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Flusulfamide Residue Detection in Chinese Kale and Cabbage

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ABSTRACT

Flusulfamide, a sulfonanilide family fungicide, is used to control clubroot caused by *Plamodiophora brassicae* Woronin in Chinese cabbage in Taiwan. To know the amounts of residues on the crops are very important in our IES (Inspection and Education System); unfortunately, information on analytical methods to determine flusulfamide residues is very rare. An analytical method was developed to detect the flusulfamide residues in Chinese kale and Chinese cabbage. The residue of sulfonanilide in vegetable samples were extracted by acetone and partitioned to dichloromethane and cleaned up by SPE (solid phase extraction). The residue was further methylated with methyl iodide. The derivatives were measured by GC-ECD and identified by GC/MS. The recovery of flusulfamide in Chinese kale and Chinese cabbage were 92.6 and 93.2% by fortification with 0.005 to 0.5 μg/g, respectively. The detection limits were 0.001 μg/g for crops. The method was applied to the determination of flusulfamide residues in crops from treated field.

Key words: flusulfamide, Chinese kale, Chinese cabbage, methylation

INTRODUCTION

Flusulfamide (2',4-dichloro-α,α,α-trifluoro-4'-nitro-*m*-toluenesulfonanilide) is a sulfonanilide family fungicide. Its mode of action is to inhibit spore germination and it is often used as a soil treatment for the control of *Plasmodiophora brassicae*, *Pythium*, *Rhizoctonia* and *Fusarium* spp.[1,2]. In Taiwan, 0.5% flusulfamide DP (Dusty Powder) is recommended for use on cabbage at a rate of 200 kg/ha and mixed with soil before planted, to control clubroot caused by *Plamodiophora brassicae* Woronin.

Not much information is available regarding residues analysis of flusulfamide. In 1997, Mitsui Toatsu Chemical Inc. (Japan) developed a gas chromatographic (GC) method to determine flusulfamide after derivatization with diazomethane by an electron capture detector to investigate the residues in potato[3]. Recently, Nakamura *et al*. developed a high performance liquid chromatography (HPLC) method without any derivative reaction to analyze the residues of flusulfamide on agricultural products, following processes through the SAX (strong anion exchange) and PSA (N-propylethylenediamine) mini column for clean up procedures[4]. Both HPLC and GC methods mention above have applied for the determination of flusulfamide in different matrices, but the lack of a specific detector and harmful reagents use, makes these methods not suitable for quantification in routine.

The objective of this study is to develop a rapid and sensitive method by using a gentler agent to derive flusulfamide as methylated residues before GC-ECD determination in Chinese kale and cabbage.

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MATERIALS AND METHODS

I. *Reagents and Materials*

Analytical standard of 99.9% flusulfamide was obtained from Mitsui. Acetone, *n*-hexane, dimethyl sulfoxide (DMSO), diethyl ether, methyl iodide, sodium chloride, sodium sulfate and sodium hydride were purchased from E. Merck (Darmstadt, Germany). Dichlormethane was purchased from Mallinckradt chrom. All the solvents were LC grade.

The florisil (1000 mg, 6mL) SPE cartridge was obtained from Agilent Co. and the silica gel (1000 mg, 6mL) SPE cartridge was obtained from J & W scientific Chemical Co.

II. *GC-ECD Analysis*

Hewlett-Packard 5890 series gas chromatograph, equipped with an electron capture detector, was used for analysis. The column was a J & W DB-608 (30 m × 0.53 mm, 0.83 μm film thickness), carrier gas and make-up gas was nitrogen at flow rate 5.8 and 30 mL/min, respectively. The temperatures of oven, injector and detector were set at 245, 270, and 300˚C respectively.

III. *GC-MS Analysis*

Hewlett-Packard 6890 model gas chromatograph equipped with a 5972 mass selective detector was used to identify the compounds. The column was a HP-5MS (5% phenyl methyl siloxane, 30 m × 0.25 mm, 0.25 μm film thickness), carrier gas was helium. The system was main-

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tained in constant flow mode (1.0 mL/min) with a pressure of 13.3 psi, and injection volume was 1.0 µL in splitless mode. The injector temperature was 200˚C, the transfer line to the MS system was set at 280˚C, the initial oven temperature was 150˚C programmed at 10˚C/min to 230˚C.

