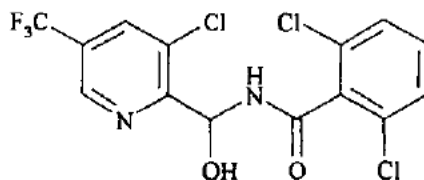
Name or Code : **AE C653711 (BAM)**
Chemical name (IUPAC) : 2,6-dichlorobenzamide
Molecular formula : $C_7H_5Cl_2NO$
Molecular weight : 190.0
Monoisotopic mass : 188.98 with 2 ^{35}Cl

Structure :



Name or Code : **RPA 427967 (hydroxy)**
Chemical name (IUPAC) :
2,6-dichloro-N-[(3-chloro-5-trifluoromethyl-2-pyridyl)(hydroxy)methyl]benzamide
Molecular formula : $C_{14}H_8Cl_3F_3N_2O_2$
Molecular weight : 399.58
Monoisotopic mass : 397.96 with 3 ^{35}Cl

Structure :



2. Analytical method principle

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

The soil samples are extracted by shaking with a mixture of water and acetonitrile 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 \pm 0.1 mg (ex Mettler AT 150)
 - . accuracy \pm 0.01 g (ex Mettler PM 2000)
- * Horizontal shaker (ex IKA Vibrax - VXR)
- * Diluter (ex Hamilton MicroLab 1000)
- * LC/MS/MS system (API 3000)

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)
- * formic acid for analysis (Merck 1.00264)
- * Water for HPLC (Millipore : Milli Q system)

3.2.2. Analytical supplies

- * Anotop filter 25 (0.2 μ m) (Merck 94003 AB)
- * HDPE Nalgene bottles 60 mL (Merck 2104-0002)
- * Common laboratory glassware

All the glassware must be rinsed with acetone and air dried before use.

- * HPLC columns :

Uptisphere HDO C18 for PCA (UP3 HDO#15M)

length :	150	mm
internal diameter :	2.0	mm
Particle size :	3	μ m

Uptisphere ODB C18 for parent compound, BAM and hydroxy (UP3 ODB#15K)

length : 150 mm
internal diameter : 2.0 mm
Particle size : 3 µm

Modified method:

Uptisphere HDO C18 for parent compound, BAM and hydroxy (UP3 HDO#10QS)

length : 100 mm
internal diameter : 2.0 mm
Particle size : 3 µm

3.3. Reagent and chemical solutions preparation

Extraction mixture : *Water - acetonitrile (30 : 70) (v/v) + 0.1% formic acid*

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

Mixture used for adjusting volume after extraction : *Water + 0.1% formic acid*

Add 1 mL of formic acid for 1L of water.

Dilution mixture used for diluting samples and for preparation of standards (calibration curve) : *Water - acetonitrile (70 : 30) (v/v) + 0.1% formic acid*

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 1B99 0002	R001739	ACS	995
AE C653711 (BAM)	AE C653711 00 1B98 0001	R001724	ACS	970
RPA 427967 (hydroxy)	AE 0608000 00 1B96 0001	MOY4232C	ACS	956

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 (70 : 30) (v/v) + 0.1% formic acid to obtain a 100 µg/L concentration, analyse the solutions obtained and compare the response, as follows :

$$\left(\frac{\text{solution 1} - \text{solution 2}}{(\text{solution 1} + \text{solution 2}) / 2} \right) \times 100\%$$

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

- Mixture solution (100 mg/L)

Pipette 10 mL of each stock solution using a class «A⁺» 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 $5\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$ 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 0.4, 0.5, 1, 5, 10, 25, 50, 75, 100 µg/L in water - acetonitrile (70 : 30) (v/v) + 0.1% formic acid.

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 $5\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$, were found to be stable at least for 5 months.

