Journal of Food and Drug Analysis

Volume 12 | Issue 3 Article 6

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Journal of Food and Drug Analysis, Vol. 12, No. 3, 2004, Pages 238-243 藥物食品分析 第十二卷 第三期

Determination of Residual Dalapon in Sugarcane by Gas Chromatograph Equipped with Electron Capture Detector (GC-ECD)

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(Received: February 12, 2004; Accepted: May 27, 2004)

ABSTRACT

A gas chromatographic method using an electron capture detector (GC-ECD) was developed for the determination of residual dalapon, esterified with methanol in sulfuric acid, in sugarcane. Dalapon in plant matrix was initially extracted with sulfuric acid solution, followed by partition extraction with diethyl ether in the presence of NaCl and esterification with 10% H_2SO_4 methanolic for GC-ECD analysis. Recovery of dalapon (0.05~0.20 ppm) spiked in sugarcane samples was carried out to obtain an average recovery of 89.7~109.4% with a coefficient of variation (CV) of 1.0~9.3% and a method detection limit (MDL) of 0.01 ppm. Peak of dalapon methyl ester was further identified by a GC-MS spectrum. The developed procedure was simple and the reagents used were slightly toxic, thus suitable for the accurate quantification of dalapon residues in crops.

Key words: pesticide, dalapon, esterification reaction, gas chromatography, electron capture detector

INTRODUCTION

Dalapon (2, 2-dichloropropionic acid), a selective systemic herbicide, is absorbed by the leaves and roots and then translocated *via* both the symplastic and apoplastic systems throughout the plants[1]. It acts by precipitation of protein, which interferes with the production of pantothenic acid[1], and leads to physiological malfunctions of plants. Dalapon for agricultural use is in sodium salt form, and the acute oral LD_{50} for chickens is 5600 mg/kg body weight. In mammals, dalapon is rapidly eliminated following oral administration. In dogs, 65-70% of a single oral dose of 500 mg is excreted within 2 hr[1].

Haloacetic acids (HAAs), similar to dalapon in structure, are the major compounds of disinfection by-products (DBPs) as a result of the chlorination of water. In addition to agricultural usage, water treatment is also an important source of dalapon in drinking water[2-4]. As a result, dalapon assay in tape water was performed simultaneously while quantifying the HAAs residues[2,4,5]. Furthermore, dalapon was suggested to be a tracer to monitor the extraction process while analyzing HAAs in water samples[3].

The tolerance level for the residue of dalapon in sugarcane is 0.1 ppm, according to "the Tolerances for the Residues of Pesticides"[6] announcement from the Department of Health, Taiwan. However, no official method is available for quantifying dalapon residue in crops in Taiwan. Commercial dalapon has been quantified by an ion-exchange HPLC-UV method[7-8], but this method is unsuitable for the residual assay of dalapon in water and plant tissues due to insufficient sensitivity. On the other hand, GC-ECD[1-3,5,9] or GC-MS[4] was suggested to be a useful tool for the residual dalapon analysis in water or plant matrices. However, there were still limitations since most of the methods were for water samples, and the extraction efficiency of analytes and the subsequent modification reactions were needed before quantitative assay. Even the improved method for determining dalapon residue in plant tissue by ECD as reported by Van Der Poll and De Vos (1980) was still complex and time-consuming.

Dalapon was derivatized through reactions with diazomethane[2], trimethylsilydiazomethane[5], methanol in strong acid[5], 3-phenylpropanol-1[9] or 1-benzyl-3-*p*-tolytriazene[1] for GC analysis. Although reactions of dalapon with diazomethane were fast and efficient, carcinogenic risks and explosive characteristics of diazomethane limited the use of diazomethane in most laboratories. Most importantly, complex and lengthy pretreatments were often conducted to improve resolution of dalapon from plant matrices. Van Der Poll and De Vos (1980) suggested complex procedures for the extraction of dalapon in plant matrices with orthophosphoric acid and phosphotungstic acid, followed by a partition extraction with ether in saturated NaCl solution and derivatization with 3-phenylpropanol-1 in boiling water bath in the presence of HCl gas. An additional clean-up process with a silica column by a large volume of organic solvent was also required.