The ion source and quadrupole temperatures were set at 230˚C and 150˚C respectively, and the ionization was done in the electron impact mode (EI) at 70 e*v*. Detection was in scanning mode, the low and high masses were set at 35.0 and 550.0, respectively.

IV. *Preparation of the Calibration Curve*

A concentration range of flusulfamide to 0.01, 0.05, 0.1, 0.5, 1.0 and 2.0 µg/mL in acetone were prepared separately. The calibration graphs were plotted subsequent to linear regression analysis of the peak area versus concentration of methylated flusulfamide.

V. *Extraction*

Twenty grams of chopped Chinese kale or cabbage samples were homogenized with 100 mL of acetone by a Polytron PT 300 mixer at 10,000 rpm for 1 min. After blending, the extracts were filtrated through a No.2 filter paper under suction, and the residual cake was washed again with acetone. The filtrate was combined and the volume made to exactly 150 mL with acetone. Fifteen mililiters aliquots of extracts were taken and transferred to a 50-mL round-bottom flask and evaporated with rotary evaporator at 40˚C until acetone was completely removed.

VI. *Liquid-Liquid Partition*

After evaporation of acetone, the crude extract was dissolved in 100 mL of dichloromethane and transferred into a 500-mL separator funnel. To the separator funnel, 100 mL of 5% sodium chloride was added. After shaking for 1 min, the dichloromethane layer was collected and the aqueous layer was again extracted with another 50 mL of dichloromethane. Dichloromethane extracts (150 mL) were combined and dehydrated with 20 g of sodium sulfate anhydrous and evaporated by rotary evaporator at 40˚C.

VII. *Clean Up by Solid Phase Extraction (SPE)*

The compositions of the Chinese kale and Chinese cabbage were determined[5]. Table 1 showed the water, carbohydrate, crude protein and fat contents in Chinese kale and Chinese cabbage. Solid phase extraction (silica gel, 1000 mg, 6 mL) was used for clean up. The samples were dissolved in a mixture of *n*-hexane/ethyl acetate (7/3, v/v) (with 5 mL for Chinese kale samples and 15 mL for cabbage samples), and applied into a silica gel cartridge. The cartridge was preconditioned with 10 mL of *n*-hexane. After 10 mL of *n*-hexane/ethyl acetate was eluted, the cartridge was eluted with 25 mL of ethyl acetate for the Chinese cabbage sample, and 10 mL of ethyl acetate for the Chinese kale sample. The eluates were evaporated to dryness by rotary evaporator at 40˚C.

VIII. *Methylation of Flusulfamide*

Two mililiters of methyl iodide/*n*-hexane (1/1, v/v) mixture was added to the dry samples and mixed well. Then 1 mL of sodium hydride/DMSO (0.1-g sodium hydride/1.0-mL DMSO) was suspended into the flask and stirred gently by hand for 1 min at room temperature. The reaction was stopped by carefully adding a few drops of water, then gradually adding another 30 mL of water to the reaction flask. The aqueous layer was transferred to a 250-mL separatory funnel, and extracted with 30 mL of *n*-hexane twice. The *n*-hexane layer was dehydrated with 15 g of anhydrous sodium sulfate and evaporated to dryness by rotary evaporator at 40˚C.

IX. *Clean-Up by Solid Phase Extraction (SPE)*

After methylation the residue was redissolved in 5 mL of *n*-hexane/diethyl ether (85/15, v/v), and loaded into a florisil cartridge which was preconditioned with 10 mL of *n*-hexane. Methylated flusulfamide was eluted with 15 mL of *n*-hexane/ethyl acetate (8/2, v/v). The eluate was evaporated to dryness in flowing nitrogen, and the final volume was made up to 1 mL with *n*-hexane before GC determination.

RESULTS AND DISCUSSION

I. *Methylation of Flusulfamide with Methyl Iodide*

Flusulfamide (Figure 1) contains a nitrogen atom that includes non-bonding electrons. This nitrogen atom has the

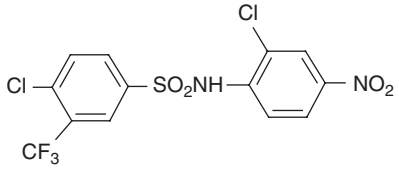

Figure 1. Chemical structure of flusulfamide.

