Note

Residue Determination Method for the Insecticide Acetamiprid in Crops by Gas Chromatography

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INTRODUCTION

An analytical method has been developed for the determination of acetamiprid, (*E*)-*N*¹-[(6-chloro-3-pyridyl)methyl]-*N*²-cyano-*N*¹-methylacetamidine (ATP) in crops by gas chromatography (GC). ATP in crops was extracted with methanol, purified by liquid-liquid partition and column chromatography and then determined by electron capture detector (ECD)-GC. The limit of detection of ATP for fruits and vegetables was 0.005 ppm and the recovery of fortified samples at 0.1 ppm was about 96% in average (80-104%). In case of green tea, the limit of detection of the compound for green tea (powder) and its leachate was 0.05 ppm and the fortified recovery rates ranged from 95 to 98%.

MATERIALS AND METHODS

1. Standard Materials

ATP used in this study was synthesized at Nippon Soda Co., Ltd. and the purity was more than 99%. The appearance of ATP is white powder and its melting point is 101-103°C (Fig. 1).

2. Reagents

The organic solvents and inorganic chemicals used in the study were guaranteed reagent purchased from Wako Pure Chemical Industries, Ltd. Florisil (Florisil PR®: non activated) used was produced by Floridon Co. Packed column (Sep Pak® C₁₈) was produced by Waters. Celite (No.545) was produced by Johns-Manville Corp.

3. Apparatus

Gas chromatograph (Model GC-14B, Shimadzu) equipped

(*E*)-*N*¹-[(6-chloro-3-pyridyl)-methyl]-*N*²-cyano-*N*¹-methylacetamidine (ATP)

Fig. 1 Structures and chemical (abbreviated) name of acetamiprid.

with an ECD was used for quantitation. Other apparatuses were a rotary evaporator (Model N-1, Tokyo Rica Kikai), a macerator (Polytron®, Kinematica GmbH), a mill (SCM-40A, SIBATA), a mechanical shaker (SR-IIw, TAIYO Co.) and balances (Model EB-3200H, Shimadzu, Model H20, Mettler). A glass tube for column chromatography was 15 mm i.d. and 300 mm length.

4. Operating Conditions of GC

A 5% PEG HT/chloromosorb W HP column (60-80 mesh, 3.2 mm i.d., 1 m length) was used for the separation. The inlet and detector temperatures were both 320°C and column temperature was 260°C. The carrier gas was nitrogen at a column head pressure of 1.5 kg/cm².

5. Preparation of Calibration Curve

Calculation of results was based on peak height measurements using calibration curve. The standard curve was obtained by direct injection of 2 µl of ATP standard (acetone) for GC in the range of 0.02 to 0.5 µg/ml. Calibration curve was prepared by plotting the peak heights vs. the weights of ATP.

6. Analytical Procedure

6.1 Sample preparation and fortification

Seven kinds of crops (9 matrices) used for fortified recovery tests were obtained from Agricultural Experiment Stations in Japan. Each matrix was cut into pieces with a kitchen knife and homogenized with a mixer. In case of the radish, two portions, the root and leaf, were used separately.

Regarding green tea (powder), leaves dried were ground with a mill.

As for fortification, the experiments were carried out at a range of 0.1 to 2 ppm (refer to Table 2).

6.2 Extraction

6.2.1 Fruits and vegetables

The subsamples (except green tea) (20 g) were homogenized with 100 ml of methanol using the macerator for 3 min and then shaken for 30 min by mechanical shaker. The homogenate was filtered through a Celite layer (1-2 cm thickness) under reduced pressure. The filter cake and the vessel was washed twice with each 25 ml of methanol and the washings were also filtered through the same Celite layer. These filtrates were combined and transferred to a 500 ml separatory funnel.

6.2.2 Green tea (Powder)

The subsample (4 g) was soaked with 16 ml distilled water for 2 hr. Then 100 ml methanol was added to the mixture and it was shaken for 30 min. The subsequent extraction manner is similar to that of fruits and vegetables.

6.2.3 Green tea (Leachate)

The subsample (4 g) was soaked in 240 ml of boiling water

for 5 min. The mixture was filtered through a filter paper and cooled to ambient temperature. The half volume of the filtrate was subjected to further analysis (corresponds to 2 g of green tea dried). Five grams of sodium chloride and 120 ml methanol were added to the filtrate and then the aqueous methanol was washed twice with each 100 ml hexane for 10 min. The hexane was discarded. The aqueous methanol was transferred to a 500 ml separatory funnel.