3.5 Samples

The method was validated with one type of soil Senas.
Some characteristics are summarised in the following table :

Soil	Country	References
Senas Untreated 0 - 10 cm	France	PR103CUFR1-0093 217094 Study :103CU n° 01-13
Senas Untreated 0 - 10 cm		PR103CUFR1-0036 217037 Study :103CU n° 01-13

4. Sample preparation procedure

4.1. Extraction procedure

- _ Accurately, weigh $W = 20.0\text{ g}$ of sample into a 60 mL plastic bottle.
- For recoveries, fortify the sample with the appropriate fortifying standard solution.
- Add 50 mL of water - acetonitrile (30 : 70) (v/v) + 0.1% formic acid
- Extract for 5 minutes by mechanical agitation at room temperature using an horizontal shaker setting at 1200 rpm.
- Centrifuge at 3600 rpm for 5 minutes at low temperature (less than 10°C). Note that the temperature is not considered as a critical parameter.
Transfer the supernatant to a 200 mL volumetric flask.
- To the solids remaining, add 50 mL of water - acetonitrile (30 : 70) (v/v) + 0.1% formic acid. Extract for 5 minutes as before.
- Centrifuge a second time as before. Transfer the supernatant to the volumetric flask.
- Make up to 200 mL with water + 0.1% formic acid and homogenize.
- This is the **final extract** ($V_f = 200\text{ mL}$).
- Before analysis by LC/MS/MS, filter this final extract with Anotop 25 - $0.2\text{ }\mu\text{m}$.