U.S. Environmental Agency (USEPA) (method 552.2)

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Journal of Food and Drug Analysis, Vol. 12, No. 3, 2004

esterified dalapon in water samples with 10% sulfuric acid methanolic solution for GC analysis. The advantages of this method included safety and cost saving. Nevertheless, there were various major drawbacks such as low recovery (about 80%) of esterified product in a medium with adequate salt level, as well as the potential damages to the GC columns as a result of the strong acidic extract[5].

In order to develop efficient extraction to derivatization reaction procedures for the assay of dalapon in plant matrices, low toxic organic reagents were used and the derivatization reactions with methanol in strong acidic solution were improved. In addition, recovery, coefficient of variation (CV), and method detection limit (MDL) of dalapon spiked in crop samples were also investigated.

MATERIALS AND METHODS

I. *Reagents*

Sulfuric acid, NaCl, methyl *tert*-butyl ether (MTBE), and anhydrous Na-sulfate were purchased from Sigma (St. Louis, MO, USA). Dalapon (2, 2-dichloropropionic acid) and dalapon methyl ester (reference standard) with a purity of 98 and 95.8%, respectively, were purchased from ChemService (West Chester, PA, USA), while methanol for chromatographic use was from J. T. Baker (de Mex, Mexico). Diethyl ether from Labscan Asia Co., Ltd. (Bangkok, Thailand) was residue grade. Derivatization vials (5 mL) with teflon screw caps were from Supleco Co. (Bellefonte, PA, USA).

II. *Preparation of Standard and Sample Solutions*

Dalapon or dalapon methyl ester (100 mg) was dissolved in MTBE and made up to 100 mL in a volumetric flask to make stock solution. The solution was further diluted with MTBE to a concentration of 0.05-0.5 μg/mL to prepare standard solution.

Fifty millimeters of de-ionized water and 1.5 mL of conc. sulfuric acid were added to homogenized (Cycle blender, Mexico) sugarcane sample (10 g, in 250-mL PP container) or that spiked with 0.05, 0.1 or 0.2 ppm of dalapon. After shaking (Model VD-12, Hsiangtai Machinery Industry Co., Ltd., Taipei, Taiwan) for 5 min, filtration through a filter paper (No. 2) was conducted with an aid of aspirator. The filter cake was pressed dry and subsequently washed twice with 20 mL of distilled water (Milli-Q System, Osaka, Japan). Water was then combined with the filtrate, followed by addition of 30 g NaCl for the partition extraction with 50 and 50 mL of ether respectively. The combined ether extracts were dried over anhydrous Na-sulfate and then concentrated to dry at reduced pressure in a rotary evaporator (Model R134, Buchi, Flawil, Switzerland). Dried residues were dissolved and made up to 2 mL with MTBE (sample solution) for the following esterification.

III. *Esterification*

One millimeter of 10% sulfuric methanolic solution was added to 2 mL of sample solution or that spiked with 0.05, 0.1 or 0.5 ppm dalapon standard solution in derivatization vial, and then the mixture was heated at 50˚C in a water bath for 2 hr. After cooling down to room temperature (about 28˚C), the mixture was added with 4 mL of 10% Na-sulfate, followed by agitation for 2 min and resting for 10 min. The upper organic solvent layer was sampled for GC analysis.

To optimize esterification reaction, 2 mL of 0.5 μg/mL dalapon standard solution was reacted with 1 mL of 10% sulfuric methanolic solution at 50˚C in a water bath for various time frames (30, 60, 90, 120, and 150 min), followed by the esterification procedures described above. Derivatization (%) = (μg of heated, esterified dalapon /1 μg of dalapon) × 100%.

