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Determination of Simazine Residue in Sugarcane by Application of Matrix Solid Phase Dispersion (MSPD) Extraction Technique

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ABSTRACT

A rapid method for the determination of simazine residue in sugarcane by matrix solid phase dispersion (MSPD) extraction and florisil cleanup was developed. This method performed extraction and cleanup in a single eluting step and significantly reduced organic solvent consumption. Two grams of homogenized sample was blended with 2 g of EnvirElut sorbent until a homogeneous mixture was observed. The mixture was transferred into a florisil cartridge, and eluted with 25 mL of dichloromethane. The eluate was evaporated to dryness, and the residue was dissolved with acetonitrile and then determined by HPLC-UV at 230 nm. Recoveries of simazine spiked in sugarcane (0.1~0.5 ppm) were determined to be 86.9~94.7%, while coefficient of variation was determined to be less than 10%. The detection limit was 0.02 ppm.

Key words: pesticide residue, sugarcane, matrix solid phase dispersion, simazine, high performance liquid chromatography

INTRODUCTION

Simazine [2-chloro-4, 6-bis(ethylamino)-1,3,5-triazine] is one of the most widely used chlorotriazine herbicides, and it is applied as a pre- and post-emergent weed control agent to improve crop yields. Simazine, an inhibitor of photosynthetic electron transport, is a selective systemic herbicide, absorbed through the roots, with translocation acropetally in the xylem, accumulating in the apical meristems and leaves[(1)]. Simazine is stable in neutral, weakly acidic and weakly alkaline media, but hydrolyzed by strong acids and bases and decomposed by UV irradiation[(1)]. The solubilities of simazine at 20˚C in water, methanol, chloroform and petroleum ether are 3.5 mg/L, 400 mg/L, 900 mg/L and 2 mg/L[(1)], respectively. According to the tolerances for the residues of pesticides[(2)] set by the Department of Health, ROC, the tolerance level for the residue of simazine in sugarcane is 0.2 ppm. Simazine is suspected as one of endocrine disruptors, a hormone-like toxin that disrupts the human endocrine system. The simazine residue in foods is quite important to be known, but there is still no official method available for analyzing simazine in crops in Taiwan.

Concerning the contamination of natural waters by leaching pesticides from the soil, many methods for the determination of simazine in soil and water have been reported[(3-9)]. The residues of simazine in plant tissues can be detected by gas chromatography (GC) equipped with NPD[(10-14)], electron capture detector[(15)], electron conductivity detector[(16-17)], MS,[(18)] or by high performance liquid chromatography[(19-20)]. These reported methods[(10-17)], including liquid-liquid partition, cleanup by ion-exchange column or other type of columns were used as a pretreatment procedure, were time-consuming and used too much solvent. The aim of this study is to develop a rapid method using a new extraction technique—matrix solid phase dispersion (MSPD) and followed by solid phase extraction (SPE), instead of traditional liquid-liquid extraction by separation funnel[(19)] to analyze the simazine residue in sugarcane.

MATERIALS AND METHODS

I. *Materials*

The peeled sugarcane samples were purchased from supermarkets or traditional markets. Bondesil-EnvirElut^{TM} bulk sorbent (#1221-4016, 40 μm, Trifunctional Octadecyl (C18/ Silica based) and Bond Elut florisil SPE cartridge (1 g, 20 mL) were both purchased from Varian (CA, USA).

II. *Reagents*

Dichloromethane and diethyl ether used in this study were residual grade. Methanol and acetonitrile were LC grade. Acetic acid, sodium acetate and sodium sulfate anhydrous were reagent grade. The standard of simazine (of purity 99%) was purchased from ChemService (Wes Chester, PA, USA).

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III. *Instruments and Analytical Conditions*

(I) *HPLC*

A Hitachi (Tokyo, Japan) HPLC system equipped with a Hitachi L-6200 pump, a Polaris C18 analytical column (25 cm × 4.6 mm i.d., 5 μm, MetaChem Technologies Inc., CA, USA), a Hitachi L-4250 UV detector was used. The UV detector was set at 230 nm. The mobile phase system was 20 mM acetic acid/sodium acetate buffer solution (pH 5.0): methanol (50:50, v/v) pumped at 1.0 mL/min. The injection volume was 20 μL.

