

METHOD NUMBER

KP-216R1

METHOD TITLE

Preliminary LC/MS/MS Analytical Method for Determination of
Assail™ 70WP Insecticide (Acetamiprid) in Crop Matrices

Data Requirement

OPPTS 860.1340

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Method Revision Notifications

The method KP-216R0 was revised to KP-216R1 on September 6, 2003.

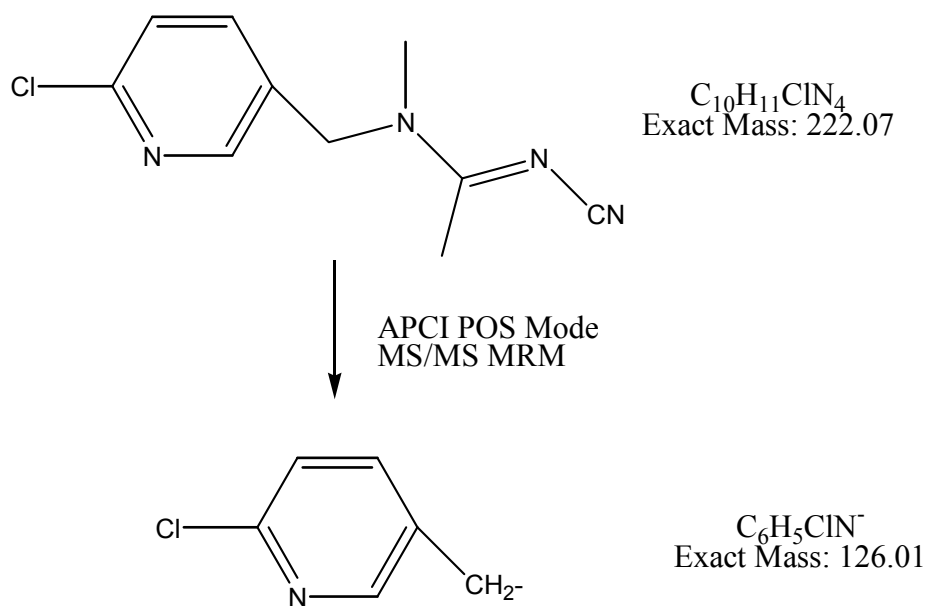
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ABSTRACT

A method has been developed for the determination of acetamiprid in various crop matrices. The method will be validated in the concentration range from 0.01 $\mu\text{g/g}$ to 1 $\mu\text{g/g}$. The analytical method employs a simple approach, involves minimum cost and time and requires commonly available equipment as requested by the guideline for monitoring purposes.

This method utilizes LC/MS/MS as the analytical instrument of choice. Sample preparation consists of extracting the analyte from crop by solvent extraction. The extract volume is adjusted to be within the calibrated volume range for instrument analysis. Quantitation is accomplished using a LC/MS/MS system with a reversed phase column for the chromatography. The MS is operated in the APCI mode which results in positively charged molecular ion of mass 223. In the MRM mode, this parent molecular ion is fragmented by collision with neutral collision gas. The resulting fragment ions generated are filtered and the product ion with $m/e=126$ is selected for quantitation. The limit of quantitation of the method is 0.01 ppm or less.



ANALYTICAL PROCEDURES

Materials

A. Solvents and Reagents.

HPLC grade solvents or better should be utilized. Other brands of HPLC grade solvents may be substituted as long as they do not produce interferences with the chromatography.

Methanol (MeOH), HPLC grade, Fisher Scientific, Pittsburgh, PA
Acetonitrile, HPLC grade, Fisher Scientific, Pittsburgh, PA

Reagents should be ACS grade or better. Other brands of ACS grade reagents may be substituted as long as they do not produce interferences with the chromatography.

Formic Acid (HCOOH), Mallinckrodt, Paris, KY

B. Miscellaneous Materials

Oasis HLB cartridges, 6cc/200mg. Waters, Milford, MA.

Extraction Solvent: Methanol : water (1:1 v/v)

Transfer Solvent: MeOH: H₂O (25:75 v/v)

Equipment

A. LC/MS/MS System

1. Waters Alliance HPLC system with cooled sample chamber and a column heater. Waters, Chromatograph Division of Millipore Corp., Milford, MA.
2. Mass Spectrometer detector, Quattro Ultima, MicroMass, Beverly, MA or equivalent.

