1. Introduction

1.1 Scope and Source of the Method

1.1.1 Scope

Acetamiprid (ATP), (E)-N¹-[(6-chloro-3-pyridyl)methyl]-N²-cyano-N¹-methylacetamidine, is an insecticide developed by Nippon Soda Co., Ltd. for control of aphids, codling moths etc. on crops.

The purpose of the study was to validate the analytical method No. NI-25-W-2 for the determination of ATP in water.

1.1.2 Source

The method No. NI-25-W-2 was developed at Nisso Chemical Analysis Service Co., Ltd. in order to determine the residues of ATP in water for EU registration (Reference 1).

1.1.3 Principle of the Method No. NI-25-W-2

ATP in water is extracted by solid phase extraction (SPE) followed by Sep-Pak C_{18} clean-up. The determination of ATP is performed by a high performance liquid chromatography (HPLC).

2. Materials and Methods

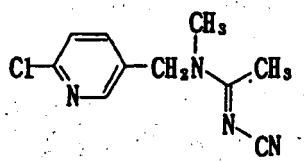
2.1 Test and Reference Substance

Common Name: Acetamiprid

Code Name: NI-25

Chemical Name: (E)-N¹-[(6-chloro-3-pyridyl)methyl]-N²-cyano-N¹-methylacetamidine

Chemical Structure:



Molecular Formula: $C_{10}H_{11}ClN_4$

Molecular Weight: 222.68

Lot No.: NNI-01

NCAS Retrieval No.: I25-064

Purity: ≧99.9% (HPLC)

2.2 Reagents and Apparatus

Acetonitrile: guranteed (Wako Pure Industries Co., Ltd.) or equivalent (eq.)

Solid phase extraction column: Mega Bond Elut® C_{18} Part No. 1225-6001 (Varian)

Packed column: C_{18} Sep-Pak plus Part No. WAT020515 (Waters)

Vacuum collection assembly: Vacuum Manifold (Waters) or eq.

Conical beaker: 50 and 300 mℓ

Pear shaped flask: 100 mℓ

Volumetric flask : 20, 25, 50, 100 mℓ

Syringe: Microliter® #710 (Hamilton) or eq.

General laboratory glass ware

Rotary evaporator: N-N Series (TOKYO RIKAKIKAI Co., LTD)

HPLC: LC-10A (Shimadzu) or eq.

2.3 Standard Solutions

2.3.1 Standard Solutions for Fortifications

ATP: 100, 10, 1, 0.2 and 0.04 ppm (μg/mℓ) in acetonitrile

Prepare a 100 μg/mℓ ATP standard solution by weighing an appropriate amount of ATP into a volumetric flask. Dissolve with acetonitrile and dilute to the mark. For example, to prepare 100 mℓ standard solution, dissolve 10 mg of ATP in a 100 mℓ volumetric flask.

Prepare a 10 μg/mℓ ATP standard solution by transferring an appropriate amount of the 100 μg/mℓ ATP standard solution with a volumetric pipet into a volumetric flask (typically 10 mℓ of 100 μg/mℓ ATP standard solution in a 100 mℓ volumetric flask). Dilute to the mark with acetonitrile.

Prepare a 1 μg/mℓ ATP standard solution by transferring an appropriate amount of the 100 μg/mℓ ATP standard solution with a volumetric pipet into a volumetric flask (typically 1 mℓ of 100 μg/mℓ ATP standard solution in a 100 mℓ volumetric flask). Dilute to the mark with acetonitrile.

2.3.1 Standard Solutions for Fortifications (Continued)

Prepare a 0.2 $\mu g/m\ell$ ATP standard solution by transferring an appropriate amount of the 1 $\mu g/m\ell$ ATP standard solution with a volumetric pipet into a volumetric flask (typically 5 $m\ell$ of 1 $\mu g/m\ell$ ATP standard solution in a 25 $m\ell$ volumetric flask). Dilute to the mark with acetonitrile.

Prepare a 0.04 $\mu g/m\ell$ ATP standard solution by transferring an appropriate amount of the 1 $\mu g/m\ell$ ATP standard solution with a volumetric pipet into a volumetric flask (typically 2 $m\ell$ of 1 $\mu g/m\ell$ ATP standard solution in a 50 $m\ell$ volumetric flask). Dilute to the mark with acetonitrile.

