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Determination of Amitraz Residue in Fruits by High Performance Liquid Chromatography

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ABSTRACT

A high performance liquid chromatographic method (HPLC) for the determination of amitraz residue in fruits has been developed. Twenty grams of sample and 2 g of sodium hydrogen carbonate were weighed and homogenized with acetone. After vacuum filtration, 1 g NaCl was added and partitioned with *n*-hexane containing 20% ethyl acetate. The organic layer was evaporated to 5 mL and applied on a florisil cartridge. The cartridge was eluted with 10 mL *n*-hexane:ethyl acetate (8:2, v/v), and the eluate was evaporated to dryness. The residue was dissolved in acetonitrile and determined by HPLC equipped with a UV detector. Separation was conducted with a Lichrosorb RP-18 column using acetonitrile: H_2O (80:20, v/v) as mobile phase to separate amitraz and interferences, and amitraz was determined at 313 nm wavelength. Recovery studies were carried out by spiking the amitraz standards to the levels of 0.25~0.75 ppm in apples and 0.1~0.3 ppm in grapefruit. The average recoveries were 88.8~92.1% and 87.2~90.9% for apples and grapefruit, respectively. The detection limit was 0.02 ppm.

Key words: **amitraz, high performance liquid chromatography (HPLC), florisil cartridge, fruit.**

INTRODUCTION

The purpose of this study was to develop an analytical method for determining amitraze residue in fruits with high recovery and good reproducibility. The detection limit of this method was expected to be lower than the tolerance levels announced by the Department of Health. Amitraz [*N*-methyl bis(2,4-xylyliminomethyl)amine], available commercially as 'Taktic' (FBC Ltd), 'Triatox' (Wellcome), and 'Bumetran' (Schering), belongs to the family of triazapentadiene[1]. Amitraz is widely used as an acaricide against mites on fruit trees, and has exceptional miticidal activity toward mites of pears, apples, and citrus fruits[2]. It is unstable in acidic media (pH<7), and slowly decomposes in prolonged storage under moist conditions[3]. The chemical structure is shown in Figure 1. Amitraz is soluble in most organic solvents, such as acetone and toluene (>

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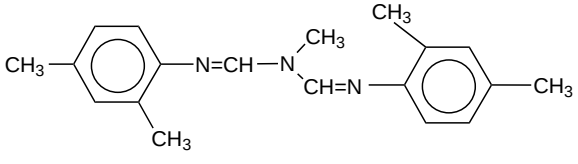
300 g/L), the solubility in water is ca. 1 mg/L at room temperature. Amitraz is a non-systemic acaricide and insecticide with contact and respiratory action. The acute oral LD_{50} to rats and mice are 800 and >1600 mg/kg, respectively[3]. Amitraz is allowed to be applied to pome and citrus crops in Taiwan according to the "Safety Tolerances of Pesticide Residues"[4] announced by the Department of Health. The tolerances for residue levels of amitraz in pome and citrus crops are 0.5 and 0.2 ppm, respectively. References about amitraz determination from agricultural products are scarce and most of them involve time-consuming sample preparation. Quantitative methods for amitraz in agicultural products have been reported using gas chromatography (GC) equipped with a nitrogen-phosphorus detector (NPD)[5], electron capture detector (ECD)[1,2] and thermionic detector[6]. Some methods[1,2] involved decomposition of amitraz and / or derivatization with heptafluorobutyric anhydride, thus requiring a complicated and time-consuming extraction procedure. Rice[7] developed a high performance liquid chromatographic method for determining amitraz formulation. Amitraz appears to undergo rapid hydrolytic degradation under acid conditions. It is very important to keep amitraz in its complete form during analysis steps applied on acid fruit samples. This paper describes a method for determining amitraz residue in apples and grapefruit by using HPLC, which is simple, and has high recovery and good reproducibility.

MATERIALS AND METHODS



Chemical name: *N*-methylbis (2,4-xylimimethyl) amine (IUPAC).
Molecular formaula: $C_{19}H_{23}N_3$.
Molecular weight: 293.

Figure 1. Chemical structure of amitraz.

I. *Materials*

The samples of apples and grapefruit were purchased from traditional markets.

II. *Chemicals*

A residue grade acetone, *n*-hexane, and ethyl acetate, LC grade acetonitrile, and a reagent grade anhydrous sodium sulfate, sodium hydrogen carbonate were used in this study. The standard of amitraz was obtained from Riedel-de Haen AG (Germany). The purity of standard was 99% as labeled.

