

1. **PRINCIPLE**

This method is based on M 3441. CL 375839 and CL 377160 residues are extracted from soil specimens using triethylamine/water/acetonitrile, 0.5/9.5/90 (v/v/v). After filtration the samples are cleaned by solid-phase extraction using C18 cartridges. Measurement is accomplished using liquid chromatography/APCI mass spectrometry (LC/MS). Results are calculated by the direct comparison of peak areas to those of external bracketing standards.

2. **VALIDATION**

Method has been validated for soil in Study 99305V and results documented in Report RLG 4603.

3. **APPARATUS**

Alternative equipment may be used if proven to be functionally equivalent.

3.1 **Assorted Glassware**

General Laboratory

3.2 **Balance**

- a. Mettler AE163 capable of weighing to the nearest 0.1 mg.
- b. Mettler PC 2000 capable of weighing to the nearest 0.01 g.

3.3 **High Performance Liquid Chromatograph/APCI Mass Selective Detector**

Hewlett Packard 1100 series HPLC attached to Finnigan Navigator LC/MS detector.

3.4 **HPLC Column**

Phenomenex-Ultracarb 5 ODS (30) 100x4.6 mm

3.5 **Solid Phase Extraction**

- a. Vac. Blot processing station.
- b. Isolute C18 (1g/6 ml)

3.6 **TECHNE Sample Concentrator**

Maintained at approximately 60°C.

3.7 Ultra-Turrax

Overhead homogeniser with 250 ml jar.

3.8 Microwave Extraction System

- a. Model MES-1000, CEM Corp.
- b. Extraction vessels and assembly.

4. REAGENTS

Precautions are to be taken to restrict exposure to all chemicals used in this method. The proper use of appropriate safety glasses/goggles, gloves, laboratory coat, ventilation and handling techniques are to be observed.

Appropriate material safety data sheets (MSDS) are to be reviewed prior to using this method.

4.1 Analytical Standards

Analytical grade, known purity, obtained from BASF Corporation, BASF Agro Research, PO Box 400, Princeton, New Jersey 08543-0400, USA.

- a. CL 375839 : 5-bromo-6,6'-dimethyl-2,2',3',4'-tetramethoxy-benzophenone.
- b. CL 377160: (3-bromo-6-methoxy-2-methylphenyl)(3'-hydroxy-2',4'-dimethoxy-6'-methylphenyl)-methanone.

Read the current version of COSHH 24 and COSHH 27 before commencing work with this material.

4.2 Solvents

Pesticide grade unless otherwise stated.

- a. Acetonitrile
- b. Methanol
- c. Dichloromethane

4.3 Chemicals

- a. Milli-Q or Analar water
- b. Acetic Acid (100%)
- c. Triethylamine

5. SOLUTIONS

5.1 Extraction Solution

Add 0.5 ml of triethylamine to 90 ml of acetonitrile and 9.5 ml of Milli-Q water. Mix well.

5.2 0.5/99.5 (v/v) Acetic Acid/Water

Add 0.5 ml of acetic acid to 99.5 ml of Milli-Q water and mix well.

5.3 10/90 (v/v) Methanol/Dichloromethane

Add 10 ml of methanol to 90 ml of dichloromethane and mix well.

5.4 50/50 (v/v) Methanol/Water

Add 50 ml of methanol to 50 ml of Milli-Q water and mix well.

5.5 Mobile Phase A

99/1 (v/v) methanol/acetic acid.

5.6 Mobile Phase B

99/1 (v/v) Milli-Q water/acetic acid.

6. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/MASS SELECTIVE DETECTOR CONDITIONS

The HPLC conditions detailed are given as a baseline for establishing the actual operating conditions used for the quantitation of CL 375839 and CL 377160 residues. These conditions may be adjusted to achieve the response (peak shape and retention time) equal to or better than that shown.

