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High Performance Chromatographic Determination of Asulam Residue in Agricultural Products

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ABSTRACT

Asulam was extracted with 0.1% acetic acid in acetonitrile, acetone, water and diethyl ether successively. Following after clean up with florisil and neutral alumina solid phase extraction cartridge, the elute was determined by HPLC with UV detector. Recoveries were carried out by spiking the standard asulam at the levels of 0.1~0.3 and 0.05~0.2 ppm to citrus and sugarcane, respectively. The average recoveries were 83.5 to 90.9 % for citrus and 80.6 to 86.7 % for sugarcane, and both detection limits was 0.01 ppm.

Key words: pesticide residue, asulam, HPLC

INTRODUCTION

Asulam, [(methyl 4-aminophenyl) sulphonyl] carbamate, a colorless crystal with a molecular weight of 230.24, is categorized into carbamates. Its chemical structure is shown in Figure 1. Asulam is an acidic compound and stable under normal conditions. It is soluble in some organic solvents such as dimethylamide, acetone, methanol, and ethanol, but barely dissolves in water except for asulam salts. It is a selective systemic herbicide used for controlling the growth of annual or perennial grasses and broad-leaf weeds[1]. According to the "Tolerances for Residues of Pesticides" announced by the Department of Health, the asulam level in citrus fruit and sugarcane is restricted to 0.2 and 0.1 ppm, respectively[2]. GC[3] and HPLC[4-6] methods are routinely used to analyze asulam residue in agricultural products. Bardlaye *et al.*[3] proposed a GC method for asulam analysis, which requires a reaction of dimethyl sulfoxide (DMSO) with sodium hydroxide to give a derivative for GC analysis. However, this method is likely to induce explosion, is time-consuming, and can provide only 50~60% recovery. Guardigli *et al.*[4] proposed a derivatization and sample clean-up method for HPLC analysis. Asulam was acetylated and hydrolyzed prior to cleanup with neutral alumina and florisil solid phase extraction cartridges. This method can give 70~80% recovery, but is still time-consuming. Lawrence[5] reported an HPLC method to analyze asulam in flour. Asulam was extracted with acetonitrile from flour and then partitioned with acetonitrile-saturated hexane. This method, however, can only be applied to flour products; while applying to other products, the interference could not be effectively removed. Kon *et al.*[6] proposed an HPLC method to analyze asulam in peaches. Acetonitrile and acetone solutions were used as extraction solvents and neutral alumina cartridge was used for clean-up. This method was capable of providing 72% recovery. Because it is time-consuming and low in recovery, a derivatization method was not considered as a suitable method for asulam analysis. An HPLC method referring to Kon *et al.*[6] was followed in this study. The purpose of this study was to develop a method with high asulam recovery, good reproducibility, and low in detection limit.

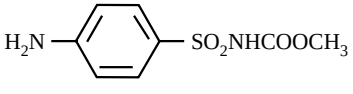

Figure 1. Chemical structure of asulam.

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MATERIALS AND METHODS

I. *Materials*

Test samples including citrus and peeled sugarcane were purchased from traditional markets.

II. *Reagents*

The residue grade acetonitrile, acetone, diethyl ether, acetic acid, methanol, n-hexane, and ethyl acetate were purchased from E. Merck (Darmstadt, F. R., Germany). Anhydrous sodium sulfate (reagent grade) was obtained from J. T. Baker (USA). Asulam standard (of purity 99%) was purchased from Riedel-de Haen AG. (Germany).

III. *Methods*

(I) *Preparation of Standard Solution*

Asulam standard (100 mg) was accurately weighed into a 100-mL volumetric flask and acetonitrile was added to the volume. The stock solution was thus prepared. As needed, the stock solution was diluted with acetonitrile to make standard solutions.

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(II) *Sample Preparation*

1. Extraction

Test samples were sliced and homogenized, and 20g homogenate was accurately weighed and then extracted twice with 60 mL of acetonitrile solution (containing 0.1% acetic acid, v/v) for 3 min. After precipitation, the clear suspension was filtered under suction. The residue and the container were then washed stepwise with 40 mL of acetonitrile containing 0.1% acetic acid, 20 mL of acetone, and 6 mL of water. The residue was further washed with 30 mL of acetonitrile. The combined filtrates were concentrated at 35˚C using a rotary evaporator to remove organic solvents, and 25 mL of diethyl ether was then added and mixed. The mixture was then transferred into a separation funnel, which was then shaken for 1 min. The separation funnel was left to stand until phase separation. Diethyl ether layer was collected and the aqueous phase was extracted with another 20 mL of diethyl ether. This extraction procedure was performed in triplicate. The combined diethyl ether filtrates were dehydrated over anhydrous sodium sulfate and then concentrated to ca. 2 mL at 35˚C using a rotary evaporator.