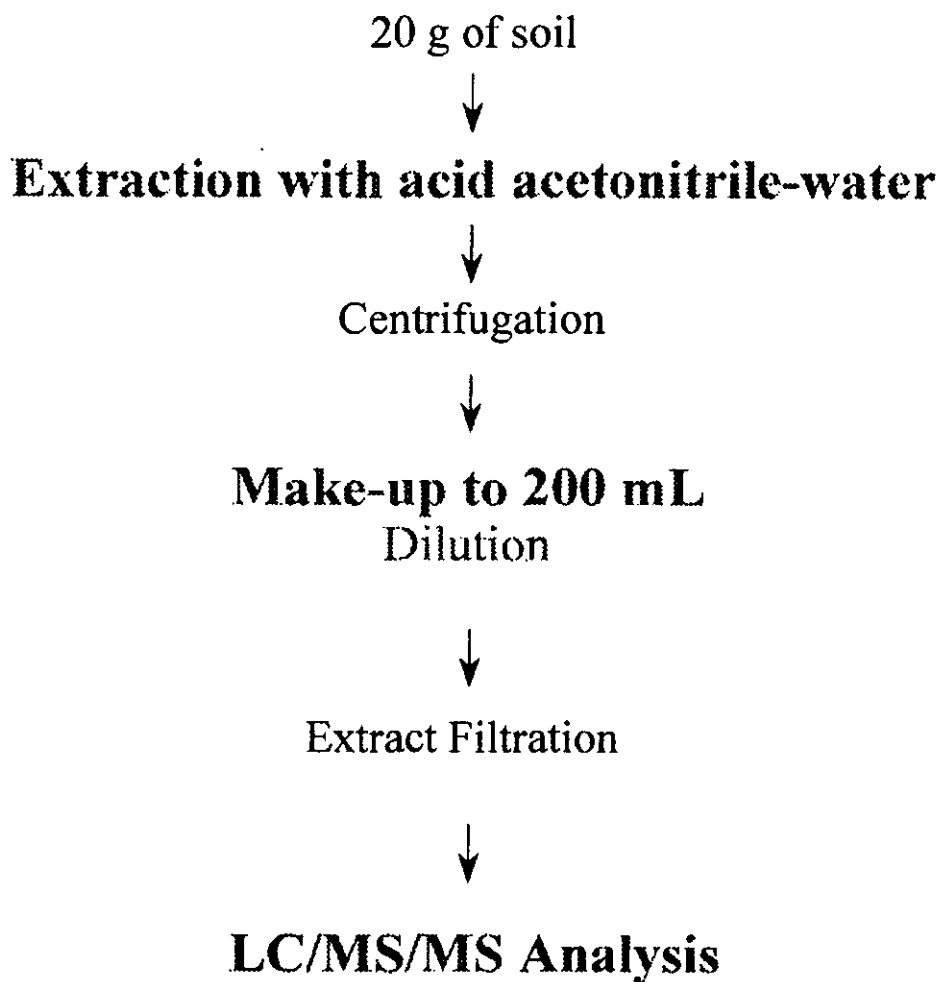
4.2. Stability of the final extracts

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

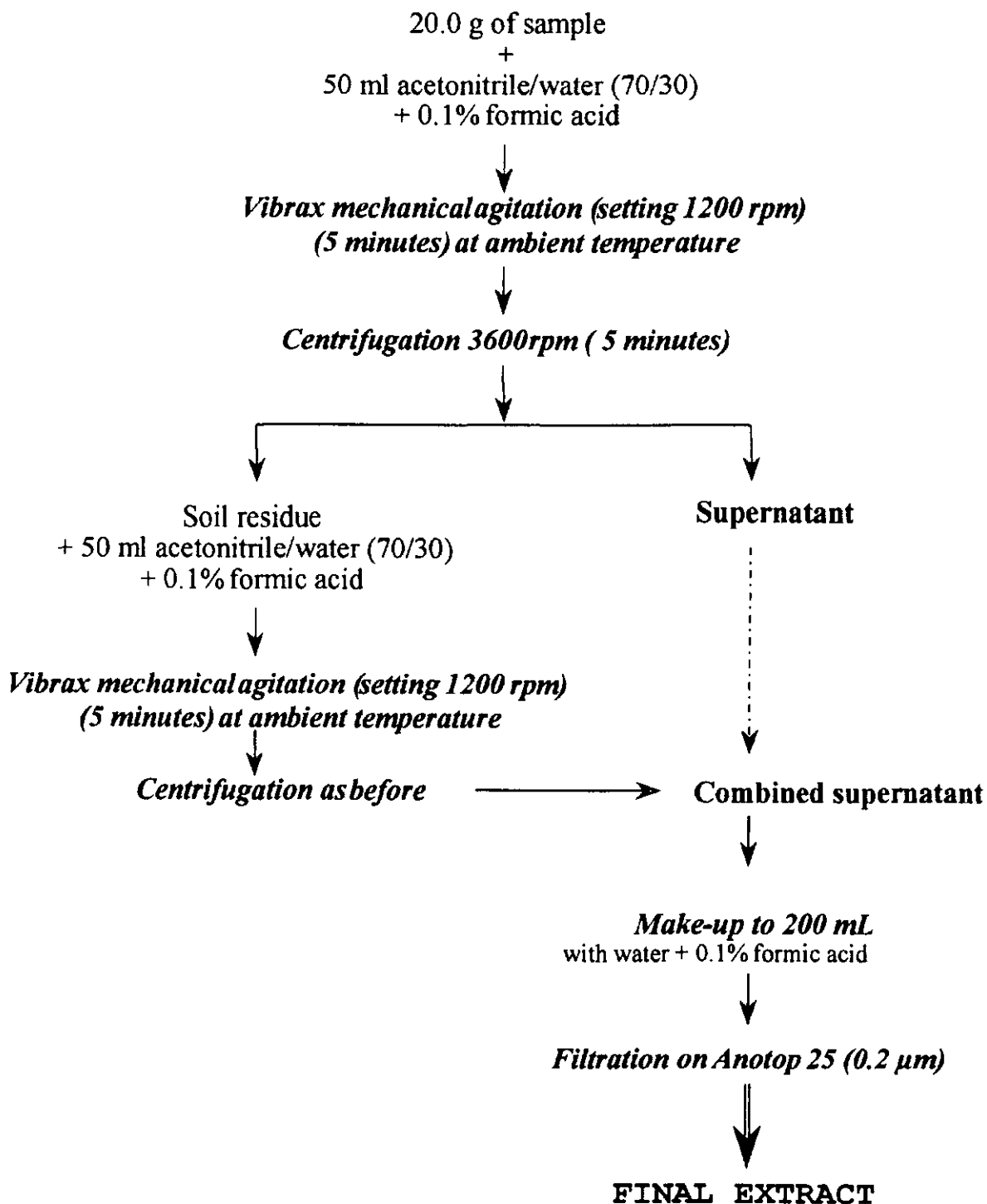
The compound PCA in all soil final extracts was found to be stable on the autosampler Peltier rack at 15 °C for a minimum of 4 days.

The compounds AE C638206, BAM and Hydroxy in all soil final extracts were found to be stable on the autosampler Peltier rack at 15 °C for a minimum of 7 days.

4.3. Schematic analytical procedure for the sample preparation



Extraction with acid mix acetonitrile-water



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), AE C657188 (PCA) and RPA 427967 (Hydroxy).

