NI-25: Method of Analysis for IM-1-4, a metabolite of NI-25, using LC/MS/MS

I. Introduction

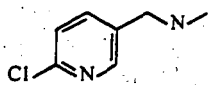
A. Scope

An analytical method is described here for the analysis of IM-1-4 in soil as defined in the Pesticide Assessment Guidelines, Subdivision O. This method has been verified during the method development stage at the spike levels of 10 ppb and 300 ppb (see result summary in Appendix), and will be more formally validated.

B. Principle

In this method, an accelerated solvent extractor (ASE) is used to extract IM-1-4 from soil samples. A soil sample is packed in a stainless steel ASE extraction cell and extracted using 0.4 N NH_4Cl in water and methanol mixture (40:60). The extract is then condensed and loaded onto a Extrelut cartridge and elute with dichloromethane. The eluted dichloromethane is dried using TurboVap and reconstituted using ACN:water mixture before injection on to LC/MS/MS. Quantification of these residues is accomplished by high performance liquid chromatography using a MS/MS detector.

C. Structure



IM-1-4

II. Materials

Reagents and Solvents were used as received from supplier, unless otherwise noted. Equivalent reagents and equipment may be substituted where appropriate.

A. Reagents, Solvents and Preparations

1. Acetonitrile, B & J, Cat. No. 015-4 or equivalent

2. Water, EM HPLC Grade, VWR Scientific Cat. No EM-WX0004-1 or equivalent
3. Sand, EM-SX0070-1, EM Science or equivalent
4. Acetic acid, EM-AX0073-13, EM Science or equivalent
5. Hydromatrix, Varian CE0A293, Varian
6. NH4Cl, EM-USP-7165, EM science or equivalent
7. Tris, 2-Amino-2-(hydroxymethyl)-1,3-propanediol, AX0945-3, EM Science or equivalent
8. 1.0N NH4Cl solution preparation: Add 12.4g Tris with 5.35g NH4Cl to 1.0L volumetric flask and bring up the volume with water. Then take 500mL of this solution and transfer to another 1.0Lvolumetric flask, add 292mL of 0.1N HCl into the flask and bring up the volume with water.

B. Equipment

1. Accelerated solvent extractor, ASE 200, Dionex
2. Analytical Balance
3. Autosampler Vials, 1 ml, clear, Wheaton, Cat. No. 223682
4. Disposable Pasteur Pipettes
5. Graduated Cylinders, appropriate sizes
6. Polypropylene Centrifuge Test Tube, 50 ml
7. Volumetric Pipettes, appropriate sizes, class A
8. Pipettes, appropriate sizes, Oxford or equivalent
9. Digital Pipettes, appropriate sizes, Eppendorf or equivalent
10. Glass collection tubes, 50 ml
11. Nylon Acrodisc filter (13 mm, 0.45 μm), Gelman No. 4426
12. Sciex API III+ LC/MS/MS system, Perkin Elmer or equivalent

13. HPLC pump, L6200, Hitachi, or equivalent

14. Autosampler, AS2000, Hitachi, or equivalent

15. HPLC column, YMC ODS-AQ, 3.0 x 150 mm, 5 µm particle size, 120A pore size

16. Extrelut cartridge, Merck 11737

17. TurboVap II, Zymark or equivalent

C. Analytical Standards

Analytical Standards available from Rhône-Poulenc Ag Company

1. IM-1-4

III. Standard Solution Preparation

A. General

1. The concentrations of standard solutions should be adjusted to account for the purity of the neat solid standards.

2. After preparation, standards should be transferred from the volumetric flasks into screw-capped amber bottles to prevent possible photodegradation.

3. Store standard solutions in the refrigerator at or below 4 °C when not in use.

B. Fortification and Calibration Standard Solutions

The following is provided as an example of how standard solutions may be prepared. Other concentrations may be used as appropriate.

1. Weigh 0.1000 g (±0.1 mg) of each analytical standard individually into 100 ml volumetric flasks. Dissolve each analytical standards in methanol (or ACN: H_20 mixture 50%) and mix well. Dilute to final volume with methanol (or ACN:H_20, 50%). Concentration of each standard is 1000 µg / ml.

2. Withdraw a 10.0 ml aliquot from each of the 1000 µg / ml individual standards and add to a 100 ml volumetric flask. Dilute to volume with methanol (ACN:H_2O, 50%). The concentration of this standard is 100 µg / ml.

3. By further dilution of the 100 µg / ml standard with methanol (or ACN:H_2O, 50%), prepare a series of standards to serve as fortification standards or calibration standards.

IV. Methods of Analysis

The tilde symbol (~) indicates 'approximately'.

The "•" symbol indicates an appropriate stopping point. Samples may be stored in freezer(< 0° C) overnight and allowed to come to room temperature before continuing.

A. Sample Preparation

1. Use samples as received from processor.
2. Weigh ~30.0 g of soil into a 50 ml centrifuge tube (See Section VIII, note).
3. Fortify as necessary and then let stand at least 10 minutes.
4. Add ~10mL of hydromatrix to soil sample, shake until well mixed.
5. Pack soil mixture into a 33 ml stainless steel extraction cell (with two filters at bottom of the cell), top the cell with sand if necessary.