Table 1. Composition of Chinese cabbage and Chinese kale (%)

Crop	Water	Carbohydrate	Crude protein	Crude fat	Ash
Chinese cabbage	96	1.8	1.1	0.2	0.7
Chinese kale	92	3.9	2.4	0.5	1.2

potential to form hydrogen bonding with other electronegative atoms in the stationary phase of the GC column, resulting in poor separation and low sensitivity. To improve the analysis, the nitrogen atom on flusulfamide should be methylated to form 4-chloro-*N*-methyl-*N*-(2-chloro-4-nitrophenyl)-3-(trifluoromethyl)benzene-sulfonamide. The analytical method developed by Mitsui Toatsu Chemical Co. used diazomethane to perform the methylation[3]. Recently, some drawbacks of the synthesis of diazomethane have been noted because *N*-methyl-*N*-nitroso-p-toluene-sulfonamide (Diazald, MNTSA) and 1-methyl-3-nitro-nitrosoguanidine (MNNG) are mutagenic. In the European Union (EU), MNNG is classified as carcinogenic[6]. Diazomethane is also classified as carcinogenic

Table 2. Recovery of Flusulfamide from Chinese cabbage and Chinese kale

Crop	Level of fortification (μg/g)	Mean of recovery (%)	RSD (%)	Method detection limit (μg/g)
Chinese cabbage	0.5	86.1	5.98	0.001
	0.05	86.4	5.72	0.001
	0.005	105.4	15.6	0.0005
Chinese kale	0.5	85.8	9.85	0.001
	0.05	95.1	0.18	0.001
	0.005	98.6	15.57	0.001

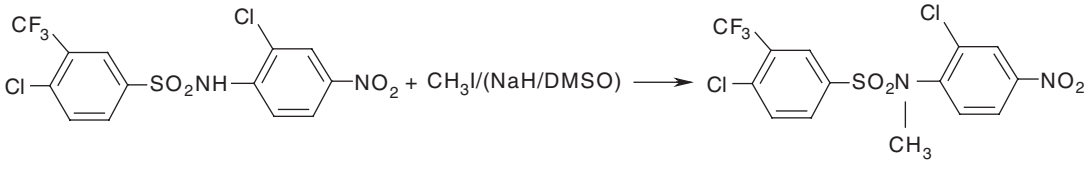

Figure 2. CH_3I methylation of flusulfamide.