- | | | |
|-----|---------------|---|
| 6.1 | <u>HPLC</u> | Hewlett Packard HP series 1100 equipped with degasser, binary pump, autosampler and column compartment. |
| 6.2 | <u>Column</u> | Phenomenex-Ultracarb 5 ODS (30) 100x4.6 mm |

6.3 HPLC Conditions

Injection volume 100 µl
Flow rate 0.5 ml/min
Run time 25 min

Mobile Phase Gradient:

Gradient time (min)	% Mobile Phase A	% Mobile Phase B
0.00	75	25
11.00	75	25
14.00	100	0
20.00	100	0
20.50	75	25

Column Temperature Ambient

6.4 Mass Spectrometer ThermoQuest aQa-Navigator MS

6.5 MS Conditions for Analysis of CL 375839 and CL 377160

Ionization mode: APCI
Polarity: Positive

Corona voltage (V): 3.5
Source voltage (V): 20
Probe temperature (°C): 300
Rf lens voltage (V): 0.2
Ion energy (eV): 1.1
Ion energy ramp (mV/amu): 0
Detector voltage (V): 1000
Low mass resolution: 12.5
High mass resolution: 12.5

Acquisition type: SIM
SIM settings: Retention window 1 0-11.5 min

Mass	Dwell time	Mass span
395.0	1 sec	0.1
397.0	1 sec	0.1

Retention window 2 11.5-15.0 min

Mass	Dwell time	Mass span
411.0	1 sec	0.1

7. PREPARATION OF STANDARD SOLUTIONS

7.1 Precautions

- a. The standard material should be weighed out according to the procedures detailed in the current version of AGF 00100.01.
- b. Ensure that solvents are at room temperature before use.
- c. Volumetric glassware previously used for 500 µg/ml solutions (or greater) should not be used for solutions for lower concentrations.
- d. Volumetric glassware should be thoroughly washed with acetone after use prior to washing up.

7.2 CL 375839 Standard

- a. Accurately weigh a known amount (approximately 10 mg ± 0.1) of CL 375839 into a 20 ml volumetric flask. Dilute to the mark with acetonitrile and mix well. Calculate and record the concentration of CL 375839, correcting for the standard purity. This solution contains nominally 500 µg/ml.
- b. Pipette 2 ml of the 500 µg/ml standard solution into a 100 ml volumetric flask. Dilute to the mark with acetonitrile and mix well. This solution contains nominally 10 µg/ml.

7.3 CL 377160 Standard

- a. Accurately weigh a known amount (approximately 10 mg ± 0.1) of CL 377160 into a 20 ml volumetric flask. Dilute to the mark with acetonitrile and mix well. Calculate and record the concentration of CL 377160, correcting for the standard purity. This solution contains nominally 500 µg/ml.
- b. Pipette 2 ml of the 500 µg/ml standard solution into a 100 ml volumetric flask. Dilute to the mark with acetonitrile and mix well. This solution contains nominally 10 µg/ml.

7.4 Mixed CL 375839 and CL 377160 Standard Solutions for Fortification of Samples

- a. 10.0 µg/ml from Sections 7.2 and 7.3.

- b. Pipette 1 ml of each 10 µg/ml standard solution into a 10 ml volumetric flask. Dilute to the mark with acetonitrile and mix well. This solution contains nominally 1.0 µg/ml CL 375839 and CL 377160.
- c. Pipette 1 ml of each 10 µg/ml standard solution into a 100-ml volumetric flask. Dilute to the mark with acetonitrile and mix well. This solution contains nominally 0.1 µg/ml CL 375839 and CL 377160.

7.5 Mixed CL 375839 and CL 377160 Standard Solutions for Linearity

- a. Pipette 1 ml of each 10 µg/ml standard solution into a 20 ml volumetric flask. Dilute to the mark with 50/50 (v/v) methanol:water and mix well. This solution contains nominally 0.5 µg/ml.
- b. Pipette 1 ml of the 0.5 µg/ml mixed standard solution into a 10 ml volumetric flask. Dilute to the mark with 50/50 (v/v) methanol:water and mix well. This solution contains nominally 0.05 µg/ml.
- c. Pipette 1 ml of the 0.5 µg/ml mixed standard solution into a 20 ml volumetric flask. Dilute to the mark with 50/50 (v/v) methanol:water and mix well. This solution contains nominally 0.025 µg/ml.
- d. Pipette 1 ml of the 0.5 µg/ml mixed standard solution into a 50 ml volumetric flask. Dilute to the mark with 50/50 (v/v) methanol:water and mix well. This solution contains nominally 0.01 µg/ml.
- e. Pipette 1 ml of the 0.5 µg/ml mixed standard solution into a 100 ml volumetric flask. Dilute to the mark with 50/50 (v/v) methanol:water and mix well. This solution contains nominally 0.005 µg/ml.
- f. Pipette 5 ml of the 0.005 µg/ml mixed standard solution into a 10 ml volumetric flask. Dilute to the mark with 50/50 (v/v) methanol:water and mix well. This solution contains nominally 0.0025 µg/ml.