2. Clean-up

The above sample solution was applied onto a 1g florisil solid phase extraction cartridge (Waters, Division of Millipore Corporation, MA, USA). Prior to loading the sample solution, the cartridge was conditioned with 10 mL of methanol and 10 mL of n-hexane. The concentration bottle was washed with another 1 mL of diethyl ether, which was then applied onto the same cartridge. Upon loading the sample, 10 mL of ethyl acetate: acetonitrile (4:1, v/v) solution was applied onto the cartridge. The washed matrix was discarded. The compound of interest in florisil cartridge was then eluted with 30 mL of methanol: acetonitrile: water (14: 5: 1, v/v/v) solution. The solvents in eluate were removed at 35˚C using a rotary evaporator.

The dry matter was dissolved in 3 mL of diethyl ether and transferred onto a 500 mg neutral alumina solid phase extraction cartridge (Waters, MA, USA), which was pre-conditioned with 10 mL of methanol and 10 mL of n-hexane. The residue in the concentration bottle was rinsed with another 1 mL of diethyl ether, which was then loaded onto the same cartridge. The cartridge was then washed with 15 mL of ethyl acetate: acetonitrile (1: 1, v/v) solution and the compound of interest was eluted with 50 mL of methanol: water (19: 1, v/v) solution. The solvents in eluate were evaporated at 35˚C using a rotary evaporator and the residue was then dissolved in 3 mL of methanol and filtered through a membrane prior to HPLC analysis.

(III) *HPLC Conditions*

Column: $C_{18,}$ 5 μm, 25 cm × 4.0 mm i.d. (E. Merck, Darmstadt, F. R. Germany)
Detector: UV detector (Shimadzu Corporation, Kyoto, Japan) set at 268nm
Mobile phase: Water: acetontirile: acetic acid (89.98: 10: 0.02, v/v/v)
Flow rate: 1.0 mL/min

(IV) *Standard Curve*

The stock solution was diluted with acetonitrile to series of concentrations ranged at 0.1~3.0 μg/mL. Twenty μL of each dilution was injected to HPLC. The standard curve was plotted based on peak area versus concentrations.

(V) *Identification and Quantification of Asulam*

The sample and standard solutions (20 μL) were separately injected to HPLC. Asulam was tentatively identified by comparing the retention time with that of the standard. Quantification of asulam in the test sample was made according to the following formula:

Asulam content (ppm) = (C × V)/M

Where C is the asulam concentration in sample solution calculated by standard curve; V is final volume of test sample after clean-up; M is the weight of test sample.

(VI) *Recovery Test*

A recovery test was performed in triplicate for each level by spiking asulam standard with 0.5-fold, 1.0-fold, or 1.5-fold tolerance level to agricultural products. Preparation of spiked and blank samples was as described in Method (II). Recovery was calculated after HPLC analysis.

(VII) *Estimation of Limit of Detection (LOD)*

The blank homogenate was spiked with 0.03, 0.02, or 0.01 ppm asulam. The spiked samples were then treated as mentioned above and analyzed by HPLC. LOD was determined based on signal to noise ratio (S/N ratio) greater than 3.

RESULTS AND DISCUSSION

I. *Preparation of Test Solution*

(I) *Extraction*

Asulam is a polar pesticide, readily soluble in a polar organic solvent, but barely dissolved in water. Therefore, some organic solvents such as acetone and methanol[1] are more favorable for extracting asulam. However, some interference together with asulam could also be extracted out when the above two solvents are employed. Acetonitrile is another choice for asulam extraction[5-6]. Because asulam is an acid compound, it is likely to form a salt, which is readily dissolved in water[1] and difficult to be extracted by organic solvents. To prevent asulam from dissociation, we chose ace-

tonitrile (containing 0.1% acetic acid) as one of the extraction solvents in this study. Acetone was used to increase recovery of asulam; while water was used to aid phase separation as well as reduce emulsion. The purpose of using diethyl ether as an extraction solvent in this study was to inhibit polar interference from being extracted, although it is not an ideal solvent for asulam extraction. The sample extract was then analyzed by HPLC-UV.