IV. *Methods*

(I) *Preparation of standard solutions*

About 10 mg of simazine was accurately weighed into a 100-mL volumetric flask and methanol was then added to the marked volume to prepare a stock solution. A series of standard solutions (0.5~5.0 μg/mL) were prepared by diluting the stock solution with methanol.

(II) *Preparation of sample solutions*

A. Liquid/liquid extraction

Traditional liquid/liquid partition method was modified from Ortiz-Gomez et al.[19]. The sugarcane sample was prepared by using a food processor and mixed thoroughly. An aliquot (10 g) of the sample was mixed with 20 mL of water, and then extracted with 50 mL of acetonitrile for 3 min. The extraction solution was then filtered under suction. The residues and container were then washed with another 30 mL of acetonitrile, which was then filtered. The filtrates were combined into an evaporation bottle and evaporated at 35˚C under vacuum. The concentrate (~30 mL) was transferred into a separation funnel in which 20 mL of 60 mM acetic acid/sodium acetate buffer solution (pH5.0) was added and extracted twice with 50 mL of diethyl ether. The combined diethyl ether layer was passed through a funnel containing sodium sulfate anhydrous and evaporated to dryness at 35~40˚C using a rotary evaporator. The residue was dissolved in 1 mL of acetonitrile and filtered through a 0.45 μm membrane prior to HPLC analysis.

B. MSPD extraction procedure

The sugarcane sample was prepared by using a food processor and mixed thoroughly. An aliquot (2 g) of the sample was placed into a glass mortar and 2 g of Bondesil-EnvirElut sorbent was added and gently blended for a few minutes using a pestle to obtain a dry-powder-like homogeneous mixture. The mixture was loaded into a Bond Elut florisil SPE column. The simazine residue was eluted four times with 5 mL of dichlormethane at a flow rate of 1~2 mL/min. The eluate was evaporated to dryness by gentle N_2 gas flow, and the residue was dissolved in 200 μL of acetonitrile and filtered through a 0.45 μm membrane prior to HPLC analysis.

(III) *Identification and quantification*

A series of concentrations of simazine standard were prepared by diluting the simazine stock solution with methanol and 20 μL of each was injected to HPLC. The standard curve of peak area verses concentration was plotted and the linear equation was calculated by linear regression. The sample and standard solutions were accurately taken and injected into HPLC according to the analytical conditions as described. The peak areas of the simazine in sample solutions were compared to those in standard solutions. The amounts of simazine in test samples were thus calculated based on the standard curve.

(IV) *Recovery test*

Recovery studies were carried out by spiking 2 g of fresh homogenized sugarcane samples with the simazine fortification solution at different levels, ranging from 0.1~0.5 ppm. Each concentration of spiked samples was prepared in triplicate. A blank sample without standard was also prepared. The preparation of sample solution by MSPD extraction method was as described. Recoveries were calculated after HPLC analysis.

(V) *Detection limit test*

A suitable amount of simazine was spiked to a homogenized sugarcane sample. The test sample solution was prepared by the MSPD method as described. The limit of detection (LOD) was estimated at a signal to noise (S/N) ratio of 3.

RESULTS AND DISCUSSION

I. *HPLC Conditions*

(I) *Wavelength determination*

The UV-VIS scanning spectrum of simazine at the concentration of 10 μg/mL in methanol was determined. A strong maximum absorbance at 225 nm, and an absorption peak at 264 nm were found. In order to reduce interference peaks in HPLC chromatogram, a higher absorbance than 225 nm, at 230 nm, which was adopted by Ortiz-Gomez et al. (1995) was selected to detect the simazine in this study.

(II) *Mobile phase determination*

A mobile phase of 20 mM acetic acid/sodium acetate buffer (pH 5): methanol (20:80, v/v) was used for determin-

ing simazine in must, and the retention time of simazine was about 1.5 min at flow rate of 1.5 mL/min[19]. In our study, different ratios of 20 mM acetic acid/sodium acetate buffer (pH 5) and methanol (20:80, 30:70, 40:60, 50:50, v/v) were tested to be mobile phases for optimization HPLC condition. To resolve the analyte and co-extractives from crops well, among the mobile phases used, a mobile phase of 20 mM acetic acid/sodium acetate buffer (pH 5): methanol (50:50, v/v) was preferred because it gave a longer retention time (9.6 min) long enough to separate the simazine from impurities in crops.