The Working Standard will be the 0.005 µg/ml standard solution.

Note: Preparation of these standard solutions may be achieved by use of alternative dilutions.

8. LINEARITY CHECK

- 8.1 Inject 100 µl aliquots of standard solutions.
- 8.2 Inject aliquots of 0.05, 0.025, 0.01, 0.005 and 0.0025 µg/ml standards.

- 8.3 Plot peak areas obtained against standard concentration to demonstrate a linear response over the range. Significant departure from linearity indicates operational problems which must be corrected before proceeding.

9. SAMPLE PREPARATION

- 9.1 Maintain all samples frozen until ready for analysis.
- 9.2 Allow frozen samples to thaw completely in an air-tight container just prior to extraction.
- 9.3 Thoroughly mix thawed samples to obtain a homogenous sample.

10. RECOVERY TEST

The ability of the instrumentation and operator to perform the procedures satisfactorily should always be demonstrated by recovery tests before analysis of unknown samples is attempted.

- 10.1 Weigh 5g (± 0.1 g) of the specimen into a microwave extraction vessel.
- 10.2 Add by pipette, a volume of standard fortification solution appropriate to the fortification level to be tested (for a 0.02 mg/kg fortification add 1 ml of the 0.1 μ g/ml standard to 5g of specimen).
- 10.3 Mix the sample and allow to stand for 15 minutes.
- 10.4 Continue with extraction and cleanup steps.

11. EXTRACTION

- 11.1 Weigh 5g of soil sample into a microwave extraction vessel.
- 11.2 Add 25 ml of extraction solvent to the soil and stir with a spatula to mix. Place the extraction vessel in the microwave extraction system. Use the following conditions for extraction:
- | | |
|----------------------------|--|
| a. Power | 20% (for 2 samples or fewer)
40% (for 4 samples)
60% (for 6 samples) etc |
| b. PSI | 50 |
| c. TAP (Time at Parameter) | 3 min |
| d. Temperature | 125°C |

Note: The microwave solvent extraction system is extremely sensitive to solvent vapour and will be temporarily disabled if vapours are detected. The rupture disk in the extraction vessel top must not be torn and all connections must be tight before starting the extraction.

- 11.3 After the extraction is completed, open the microwave door and let extraction vessel cool to about 45°C before disconnecting and removing the vessels from the microwave.
- 11.4 After being removed from the microwave, allow the extraction vessel to reach approximately room temperature. Gently uncap the extraction vessel so as not to disturb the soil bed and carefully transfer a 5 ml aliquot of the extract into a 50 ml beaker.
- 11.5 Add 5 ml of 0.5% acetic acid in Milli-Q water to the extract in the beaker and swirl to mix.

12. SOLID PHASE EXTRACTION (SPE)

- 12.1 Using a Vac Elut sample processing station, prepare a C18 cartridge (1g/6 ml) by washing with one column volume each, in this order, of dichloromethane, methanol and Milli-Q water. (Do not allow the SPE column to go dry during any of the loading or washing steps).
- 12.2 Add the solution from Step 11.5 to the cartridge and load the sample at a flow rate of approximately 2-3 drops per second. Discard the eluate.
- 12.3 Wash the 50 ml beaker with 5 ml of Milli-Q water and pass through the cartridge. Once the rinsing is completed, turn the vacuum to the maximum for approximately 1 minute to dry the cartridge.
- 12.4 Elute the C18 cartridge with 5 ml of 10/90 (v/v) methanol in dichloromethane, collecting the eluant in a scintillation vial. Evaporate the eluant to dryness using the Techne sample/concentrator with a nitrogen stream and temperature setting at about 60°C. Remove the vial as soon as it dries.
- 12.5 **Immediately** add 2 ml of methanol to the scintillation vial, cap tightly and vortex for 10 seconds. Place the scintillation vial back on the heating surface (60°C) of the Techne sample/concentrator for 15 minutes, vortex for 10 seconds and let cool to room temperature. This procedure is required to ensure that the residues are completely dissolved.
- 12.6 Add 2 ml of Milli-Q water to the vial, cap and vortex for 10 seconds. Transfer an aliquot into a HPLC vial for LC/MS analysis.

