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High Performance Liquid Chromatographic Determination of Maleic Hydrazide Residue in Potatoes

WAN-CHEN LEE, TSUNG-LIN LI*, PI-CHIOU CHANG AND SHIN-SHOU CHOU

National Laboratories of Foods and Drugs, Department of Health, Executive Yuan
161-2, Kuen Yang Street, Nankang 115, Taipei, Taiwan, R.O.C.

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ABSTRACT

A reliable and fast method was developed using high performance liquid chromatography equipped with a fluorescence detector for separating and quantifying the residues of maleic hydrazide in potatoes. The method started with methanol extraction, followed by filtration or centrifugation, and concentration to dryness. The remaining was reconstituted with water for cleanup, which was applied to a SCX solid phase extraction cartridge (a strong cation exchanger). Average recoveries of potatoes spiked with 10~20 ppm maleic hydrazide were in the range of 87.8~95.7%, and their coefficients of variation were in the range of 2.1~4.0%. The limit of detection was 0.5 ppm.

Key words: pesticide residue, maleic hydrazide, HPLC

INTRODUCTION

Maleic hydrazide functions as a growth regulator acting especially on root vegetables. Maleic hydrazide is applied as over-the-top foliar spray when the foliage is still in a good condition. Routinely, it is also used as a sprouting inhibitor for storage[1~3]. According to the "Tolerances for Residues of Pesticides" published by the Department of Health, Taiwan, the tolerance level of maleic hydrazide in potatoes is 15 ppm[1]. Table 1 is the summary relating to chemical structure, physical-chemical properties and reaction mechanisms of maleic hydrazide[2].

High performance liquid chromatography (HPLC) and gas chromatography (GC) have been developed for determining the residues of maleic hydrazide in agricultural products. Methods with HPLC-UV in determining agricultural products such as potato, onion, tobacco, garlic bulbs, and processed food such as potato chips have been reported. Further reducing interferences in potato and onion samples with an ion-exchange solid phase cartridge was introduced by Vadukul[4]. Newsome, on the other hand, took advantage of anion exchange chromatography to quantify maleic hydrazide and β-D-glucoside in various samples[5]. In addition, a very specific method using a fluorescence detector was studied by Kubilius and Bushway[6].

In GC methods, derivatization is an inevitable measurement needed before applying to GC. Terashi *et al* derivatized maleic hydrazide with dimethyl sulphate, which was then analyzed by a nitrogen-phosphorous detector[7]. King converted the maleic hydrazide residues in potatoes to a volatile Diels-Alder adduct which were determined by electron capture detection[8].

This study took advantage of the approaches mentioned above to develop a new method, which will satisfy all around requirements.

* Author for correspondence. Tel: 02-26531262;
Fax: 02-26531256; E-mail:lee1464@nlfd.gov.tw

MATERIALS AND METHODS

I. *Materials*

Potato samples were purchased from traditional markets and supermarkets.

II. *Reagents*

Methanol and acetonitrile were residue grade. Phosphoric acid and anhydrous sodium hydrogen phosphate were reagent grade. Maleic hydrazide standard was obtained from Riedel-de Haen, AG (Germany) with 99 % purity.

III. *Methods*

(I) *Preparation of Standard Solutions*

Maleic hydrazide (100 mg) was accurately weighed into a 100-mL volumetric flask. Methanol was then added up to the mark as stock solution. As needed, the stock solution was diluted with water in various working solutions.

(II) *Sample Preparation*

1. Extraction

Test samples were sliced and homogenized. Ten grams of each sample were aliquoted into a separation funnel, extracted twice with 30 mL methanol and the funnel rigorously shaken 3 min for each extraction. The pooled solution was then filtrated or centrifuged (3500 rpm, 5 min), and then concentrated with a rotary evaporator at 35˚C to dryness. The

remaining was reconstituted with 2 mL of water for cleanup.

2. Cleanup

Aliquot 1 mL of reconstituted sample was applied to a 1 g of SCX solid phase extraction cartridge (a strong cation exchanger; Waters, Division of Millipore Corporation, MA, USA), which had been equilibrated first with 5 mL of methanol and followed by 5 mL of 0.001 N NaOH solution. After loading the sample, elute the cartridge is eluted with 2 mL of 0.001 N NaOH solution. Water is added to the elute to 2 mL, and then filtered with a nylon membrane prior to HPLC analysis.