(II) *Clean-up by Solid Phase Extraction*

Using diethyl ether to extract asulam from agricultural products was capable of minimizes polar interference; however, the further clean-up procedure was still necessary because the liquid-liquid extraction was unable to achieve a satisfactory result. In our preliminary study, we found that clean-up using a 500-mg florisil cartridge was not enough to accommodate sample solution. Instead, we used a tandem 500-mg cartridge or a 1-g cartridge that allowed most of interference to be removed and remained a satisfactory recovery of asulam. However, trace interference still affected the quantification of asulam. In this study, a further clean-up using a neutral alumina cartridge was performed to effectively eliminate interference peaks that appeared on the HPLC chromatogram.

(III) *Vacuum Evaporation*

It happened that acetonitrile suddenly began boiling during vacuum evaporation. Keeping the temperature under 35˚C, using a larger concentration bottle, or adjusting the vacuum intensity can improve this situation. In addition, evaporation to dryness should be avoided because it could lead to a difficulty in dissolving asulam from dry matter. In this study, we spiked some water to elution solvent to minimize solvent to be dried out during concentration.

II. *HPLC Conditions*

The wavelengths at 254, 268, and 280 nm were reported to detect asulam as using HPLC-UV analysis[5-6]. UV scanning data showed that maximum absorption of asulam appeared at 268 nm. We also found that the S/N ratio at 268 nm was greater than at 254 nm and 280 nm, and no interference peak was observed as using 268 nm detection. Therefore, we selected UV 268 nm as a detection wavelength in this study.

Because of the acid characteristic of asulam (pKa = 4.825), the acidity of mobile phase should be adjusted to prevent asulam from dissociation and peak tailing[5-6]. In our preliminary study, a chromatographic condition referring to Lawrence *et al*.[5] was followed. We used a Lichrosorb RP-8 HPLC column and mobile phase, water: acetonitrile: acetic acid (79.98: 20: 0.02, v/v/v) preliminarily. The asulam peak appeared at about 5 min but with poor peak resolution and the interference peaks existed on the chromatogram. The HPLC conditions were then changed as follows. The analytical column was switched to a C_{18} column and the mobile phase composition was changed to water: acetonitrile: acetic acid (89.98: 10: 0.02). The above modifications made the retention time of the asulam peak move to 13.6 min without any interference peaks appearing. These HPLC conditions were therefore adopted in this study. The HPLC chromatograms of asulam extracted from spiked citrus fruit and sugarcane are shown in Figure 2 and 3, respectively.

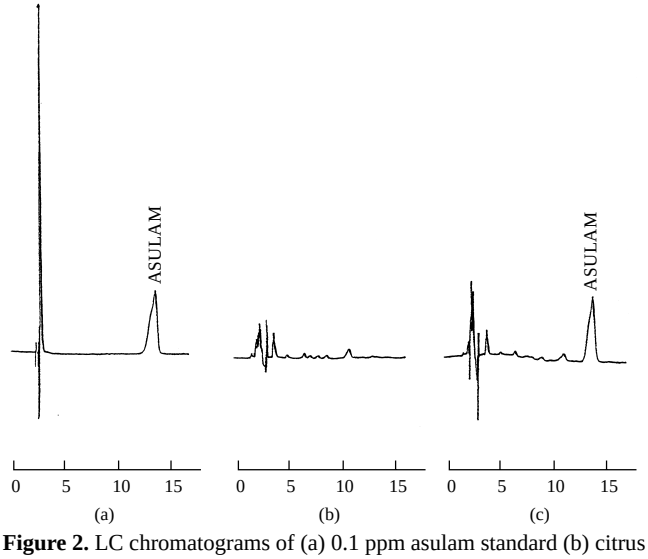

Figure 2. LC chromatograms of (a) 0.1 ppm asulam standard (b) citrus sample, blank (c) citrus fruit sample, spiked with 0.1 ppm asulam.

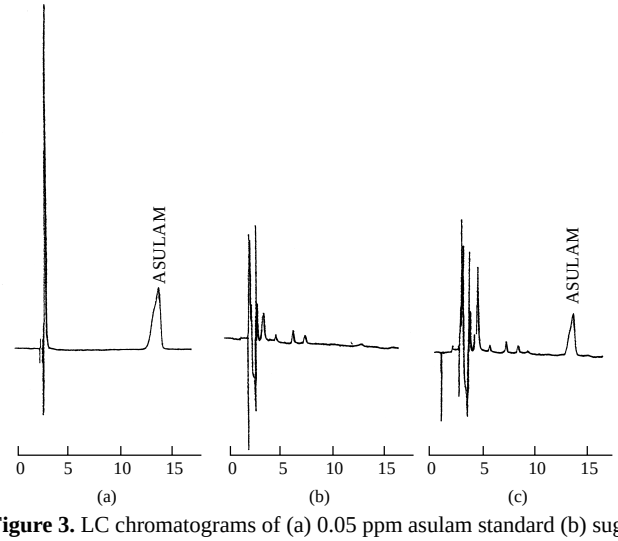

Figure 3. LC chromatograms of (a) 0.05 ppm asulam standard (b) sugarcane sample, blank (c) sugarcane sample, spiked with 0.05 ppm asulam.