III. *Methods*

(I) *Preparation of Standard Solutions*

One hundred mg of sodium amitraz was accurately weighed into a 100 mL volumetric flask and dissolved to volume in acetonitrile as a stock solution. This solution was then diluted to a final concentration of 10 μg/mL with acetonitrile as a standard solution.

(II) *Sample Preparation*

1. *Extraction*

Twenty grams of sample, 2 g of sodium hydrogen carbonate and 60 mL acetone were added in a blender jar. The sample was macerated for 5 min, and the extract was vacuum-filtered through filter paper. After filtration, the pellets and container were washed with 20 mL of acetone, which was then filtered. The combined filtrates were transferred into a separation funnel in which 1 g NaCl was added and extracted twice with *n*-hexane: ethyl acetate (8:2, v/v) 40 mL for 2 min. The combined organic phase was passed through a funnel containing anhydrous sodium sulfate and evaporated to dryness at 35~40°C using a rotary evaporator. The residue was dissolved in 5 mL of *n*-hexane: ethyl acetate (8:2, v/v) and applied 2 mL on a florisil cartridge for cleanup.

2. *Cleanup*

The sample was applied on a Sep-Pack florisil cartridge (910 mg) which was rinsed with 5 mL *n*-hexance before loading the sample. The cartridge was eluted with 10 mL *n*-hexane:ethyl acetate (8:2, v/v) and the eluate was evaporated to dryness. The residue was dissolved in 2 mL of acetonitrile and filtered through a 0.45 μm nylon membrane prior to HPLC analysis.

III. *High Performance Liquid Chromatography (HPLC) Analysis*

HPLC analysis was carried out by using a Hitachi HPLC system (Japan) equipped with a L-6200 liquid pump system, a L-4250 UV-VIS detector, and a C-R4A Chromatograph data management system. The separation was performed on a Merck Lichrosorb RP-18 column (5 μm, 250 x 4.0 mm i.d.). A mobile phase of acetonitrile: H_2O (80:20, v/v) pumped at a flow rate of 0.9 mL/min was used. Detection was carried out on a UV detector set at 313 nm. The injection volume was 20 μL.

IV. *Gas Chromatography / Electron Impact-Mass Spectrometry (GC / EI-MS) Analysis*

GC / MS analysis was performed by using a HP-5890 series II GC equipped with a HP 5970B quadrupole mass selective detector (MSD) and a HP 340C ChemStation data management system. A J&W Scientific HP-1 column (25 m × 0.25 mm i.d.) was used. The column oven temperature was held at 50°C for 2 min and then programmed to 320°C at 15°C/min. Both temperatures of injection port and interface to MSD were 250°C. The injection volume was 1 μL. The carrier gas was helium and the head pressure was adjusted to 8