Store standard solutions in a refrigerator (0~9°C).

2.3.2 Standard Solutions for HPLC Determination

ATP: 1.0, 0.1, 0.05, 0.02, 0.01 and 0.005 ppm ($\mu g/m\ell$) in mobile phase solution for HPLC (acetonitrile / water = 3 + 7, v/v), MPS.

Prepare a 100 $\mu g/m\ell$ ATP standard solution by weighing an appropriate amount of ATP into a volumetric flask. Dissolve with acetonitrile and dilute to the mark. For example, to prepare 100 $m\ell$ standard solution, dissolve 10 mg of ATP in a 100 $m\ell$ volumetric flask.

Prepare a 1 $\mu g/m\ell$ ATP standard solution by transferring an appropriate amount of the 100 $\mu g/m\ell$ ATP standard solution with a volumetric pipet into a volumetric flask (typically 1 $m\ell$ of 100 $\mu g/m\ell$ ATP standard solution in a 100 $m\ell$ volumetric flask). Dilute to the mark with MPS.

Prepare a 0.1 $\mu g/m\ell$ ATP standard solution by transferring an appropriate amount of the 1 $\mu g/m\ell$ ATP standard solution with a volumetric pipet into a volumetric flask (typically 2 $m\ell$ of 1 $\mu g/m\ell$ ATP standard solution in a 20 $m\ell$ volumetric flask). Dilute to the mark with MPS.

Prepare a 0.05 $\mu g/m\ell$ ATP standard solution by transferring an appropriate amount of the 1 $\mu g/m\ell$ ATP standard solution with a volumetric pipet into a volumetric flask (typically 1 $m\ell$ of 1 $\mu g/m\ell$ ATP standard solution in a 20 $m\ell$ volumetric flask). Dilute to the mark with MPS.

2.3.2 Standard Solutions for HPLC Determination (Continued)

Prepare a 0.02 $\mu g/ml$ ATP standard solution by transferring an appropriate amount of the 1 $\mu g/ml$ ATP standard solution with a volumetric pipet into a volumetric flask (typically 0.4 ml of 1 $\mu g/ml$ ATP standard solution in a 20 ml volumetric flask). Dilute to the mark with MPS.

Prepare a 0.01 $\mu g/ml$ ATP standard solution by transferring an appropriate amount of the 0.1 $\mu g/ml$ ATP standard solution with a volumetric pipet into a volumetric flask (typically 2 ml of 0.1 $\mu g/ml$ ATP standard solution in a 20 ml volumetric flask). Dilute to the mark with MPS.

Prepare a 0.005 $\mu g/ml$ ATP standard solution by transferring an appropriate amount of the 0.05 $\mu g/ml$ ATP standard solution with a volumetric pipet into a volumetric flask (typically 2 ml of 0.05 $\mu g/ml$ ATP standard solution in a 20 ml volumetric flask). Dilute to the mark with MPS.

2.3.3 Standard Solutions for GC-MS Determination

ATP: 0.2, 0.1, 0.05, 0.02 and 0.01 ppm ($\mu g/ml$) in acetone

Prepare a 1 $\mu g/ml$ ATP standard solution by concentrating an appropriate amount of the 100 $\mu g/ml$ ATP standard solution (acetonitle) under reduced pressure (typically 1 ml of 100 $\mu g/ml$ ATP standard solution in a 50 ml pear shaped flask). Dissolve the residue twice with each 10 ml acetone and transfer it into a 100 ml volumetric flask. Dilute to the mark with acetone.

Prepare a 0.2 $\mu g/ml$ ATP standard solution by transferring an appropriate amount of the 1 $\mu g/ml$ ATP standard solution with a volumetric pipet into a volumetric flask (typically 10 ml of 1 $\mu g/ml$ ATP standard solution in a 50 ml volumetric flask). Dilute to the mark with acetone.