Continue to 6.3 (line 5).

6.3 Liquid-liquid partition

Five percents of sodium chloride solution (150 ml) was added to the filtrate. The aqueous methanol solution was shaken twice with each 100 ml hexane for 10 min. The hexane layer was discarded. The aqueous methanol was transferred to another 500 ml separatory funnel. The aqueous methanol was shaken twice with each 100 ml dichloromethane (DCM) for 10 min. In case of summer orange and dry matrices, the DCM layer was washed with 100 ml of 0.05 N alkaline solution for 5 min. The alkaline solution was discarded. The DCM solution was passed through a filter paper with anhydrous sodium sulfate and collected in a 300 ml round bottomed flask. One gram of Florisil PR was added to the DCM layer and the DCM solution was concentrated to dryness in a water bath at *ca.* 40°C under reduced pressure.

6.4 Column chromatography

The residue with 1 g Florisil PR was transferred onto the top of the column packed with Florisil PR (9 g) with the aid of hexane. The vessel was rinsed twice with each 10 ml of a mixed solvent of acetone and hexane (20+80, v/v), and the washings were charged to the column. The eluate was discarded. The same mixed solvent described above (130 ml) was passed through the Florisil PR column and the eluate was discarded. Then ATP was eluted with a mixed solvent of acetone and hexane (50+50, v/v, 120 ml). The eluate was collected and concentrated to about 10 ml in a water bath at *ca.* 40°C under reduced pressure. The concentrate except summer orange and dry matrices was transferred to a 100 ml of pear shaped flask and dried up under the same manner described above. With regard to summer orange and dry matrices, the eluate collected from Florisil PR column was concentrated to dryness in a water bath at *ca.* 40°C under reduced pressure. The residue was dissolved in 5 ml of distilled water and charged on the top of Sep Pak® C₁₈ pretreated with 20 ml of methanol and then with 20 ml distilled water. The vessel was rinsed three more times with each 5 ml distilled water. Each rinsing was charged on the C₁₈ cartridge and eluted. These water eluates were discarded. The vessel was washed with 30 ml of 15% acetonitrile and the washings were charged on the top of the C₁₈ cartridge. ATP was eluted with the 30 ml of the 15% acetonitrile solution and the eluate was collected and then concentrated to dryness in a water bath at 40–50°C under reduced pressure.

6.5 GC determination

The residue was dissolved in a certain volume of acetone.

Two microliters of the solution was injected into the GC. The residue amount of ATP was calculated by the calibration curve prepared previously. Detector linearity, from 0.04 to 1.0 ng, was checked daily for ATP.

RESULTS AND DISCUSSION

1. Extraction Solvent

Methanol, a mixed solvent of methanol and 0.1 M HCl (75+25, v/v), acetonitrile and acetone were checked as extraction solvents for ATP.

The residue amount obtained from methanol was a little higher than that from the other solvents so that methanol was used as the extraction solvent in this study¹⁾ (Table 1).

2. Clean Up

2.1 Liquid-liquid partition

The liquid-liquid partitions were investigated between 50% aqueous methanol and hexane or DCM. Since ATP was not transferred to hexane but transferred to DCM quantitatively, DCM was adopted as extraction solvent of ATP from 50% aqueous methanol. Whereas hexane was used in order to remove interferences in 50% aqueous methanol from crops. ATP in DCM was not transferred to diluted alkaline solution (0.05 N) so that the DCM layer was washed by the alkaline solution as a clean up procedure for summer orange and dry matrices.

2.2 Column chromatography

Florisil PR was checked in combination with two kinds of mixed solvents of methanol and DCM (2.5–5.0+97.5–95, v/v), acetone and hexane (20–50+80–50, v/v). ATP was eluted under both conditions, but the mixed solvent of acetone and hexane was more effective to remove interferences from crops.

For residue analyses of ATP in summer orange and dry matrices, interferences could not be removed sufficiently by liquid-liquid partitions and Florisil PR column chromatography. Reverse phased column (Sep Pak® C₁₈) was used as an additional clean up procedure to remove the interferences from summer orange and dry matrices.