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 quaternary Hewlett Packard series 1100 Software ANALYST NT Version 1.1
Ionisation and MS Mode :	TIS (TurboIon Spray) Positive and Negative ion mode MS/MS with multiple reaction monitoring (MRM).
Ion Spray Voltage :	5500 V (positive mode for AE C638206, BAM and Hydroxy) - 5500 V (negative mode for PCA)
Orifice Voltage :	41 V (BAM, period 1, positive mode) 20 V (Hydroxy, period 2, experiment 1, positive mode) 51 V (AE C638206, period 2, experiment 2, positive mode) -35 V (PCA, negative mode)
Nebulizer setting (air) :	8 (positive mode) 6 (negative mode)
Curtain Gas Setting (nitrogen) :	8
Auxiliary gas (air) :	gas 2 : 8 L/min, 475 °C
Collision Gas Setting (nitrogen):	9
Collision Energy :	25 V (BAM) 13 V (initial) or 25 V (modified method) (Hydroxy) 31 V (AE C638206) -14 V (PCA)

Mass Transitions (Dwell times in milli seconds) :

Positive mode :	BAM : 190.0/173.0 (600 ms)
	Hydroxy : 399.0/381.0 (400 ms) (initial) or 399.0/173.0 (modified method) (400 ms)
	AE C638206 : 383.0/173.0 (200 ms)
Negative mode :	PCA : 224.0/180.0 (600 ms)
Column :	ambient temperature
Positive mode :	Uptisphere ODB C18, 150 x 2 mm, 3 µm (initial) Uptisphere HDO C18, 100 x 2 mm, 3 µm (modified)
Negative mode :	Uptisphere HDO C18, 150 x 2 mm, 3 µm
Mobile phase flow rate :	0.2 or 0.3 mL/min no split
Mobile phase composition :	
Positive mode :	start gradient : 30 % acetonitrile / 70 % water start gradient : 30 % acetonitrile / 70 % water with 0.1 % HCOOH in water (modified method)
Negative mode :	isocratic : 40 % acetonitrile / 60 % water
Injection volume :	50 or 30 µL
Retention times :	about 2.5 min for BAM about 7.2 min for Hydroxy about 8.1 min for AE C638206 } with modified method about 5.6 min for PCA

Remark : for PCA, we have noticed a retention time variation between different interday batches, but it is stable within intraday batch.

Retention times may vary from those presented above (see Appendix 1).

Note the indicated LC/MS/MS parameters are guidelines and should be optimized for the instrument and column actually used.

Instrument parameters and mobile phase compositions may be adjusted to improve separation from interfering peaks.

That is the reason why some changes have been made within the method.

A summary of different changes is presented below :

Changes	Initial method	Modified method
LC column	ODB C18, 150 x 2 mm, 3µm	HDO C18, 100 x 2 mm, 3µm
Mobile phase	Water	Water + 0.1 % HCOOH
MRM transition	399 > 381	399 > 173

These changes have been done in order to improve recoveries for hydroxy.

All parameters, used in the modified method, are given in appendices 11 and 12 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 5 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 µg/L with least squares method) and plotted for each compound (See Appendix 4).

The final concentration in µg/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 1.1) 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 :

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

A dilution of sample final extracts fortified at the highest level (100LQ = 0.500 mg/kg) concentration was necessary and $f_D = 10$.

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 $\mu\text{g}/\text{kg}$ of the compound was calculated, using the following formula :

$$C_{\text{sample}} (\mu\text{g}/\text{kg}) = \frac{Vf}{W} \times f_D \times C_{\text{final extract}} (\mu\text{g}/\text{L})$$

where :