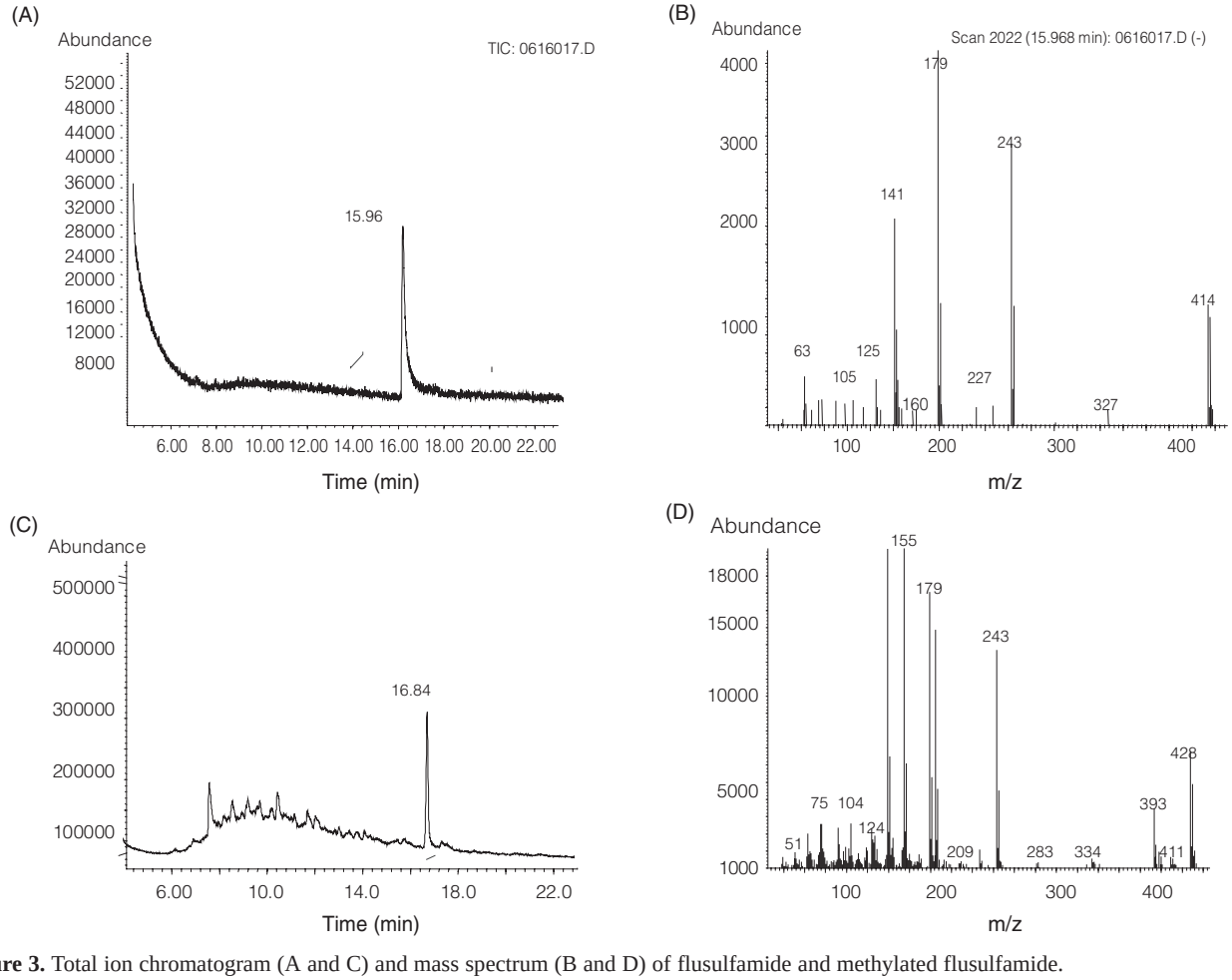

Figure 3. Total ion chromatogram (A and C) and mass spectrum (B and D) of flusulfamide and methylated flusulfamide. (A) and (B) flusulfamide, 10 μg/mL, (C) and (D) flusulfamide derivative, 100 μg/mL.

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and furthermore it can explode in contact with ground glass or when heated above 90˚C[7]. Accordingly, methyl iodide was used as the methylation reagent in this study. Fast methyl iodide reactions proceeded S_N2 nucleophilic substitution, which was supported by a bipolar aprotic solvent like dimethyl sulfoxide (DMSO). The sulfoxide promised high reaction rates in the presence of an additional strong base, such as NaH[8]. The reaction happened even at room temperature (Figure 2).

II. *Confirmation of Methylation by GC/MS*

Figure 3 shows the total ion chromatogram and mass spectrum of flusulfamide and methylated flusulfamide. On the mass spectrum, the parent ion peak of flusulfamide appeared at m/z 414 and the parent ion peak of methylated flusulfamide appeared at 428. The identification of the methylated derivative was therefore confirmed.

III. *Calibration Curve and Gas Chromatography*

From the calibration curve of methylated derivative, the correlation coefficient was calculated and r^2 was 0.9999. Figures 4 and 5 show the GC chromatograms of flusulfamide after the methylation of methyl iodide, and of samples spiked with 0.5 μg/g flusulfamide, respectively. The retention time was 10.5 min. This study also shows almost no interference from the Chinese kale and cabbage matrices, even after methyl iodide methylation.

IV. *Recovery and Detection Limits of Flusulfamide in Chinese Kale and Cabbage*

Several criteria must be considered in assessing the performance of an analytical method before it is employed in routine usage. For example, when the spiked amount is five times the limit of determination, the recoveries should be in the 70-110% range, with relative standard deviations of < 20%. The levels of fortification in this study were 0.005, 0.05 and 0.5 μg/g, based on the estimated tolerance of the Chinese kale and cabbage at the 0.05 ppm level. Values ten times higher and lower than this tolerance level were chosen to assess the performance of this method. Table 2 shows the recoveries of flusulfamide using the above mentioned analytical procedure. The mean recovery rates of the Chinese kale and Chinese cabbage were 93.2 and 92.6%; the mean S.D. were 8.2 and 8.8%, respectively, and the detection limits of the method were 0.0005 ~ 0.001 μg/g. The lower level (0.005 μg /g) of flusulfamide has a higher recovery rate than other's, the reason was probably due to 0.005 μg/g caused the signal to be very close to the limits of detection of the analytical instrument. At this