(III) *HPLC Analysis*

HPLC analysis was carried out on a Shimadzu HPLC system (Japan), which included a L-6200 pump, a CBM-10A communication module and a SIL-10A auto-injector. Analysis was monitored by a fluorescence detector (RF-10 AXL) at wavelength 303 and 400 nm for excitation and emission, respectively. All data were stored and treated with a SISC commercial application program (Taiwan).

The separation was performed on a Luna C_{18} column (25 cm × 4.6 mm id, 5 μm coating) with a 0.5 mL/min flow-rate under the pressure limit of 300 psi.

(IV) *Mobile Phase Solution*

Mix acetonitrile with 0.04% phosphoric acid buffer solution (3/97, v/v) as mobile phase solution. Filter the solution before use.

(V) *Standard Curve*

The stock solution was diluted with water to a series of concentrations ranging from 1~10 μg/mL. Twenty μL of each dilution was injected to HPLC. The standard curve was plotted based on peak area versus concentrations.

(VI) *Identification and Quantification Analysis*

The sample and standard solutions (20 μL) were alternately injected to HPLC. Comparing the retention time with that of the standard tentatively identified maleic hydrazide. Quantification of maleic hydrazide in test sample was made according to the following formula:

$$\text{Maleic hydrazide content (ppm)} = (C \times V \times 2)/M$$

Where C is the maleic hydrazide concentration in sample solution calculated by standard curve; V is the final volume of test sample after cleanup; M is the sample weight.

(VII) *Recovery Test*

A recovery test was triplicated in each level of spiking with 10, 15 or 20 ppm standard to test samples. Preparation of spiked and blank samples was the same as Method (II).

(VIII) *Estimation of Limit of Test*

The homogenate blank samples were spiked with 0.1, 0.3 or 0.5 maleic hydrazide. The spiked samples were then treated as method mentioned above and analyzed by HPLC.

Table 1. Chemical structure, physical-chemical properties, and reaction mechanisms of maleic hydrazide[1]

Chemical structure	
Chemical names	1,2-dihydropyridazine-3,6-dione(IUPAC) 1,2-dihydro-3,6-pyridazinedione(CA)
Trade names	Royal maleic hydrazide -30(Unironyal) Regulox(Burts&Harvey), Mazide (Synchemicals)
Chemical family	Pyridazine
Physical-chemical properties	MW: 112.10 Molecular formula: $C_4H_4N_2O_2$ Physical form: Colourless crystals Melting point: 292~298˚C Vapor pressure: Non-volatile Stability: Stable to hydrolysis, but decomposed by oxidizing agents and strong acids. Forms water-soluble alkali-metal and amine salts, but in hard water, the calcium salt is precipitated Solubility: In water 6 g/kg (25˚C), in dimethylformamide 24, ethanol, acetone and xylene<1 g/kg (25˚C). Diethanolamine salt 700, potassium salt 400, sodium salt 200 in water g/kg (25˚C).
Mode of action	Plant growth regulator, absorbed by the leaves and roots, with translocation in the xylem and phloem. Inhibits cell division in the meristemic regions, but not cell extension. Also used as herbicidal activity.
Toxicity to mammals	Acute oral LD50 for rats > 5000 (acid), 6950 (sodium salt), 3900 (potassium salt), 2340 (diethanolamine salt) mg/kg.Non-oncogenic and non-mutagenic.
Degradation and metabolism	Environmental: Half-life in soil is ca.2~8 weeks. Rapid photochemical degradation occurs in water.In plants: Various acids e.g. succinic, fumaric and maleic, are found as metabolites in plants.

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LOD was determined based on signal to noise ratio (S/N ratio) no less than 3.

(IX) *GC/Electron Impact Mass Spectrometer (GC/EIMS) Analysis*

1. Derivatization

The derivatization method was similar to that described by Terashi *et al*[7]. Twenty mL of 2.4 N HCl solution was added to 10 g of grounded potato and shaken thoroughly for 30 min. Five mL of 10 N NaOH solution was added followed with 1 mL dimethyl sulfate, and the combinations shaken rigorously for 30 min. The solution was then extracted twice with ethyl acetate (50 mL). The pooled ethyl acetate layers were dehydrated over an anhydrous sodium sulfate and evaporated by rotary evaporator under 40˚C to dryness. The remaining was then dissolved in 1 mL of n-hexane and applied onto GC/MS.

