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Contributed Paper

HS-SPME for the Determination of Phthalate Esters in Vegetable Oil and Soft Drink Samples

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ABSTRACT

A simple and environmental friendly method using headspace solid-phase microextraction (HS-SPME) determination of four phthalate esters in vegetable oil and soft drink samples were investigated. The extraction temperature, extraction time and desorption time were evaluated by comparing two types of commercial SPME fibers; polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber and polyethylene glycol (PEG) fiber. The PDMS/DVB fiber showed better response than PEG fiber. The linearity, limit of detection, repeatability and reproducibility were validated. The enrichment factor (EF) for oil samples and soft drink samples were in the range of 11-17, 37-848, respectively. The percentage recoveries by spiking samples with standard solution were also examined, the result found in the range of 84.5-102.1 % with the RSD less than 10 %. The proposed method was successfully applied for trace analysis of four phthalate esters in vegetable oil and soft drink samples.

Keywords: headspace solid-phase microextraction, sample preparation, gas chromatography, vegetable oil, soft drink

1. INTRODUCTION

Vegetable oils are popular raw materials for cooking, especially when stir frying, deep frying and preparing salad dressing. Most vegetable oils are packaged in plastic containers. Polyethylene terephthalate (PET) bottles are quite popular. Many plastics are blended with additives to alter and improve their properties. They are plasticized mainly with bis(2-ethylhexyl) phthalate ester (DEHP) [1-2]. Phthalate esters do not chemically bond to plastic products, so they could be released and migrate into food, especially fatty food like vegetable oil. The discovery of some phthalate esters, such as diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP) and DEHP in food, demonstrates the ability of these esters to interfere with food products [3]. Research has detected phthalate esters in vegetable oils [4-10]. Phthalate esters have been found in human tissue and some of

these compounds cause allergic reactions, dermatitis, respiratory disease, liver and kidney damage and cancer-causing possibilities [11-16]. The US Environmental Protection Agency (EPA) has set the Maximum Admissible Concentration (MAC) for DEHP at 6 μg L^{-1}in drinking water. The European Food Safety Authority (EFSA) has specified 0.01 and 0.05 mg kg^{-1} body weight per day for DnBP and DEHP, respectively [17-19].

Trace analysis of phthalate esters may generally pose a serious problem due to the high risk of contamination from the environment. Further difficulties are encountered separating some matrices such as beer [20-21], milk [22-25], wine [26-28] and especially, viscous edible oils [4-10]. Preconcentration methods for trace analysis of phthalate esters in edible oil samples is necessary. Various extraction methods have been developed for the preconcentration of phthalate esters such as liquid-liquid extraction (LLE) [5-6], solid-phase extraction (SPE) [10], solid-phase microextraction (SPME) [4, 8]. The headspace solid-phase microextraction (HS-SPME) is solvent free, avoids matrix contamination and environmental friendly method for the extraction of analytes.

Therefore, this study was to investigate the applicability of the HS-SPME method for extraction of phthalate esters; DMP, DEP, DnBP and DEHP in vegetable oil and soft drink samples by GC-FID using polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber and polyethylene glycol (PEG) fiber. The effects of various experimental parameters on the extraction performance of the target analytes, such as extraction temperature, extraction time, desorption time and salt addition were optimized. The resulting method was validated for the extraction of four phthalate esters in vegetable oil and soft drink samples.

2. MATERIALS AND METHODS

2.1 Chemicals and Materials

Dimethyl phthalate (DMP) and diethyl phthalate (DEP) were obtained from Fluka (Switzerland). Di-*n*-butyl phthalate (DnBP) and di(2-ethylhexyl) phthalate (DEHP) were obtained from Supelco (USA). All organic solvents were HPLC grade from Merck (Germany). NaCl, Na_2SO_4, CH_3COONa, $NaNO_3$ and $(NH_4)_2SO_4$ were AR grade from UNIVAR (Australia). The individual standard stock solutions were prepared in ethyl acetate and stored at 4 °C. The working standard solutions were prepared daily by diluting a stock standard solution with ethyl acetate to the required concentrations. All chemicals were prepared in a glass apparatus and stored in a glass bottle.

The commercial SPME devices, 60 μm polyethylene glycol (PEG) and 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), were purchased from Supelco (USA). The new fibers were equilibrated at recommended temperatures of 240 °C for PEG fiber and 250 °C for PDMS/DVB fiber prior to use in a GC injector for 30 min

2.2 Instrumentation

The experiments were carried on a GC-2014 gas chromatograph (Shimadzu, Japan) equipped with a DB-17 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and a flame ionization detector (FID). The injector temperature was 200 °C and the detector temperature was 270 °C. The temperature program was started from 150 °C and held for 5 min, then ramped to 270 °C with the rate of 15 °C/min and held for 15 min. The injection volume was 1.0 μL in split mode.

2.3 Sample and Extraction Procedure

Vegetable oil and soft drink samples, packed in plastic containers and stored at room temperature were bought at a local market in Maha Sarakham Province, Thailand. Soft drink samples were degassed in an ultrasonic bath for 20 min before extraction process.

The 5 mL of vegetable oil samples were spiked with 200 ng mL^{-1} of each phthalate ester standard solutions, then placed in a 10 mL amber glass vial. The vial was sealed with a polyetrafluoroethylene (PTFE) faced septum cap. The SPME fiber was then exposed to the headspace at various conditions: extraction temperature (50-90 °C), extraction time (10-50 min), desorption time (1-10 min) and salt addition. The samples were continuously stirred at constant rate. The extraction process was allowed to the equilibrium of analytes between the sample phase and the headspace, and immediately inserted into the injection port of gas chromatograph at a desorption temperature of 200 °C. The experimental parameters were compared between PDMS/DVB and PEG fiber.