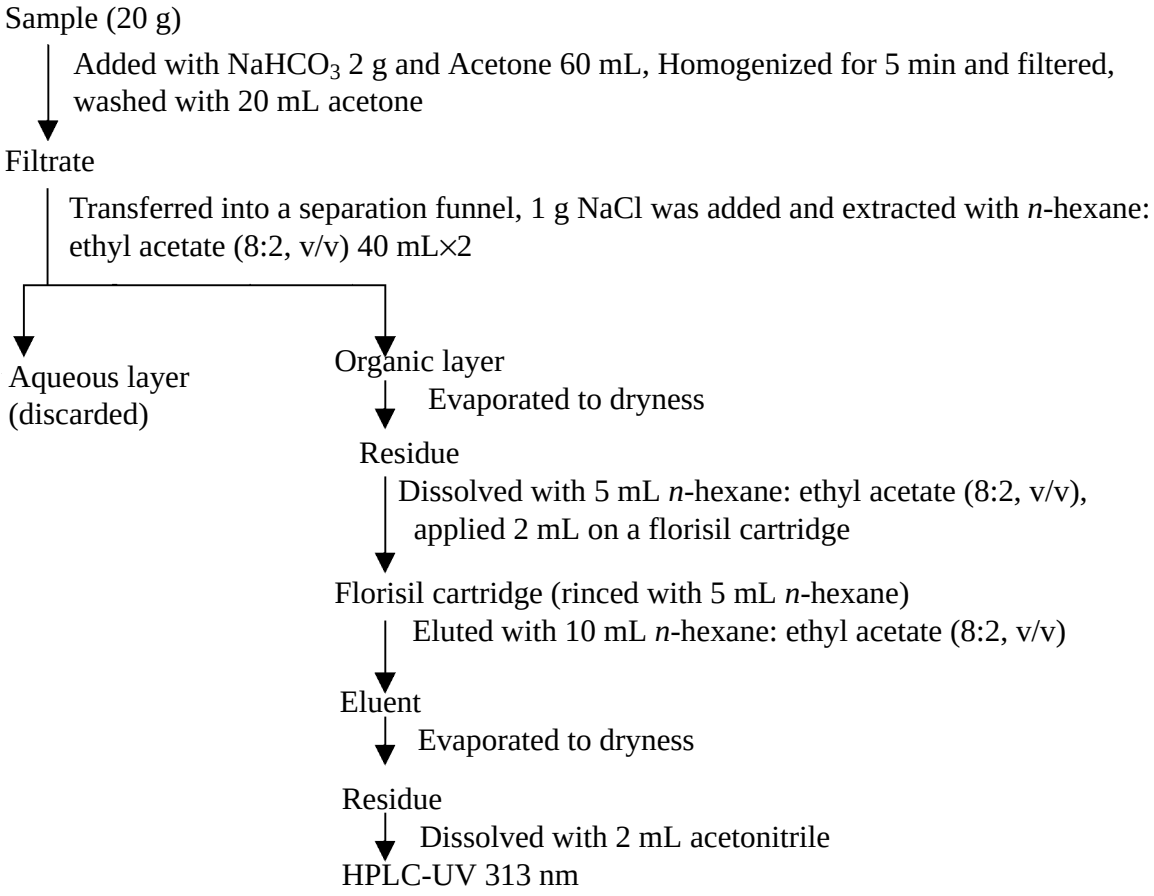


Figure 2. Analytical procedure for determining amitraz residue in apples and grapefruit.

psi.

V. *Detection Limit Test*

Fruit samples were spiked with different concentration levels of amitraz standard followed by our analysis procedures and determined by HPLC. The detection limit was evaluated by the peak signal/noise (S/N) ratio. An S/N ratio greater than 3 was considered as a detectable peak.

RESULTS AND DISCUSSION

I. *Extraction*

Nakamura *et al.*[5] developed a method for determining 8 acaricides (including amitraz) in samples including fruits, vegetables, and brown rice. Samples were homogenized with acetone or acetone containing 30% of water, NaCl was added and extracted with *n*-hexane containing 20% ethyl acetate. Following this extraction procedure and applied on apple and grapefruit samples, a low recovery (<10%) of amitraz was obtained. Amitraz is unstable in acidic media (pH<7). The pH of apple and grapefruit juice is below 4. We found amitraz appears to undergo rapid hydrolytic degradation during this extraction procedure for these types of fruits. The pH and water content are the key points for keeping amitraz in a complete form during hydrolysis. Our preliminary study showed that when 0.1 M ammonia buffer (pH 11) solution was added to the fruit samples (1:1, w/v) before homogenization, the recovery of amitraz in fruits increased to 60%. Adding sodium hydrogen carbonate before homogenization and no water added during extraction and cleanup steps can keep amitraz stable in the analysis procedure and raise the recovery of amitraz in fruits up to over 80%. The extraction solvents for amitraz analysis in references were dichloromethane[9], benzenze [6], acetone and *n*-hexane: ethyl acetate (8:2, v/v)[5]. Dichloromethane and benzene are carcinogens and highly toxic. Because of these factors, we decided to substitute dichloromethane and benzene with acetone and *n*-hexane: ethyl acetate (8:2, v/v). The extraction and cleanup procedures in our experiments are shown in figure 2.

II. *Cleanup Operation*

A cleanup operation was used to prevent UV

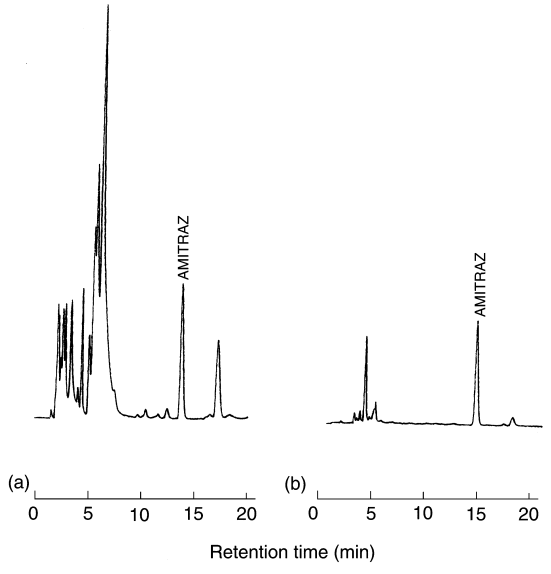


Figure 3. HPLC chromatograms of amitraz spiked into apples (a) before and (b) after cleanup by Sep-Pak florisil cartridge.
HPLC column: Lichrosorb RP-18; mobile phase: CH_3CN:H_2O (80:20, v/v); flow rate: 0.9 mL/min; UV detector: 313 nm.