2. Condition

Analysis was performed using an HP-5890 series II GC equipped with an HP-5970B quadruple mass selective detector (Hewlett-Packard Company, USA), which was controlled by a HP-340C ChemStation data management system. An analysis capillary column (HP-5, 30 m × 0.25 mm id, 0.25 μm coating) was used and preceded with an oven temperature program, which initiated at 50˚C for 2 min, heated up in the rate of 20˚C/min to 250˚C and leveled off at 250˚C for 15 min. Both temperatures of injection port and interface to MSD were all maintained at 250˚C. Head pressure was adjusted at 8 psi with helium as carrier gas. One μL of sample was injected into the GC/MS.

RESULTS AND DISCUSSION

I. *Preparation of Test Solutions*

(I) *Extraction*

Maleic hydrazide, a polar compound, is highly soluble in methanol[2~6, 9]. However, many co-extractants accompanying with maleic hydrazide were found while extracting with methanol alone. Cleanup with acetonitrile and n-hexane (1/4, v/v) followed by water, methanol and n-hexane (1/1/1, v/v/v) were referred to removing non-polar interferences from the potato matrix[9]. However, this study showed that this practice was not better than using methanol only.

(II) *Solid Phase Extraction*

The C_{18}[3, 6] or SCX[4] solid phase extraction cartridges have been applied to further cleanup maleic hydrazide from interferences. In our study, the results showed that C_{18} cartridges (0.5 or 1 g) failed to separate maleic hydrazide from