IV. *Chromatographic Conditions*

Gas chromatograph (Varian 3400, Varian Technologies, Walnut Creek, CA, USA) equipped with a separation column of DB-1 (0.53 mm in inner diameter, 30 m in length, 0.83 μm in film thickness), an auto sample injector of CombiPAL (CTC Analytics, Switzerland), and an electron capture detector (ECD) (J & W Scientific, CA, USA) was used. Data was processed using a Chromatography Data Station Software, Version III (Scientific Information Service Corporation, ROC). The Chromatographic conditions were as follows: carrier gas, N_2 (10 mL/min); temperature of the injection port, 250˚C; temperature of detector, 300˚C; sample injection volume, 1 μL; injection mode, splitless. The oven temperature was programmed as follows: 50˚C for 10 min, 30˚C/min up to 280˚C, held for 3 min.

One microliter of dalapon methyl ester standard solution with 0.1, 0.2, 0.4, and 0.5 μg/mL was used to construct the calibration curve (y =1115735 × – 5366, r = 0.9992); where x is analyte concentration, y is peak area, and r is correlation coefficient. Dalapon content (ppm) in sugarcane sample was calculated according the following equation:

$$\text{Dalapon content in sample (ppm)} = (C \times V)/M \times F$$

where C, amount of derivatized dalapon (μg/mL) calculated from the calibration curve constructed with standard solution of dalapon methyl ester; V is volume (mL) of sample solution; M is sample weight (g); F is molecular weight conversion factor (143/157).

V. *Recovery Test and Coefficient of Variations (CV)*

Homogenized sugarcane sample (10 g) spiked with 0.05, 0.1 or 0.5 ppm of dalapon was used for fortification analysis to evaluate the recovery (%) and determine the coefficient of variation (CV, %) of dalapon. Average

recovery and coefficient of variations (CV) were determined by triplicate samples.

VI. *Method Detection Limit (MDL)*

Dalapon was spiked to a homogenized sugarcane blank sample and the MDL of dalapon was estimated on the basis of signal/noise (S/N) > 3[11].

VII. *Gas Chromatography/Ion Trap Mass Spectrometry*

Gas chromatograph (Varian 3800) equipped with a mass detector (Varian Saturn 2200), cp-sil 8 CB lowbleed/MS column (inner diameter 0.25 mm × 30 m, Varian, USA) and Saturn Ws software (ver 5.5 kit) was used. The GC/MSD conditions were as follows: carrier gas, He (1 mL/min); ionization energy, 70 eV; temperature of the injection port, 250˚C; MSD interface temperature, 280˚C; sample injection volume, 1 μL; the oven temperature was programmed as follows: 50˚C for 10 min, 15˚C/min up to 270˚C.

RESULTS AND DISCUSSION

I. *Chromatogram of Dalapon Methyl Ester*

Esterification with 10% sulfuric acid methanolic solution increased the evaporability of dalapon, which thus became available for GC analysis[5]. In the presence of sulfuric acid, carboxyl group in dalapon (MW 143) reacted with methanol and the dalapon methyl ester (MW 157) was formed. Dalapon methyl ester (0.1 μg/mL), which was separated by DB-1 column and detected by ECD, showed a sharp and symmetrical peak at a retention time of about 5.7 min (Figure 1).