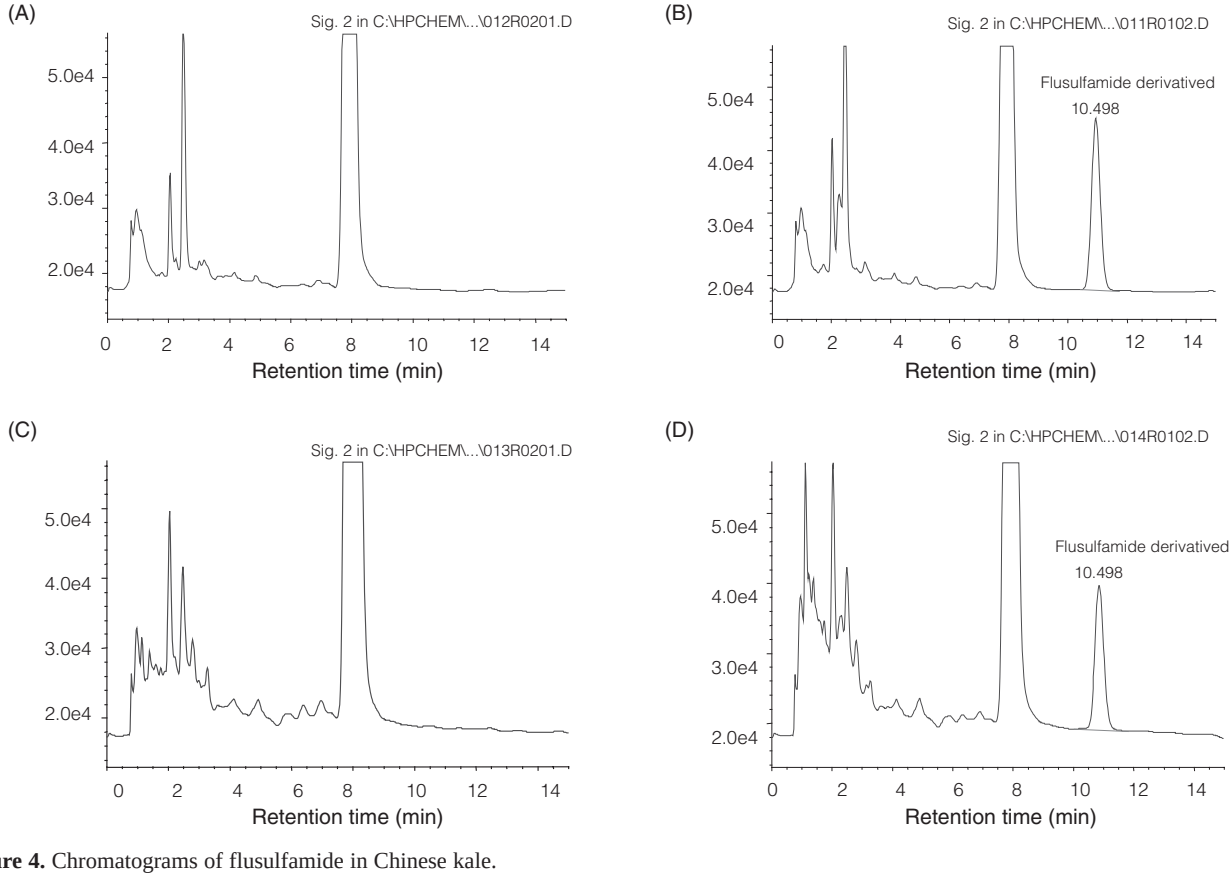

Figure 4. Chromatograms of flusulfamide in Chinese kale.
(A) Methylation reagent only. (Blank); (B) Derivative flusulfamide standard solution, 1 ng; (C) Untreated Chinese kale; (D) Chinese kale fortified with 0.5 μg/g flusulfamide.