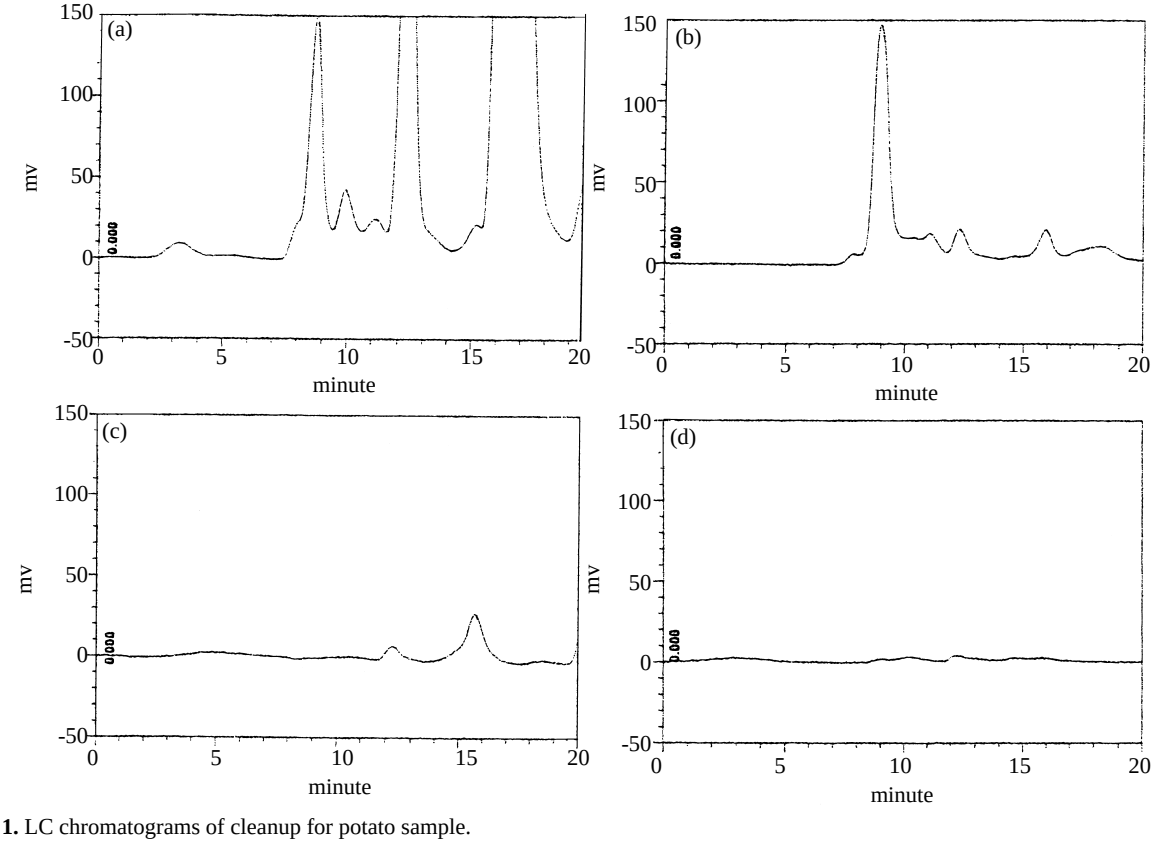

Figure 1. LC chromatograms of cleanup for potato sample.
(a) before cleanup (b) cleanup with 1 g of C18 cartridge (c) cleanup with 500 mg of SCX cartridge (d) cleanup with 1 g of SCX cartridge.

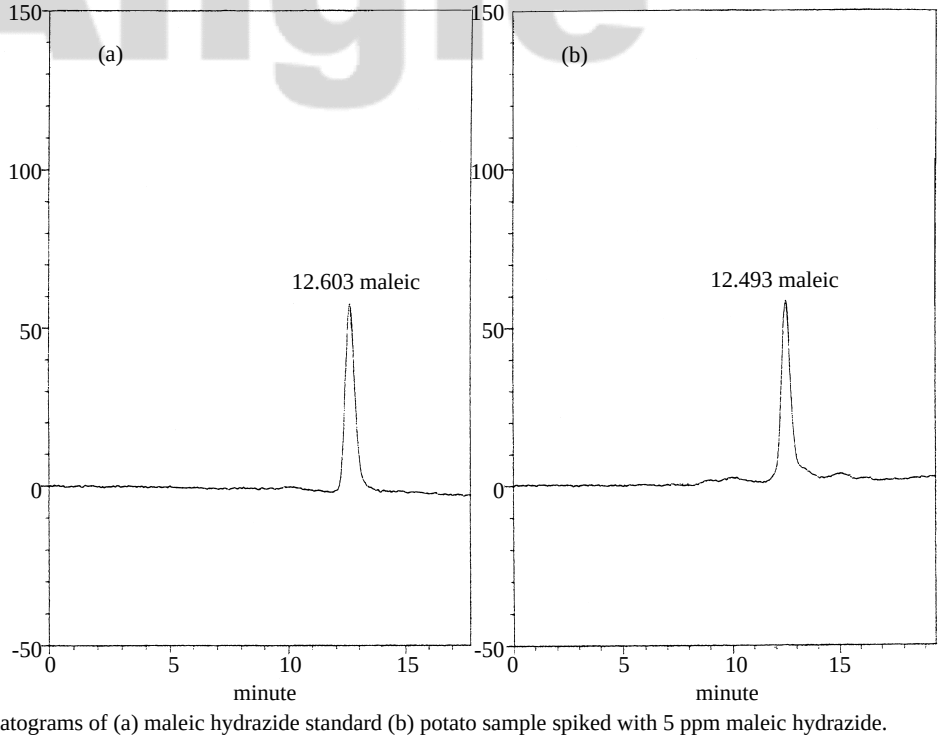

Figure 2. LC chromatograms of (a) maleic hydrazide standard (b) potato sample spiked with 5 ppm maleic hydrazide.

Table 2. Recoveries of maleic hydrazide spiked in potato samples

Sample	Spiked level (ppm)	Recovery[a] (%)
Potato	10	95.7(3.3)[b]
	15	91.6(4.0)
	20	87.8(2.1)

[a] average of triplicate.
[b] value in parenthesis is coefficient of variation (CV, %).

those interferences. We found the problem was solved by using 1 g of SCX cartridges. However, 0.5 g of SCX cartridges is not recommended in this case due to their insufficient capacity (Figure 1).

II. *HPLC Conditions*

(I) *Detection*

Analyzing maleic hydrazide using HPLC[3~6, 9] or GC[7~8] has been reported. However, employing HPLC is prevailingly regarded as more convenient than employing GC in this case. The maximum absorbance of maleic hydrazide was observed at 303 nm, but the wavelengths at 313 and 330 nm were reported to analyze maleic hydrazide as well[3~5, 9]. Nevertheless, those unwanted peaks still exist, which may much skew the correctness of quantification. A highly selective and sensitive fluorescences method based on the specific excitation and emission energy observed at 303 and 400 nm[6], respectively, of maleic hydrazide was therefore developed for this purpose. A reliable quantification from an intensive and symmetric peak without interference was the result of this study.