13. LC/MS ANALYSIS

- 13.1 After obtaining a stable chromatographic response inject in sequence a 100 µl aliquot of the working standard (0.005 µg/ml), 100 µl aliquot of a sample and another 100µl aliquot of the working standard.
- 13.2 Compare the peak response or area of the sample with those of the 0.005 µg/ml standards injected before and after the sample (bracketing standards).
- 13.3 If the sample peak exceeds that of the linearity standards, dilute to an appropriate volume and re-inject.
- 13.4 Make a standard injection after every sample and use the average peak height of the standard injection before and after the sample injections for quantitation.

14. CALCULATIONS

For each sample calculation, use the sample peak area and the average peak area measurement of the working standard obtained before and after the sample injections as follows:

To calculate residue as µg in final reconstitution volume:

$$R (\text{Residue in final volume } \mu\text{g}) = \frac{A(\text{SAMP}) \times V \times C(\text{STD})}{A(\text{STD})}$$

To calculate the residue in mg/kg:

$$\text{mg/kg} = R \times \frac{V1}{V2} \times \frac{1}{W}$$

To calculate the % recovery:

$$\% \text{ RECOVERY} = \frac{R(\text{FOUND})}{FV \times FC} \times \frac{V1}{V2} \times 100$$

Where:

- A(SAMP) = Peak area of sample
- A(STD) = Average peak area of bracketing, working standard
- C(STD) = Concentration of working standard (0.005 µg/ml)
- V = Reconstitution volume of final specimen for analysis
- V1 = Volume of extraction solvent in millilitres (25 ml)
- V2 = Aliquot of extract taken for analysis in millilitres (5.0 ml)
- W = Sample weight (5.0g)
- FV = Fortification volume in millilitres
- FC = Fortification concentration (of standard solution added) in micrograms per millilitre.

Standard Operating Procedure

Department: Residues Analysis

SOP No: RLA 12636.00

Supersedes: New

Title: Method to confirm the Specificity of Analytical Procedure RLA 12618 (Determination of CL 375839 [BAS 560F] and CL 377160 Residues in Soil using LC-MS)

Signatures:

Author: K Richardson
K Richardson, Principal Scientist

3 July 01
Date

Approval: G Taylor
G Taylor, Manager, Residues Analysis

3rd July 01
Date

Review Summary

Review Requirement: Prior to use in a new study

Review Date	Action Taken	Re-Issue No

1. **PRINCIPLE**

This is a qualitative method, which is used to confirm the specificity of method RLA 12618.

The specificity is determined using an alternate detection method (LC/MS/MS).

The procedures for specimen preparation, extraction and cleanup are the same as those described in RLA 12618.

CL 375839 (BAS 560F) and CL 377160 residues are extracted from soil specimens using triethylamine/water/acetonitrile, 0.5/9.5/90 (v/v/v). After filtration the samples are cleaned by solid-phase extraction using C18 cartridges.

The current version of method RLA 12618 should be used to quantitatively measure CL 375839 and CL 377160 residues in soil to an LOQ of 0.02mg/kg.

2. **VALIDATION**

Method has not been validated for the quantitative measurement of CL 375839 and CL 377160 residues in soil. The procedure is tested using authentic reference chemicals.

3. **APPARATUS**

Alternative equipment may be used if proven to be functionally equivalent.

3.1 **General Laboratory Glassware**

- a. Assorted beakers, graduated cylinders, volumetric flasks and volumetric pipettes.
- b. Glass vials.

3.2 **Balance**

- a. Mettler AE163 capable of weighing to the nearest 0.1 mg.
- b. Mettler PC 2000 capable of weighing to the nearest 0.01 g.