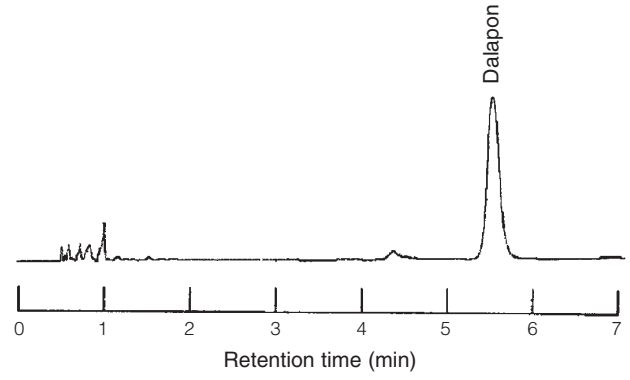

Figure 1. Gas chromatogram of 0.1 μg/mL dalapon methyl ester standard separated with a DB-1 column and detected by an electron capture detector. GC conditions: column temperature, 50˚C, 10 min, 30˚C/min, 280˚C, 3 min; injector temperature, 250˚C; detector temperature, 300˚C.

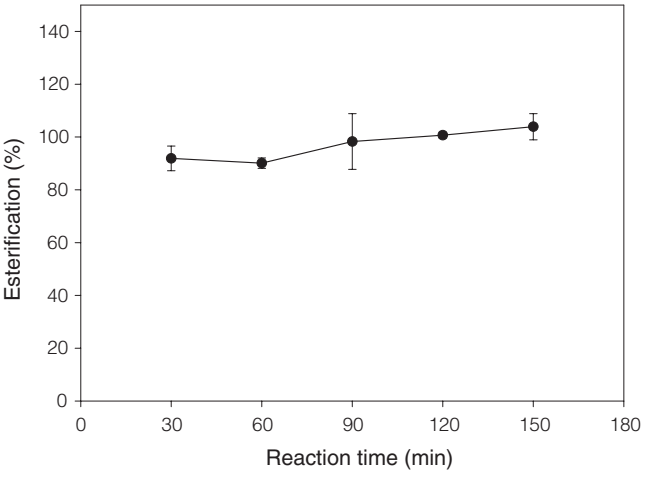

Figure 2. Esterification percentage of dalapon into dalapon methyl ester at 50˚C for different time frame. Bars in the figure represent the standard deviation.

II. *Optimal Derivatization Conditions of Dalapon*

Standard solution (0.5 μg/mL, 2 mL) of dalapon added with 10% sulfuric acid methanolic solution was heated separately at 50˚C for 30, 60, 90, 120, or 150 min. Afterwards, 10% sodium sulfate solution was added to the solution for clean-up and to reduction of the potential degradation of GC column. It was observed that the derivatization percentage (%) of dalapon heated for 30 and 60 min was about 95%, but the percentage increased to about 100% at the extended reaction times of 120 and 150 min (Figure 2). The observed derivatization efficiency was clearly much higher than that reported by Pawlecki-Vonderheide *et al*. (1997) who esterified dalapon using trimethylsilydiazomethane or acidic methanol as the derivatizing agent and obtained an average esterification efficiency of 91 and 79%, respectively. Therefore, heating time of 120 min at 50˚C was suggested in the following experiments.

Residual dalapon in water was reacted with diazomethane solution or gaseous diazomethane from a generator and then quantified by a GC-ECD[2]. However, appropriate safety equipment to minimize skin contact and inhalation hazards was suggested. Dalapon esters prepared with methanol in the presence of strong acids might cause a GC column degradation without a proper neutralization treatment of acidic extracts[5]. Van Der Poll and De Vos (1980) adopted a lengthy derivatization procedure which consisted of addition of 3-phenylpropanol-1 to the ether extract of dalapon which was prepared in the presence of phosphoric acid, phosphotungstic acid, and NaCl, performing esterification with the aids of dry HCl gas and heating. Another derivatizing agent, 1-benzyl-3-*P*-tolytriazene, was used by Tsukioka and Shimizu (1985) to form dalapon-benzylated derivative for GC and mass analysis.