(II) *Column & Mobile Phase*

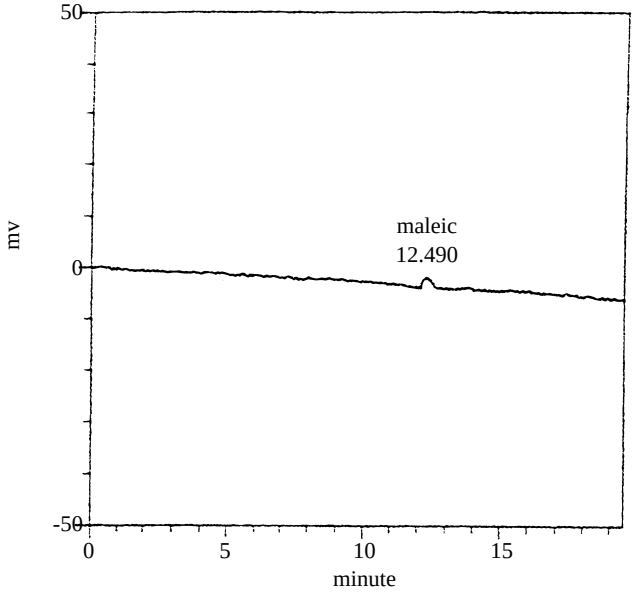

Figure 3. LC chromatogram of the detection limit for potato sample spiked with 0.5 ppm maleic hydrazide.

The NH_2P_{50}[9] and C_{18}[4~6] columns were mainly considered for the analysis of maleic hydrazide. Our studies, however, showed that the performance of NH_2P_{50} column was not comparable to that of C_{18} column; the former did cause a tailing and splitting phenomenon. Thus, a C_{18} column was adopted throughout this study.

Due to the comparatively polar and acidic (pKa = 5.65) properties of maleic hydrazide, we acidified the mobile solution with phosphoric acid to the resulting pH below pKa in order to prevent the dissociation of protons from maleic

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hydrazide, otherwise bringing about peak tailing while running HPLC[4~6]. For assuring a reproducible result, an acid-resistant C_{18} column was therefore required. Moreover, we found that the mobile phase solution fortified with 5 mM TBA (tetra-n-butyl-ammonium phosphate) solution as coupled ions was also able to give a sound peak shape and acceptable resolution instead of adding 0.04% phosphoric acid. But, we added acid rather than coupled ions, which yielded a reasonable retention time at 12.5 min and an idea peak shape and comparable resolution (Figure 2), in addition, a prolonged column life.

III. *Standard Curve*

By applying the method described above to real practices, a standard curve, Y = 32694.9313X – 3271.0522, with regression coefficient of 0.9992 was obtained, which presented an adequate linearity.

IV. *Recovery Test*

The recoveries of maleic hydrazide from spiked potatoes samples are shown in Table 2. The average recoveries spiked with 10~20 ppm of maleic hydrazide range from 87.8 to 95.7% with coefficient of variation from 2.1 to 4.0% in which the results presented a satisfactory recovery and reproducibility.

V. *LOD Estimation*

Based upon the minimum requirement of S/N ratio being no less than 3, the LOD of maleic hydrazide in potatoes was estimated as 0.5 ppm (Figure 3) of which the LOD was much below the official tolerance levels. The method devel-