Prepare a 0.1 $\mu g/ml$ ATP standard solution by transferring an appropriate amount of the 1 $\mu g/ml$ ATP standard solution with a volumetric pipet into a volumetric flask (typically 5 ml of 1 $\mu g/ml$ ATP standard solution in a 50 ml volumetric flask). Dilute to the mark with acetone.

2.3.3 Standard Solutions for GC-MS Determination (Continued)

Prepare a 0.05 $\mu g/m\ell$ ATP standard solution by transferring an appropriate amount of the 1 $\mu g/m\ell$ ATP standard solution with a volumetric pipet into a volumetric flask (typically 2.5 $m\ell$ of 1 $\mu g/m\ell$ ATP standard solution in a 50 $m\ell$ volumetric flask). Dilute to the mark with acetone.

Prepare a 0.02 $\mu g/m\ell$ ATP standard solution by transferring an appropriate amount of the 0.2 $\mu g/m\ell$ ATP standard solution with a volumetric pipet into a volumetric flask (typically 5 $m\ell$ of 0.2 $\mu g/m\ell$ ATP standard solution in a 50 $m\ell$ volumetric flask). Dilute to the mark with acetone.

Prepare a 0.01 $\mu g/m\ell$ ATP standard solution by transferring an appropriate amount of the 0.1 $\mu g/m\ell$ ATP standard solution with a volumetric pipet into a volumetric flask (typically 5 $m\ell$ of 0.1 $\mu g/m\ell$ ATP standard solution in a 50 $m\ell$ volumetric flask). Dilute to the mark with acetone.

2.3.4 Stability of Standard Solution

Standard solutions of ATP for fortifications and HPLC determination are kept in a refrigerator for definite periods (old standard solutions).

A 100 $\mu g/m\ell$ ATP standard solution (acetonitrile), 3 concentrations of ATP standard solutions for fortification (10, 1 and 0.04 $\mu g/m\ell$) and 3 concentrations of ATP standard solutions for HPLC determination (0.1, 0.01 and 0.005 $\mu g/m\ell$) are prepared newly after finishing the validation experiment (new standard solutions).

Inject the proper volume of new and corresponding old standard solutions into the HPLC. Compare the peak areas of the standards to evaluate the stability of the old standard solutions.

2.4 Analytical Procedure

The following procedures are for drinking water, ground water and surface water. The schematic of analytical procedure for ATP in the waters are presented in Section 8.

2.4.1 Sample Preparation

Drinking water and ground water were collected at the performing laboratory, and surface water was collected from Sakawa river near the performing laboratory.

2.4.2 Fortification and Extraction

Weigh 200 mℓ (g) of water into a 300 mℓ conical beaker. As for fortification test, an appropriate volume of ATP standard solution is added to a control sample by a volumetric pipet.

For example, pipet 0.5 mℓ of the 0.04 μg/mℓ standard solution of ATP into the control sample for 0.1 ppb fortification test.

Wash a Mega Bond Elut C_{18} with 5 mℓ acetonitrile and then 5 mℓ distilled water at a flow rate of ca. 5 mℓ/min.

Pass the 200 mℓ water sample through a Mega Bond Elut C_{18} at a flow rate of ca. 5 mℓ/min. Discard the eluate.

Wash the packed column with 10 mℓ distilled water at a flow rate of ca. 5 mℓ/min. Discard the eluate.

Elute ATP with 30 mℓ mixed solvent of acetonitrile and water (3 + 17, v/v) at a flow rate of ca. 2 to 3 mℓ/min. Collect this eluate in a 100 mℓ pear shaped flask.

Evaporate the eluate to dryness under reduced pressure.

2.4.3 Sep-Pak C_{18} Clean-up

Wash a Sep-Pak C_{18} cartridge with 5 mℓ acetonitrile and then 5 mℓ distilled water at a flow rate of ca. 5 mℓ/min.

Dissolve the residue in 10 mℓ distilled water and pass it through the Sep-Pak C_{18} at a flow rate of ca. 2 to 3 mℓ/min. Discard the eluate.

Elute ATP with 30 mℓ of acetonitrile and water (3 + 17, v/v) at a flow rate of ca. 2 to 3 mℓ/min. Collect this eluate to a 100 mℓ pear shaped flask.