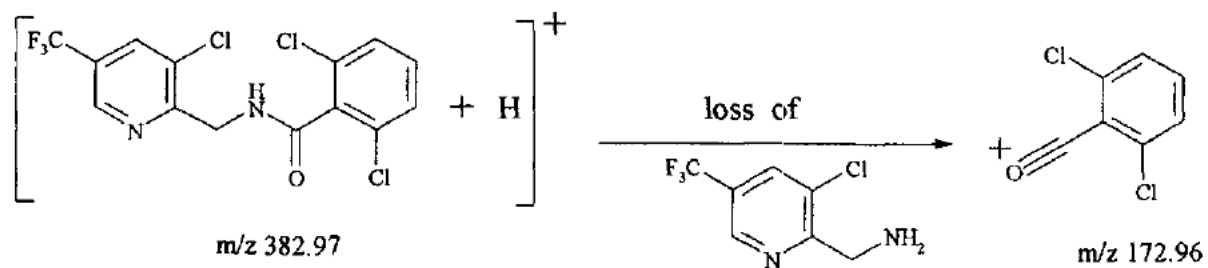
- W** : weight of the sample (20 g)
- Vf** : volume of the final extract (200 mL)
- f_D** : dilution factor

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

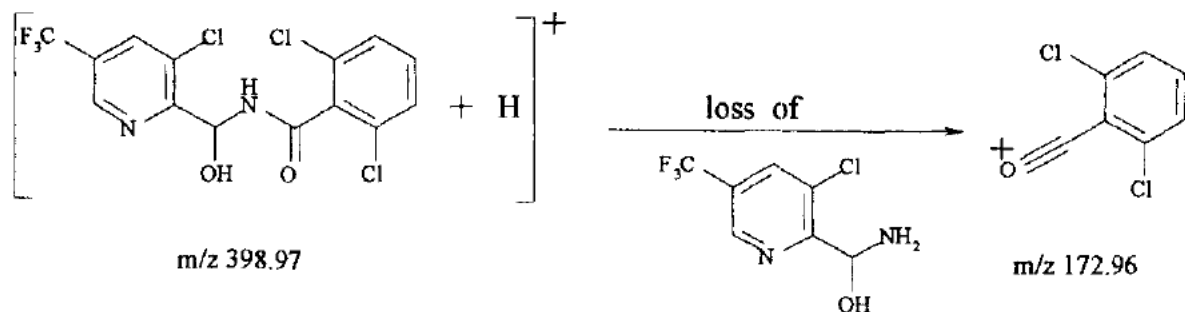
Appendix 10

LC/MS/MS fragmentation

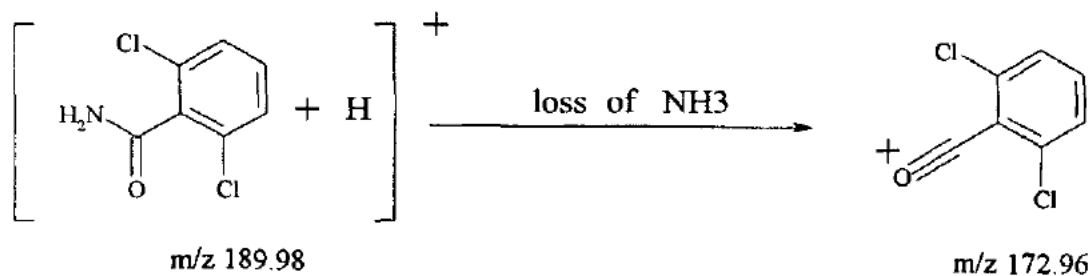
AE C638206



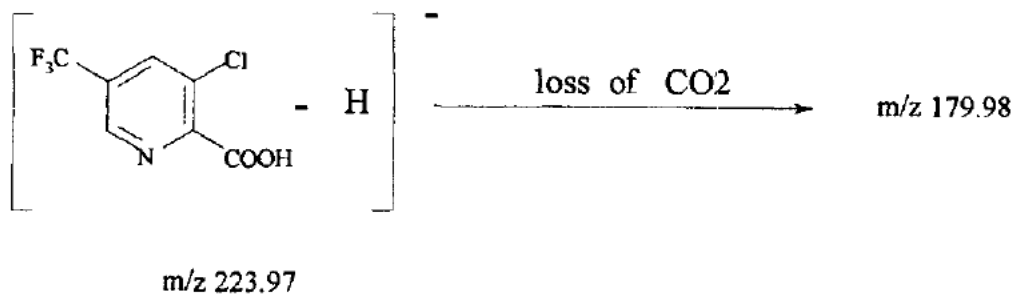
Hydroxy



BAM



PCA



Appendix 11

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

Acquisition Method Properties

Source : TurboIonSpray
Comment:
Duration (min): 13.007
Synchronization Mode: LC Sync
Auto-Equilibration: Off