B. Sample Extraction

1. Load the extraction cells onto ASE system.
2. Extract samples using the ASE conditions described in this method.
3. After extraction finished, adjust the final volume of extract to 60 mL.(•)

C. Sample Clean-up

1. Transfer 30 mL of extract into TurboVap tube (using graduate cylinder) and rinsing cylinder wall with some methanol.
2. TurboVap at ~50°C & 1 bar to evaporate all the methanol out of the extract (final volume is about 10 mL).

3. Transfer the extract onto an Extrelut cartridge gradually, rinse the test tube with small amount of water if necessary.

4. Let the cartridge sit at least 20 min to absorb all the aqueous.

5. Elute with a total volume of ~75 mL of dichloromethane onto the Extraelut cartridge, and collect all the fractions. (It may be easy to separate 75 mL into three 25 mL fractions, and use the first fraction to rinse the Turbo-Vap tube used in step C.3, and sonicate if necessary).

6. Add about ~0.5 mL ethylene glycol into dichloromethane. Evaporate all the dichloromethane using TurboVap at ~30 °C and 0.8 bar. (small amount of ethylene glycol will remain in the tube)

7. Dilute the extract in TurboVap tube using 50:50 ACN:water mixture to desired concentration (may first dissolve extract in 100% ACN, then mix with equal amount of water).

8. Sonicate and filter into LC/MS vials for LC/MS/MS analysis.

V. ASE Method

Method for IM-1-4:

Temperature:	100 °C
Pressure:	1500 psi
Preheat:	0 min with valve c
Heat up time:	5 min
Static time:	15 min
Static cycle	3 times
Flush volume:	90% of cell
Purge Time:	140 sec
Solvent #A:	1.0 N NH4Cl in water
Solvent #B:	methanol
Solvent mixing ratio:	A/B 40:60

VI. LC/MS/MS

A. Instrumentation

Instrument used:	Perkin Elmer Sciex API III+ LC/MS/MS system Hitachi L6200 HPLC pump PE Turbo IonSpray Electrospray Interface. Hitachi AS2000 autosampler

B. Conditions

Ionization:	Electrospray (TurboIonSpray), positive ion mode
Curtain gas flow:	Nitrogen at ~1.2 L/min
Nebulizer pressure:	55 psi
Turbo IonSpray Settings:	Heated air at ~4.75 L/min, 500^0 C
MS Mode:	MS/MS with multiple reaction monitoring (MRM)
Orifice voltage:	50 V
Collision gas:	Argon at approximately 275 x 10^{13} atoms/cm^2
Collision energy (R2-R0):	13V - 30V = -17V
Mass Transitions:	IM-1-4: 157/126
Column:	YMC ODS-AQ, 3.0 x 150mm, 5µm particle size, 120A pore size
Mobile phase flow rate:	0.5 ml/min split to ~150µl/min
Mobile phase composition:	40% Acetonitrile / 60% (1.0% Acetic acid in Water)
Injection volume:	25µl
Retention times:	See Chromatograms and data reports

VII. Quantification of Residues

A. Calibration Curves

1. Linear regression should be used to generate a calibration curve for the analyte. At least four different standard concentrations should be run with each set of samples. Standards should be interspersed with samples to compensate for any minor change in instrument response. Extracts should be diluted such that the peak areas obtained are within the area range between the lowest and highest standards injected.

2. Linear regression coefficients should be calculated from 'peak area' (or 'peak height') versus 'nanogram / ml injected'. Data from the analytical standards should be fit to the linear equation, $y = a + bx$.

where: y = peak area or height
a = calibration line intercept
b = calibration line slope
x = conc of analyte in inj soln

B. Quantification of Residues

1. IM-1-4 should be quantified by comparison to its standard curves obtained from a linear regression analysis of the data.

2. Equations

2.1 Concentration of analyte in sample in ppb (parts per billion).

$$z = (y - a) / b \times c / d$$

where: y = peak area (or height), response of analyte of interest
a = intercept of calibration line from linear regression (area or height)
b = slope of calibration curve from linear regression (response per ng/ml)
c = final volume of sample (ml)
d = sample weight (g)
z = conc of analyte in sample (ppb)

2.2 Corrected concentration of analyte in sample in ppb.

$$Z' = z \times C$$

where: Z' = corrected concentration
z = concentration found from curve
C = conversion factor

2.3 Percent recovery

$$\% \text{ recovery} = \frac{(\text{ppb found in fort sample} - \text{ppb found in UTC})}{\text{actual fortification level in ppb}} \times 100\%$$

VIII. Comments and Notes

Ethylene glycol: The present of ethylene glycol in final extract may cause signal depression in MS/MS detector for IM-1-4. There are two ways to get around this problem: 1. Dilute the final extract as much as possible; 2. Prepare the same concentration of ethylene glycol in the standards.

LC/MS/MS conditions could be modified for better sensitivity and selectivity.

ASE conditions may be modified for better extraction for each type of soil.

If a certain type of soil causes clogging in extraction cell, or the soil is very wet and difficult to mix with dispersion agent, using smaller soil sample size (such as 15 g) and more dispersion agent is recommended.