Concentrate to dryness under reduced pressure using a rotary evaporator.

Dissolve each sample in an appropriate volume of MPS (typically 2.0 mℓ for 0.1 ppb fortification and control samples).

2.5 Instrumentation

2.5.1 Operating Conditions of HPLC

Instrument: LC-10A (Shimadzu)

Column: Inertsil ODS-3 4.6 mm i.d. 150 mm length (GL Sciences Inc.)

Mobile Phase: acetonitrile + distilled Water = 3 + 7 (v/v)

Column Temp.: 40°C

Flow rate: 1.0 mℓ/min

Detector: UV

Wavelength: 248 nm

2.5.2 Calibration Procedure

Calculation of results is based on peak area measurements. The calibration curve is obtained by direct injections of 50 $\mu\ell$ of the 0.1, 0.05, 0.02, 0.01 and 0.005 $\mu g/m\ell$ standard solutions to HPLC. Prepare the calibration curve by plotting the peak area vs. the weight of ATP.

2.5.3 Sample Analysis

Establish the stability of the detection response by injecting standard solutions of several concentrations. For analysis, alternate samples and standards.

For each set, the set should begin and end with standard injections, and each standard solution should be injected at least in duplicate.

2.6 Confirmatory Techniques

In order to confirm the reliability of this HPLC method, representative control and fortified samples were also measured by GC-MS as mentioned below, and the results by GC-MS are compared with those by HPLC. Typical GC-MS chromatograms of standard, control and fortified samples are presented in Appendix A.

2.6.1 Operating Conditions of GC-MS

Instrument: Shimadzu GC-MS GC-17A/QP-5000

GC Condition

Column: HP-5MS (0.25 mm i.d., 30 m length, 0.25 μm film thickness; HP)
Injection Temperature: 300℃
Column Temperature: Initial = 100℃ Hold Time = 2 min
Rising = 100℃ to 300℃ (20℃/min)
Final = 300℃ Hold Time = 2.0 min
Carrier gas: He
Carrier gas pressure: Initial = 110 kPa Hold Time = 2 min
Rising = 110 kPa to 200 kPa (10 kPa/min)
Final = 200 kPa Hold Time = 3.0 min
Injection mode: Splitless

MS Condition

Interface Temp.: 300℃
Ionization energy: 70 eV
Analytical mode: EI SIM
Monitoring: m/z 152 (Target), 126 (Reference)

2.6.2 Calibration Curve

Calculation of results is based on peak area measurements using a calibration curve. The calibration curve is obtained by injections of 2 $\mu\ell$ of 0.2, 0.1, 0.05, 0.02 and 0.01 $\mu g/m\ell$ acetone standard solutions to GC-MS. Prepare a calibration curve by plotting the peak area vs. the weight of ATP using a linear fit.

2.6.3 Sample Analysis (Continued from section 2.4.3, line 7)

Dissolve each sample in an appropriate volume of acetone with a volumetric pipet (typically 1.0$m\ell$ for 0.1 ppb fortification and control sample) instead of MPS. Inject 2.0 $\mu\ell$ of sample solution to GC-MS.

2.7 Time Required for Analysis

The time required for a set of 5 samples, 2 recoveries and a control is about 12 hours including HPLC determination and data analysis.

2.8 Potential Problems and Helpful Hints

1) Particular attention should be given to not allow Mega Bond Elut and Sep-Pak cartridges to go dry before addition of the sample.
2) As the performance of solid phase extraction sorbents can vary from lot to lot, it is highly recommended to check the column performance of any new lot with an ATP standard.
3) Flow rate of each packed column described in Sections 2.4.2 and 2.4.3 have to be maintained at 2 to 3 $m\ell$/min.

3. Method of Calculation

3.1 Principle

Calculation of results is based on peak area measurements. A calibration curve is calculated by linear regression analysis of peak area vs. ng ATP injected. The residue of ATP is calculated from the calibration curve and the equation shown in Section 3.2. An example residue calculation is presented in Section 9. The calculation should be made by a suitable computer program.

At least one fortification and one control sample (untreated) are run with each set of samples. Recoveries % (S) is determined as described in Section 3.3.