3. GC Conditions

GC condition was investigated by ECD in combination with several kinds of packing materials to get a sharp peak, minimum detectable amount of ATP and separation of ATP

Table 1 Investigation for extraction solvent of ATP from crops (unit : ppm).

Crop	Solvent			
	MeOH	0.1 M HCl-MeOH (25+75, v/v)	Acetonitrile	Acetone
Eggplant	0.46	0.41	0.37	0.34
Pear	0.43	0.40	0.41	0.37
Green pepper	0.78	0.71	0.80	0.72

Figures are average of duplicates.

Table 2 Fortified recoveries of ATP in 12 matrices by Parent Method.

Crop	Sample weight (g)	Amount added (μg)	Recovery (%) ^{a)}
Cabbage	20	2	102
Potato	20	2	90
Radish (Leaf)	20	2	100
Radish (Root)	20	2	95
Grape	20	2	99
Summer orange (Whole body)	20	2	80
Apple	20	2	98
Strawberry	20	2	104
Green pepper	20	2	98
Eggplant	20	2	98
Green tea (Powder)	4	2	95
Green tea (Leachate)	2	1	98

^{a)} Figures are the average of duplicates.

from interferences. As a result, 5% PEG-HT/Chromosorb W HP was the best packing material. Other than these packed column conditions, a few kinds of capillary columns (widebore type) were checked with ECD-GC. DB-17 (0.53 mm i.d., 30 m length, 1.0 μm film thickness, produced by J & W Scientific) and BPX-35 (0.25 mm i.d., 25 m length, 0.25 μm film thickness, produced by SGE) gave a good result from the view points described above.

Calibration curves were prepared by a linear and a quadratic equations. The coefficient of correlation of the quadratic equation was higher than that of the linear equation.

4. Limit of Detection and Fortified Recovery

4.1 Limit of detection

The minimum detectable amount of ATP was 0.04 ng. The limit of detection was 0.005 ppm (fruits and vegetables) when 20 g of sample was used and injected 2 μl into GC from the final solution (5 ml).

4.2 Fortified recovery

The recovery of ATP fortified at 0.1 ppm to 10 kinds of representative crops (12 matrices) was 96% in average (Table 2).

Fortified recovery tests at 0.005 and 0.05 ppm (green tea) gave clear peak of ATP for all matrices (Figs. 2 and 3).

5. Others

5.1 HPLC determination

The maximal absorption of ATP in distilled water was at 246 nm and the molecular extinction coefficient was $\text{ca. } 2 \times 10^4$. It was possible to adopt a HPLC with UV detector for determination of ATP. Normal phased column, Zorbax BP NH₂ in combination with methanol/hexane/DCM (0.4+10+90, v/v) was available for the residue analyses of ATP in many kinds of crops. For reversed phased column, Supelco SIL LC-18 in combination with methanol/water (3+7, v/v) was also available. But chromatograms obtained from GC

Fig. 2 Examples of GC chromatograms for standard ATP. (a) Minimum detectable amount of ATP 0.04 ng (2 μl). (b) ATP 1.0 ng (2 μl). (c) Reagent blank (2 $\mu\text{l}/5\text{ ml}$).

Fig. 3 Examples of GC chromatograms for cabbage. (a) Cabbage control (2 $\mu\text{l}/5\text{ ml}$). (b) Fortified recovery of ATP at 0.1 ppm (2 $\mu\text{l}/5\text{ ml}$).

is better than that from HPLC.

The minimum detectable amount of ATP was 1.0 ng. The limit of detection was 0.005 ppm (fruits and vegetables) when 20 g of sample was used and the final solution was adjusted to 2 ml and 20 μl of the final solution was injected into a HPLC.

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REFERENCES

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要 約

ガスクロマトグラフィーによる作物中の殺虫剤アセタミプリドの残留分析法

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新規殺虫剤アセタミプリド (*E*)-*N*¹-[6-chloro-3-pyridyl)meth-

yl]-*N*²-cyano-*N*¹-methylacetamidine の作物残留分析法を検討した。その結果, 作物中のアセタミプリドをメタノールで抽出後, 液々分配およびカラムクロマトグラフィーにより精製した後, ECD-GC により定量する方法を確立した。検出限界は果物・野菜類で 0.005 ppm, 茶で 0.05 ppm であり添加回収率 (0.1 ppm) は 80~104% であった。