3.3 **LC/MS/MS**

Jasco pumps (PU-980) and Jasco autosampler (AS-950) attached to MicroMass Quattro triple quadrupole mass spectrometer

3.4 HPLC Column

Phenomenex-Ultracarb 5 ODS (30) 100x4.6 mm

3.5 Solid Phase Extraction

- a. Vac Elut processing station.
- b. Isolute C18 (1g/6 mL)

3.6 TECHNE Sample Concentrator

Maintained at approximately 60°C.

3.7 Ultra-Turrax

Overhead homogeniser with 250 mL jar.

3.8 Microwave Extraction System

- a. Model MES-1000, CEM Corp.
- b. Extraction vessels and assembly.

4. REAGENTS

Precautions are to be taken to restrict exposure to all chemicals used in this method. The proper use of appropriate safety glasses/goggles, gloves, laboratory coat, ventilation and handling techniques are to be observed.

Appropriate material safety data sheets (MSDS) are to be reviewed prior to using this method.

4.1 Analytical Standards

Analytical grade, known purity, obtained from BASF Corporation, BASF Agro Research, PO Box 400, Princeton, New Jersey 08543-0400, USA.

- a. CL 375839: 5-bromo-6,6'-dimethyl-2,2',3',4'-tetramethoxy-benzophenone.
- b. CL 377160: (3-bromo-6-methoxy-2-methylphenyl)(3'-hydroxy-2',4'-dimethoxy-6'-methylphenyl)-methanone.

Read the current version of COSHH 24 and COSHH 27 before commencing work with this material.

4.2 Solvents

Pesticide grade unless otherwise stated.

- a. Acetonitrile
- b. Methanol
- c. Dichloromethane

4.3 Chemicals

- a. Milli-Q or Analar water
- b. Acetic Acid (100%)
- c. Triethylamine

5. SOLUTIONS

5.1 Extraction Solution

Add 0.5 mL of triethylamine to 90 mL of acetonitrile and 9.5 mL of Milli-Q water. Mix well.

5.2 0.5/99.5 (v/v) Acetic Acid/Water

Add 0.5 mL of acetic acid to 99.5 mL of Milli-Q water and mix well.

5.3 10/90 (v/v) Methanol/Dichloromethane

Add 10 mL of methanol to 90 mL of dichloromethane and mix well.

5.4 50/50 (v/v) Methanol/Water

Add 50 mL of methanol to 50 mL of Milli-Q water and mix well.

5.5 Mobile Phase A

99/1 (v/v) Methanol/acetic acid.

5.6 Mobile Phase B

99/1 (v/v) Milli-Q water/acetic acid.

6. LC/MS/MS CONDITIONS

The conditions detailed are given as a baseline for establishing the actual operating conditions used for the measurement of CL-375839 and CL-377160. These conditions may be adjusted to optimise both the chromatography and response of the mass spectrometer.

HPLC : Jasco pumps (PU-980) and Jasco autosampler (AS-950).

Column: Phenomenex-Ultracarb 5 ODS (30) 100x4.6 mm

HPLC Conditions:

Injection volume 100 µl
 Flow rate 0.5 mL/min (split approximately 5:1, waste: MS)
 Run time 25 min

Mobile Phase Gradient:

Gradient time (min)	% Mobile Phase A	% Mobile Phase B
0.00	75	25
11.00	75	25
14.00	100	0
20.00	100	0
21.00	75	25

Column Temperature: Ambient

Mass Spectrometer: MicroMass Quattro triple quadrupole

MS Conditions for Analysis of CL-375839 and CL-377160:

Ionization mode: Electrospray
 Polarity: Positive
 Capillary voltage: 3.97
 HV lens: 0.84
 Cone voltage: 17
 Lens 3: 20
 Source temperature (°C): 150
 Energy Filter: 1.1
 Lens 5: 333
 Lens 6: 9
 Ion energy: MS1: 3.1 MS2: 5.4
 Collision energy: 16

Lens 7: 237
 Lens 8: 47
 Lens 9: 19
 Gas Cell: 1.3×10^3
 Multiplier: MS1: 788 MS2: 755
 Low mass resolution: MS1: 11.0 MS2: 15.0
 High mass resolution: MS1: 11.0 MS2: 15.0

Acquisition type: MRM

Retention window: 0-25 min

Transitions*	Dwell time	Mass span
395.0-195.0	2 sec	0.1
397.0-195.0	2 sec	0.1
409.0-209.0	2 sec	0.1
411.0-228.0	2 sec	0.1

* Daughter ion spectra should be obtained to verify transitions when using different instrumentation. Exact transitions should be determined during optimisation of the instrument.