3.2 Calculation of Residue

The residue of ATP (R) in ppb is calculated with the following formula:

$$R\ (ppb) = \frac{W \times Vf}{G \times Vi}$$

G = Sample weight (g) (corresponds to sample volume (mℓ) used)
Vi = Injection volume (mℓ)
Vf = Final volume (mℓ) W = Amount of ATP from calibration curve (ng)

3.3 Calculation of Recoveries

The fortification recoveries (S) for ATP in % is calculated from the following equation:

$$\text{Residue amount of ATP } (R') = \frac{W \times Vf}{G \times Vi}\ (ppb)$$

$$S\ (\%\ \text{recovery}) = \frac{R'\ (ppb) \times 100}{\text{ppb ATP added}}$$

G = Sample volume (g) (corresponds to sample volume (mℓ) used)
Vi = Injection volume (mℓ)
Vf = Final volume (mℓ) W = Amount of ATP from calibration curve (ng)

An example recovery calculation is presented in Section 10.

8. Schematic of Analytical Procedure for ATP in Water

Matrix (Water) 200 mℓ

↓ (pretreatment: wash Mega Bond Elut C_{18} with 5 mℓ acetonitrile and then 5 mℓ distilled water)

pass through a packed column (Mega Bond Elut C_{18}, Part No. 1225-6001: Varian) at a flow rate of ca. 5 mℓ/min

↓

discard the eluate

↓ wash the packed column with 10 mℓ of distilled water at a flow rate of ca. 5 mℓ/min

discard the washings

↓ elute ATP with 30 mℓ of a mixed solvent (CH_3CN + H_2O = 3 + 17, v/v) at a flow rate of ca. 2~3 mℓ

collect the eluate and concentrate it to dryness under reduced pressure

↓ (pretreatment: rinse Sep-Pak C_{18} Plus with 5 mℓ acetonitrile and then 5 mℓ distilled water)

↓ dissolve the residue with 10 mℓ distilled water and pass through a packed column (Sep-Pak C_{18} Plus, Part No. WAT020515: Waters) at a flow rate of ca. 2~3 mℓ/min

discard the eluate

↓ elute ATP with 30 mℓ of the same mixed solvent shown above at a flow rate of ca. 2~3 mℓ/min

collect the eluate and concentrate it to dryness under reduced pressure

↓

dissolve the residue in a certain volume (≧2 mℓ) of the HPLC mobile phase

↓

inject 50 μℓ to HPLC

9. Example Residue Calculation

Lab Code No. D-F10, Control water (Drinking) fortified with 50 ppb of ATP

The residue of ATP in ppb (R) is calculated with the following formula:

$$R = \frac{W \times Vf}{G \times Vi}$$

G = Sample volume (g) (corresponds to Sample volume (mℓ) used)
Vi = Injection volume (mℓ) = 0.050
Vf = Final volume (mℓ) = 100 W = Amount of ATP from calibration curve (ng)

To determine amount of ATP (refer Tables 3 and 6)
If the Y-axis is peak area and X-axis is amount of ATP, then

$$\text{Amount of ATP (ng)} = \frac{\text{Peak Area} - b}{a}$$

calibration curve : $Y = ax + b = 2271.6X - 10.206$
Amount of ATP (ng) ≥ 0

$$\text{Amount of ATP (ng)} = \frac{10739 - (-10.206)}{2271.6} = 4.732 \text{ ng}$$

$$\text{Residue amount of ATP} = \frac{100 \text{ mℓ} \times 4.732 \text{ ng}}{200 \text{ g} \times 0.050 \text{ mℓ}} = 47.32 \text{ ppb}$$

10. Example Recovery Calculation

Lab Code No. D-F10, Control water (Drinking) fortified with 50 ppb of ATP

The fortification recovery (S) for ATP in % is calculated from the following equation.

$$S\ (\%\ \text{recovery}) = \frac{\text{Residue (ppb)} \times 100}{\text{Fortified level of compound (ppb)}}$$

Fortified level = 50 ppb

Residue = 47.32 ppb

$$S = \frac{47.32\ \text{ppb} \times 100}{50\ \text{ppb}} = 95\%$$