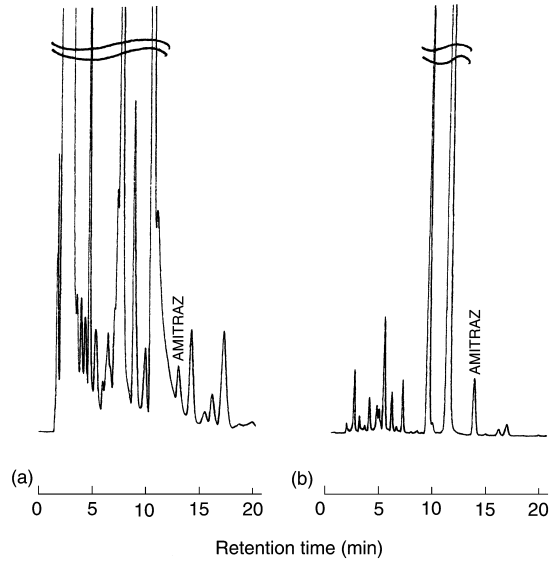


Figure 4. HPLC chromatograms of amitraz spiked into grapefruit (a) before cleanup and (b) after cleanup by Sep-Pak florisil cartridge.
HPLC conditions are shown in Figure 3.

Table 1. Recoveries of amitraz spiked into apples and grapefruit

Sample(Crop type)	Spiked level(ppm)	Recovery [a](%)
Apple	0.25	91.8(7.6) [b]
(Pome)	0.50	88.8(7.8)
	0.75	92.1(5.6)
Grapefruit	0.1	87.2(3.0)
(Citrus)	0.2	87.2(5.6)
	0.3	90.9(4.0)

[a]: average of triplicate.

[b]: value in the parenthesis is coefficient of variation (CV, %).

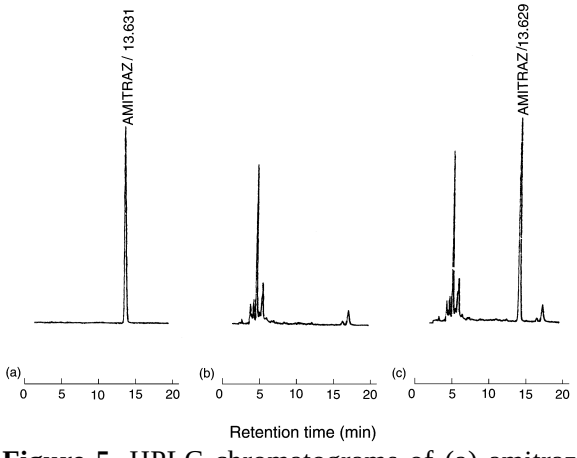


Figure 5. HPLC chromatograms of (a) amitraz standard (b) apple blank (c) apple spiked with 0.5 ppm amitraz.
HPLC conditions are shown in Figure 3.

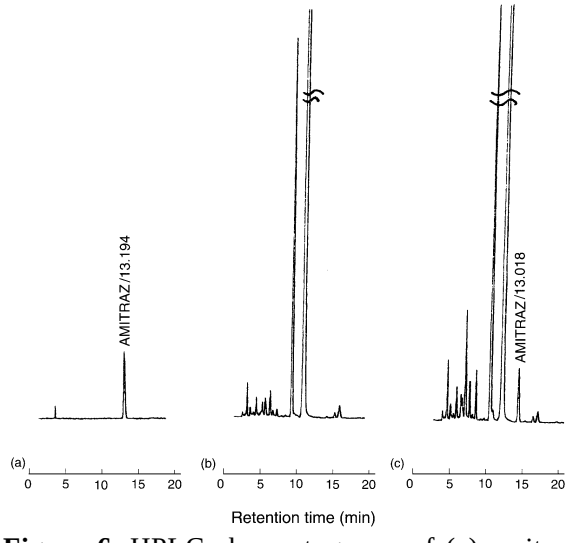


Figure 6. HPLC chromatograms of (a) amitraz standard (b) grapefruit blank (c) grapefruit spiked with 0.5 ppm amitraz.
HPLC conditions are shown in Figure 3.