Mass Spectrometer Method Properties

Period: 1
Duration: 4.003 mins
Cycle Time: 0.602 secs
Cycles: 397
Period Delay: 0.00 secs

Period: 1 Experiment: 1
Scan Mode: None
Scan Type: Positive MRM
Resolution Q1:UNIT
Resolution Q3:LOW
Intensity Thres.: 0.00 cps
Smart Settling: Off
Settling Time: 0.00 ms
MR Pause: 2.00 ms
MCA: No
NEB: 8.00
CUR: 8.00
IS: 5500.00
TEM: 475.00
CAD: 9.00
Step Size: 0.00 amu

Q1 Mass (amu)	Q3 Mass	Time (msec)	Param	Start	Stop
190.00	173.00	600.00	DP	41.00	41.00
BAM			FP	180.00	180.00
			EP	-10.00	-10.00
			CE	25.00	25.00
			CXP	12.00	12.00

Appendix 11 (continued)**Details of parameters used for positive LC/MS/MS mode
Run for BAM, AE C638206 and Hydroxy**

Period: 2
 Duration: 9.004 mins
 Cycle Time: 0.607 secs
 # Cycles: 890
 Period Delay: 0.00 secs

Period: 2 Experiment: 1
 Scan Mode: None
 Scan Type: Positive MRM
 Resolution Q1:UNIT
 Resolution Q3:UNIT
 Intensity Thres.: 0.00 cps
 Smart Settling: Off
 Settling Time:0.00 ms
 MR Pause: 2.00 ms
 MCA: No
 NEB: 8.00
 CUR: 8.00
 IS: 5500.00
 TEM: 475.00
 CAD: 9.00
 Step Size: 0.00 amu

Q1 Mass (amu)	Q3 Mass	Time (msec)	Param	Start	Stop
399.00	173.00	400.00	DP	20.00	20.00
Hydroxy (RPA 427967)			FP	130.00	130.00
			EP	-10.00	-10.00
			CE	25.00	25.00
			CXP	12.00	12.00

Period: 2 Experiment: 2
 Scan Mode: None
 Scan Type: Positive MRM
 Resolution Q1:UNIT
 Resolution Q3:UNIT
 Intensity Thres.: 0.00 cps
 Smart Settling: Off
 Settling Time:0.00 ms
 MR Pause: 5.00 ms
 MCA: No
 NEB: 8.00
 CUR: 8.00
 IS: 5500.00
 TEM: 475.00
 CAD: 9.00
 Step Size: 0.00 amu

Q1 Mass (amu)	Q3 Mass	Time (msec)	Param	Start	Stop
383.00	173.00	200.00	DP	51.00	51.00
AE C638206			FP	275.00	275.00
			EP	-10.00	-10.00
			CE	31.00	31.00
			CXP	10.00	10.00

Appendix 11 (continued)**Details of parameters used for positive LC/MS/MS mode
Run for BAM, AE C638206 and Hydroxy**

Column : SMAR19 :Uptisphere HDO C18 100 x 2mm 3µm

HP 1100 LC Pump Method Properties

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 Water + 0.1 % HCOOH
 Right Solvent: B1 ACN

Total Time min)	Flow Rate µl/min)	A (%)	B (%)
0	0.0	300.0	70.0 30.0
1	2.0	300.0	55.0 45.0
2	2.2	300.0	30.0 70.0
3	6.2	300.0	30.0 70.0
4	6.3	300.0	70.0 30.0
5	13.0	300.0	70.0 30.0

HP 1100 LC Pump Method Properties

Compressibility: 75.0
 Dead Volume (µl): 40.0
 Stroke Volume (µl): -1.0

Total Time min)	Flow Rate µl/min)	A (%)	B (%)	C (%)	D (%)
0	0.0	200.0	0.0	50.0	50.0 0.0
1	13.0	200.0	0.0	50.0	50.0 0.0

B = ACN
 C = Water + 0.1 % HCOOH

HP 1100 Autosampler Properties

Syringe Size (µl): 100
 Injection Volume (µl): 50.00
 Sample Speed (µl/min): 300
 Needle Level (mm): 0.00
 Wash Vial Number: 100
 Wash Rack Number: 1