II. *Standard Curve*

A standard curve was plotted (5 points) with standard solutions of 0.5~5 μg/mL analyzed by HPLC at 230 nm and shown in Figure 1. The regression equation was Y = 54761X + 870. A correlation coefficient (r) of 0.9999 throughout the range of simazine concentrations indicated good linearity.

III. *Preparation of Sample Solutions*

Matrix solid phase dispersion (MSPD) can be regarded as a valuable alternative to the more classic sample preparation methods because it allows a significant reduction in both the sample size and solvent consumption needed for pesticide analysis. In order to demonstrate the suitability of MSPD technique for determining simazine residue in sugarcane samples, comparisons of the HPLC chromatograms obtained by the MSPD method and traditional liquid/liquid partition method modified from Ortiz-Gomez et al.[19] were done. The HPLC chromatograms of sample blank and fortified sample obtained from traditional liquid/liquid partition method and MSPD method were shown in Figure 2 and Figure 3. These chromatograms had shown that the cleaner extracts were obtained from the MSPD method in this study as a result of the combination of MSPD extraction technique and SPE cleanup column. The blending of sugarcane tissue with Bondesil-EnvirElut™ bulk sorbent dispersed the tissue and allowed an efficient extraction of the pesticides into the dichloromethane eluting solvent, resulting in high recoveries. The residual interfering compounds present in the dichloromethane eluting from the sorbent/matrix blend were removed as the dichloromethane passed through the florisil cartridge. There were no interfering peaks in the simazine elution in the sample blank shown in Figure 3 (A). From the results of our study, the developed method combined MSPD extraction technique and SPE cleanup in a single eluting step showed high recoveries and good cleanup performance.

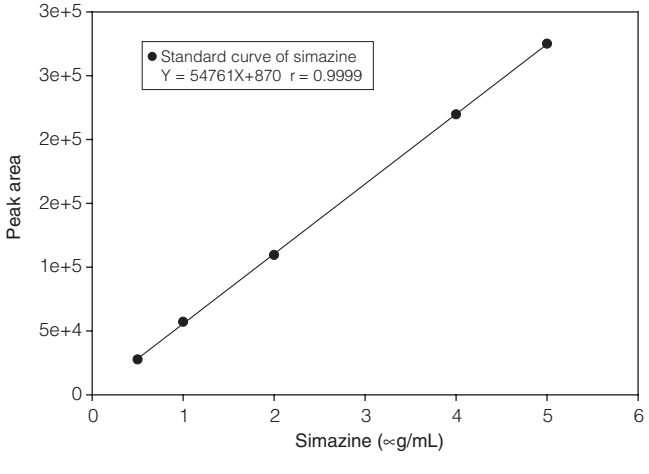

Figure 1. Standard curve of simazine.

IV. *Fortification Recovery Test*

The recoveries of simazine from sugarcane spiked with 0.1~0.5 ppm were listed in Table 1. The recoveries were ranged 86.9~94.7% with 5.9~9.6% coefficient of variation. The data showed both satisfactory recoveries and repeatibilities.

V. *Detection Limit Test*

Based on S/N of 3, the detection limit was determined to be 0.02 ppm. The detection limit of the proposed MSPD method (0.02 ppm) was 1/10 of the maximum residue limit