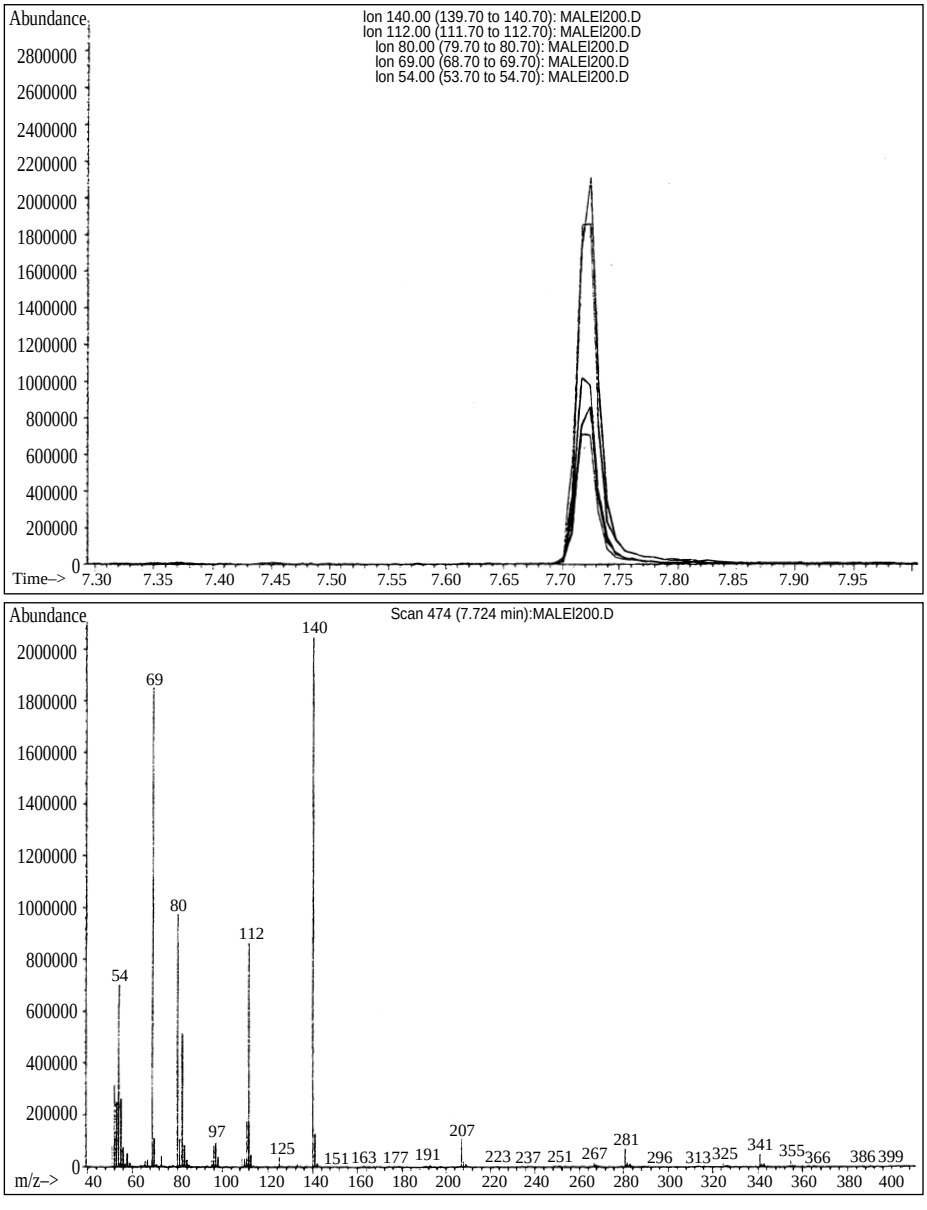

Figure 4. GC-MS spectrum of derivatized maleic hydrazide.

oped is therefore in a position as a candidate of an official method to monitor the maleic hydrazide residues in potatoes.

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

GC-MS was not used for analyzing maleic hydrazide in routine exercise due to the time-consuming procedures of derivatizing maleic hydrazide. It was used to confirm our results obtained by this quick method developed. The molecular weight of the original maleic hydrazide is 112.10, which derivatized molecular ion m/z at 140 represents the base peak. Furthermore, the m/z 112 fragment is due to the losing of carbonyl (-CO) group from the m/z 140 ion, followed by losing $-CH_3OH$ or $-CH_3N_2$ fragment which gives the fragment at m/z 80 or m/z 69 (Figure 4).

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以高效液相層析儀檢測馬鈴薯中抑芽素殘留量

李婉媜　李宗璘*　張碧秋　周薰修

行政院衛生署藥物食品檢驗局
台北市南港區昆陽街 161-2號

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摘　要

以甲醇萃取馬鈴薯中抑芽素之殘留量，經過濾或離心，抽出液以減壓濃縮至乾，殘留物以水溶解後，以SCX固相萃取匣（a strong cation exchanger）淨化之，所得檢液以高效液相層析儀配合螢光檢出器偵測。抑芽素添加10~20 ppm檢體濃度於馬鈴薯中之平均回收率為87.8~95.7％，變異係數為2.1~4.0%。本方法操作簡便、回收率高且再現性良好，最低檢出限量為0.5 ppm。

關鍵詞：農藥殘留量，抑芽素，高效液相層析