Valco Valve Method Properties
Valco Valve Diverter

Total Time (min)	
1	0.0 waste
2	1.0 MS
3	4.0 waste
4	6.2 MS
5	9.0 waste

Appendix 11 (continued)**Details of parameters used for positive LC/MS/MS mode
Run for BAM, AE C638206 and Hydroxy**

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		
Gas 2 Manifold Setting		Valco valve	Column selector
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	
Pumps		Source	TurboIonSpray
Binary pump HP1100	2770 psi	Device n°	1164
Flow	0.3 mL/min	Horizontal Position	+ 6
A2 = H2O + 0.1 % HCOOH	70 %	Lateral Position	- 7
B1 = ACN	30 %		
Linear gradient		Mass Spectrometer	
		Device n°	1194
Quaternary pump HP1100	105 psi		
Flow	0.2 mL/min	Column	
A	-	Precolumn	SMPREAR2
B = ACN	50 %	Column	SMAR 19-1
C = H2O + 0.1 % HCOOH	50 %	Uptisphere HDO C18 100 x 2.0 mm 3µm	
D	-		
Isocratic mode			

Nota Bene : all these parameters correspond to the modified method.

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

Appendix 12

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

Acquisition Method Properties

Source : TurboIonSpray
Comment:
Duration (min): 6.000
Synchronization Mode: LC Sync
Auto-Equilibration: Off

Mass Spectrometer Method Properties

Period: 1
Duration: 6.000 mins
Cycle Time: 0.602 secs
Cycles: 598
Period Delay: 0.00 secs

Period: 1 Experiment: 1
Scan Mode: None
Scan Type: Negative MRM
Resolution Q1:UNIT
Resolution Q3:UNIT
Intensity Thres.: 0.00 cps
Smart Settling: Off
Settling Time: 0.00 ms
MR Pause: 2.00 ms
MCA: No
NEB: 6.00
CUR: 8.00
IS: -5500.00
TEM: 475.00
CAD: 9.00
Step Size: 0.00 amu

Q1 Mass (amu)	Q3 Mass	Time (msec)	Param	Start	Stop
224.00	180.00	600.00	DP	-31.00	-31.00
			FP	-170.00	-170.00
			EP	10.00	10.00
			CE	-14.00	-14.00
			CXP	-8.00	-8.00

Appendix 12 (continued)**Details of parameters used for negative LC/MS/MS mode
Run for PCA**

Column : SMAR2 :Uptisphere HDO C18 150 x 2mm 3µm

HP 1100 LC Pump Method Properties

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 WATER
 Right Solvent: B1 ACN

Total Time (min)	Flow Rate (µl/min)	A (%)	B (%)
0	0.0	300.0	60.0 40.0
1	6.0	300.0	60.0 40.0

HP 1100 Autosampler Properties

Syringe Size (µl): 100
 Injection Volume (µl): 30.00
 Sample Speed (µl/min): 300
 Needle Level (mm): 0.00
 Wash Vial Number: 100
 Wash Rack Number: 1

HP 1100 LC Pump Method Properties

Compressibility: 75.0
 Dead Volume (µl): 40.0
 Stroke Volume (µl): -1.0

Total Time (min)	Flow Rate (µl/min)	A (%)	B (%)	C (%)	D (%)
A = WATER B = ACN					
0	0.0	300.0	60.0 40.0	0.0	0.0
1	6.0	300.0	60.0 40.0	0.0	0.0

Valco Valve Method Properties
 Valco Valve Diverter

Total Time (min)
 1 0.0 waste
 2 2.7 MS
 3 5.5 waste

Appendix 12 (continued)**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		
Gas 2 Manifold Setting		Valco valve	Column selector
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	
Pumps		Source	TurboIonSpray
Binary pump HP1100	3510 psi	Device n°	1164
Flow	0.3 mL/min	Horizontal Position	+ 6
A1 = H2O	60 %	Lateral Position	- 7
B1 = ACN	40 %		
Isocratic mode		Mass Spectrometer	
		Device n°	1194
Quaternary pump HP1100	190 psi	Column	
Flow	0.3 mL/min	Precolumn	SMPREAR2
A = H2O	60 %	Column	SMAR 19-1
B = ACN	40 %	Uptisphere HDO C18 150 x 2.0 mm 3µm	
C	-		
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.