III. *Establishment of Pretreatment*

In order to facilitate the partition extraction of dalapon in plant matrices by ether or MTBE, NaCl[5,9-10] and strong

Journal of Food and Drug Analysis, Vol. 12, No. 3, 2004

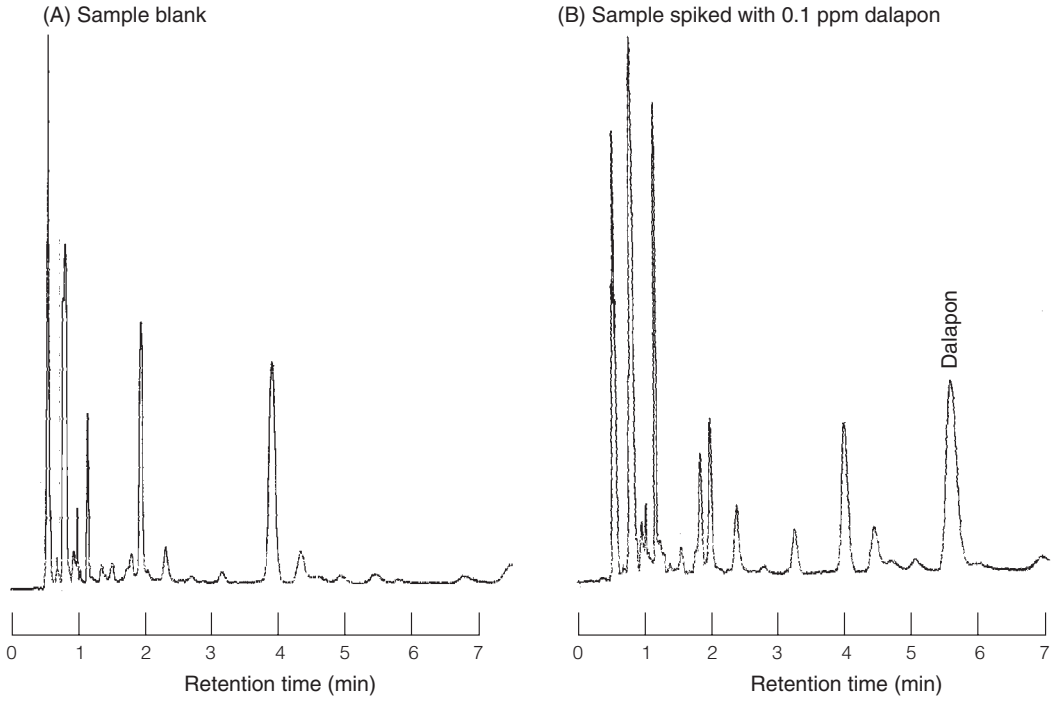

Figure 3. Gas chromatograms of sugarcane sample blank (A) and sugarcane sample spiked with 0.1 ppm dalapon (B). GC conditions: column, DB-1; detector, electron capture detector; column temperature, 50˚C, 10 min, 30˚C/min, 280˚C, 3 min; injector temperature, 250˚C; detector temperature, 300˚C.

acids such as phosphoric acid, phosphotungstic acid[9] and sulfuric acid[4-5,10] were added the solution. As the result, ionic strength of the aqueous phase was increased and the pH value of matrix homogenates was lower to less than 2, respectively, to insure that the target analytes were in acid forms. In addition, emulsion formation during partition extraction with ether was also avoidable and thus, resulted in the sharp distinction between organic and aqueous phases[5,10].

In the present study, similar procedures were performed to extract dalapon in sugarcane matrix, followed by esterification to form dalapon methyl ester. Comparing with the sample blank in Figure 3A, a sharp peak of dalapon derivative (0.1 ppm) was observed at a retention time of 5.761 min (Figure 3B), suggesting the efficiency of extraction and esterification of dalapon in the pretreatment. No significant impurity peaks appeared in the chromatogram during the esterification reaction. Van Der Poll and De Vos (1980) proposed complex pretreatments of dalapon in plant tissues and derivatization reactions with 3-phenylpropanol-1 in the presence of saturated HCl gas. However, a following clean-up process was needed before GC analysis, thereby consuming a large quantity of organic solvent.

