NI-25: Method of Analysis for NI-25 and its Metabolite, IC-0 using LC/MS/MS

I. Introduction

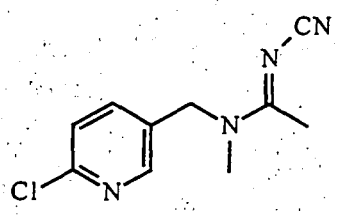
A. Scope

An analytical method is described here for the analysis of NI-25 and its metabolite, IC-0 in soil as defined in the Pesticide Assessment Guidelines, Subdivision O. This method has been verified during the method development stage for four different soils at the spike levels of 10 ppb and 300 ppb (see result summary in Appendix), and will be more formally validated.

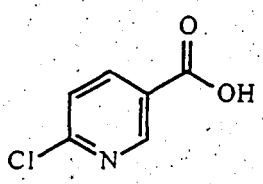
B. Principle

In this method, an accelerated solvent extractor (ASE) is used to extract NI-25 and its metabolite, IC-0 from soil samples. 30 grams of soil samples are mixed with dispersing agents such as sand and hydromatrix, packed into ASE stainless steel extraction cells and extracted using 50:50 acetonitrile and water mix at 100 °C and 1500 psi. The obtained extraction solution is then diluted and filtered before direct injection on to LC/MS/MS. Quantification of these residues is accomplished by high performance liquid chromatography using a MS/MS detector.

C. Structures



NI-25



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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, Part No. 0019-8003, Varian

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

C. Analytical Standards

Analytical Standards available from Rhône-Poulenc Ag Company

1. NI-25
2. IC-0

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 (or ACN:H_20, 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_20, 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 sample$_s$ 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, dilute sample if necessary and pass sample extract through a Gelman 0.45 um filter using a syringe and aliquot into LC vial (dilutions may be needed for high concentration samples).

V. ASE Method

Method for NI-25, IC-O:

Temperature:	100 °C
Pressure:	1500 psi
Preheat:	0 min with valve c
Heat up time:	5 min
Static time:	5 min
Static cycle	3 times
Flush volume:	80% of cell
Purge Time:	180 sec
Solvent #A:	ACN
Solvent #B:	water
Solvent mixing ratio:	A/B 50:50

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 ~1.0 L/min
Nebulizer pressure:	55 psi
Turbo IonSpray Settings:	Heated air at ~5.25 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:	NI-25: 223/126 IC0: 158/122
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. NI-25 and IC-0 should be quantified by comparison to their 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

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

VIII. Comments and Notes

NI-25: MS signal enhancement was observed at LOQ levels (10 ppb) for all soils when final extracts were not sufficiently diluted. Typical dilution volume for 60 mL final ASE extract is 240 mL, or four fold dilution in order to get rid of such interference.

IC-0: Avoid high temperature over 100°C.

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

ASE conditions can be modified for better extraction recovery.

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.