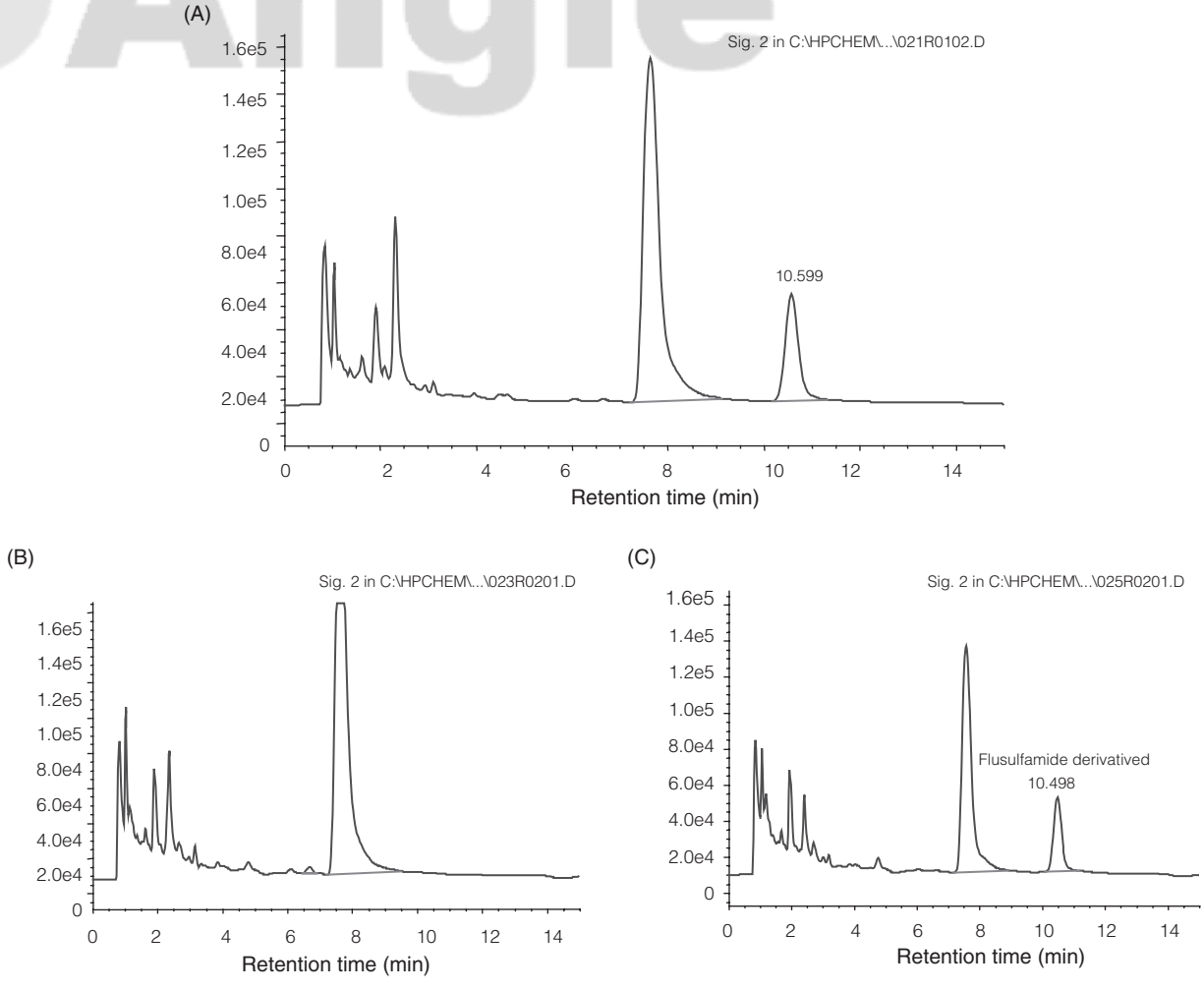

Figure 5. Chromatograms of flusulfamide in Chinese cabbage.
(A) Derivative flusulfamide standard solution, 1 ng; (B) Untreated Chinese cabbage; (C) Chinese cabbage fortified with 0.5 μg/g flusulfamide.

level, the noise most likely coming from the instrument will affect to a large extent the expression of the actual signal of the samples, and result in the responses of the lower level samples to be higher than the standard. This could explain why the variation was also higher than other levels. All of these results showed that this method is suited to be used for routine analysis of the residues of flusulfamide on the crops.

CONCLUSION

Fungicide flusulfamide contains halogen elements like fluoride and chloride that can be easily detected by GC-ECD. However, the affinity of 4-nitrophenyl moiety to the stationary phase caused poor column separation. Therefore, flusulfamide must be methylated before GC determination. Previously, diazomethane was used for methylation. In this study, methyl iodide was used to replace the diazomethane to avoid potential dangers caused by diazomethane. The methylated product was confirmed by GC/MS in this study.

The limit of detection was 0.001 μg/g on the Chinese kale and the Chinese cabbage after methylation and clean up, which limit was lower than that of the method developed by Mitsui Toatsu Chemicals in 1997 (0.005 mg/kg), suggesting that the method proposed herein is more sensitive and suitable for analyzing residues in crops.

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