interference from co-extractives when a method of reverse phase HPLC was carried out. Florisil cartridge, silica cartridge and C_{18} cartridges were tested for cleanup effect and the florisil cartridge was found to have the best cleanup effect with high recovery. *n*-Hexane: ethyl acetate (9:1, v/v) , *n*-Hexane: ethyl acetate (8:2, v/v) and *n*-Hexane: ethyl acetate (7:3, v/v) were tested for eluting solvent, and *n*-Hexane: ethyl acetate (8:2, v/v) was chosen for its best performance as the eluting solvent system. Figures 3 and 4 demonstrate the cleanup effect of the florisil cartridge for HPLC determination of amitraz in apples and grapefruit.

III. *HPLC Conditions*

The HPLC conditions for amitraz analysis were adopted from the findings of Rice[7] with slight modifications. Acetonitrile: H_2O (80:20, v/v) as mobile phase was able to yield a peak with retention time at ca. 13 min, which completely separated amitraz and interference peaks.

IV. *Fortification Recovery Test*

Table 1 gives the recoveries of fortified amitraz at the levels of 0.25-0.75 ppm for apples and 0.1-0.3 ppm for grapefruit. The average recoveries for apples and grapefruit ranged from 88.8 to 92.1% and 87.2-90.9%, respectively, and the coefficients of variation for both ranged from 3.0-7.8%. The results showed satisfactory recovery

and reproducibility. Figure 5 and Figure 6 show the HPLC chromatograms of amitraz in apples and grapefruit, respectively. The compounds of interest were well resolved from other co-extractives as compared to the sample blanks without fortification of amitraz . These results indicate that the method used in this study provides a satisfactory cleanup.

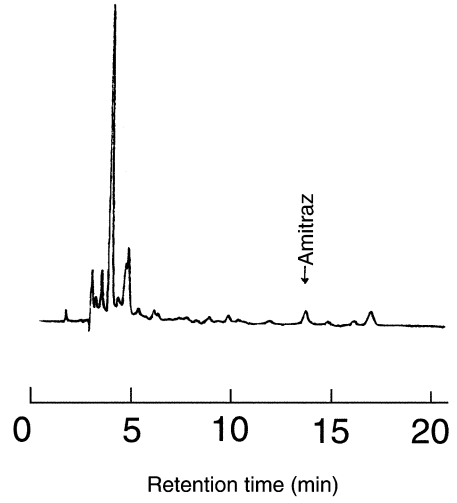


Figure 7. HPLC chromatogram of apples spiked with 0.02 ppm amitraz (the detection limit).
HPLC conditions are shown in Figure 3.

V. *Detection Limit Test*

By using the methods described above, the detection limit of amitraz for tested sample was taken to be 0.02 ppm (Figure 7), which were lower than the tolerance levels announced by the Department of Health. This indicates that the method developed in our laboratory was sensitive and could be used as a official method to determine amitraz in pome and citrus crops.

VI. *Gas Chromatography-Mass Spectrometry (GC-MS) Analysis*

Amitraz can be analyzed by GC-MS and its mass spectrum is shown in Figure 8. The fragments of m/z 294, 162 and 121 are suggested to be the ions for the selected ion monitoring (SIM) detection. This GC-MS is used for the further identification purpose.