IV. *Recovery, MDL, and CV*

To evaluate the recovery and reproducibility, homogenized sugarcane sample was spiked with 0.05, 0.10 or 0.20 ppm of dalapon and the results are shown in Table 1.

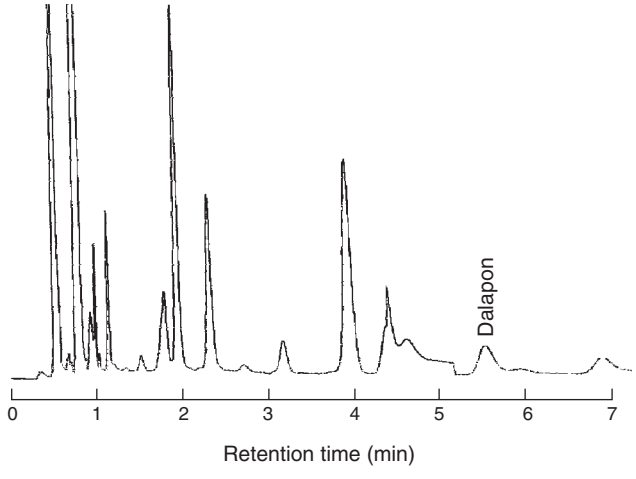

Figure 4. Gas chromatogram of sugarcane sample containing 0.01 ppm dalapon. GC conditions: column, DB-1; detector, electron capture detector; column temperature, 50˚C, 10 min, 30˚C/min, 280˚C, 3 min; injector temperature, 250˚C; detector temperature, 300˚C.

Table 1. Recoveries[a] of dalapon spiked in sugarcane sample

Sample	Spiked level (ppm)	Recovery (%)[a]
Sugarcane	0.05	109.4 ± 2.1 (9.3)[b]
	0.10	90.7 ± 1.5 (4.8)
	0.20	89.7 ± 1.3 (1.0)

Each value is the average of three triplicate results.
[a]Recovery (%) = 100 % × (Level of dalapon in spiked sample - Level of dalapon in blank sample)/Level of dalapon in spiked sample.
[b]Value in the parenthesis is coefficient of variation (CV, %).

Average recovery was estimated to be 89.7-109.4% with a CV of 1.0-9.3%, suggesting the efficiency of the procedures presented in this study. On the other hand, MDL of dalapon derivative to give a signal three-times the noise (S/N > 3) was 0.01 ppm (Figure 4), lower than that (0.02 ppm) reported by Van Der Poll and De Vos (1980).

V. *GC-MS Analysis*

Figure 5 shows the GC-RIC (reconstructed ion current chromatography) of dalapon methyl ester standard, sugarcane sample blank, sugarcane sample spiked with 0.2 ppm dalapon, and the mass spectrum of dalapon methyl ester. Apparently, no interfering peaks were observed on GC-RIC of sample blank. The ion at m/z 157 was the molecular ion (M^+) of dalapon methyl ester, while the major fragment ion at m/z 97 ($CH_3CCl_2^+$) was derived from the detachment of the $COOCH_3$ group from the molecule. A peak at m/z 61 was attributed to the further fragmentation and the loss of Cl of the major fragment ion. The GC-MS spectrum was available as reference for further confirmation. Notably, matching quality was above 65% in comparison of the MS spectrum of dalapon methyl ester in standard solution and sugarcane sample.

In conclusion, public concepts over the toxic properties of chemical reagents used during the esterification of dalapon have prompted investigations on the alternative derivatization techniques. As a result, a simple extraction and esterification procedure was developed with a high esterification percentage, recovery, and acceptable MDL of dalapon. Additionally, the potential GC column degradation was minimized by the modification of Na-sulfate solution treatment of the methylated acidic ester extracts.