B. SPE vacuum manifold, Supelco, Bellefonte, PA or equivalent.

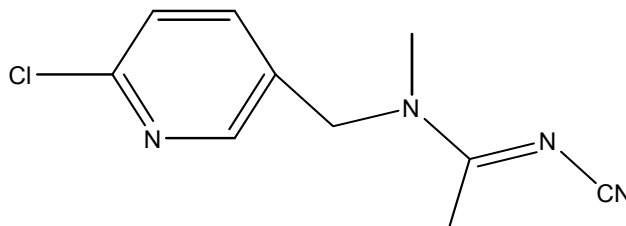
C. Adjustable pipettes, assorted sizes

D. Glassware

1. Graduated test tubes 13 mm x 100 mm or regular test tubes calibrated at 10 ml.
2. Graduated cylinders, 250 ml and assorted.
3. Beakers, various sizes.
4. Class A volumetric flasks, assorted sizes
5. Autosampler vials, Alltech Assoc., Inc., Deerfield, IL

Test Substances

Common Name: acetamiprid
CAS Number: 135410-20-7
Chemical Name: (*E*)-*N*-[(6-chloro-3-pyridinyl)methyl]-*N*1-cyano-*N*1-methyl
ethanimidamide



$C_{10}H_{11}ClN_4$
Exact Mass: 222.07

Acetamiprid used as a standard should be considered a chemical hazard. All caution should be exercised when handling pure material or concentrated stock solutions. Avoid skin contact and inhalation. See Material Safety Data Sheet documentation accompanying standard shipment.

STANDARDS PREPARATION

Preparation of acetamiprid stock standard

- Weigh 0.0100 g (with appropriate purity adjustment) of solid acetamiprid material in a glass weigh boat and transfer to a 100 ml volumetric flask using methanol. Avoid use of plastics for the weighing of compounds and storing of reagents. The small amounts of plasticizer leaching into the sample may have a detrimental effect on the analysis.)
- Half fill the volumetric flask with methanol and agitate gently until standards are completely dissolved.
- Dilute to volume with methanol and mix by inverting several times. Final concentration = 100 $\mu\text{g/ml}$.
- The concentrated standard solution 100 $\mu\text{g/ml}$ should be prepared annually when stored at 0-7 $^{\circ}\text{C}$.

Preparation of Fortification Standards

Fortification standards (from 0.5 to 10 $\mu\text{g/ml}$) should be prepared on a monthly basis by dilution of the concentrated standard solution with methanol. Store in a refrigerator at 0-7 $^{\circ}\text{C}$.

Preparation of Calibration Standards

Prepare calibration standards weekly by dilution of the fortification standards with 20% acetonitrile in water. Store in refrigerator at 0-7 $^{\circ}\text{C}$. A typical range of concentrations for the calibration standards is 0.5, 1.0, 2.5, 5.0 and 10.0 ng/mL.

SAMPLE EXTRACTION PROCEDURE

1. Weigh approximately 20-40 g (depending on the matrix) of randomly sampled crop to the nearest 0.01 g into a 250 ml HDPE, PTFE, or glass bottle for extraction by shaking or blending .
2. For the preparation of analytical recovery samples, fortify control samples in the extraction vessel by pipetting a known volume and concentration of the fortification standard onto the sample matrix.
3. Add 75 ml of extraction solvent: MeOH : water (1:1 v/v).
4. If blending sample, blend the sample using a Cyclone for 3 minutes at moderate speed. If shaking the sample, place in a shaker and shake at moderate – high speed for approximately 10 minutes.
5. Centrifuge sample in a centrifuge at 3,000 to 4,000 rpm for 5 minutes depending on the sample matrix. If sample is not extracted in a container suitable for centrifugation, transfer to a centrifuge bottle (250 ml) with liberal rinses of extraction solvent.
6. Separation of the extraction solvent from the solid sample matrix may be accomplished by filtration through a Buchner funnel or decantation.

If filtration is used: seat a #4 Whatmen filter with a small amount of MeOH/water (25/75) into a Buchner funnel that has been secured onto a vacuum graduate cylinder while drawing a vacuum through the funnel and cylinder assembly. Top the filter paper with a thin layer of Celite 545 filter aid. Decant the supernatant extract to the Buchner funnel. Collect filtrate in a 250 ml graduate cylinder.

7. Re-extract the sample with 75 ml of extraction solvent once more and separation of extract from matrix as outlined in steps 4-6. Centrifugation may not be necessary for certain matrices. Combine the liquid phases from both extractions into the collection cylinder.

Note: When analyzing dry matrices such as hay, forage, etc., two additional extractions may be used with 50-75 ml of extraction solvent after the first extraction. In this case, the centrifuge step will be needed after each extraction step.

8. Rinse the extraction cap and bottle and include in the collection. Perform a final rinse of the filter cake in the Buchner funnel with a small amount of transfer solvent (25:75 MeOH:water) and allow for collection of the filtrate until the cake is nearly dry.
9. Dilute the total volume to 200 ml with transfer solvent and mix well.