7. PREPARATION OF STANDARD SOLUTIONS

7.1 Precautions

- The standard material should be weighed out according to the procedures detailed in the current version of AGF.00100.01.
- Ensure that solvents are at room temperature before use.
- Volumetric glassware previously used for 500 µg/mL solutions (or greater) should not be used for solutions for lower concentrations.
- Volumetric glassware should be thoroughly washed with acetone after use prior to washing up.

7.2 CL 375839 Standard

- Accurately weigh a known amount (approximately 10 mg ± 0.1) of CL 375839 into a 20 mL volumetric flask. Dilute to the mark with acetonitrile and mix well. Calculate and record the exact concentration of CL 375839, correcting for the standard purity. This solution contains nominally 500 µg/mL.

- b. Pipette 2 mL of the 500 µg/mL standard solution into a 100 mL volumetric flask. Dilute to the mark with acetonitrile and mix well. This solution contains nominally 10 µg/mL.

7.3 CL 377160 Standard

- a. Accurately weigh a known amount (approximately 10 mg ± 0.1) of CL 377160 into a 20 mL volumetric flask. Dilute to the mark with acetonitrile and mix well. Calculate and record the exact concentration of CL 377160, correcting for the standard purity. This solution contains nominally 500 µg/mL.
- b. Pipette 2 mL of the 500 µg/mL standard solution into a 100 mL volumetric flask. Dilute to the mark with acetonitrile and mix well. This solution contains nominally 10 µg/mL.

7.4 Mixed CL 375839 and CL 377160 Standard Solutions for Fortification of Specimens

- a. 10.0 µg/mL from Sections 7.2 and 7.3.
- b. Pipette 1 mL of each 10 µg/mL standard solution into a 10 mL volumetric flask. Dilute to the mark with acetonitrile and mix well. This solution contains nominally 1.0 µg/mL CL 375839 and CL 377160.

7.5 Mixed CL 375839 and CL 377160 Standard Solutions for Linearity

- a. Pipette 1 mL of each 10 µg/mL standard solution into a 20 mL volumetric flask. Dilute to the mark with 50/50 (v/v) methanol:water and mix well. This solution contains nominally 0.5 µg/mL.
- b. Pipette 1 mL of 0.5 µg/mL mixed standard solution into a 2 mL volumetric flask. Dilute to the mark with 50/50 (v/v) methanol: water and mix well. This solution contains nominally 0.25 µg/mL.
- c. Pipette 1 mL of 0.5 µg/mL mixed standard solution into a 5 mL volumetric flask. Dilute to the mark with 50/50 (v/v) methanol: water and mix well. This solution contains nominally 0.1 µg/mL.
- d. Pipette 1 mL of the 0.5 µg/mL mixed standard solution into a 10 mL volumetric flask. Dilute to the mark with 50/50 (v/v) methanol:water and mix well. This solution contains nominally 0.05 µg/mL.

- e. Pipette 1 mL of the 0.5 µg/mL mixed standard solution into a 20 mL volumetric flask. Dilute to the mark with 50/50 (v/v) methanol:water and mix well. This solution contains nominally 0.025 µg/mL.

The Working Standard will be the 0.05 µg/mL standard solution.

Note: Preparation of these standard solutions may be achieved by use of alternative dilutions

8. LINEARITY CHECK

- 8.1 Inject 100 µl aliquots of standard solutions.
- 8.2 Inject aliquots of 0.5, 0.25, 0.1, 0.05 and 0.025 µg/mL standards.
- 8.3 Plot peak areas obtained against standard concentration to demonstrate a linear response over the range. Significant departure from linearity indicates operational problems which must be corrected before proceeding.

9. SAMPLE PREPARATION

- 9.1 Maintain all samples frozen until ready for analysis.
- 9.2 Allow frozen samples to thaw completely in an air-tight container just prior to extraction.
- 9.3 Thoroughly mix thawed samples to obtain a homogenous sample.