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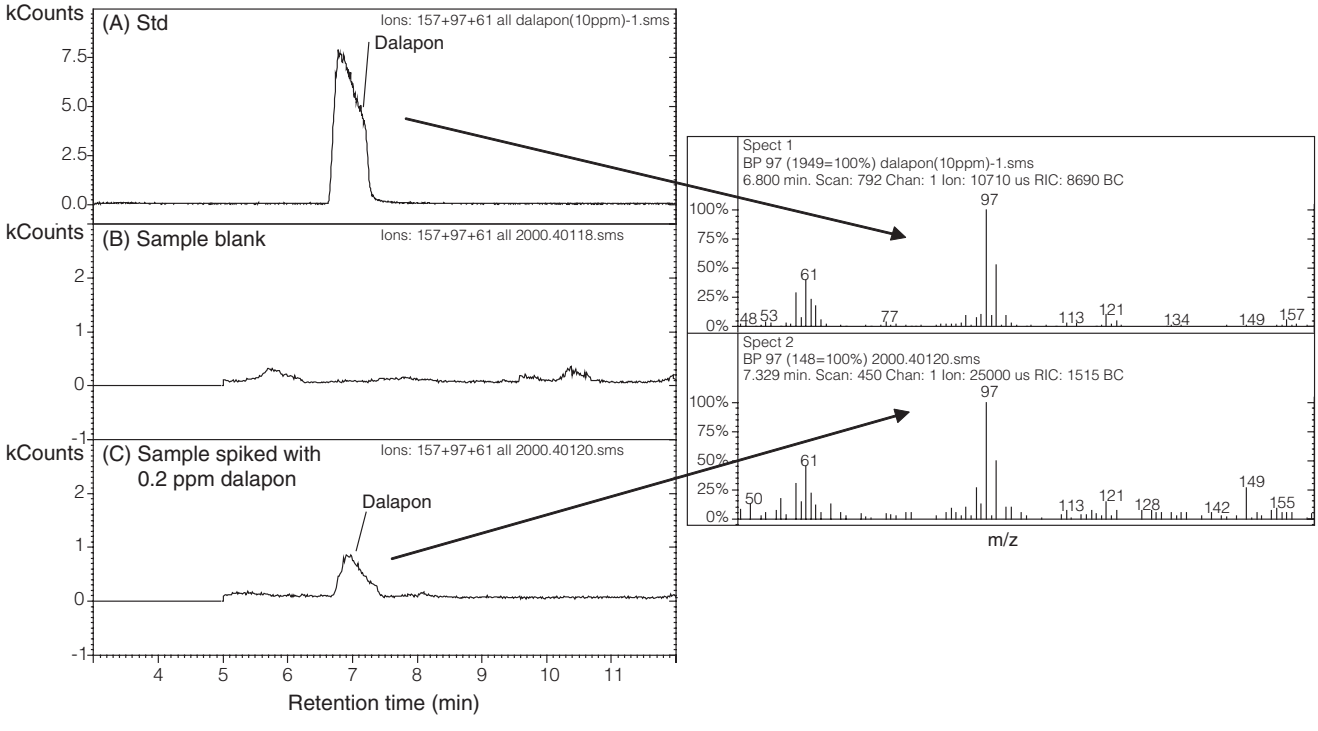

Figure 5. GC-RIC (reconstructed ion current chromatography) of dalapon methyl ester standard (10 μg/mL) (A), sugarcane sample blank (B), and sugarcane sample spiked with 0.2 ppm dalapon (C). MS spectra of dalapon methyl ester peak in Figure 5 (A) and Figure 5 (C) are shown on the right.

GC-MSD conditions: column, cp-sil 8 CB; initial temperature, 50˚C; initial time, 10 min; 15˚C/min, final temperature, 270˚C; injector port temperature, 250˚C; MSD interface temperature, 280˚C; ionization energy: 70 eV.

Journal of Food and Drug Analysis, Vol. 12, No. 3, 2004

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