SOLID PHASE EXTRACTION PROCEDURE

Waters Oasis HLB, 6cc/200mg cartridges are used in the procedure. Other reversed phase cartridges may be used as long as they are checked to ensure good recoveries of acetamiprid. The flow rate should be controlled between 1.0-3.0 ml/minute. Volume of extract loaded on the cartridge and the final volume could be various depending on the instrument sensitivity and matrices.

1. Pre-rinse the cartridge with 5 ml acetonitrile followed by 5 ml of acetonitrile:water 20:80 solution.
2. Transfer 10 ml extract to the cartridge. Rinse the test tube with ~3 ml of acetonitrile:water 20:80 solution and add to the cartridge.
3. Elute the cartridge with 5 ml acetonitrile and collect in a graduated test tube.
4. Bring the volume of the test tube to 10.0 ml with water, mix the contents.
5. Filter the extract through a 0.45 μm x 25 mm PTFE or Nylon syringe filter. Sample is ready for LC/MS/MS analysis.

LC/MS/MS

Analysis of samples will be performed using LC/MS/MS system. A diverter switch valve may be utilized. In this case sample will be diverted to waste for the first two minutes after each run starts. The diverter is switched to the mass spectrometer after two minutes.

Preparation of Mobile Phase:

1. Dilute 1 ml formic acid to 1 liter with water.
2. Methanol, HPLC grade or better.

HPLC Column Conditions:

Mobile and/or stationary phase conditions may be changed slightly (depending on interferences) in order to optimize chromatographic selectivity and/or efficiency.

1. Column: Betasil C8, 100 x 4.6 mm, 5 μ , ThermoHypersil-Keystone, Bellefonte, PA or equivalent.
2. Column Temperature: 28°C
3. Injection Volume: 25-100 μl

 Mass Spectrometer

APCI or electrospray can be used for the analysis. Tuning and optimization on mass spectrometer should be performed by the operator occasionally to ensure good sensitivity. The following instrument parameters utilizing APCI interface on MicroMass Ultima should be used as a general guidance.

Ionization Mode	APCI
Scan Type	Positive MRM
Source Temperature	130°C
Desolvation Temp	450°C
Corona	1.0 μ A
Cone Voltage	35 V
Cone Gas Flow	145 L/Hr
Desolvation Gas Flow	738 L/Hr
Entrance	25
Collision	Argon with energy 20
Retention Time	approximate 6.5 minute
Run Time	10 minutes
Q ₁ mass	223
Q ₃ mass:	126

Mobile phase gradient

Time min	Flow Rate ml/min	MeOH	0.1%HCOOH in water
0.0	0.75	20	80
1.0	0.75	20	80
6.0	0.75	99	1
7.0	0.75	99	1
7.5	0.75	20	80

HPLC QUANTITATION PROCEDURES

Standardization.

1. A linear regression response curve should be generated for every analytical run.
2. The curve should be generated by injections of at least four different concentrations of standards through the entire run. The standards should be injected prior to and immediately after a sequence of samples. If the sample set is >5 then an occasional standard should be analyzed between samples to demonstrate response stability.
3. A standard that represents a residue of 50% of the method's lower limit of quantitation (LOQ) for each analyte must be among those standards injected.

- Any sample residue which exceeds the highest standard injected must be appropriately diluted and re-injected with a standard set such that it falls within the linear response range of the standards injected, or injected at a lower volume such that it falls within the linear response.

Calculations

Quantitation of acetamidrid is dependent on: sample size, the final sample volume, and the final dilution volume. To standardize the analysis, all samples of a given matrix should be analyzed using the same sample volume, brought to the same final sample volume, and analyzed on the LC/MS/MS with a standard injection volume. Dilution factors to achieve quantifiable concentrations for LC/MS/MS should be the only volume variables. With these standardizing assumptions, the following equation may be used to obtain parts per million values for acetamidrid:

$$ppm = \frac{C \times E \times V \times \text{Dilution Factor}}{1000 \times W \times V'}$$

Where:

- E = final sample volume for HPLC in milliliters.
- C = concentration (ng/ml) as interpolated mathematically from a linear regression curve of the compound's HPLC response versus concentration of standard injected.
- V = Total extract volume in milliliters.
- V' = Aliquot taken for SPE procedure in milliliters
- W = original sample weight in grams.

The dilution factor is based on the ratio of the final dilution volume to the volume of any aliquot(s) removed from the final HPLC extract.

Example:

A 25 g sample was analyzed. After extraction, it is brought to final volume of 200.0 ml for LC/MS/MS analysis. 10 ml of extract was cleaned in a SPE and the analyte was eluted and diluted to 10 ml for the instrument analysis

$$ppm = \frac{C \times 10 \times 200}{1000 \times 25 \times 10}$$

Where:

- C = The interpolated concentration value from a linear regression curve prepared from co-chromatographed standards and entered into the linear regression with the units of ng/ml.