10. RECOVERY TEST

The ability of the instrumentation and operator to perform the procedures satisfactorily is demonstrated by recovery tests.

- 10.1 Weigh 5g (±0.1g) of the specimen into a microwave extraction vessel.
- 10.2 Add by pipette, a volume of standard fortification solution appropriate to the fortification level to be tested (for a 0.2 mg/kg fortification add 1 mL of the 1.0 µg/mL standard to 5g of specimen).
- 10.3 Mix the sample and allow to stand for 15 minutes.
- 10.4 Continue with extraction and cleanup steps.

11. EXTRACTION

- 11.1 Weigh 5g of soil sample into a microwave extraction vessel.
- 11.2 Add 25 mL of extraction solvent to the soil and stir with a spatula to mix. Place the extraction vessel in the microwave extraction system. Use the following conditions for extraction:
- | | |
|----------------------------|--|
| a. Power | 20% (for 2 samples or fewer)
40% (for 4 samples)
60% (for 6 samples) etc |
| b. PSI | 50 |
| c. TAP (Time at Parameter) | 3 min |
| d. Temperature | 125°C |

Note: The microwave solvent extraction system is extremely sensitive to solvent vapour and will be temporarily disabled if vapours are detected. The rupture disk in the extraction vessel top must not be torn and all connections must be tight before starting the extraction.

- 11.3 After the extraction is completed, open the microwave door and let extraction vessel cool to about 45°C before disconnecting and removing the vessels from the microwave.
- 11.4 After being removed from the microwave, allow the extraction vessel to reach approximately room temperature. Gently uncap the extraction vessel so as not to disturb the soil bed and carefully transfer a 5 mL aliquot of the extract into a 50 mL beaker.
- 11.5 Add 5 mL of 0.5% acetic acid in Milli-Q water to the extract in the beaker and swirl to mix.

12. SOLID PHASE EXTRACTION (SPE)

- 12.1 Using a Vac Elut sample processing station, prepare a C18 cartridge (1g/6 mL) by washing with one column volume each, in this order, of dichloromethane, methanol and Milli-Q water. (Do not allow the SPE column to go dry during any of the loading or washing steps).
- 12.2 Add the solution from Step 11.5 to the cartridge and load the sample at a flow rate of approximately 2-3 drops per second. Discard the eluate.
- 12.3 Wash the 50 mL beaker with 5 mL of Milli-Q water and pass through the cartridge. Once the rinsing is completed, turn the vacuum to the maximum for approximately 1 minute to dry the cartridge.

- 12.4 Elute the C18 cartridge with 5 mL of 10/90 (v/v) methanol in dichloromethane, collecting the eluant in a scintillation vial. Evaporate the eluant to dryness using the Techne sample/concentrator with a nitrogen stream and temperature setting at about 60°C. Remove the vial as soon as the eluant dries.
- 12.5 **Immediately** add 2 mL of methanol to the scintillation vial, cap tightly and vortex for 10 seconds. Place the scintillation vial back on the heating surface (60°C) of the Techne sample/concentrator for 15 minutes, vortex for 10 seconds and let cool to room temperature. This procedure is required to ensure that the residues are completely dissolved.
- 12.6 Add 2 mL of Milli-Q water to the vial, cap and vortex for 10 seconds. Transfer an aliquot into a HPLC vial for LC/MS/MS analysis.

13. LC/MS/MS ANALYSIS

- 13.1 Inject a 100 µL aliquot of a mixed standard solution (1mg/mL) to obtain daughter ion spectra of CL375839 and CL 3771660.
- 13.2 After obtaining a stable chromatographic response inject in a 100 µl aliquot of the mixed working standard (0.05 µg/mL) in MRM mode
- 13.3 Inject 100 µL aliquots of control and fortified specimens in MRM mode.
- 13.4 Compare the retention times of the peaks produced by the 0.05 µg/mL standard solution with that obtained from control and fortified specimens. If the retention times of these match and there is no significant peak of equivalent retention time in the control specimen then the specificity of the method is confirmed.

Note: The LC/MS analysis described in the current method RLA 12618 should be used for quantitative determinations of fortified specimens to confirm that the procedures have been followed correctly.