REFERENCES

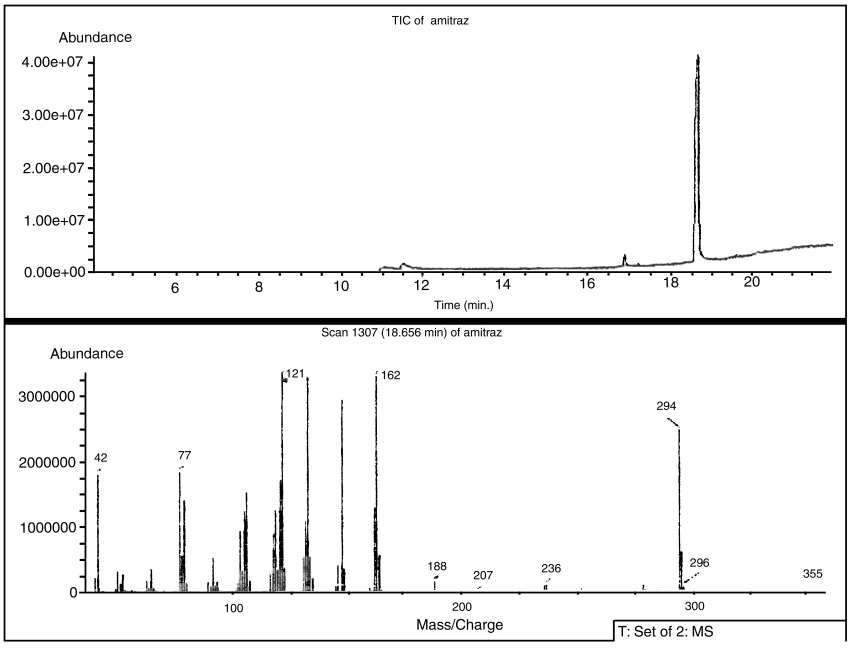


Figure 8. GC-MS spectrum of amitraz standard.
GC-MSD conditions: Column: HP-1 ; Initial temp.: 50°C; Initial time: 2 min; Rate: 15°C/ min; Final temp.: 320°C; Final time: 20 min; MSD interface temp.: 250°C; Injector temp.: 250°C.

1. Taccheo, M. B., Paoli, M. D. and Spessotto, C. 1988. Determination of total amitraz residue in honey by electron capture capillary gas chromatography-a simplified method. Pestic. Sci. 23:59-64.
2. Hornish, R. E., Clasby, M. A., Nappier, J. L., Nappier, J. M. and Hoffman, G. A. 1984. Total residue analysis of amitraz [1,5-bis(2,4-dimethylphenyl)-3-methyl-1,3,5-triazapenta-1,4-diene] residues in fruit and soil samples by electron capture gas chromatography. J. Agric. Food Chem. 32:1219-1223.
3. Royal Society of Chemistry. 1987. The Agrochemicals Handbook. 2nd ed. Unwin Brothers Limited, Surrey, U.K.
4. Department of Health, Executive Yuan. 1987. Tolerances for Residues of Pesticides. Announcement No. 86022970.
5. Nakamura, Y., Hasegawa, Y., Tonogai, Y. and Ito, Y. 1989. Simultaneous determination of 8 kinds of acaricides in agricultural products by gas chromatography. Eisei Kagaku 35: 466-475.
6. Iwata, Y., Walker, G. P., O'Neal, J. R. and Barkley, J. H. 1985. Residues of acephate, amitraz, chlorpyrifos and formetanate hydrochloride on and in fruits after low-volume application to orange trees. Pestic. Sci. 16:172-178.
7. Rice, L. G. 1984. Rapid separation of pesticides by high-performance liquid chromatography with 3-μm columns. J. Chromatogr. 317: 523-526.
8. Ameno, K., Fuke, C., Ameno, S., Kiriu, T., Shinohara, T. and Ijiri, I. 1991. A rapid and sensitive quantitation of amitraz in plasma by gas chromatography with nitrogen-phosphorus detection and its application for pharmacokinetics. J. Anal. Toxicol. 15: 116-118.
9. Berzas Nevado, J. J., Mahedero, M. C., Olibares, J. A. and Salinas, F. 1991. Determination of amitraz in honey by first-derivative spectrophotometry. Intern. J. Environ. Anal. Chem. 43: 187~194.

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以高效液相層析檢測水果中三亞蟎殘留量

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摘　要

本研究發展出以高效液相層析法檢驗水果中三亞蟎殘留量之方法。稱取20 g檢體及2 g碳酸氫鈉，以丙酮均質抽取，抽取液抽氣過濾後加入1 g氯化鈉，以正己烷：乙酸乙酯 (8:2, v/v) 溶液萃取，有機溶媒層減壓濃縮至5 mL後注入矽酸鎂固相萃取匣，以10 mL正己烷：乙酸乙酯 (8:2, v/v) 溶液沖提，沖提液減壓濃縮至乾以乙腈定容並以HPLC附UV檢出器分析。層析管為Lichrosorb RP-18，移動相為乙腈：水 (80:20, v/v)，UV檢出器設定為313 nm。添加三亞蟎於蘋果中，檢體濃度為0.25～0.75 ppm，所得平均回收率為88.8～92.1%；添加於葡萄柚中，檢體濃度為0.1～0.3 ppm，所得平均回收率為87.2～90.9%。本檢驗方法之最低檢出限量為0.02 ppm。

關鍵詞：三亞蟎，高效液相層析，矽酸鎂固相萃取匣，水果。