Table 1. Recoveries of asulam in spiked agricultural products

Sample (agricultural products type)	Spiked level (ppm)	Recovery[a](%)
Citrus fruit	0.1	90.9(2.3)[b]
(Citrus)	0.2	89.4(5.4)
	0.3	83.5(0.8)
Sugar stem	0.05	81.3(8.8)
(Sugarcane)	0.1	80.6(6.5)
	0.2	86.7(2.9)

[a] average of triplicate.

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

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III. *Standard Curve*

By using the method described above, a calibration curve, Y = 77573.64X-2703.69, with regression coefficient of 0.9998 was obtained. It showed a satisfactory linearity.

IV. *Recovery Test*

The recovery of asulam from spiked citrus and sugarcane is shown in Table 1. The average recoveries from citrus spiked with 0.1~0.3 ppm asulam were in the range of 83.5~90.9% with coefficient of variation 0.8~5.4%. The average recoveries of asulam from sugarcane spiked with 0.05~0.2 ppm asulam were in the range of 80.6~86.7% with coefficient of variation 2.9~8.8%.

V. *LOD Estimation*

Asulam in agricultural products was analyzed as described above. On the basis of S/N ratio greater than 3, LOD of asulam in both citrus and sugarcane was determined to be 0.01 ppm (Figure 4), which is lower than the announced tolerance levels. This result indicates that the developed method is sensitive enough to be an official method for monitoring asulam residue in agricultural products.

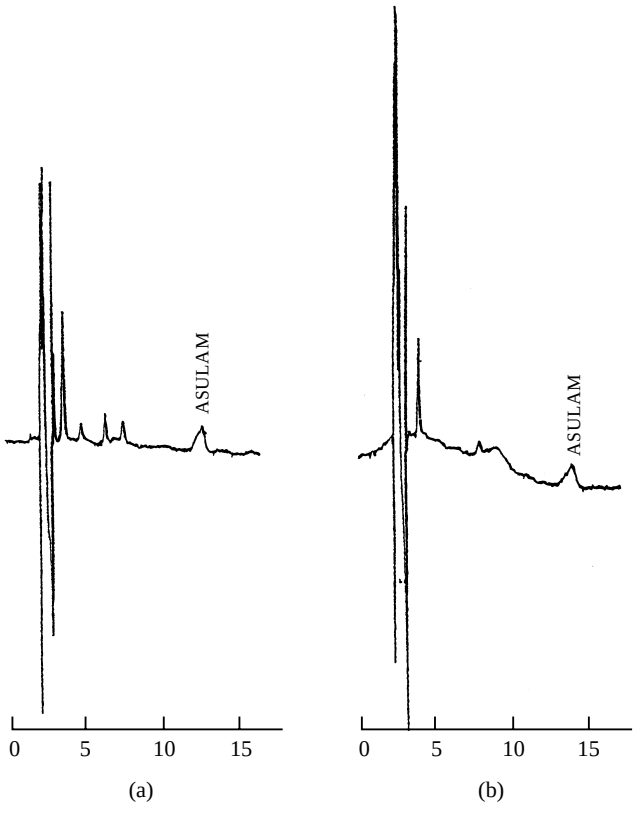

Figure 4. LC chromatograms of the detection limit of asulam in (a) citrus sample (b) sugarcane sample, spiked with 0.01 ppm.

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以高效液相層析儀檢測農產品中亞速爛殘留量

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摘　要

依序以含0.1%醋酸之乙腈(v/v)溶液、丙酮、水及乙醚萃取農產品中亞速爛，再以矽酸鎂固相萃取匣及中性氧化鋁固相萃取匣淨化之，所得檢液以高效液相層析儀配合紫外光檢出器檢測。亞速爛添加0.1　0.3 ppm濃度於柑橘中之平均回收率為83.5　90.9%，添加0.05　0.2 ppm濃度於甘蔗中之平均回收率為80.6　86.7%，最低檢出量均為0.01 ppm。

關鍵詞：農藥殘留量，亞速爛，高效液相層析