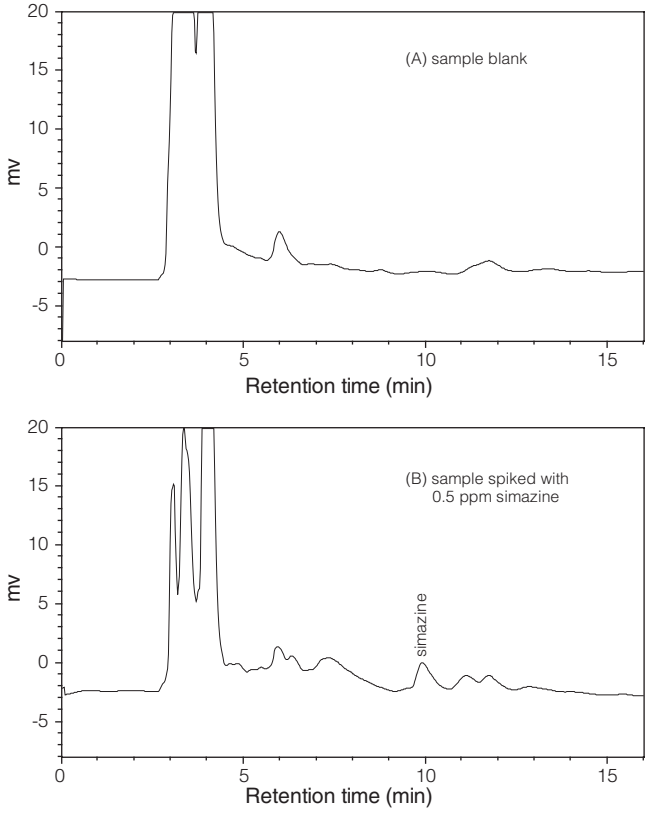

Figure 2. HPLC chromatograms for simazine analysis in sugarcane by traditional liquid/liquid partition method. (A) sample blank (B) sample spiked with 0.5 ppm simazine.
HPLC conditions: Column: Polaris C18; Mobile phase: MeOH: pH 5.0 acetate buffer (50:50, v/v); Flow rate: 1.0 mL/min; Detector: UV 230 nm.

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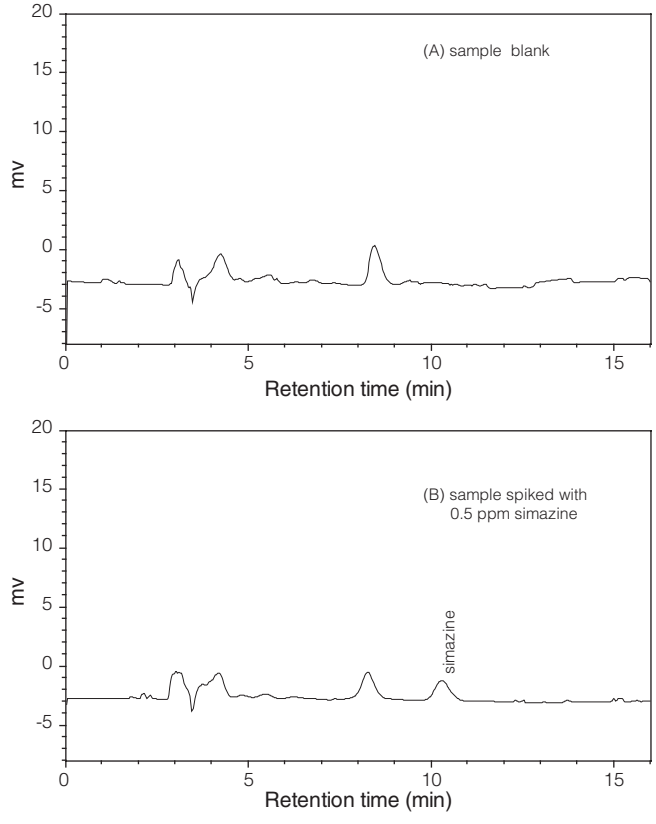

Figure 3. HPLC chromatograms for simazine analysis in sugarcane by MSPD method. (A) sample blank (B) sample spiked with 0.5 ppm simazine.
HPLC conditions were the same as in Figure 2.

of simazine in sugarcane (0.2 ppm) set by our government, indicating the proposed method is sensitive enough to be an official method for simazine determination.

CONCLUSION

Compared to the traditional liquid/liquid extraction technique, MSPD is faster, less labor intensive and requires lower solvent consumption. In addition, the proposed method in this study combined MSPD extraction technique and SPE clean-up cartridge, performed good extraction and clean-up in a single eluting step. The proposed method associated with HPLC is an appropriate methodology for routine simazine analysis in sugarcane samples.

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Table 1. Recoveries of simazine spiked into sugarcane

Sample	Spiked level (ppm)	Recovery[a] (%)
Sugacane	0.1	94.7 (9.6)[b]
	0.2	91.6 (6.4)
	0.5	86.9 (5.9)

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

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