The optimum condition for vegetable oil sample was applied to determination of four phthalate esters in soft drink samples

3. RESULTS AND DISCUSSION

The experimental parameters were optimized in oil matrix samples. A high extraction temperature is desirable to accelerate the release of analytes from the oil matrix and increase the concentration of the phthalate esters in the headspace. The effect of temperature in the extraction efficiency in the term of peak area was investigated varying from 50 to 90 °C with a constant extraction time of 30 min and desorption time of 10 min at 200 °C. All analytes are less extracted at a low temperature because they have a high boiling point so that less evaporation. Attending to the expected behavior of the phthalate esters increasing the temperature improved the mobility of the phthalate esters in oil sample through gas phase and better response were obtained until 80 °C, as shown in Figure 1. The ability of the HS-SPME fiber to absorb the tested phthalate esters was decreased at 90 °C. It might be the partition coefficients to the extraction phase are decreased. Another reason is the loss of the target analytes in the solution at higher temperature [29]. In addition, the solution in the vial is boiling at the temperature higher than 90 °C. Therefore, the optimum extraction temperature is set at 80 °C for all analytes. The effect of extraction temperature is the same result in both fibers.

HS-SPME method has maximum sensitivity when the equilibrium point is reached. The effect of extraction time was investigated varying from 10-50 min with a constant extraction temperature of 80 °C and desorption time of 10 min at 200 °C. The mean effects of extraction time on HS-SPME technique is shown in Figure 2. Before 30 min of extraction time, all analytes have less extraction efficiency due to the fiber having a low absorption rate. It may take longer time to absorb completely. After absorption is completed, some analytes could be desorbing into the headspace phase and come down into liquid phased, so the extraction efficiency is decreasing at long extraction time. This may be caused by the phase not being in equilibrium. Thus, the optimum extraction time was set at 30 min for all analytes. The effect of extraction time had the same result in both fibers.

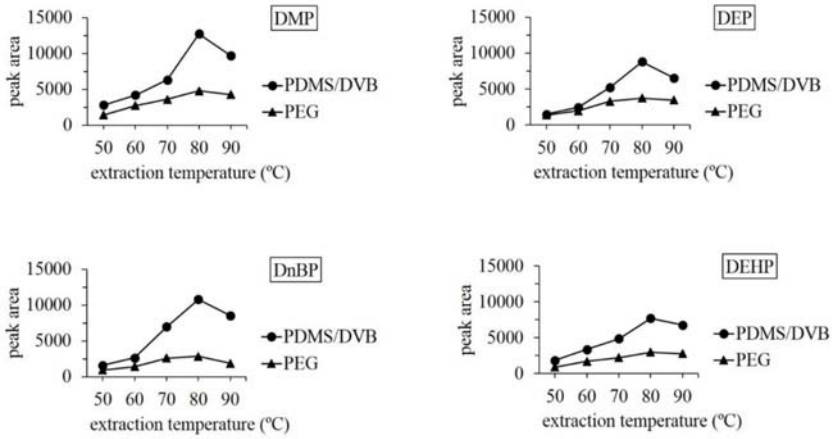

Figure 1. Effect of extraction temperature on HS-SPME of spiked 200 ng mL^{-1} of four phthalate esters in vegetable oil sample with extraction time of 30 min, desorption time of 10 min at 200 °C.

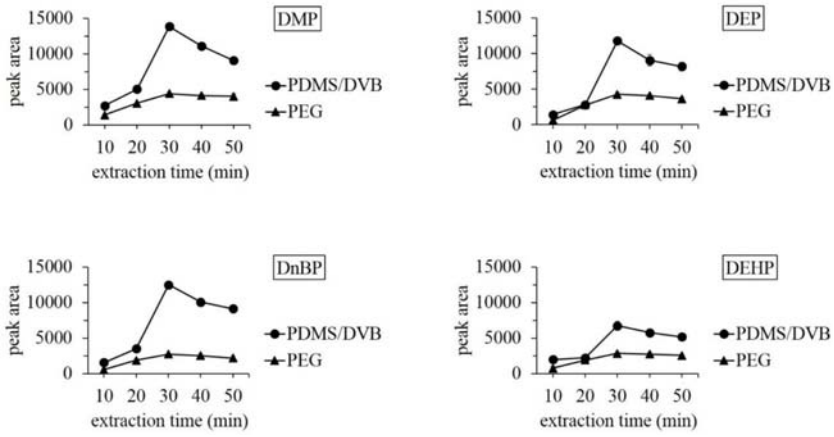

Figure 2. Effect of extraction time on HS-SPME of spiked 200 ng mL^{-1} of four phthalate esters in vegetable oil sample with extraction temperature of 80 °C, desorption time of 10 min at 200 °C.

The serious problem in the analysis by the HS-SPME method is memory effect, that analytes are non-completely desorbed, and some left in the fiber and may give false signals in subsequent analysis. Therefore, the temperature and time needed for complete desorption of analytes from a fiber were determined. The experiments were carried out at desorption time ranging from 1-10 min at desorption temperature 200 °C. The result showed that most of the target analytes were desorbed during a 4 min period and 6 min period for PDMS/DVB fiber and for PEG fiber, respectively. After that the peak showed a long tailing effect indicating that the extraction efficiently decreased, as shown in Figure 3.

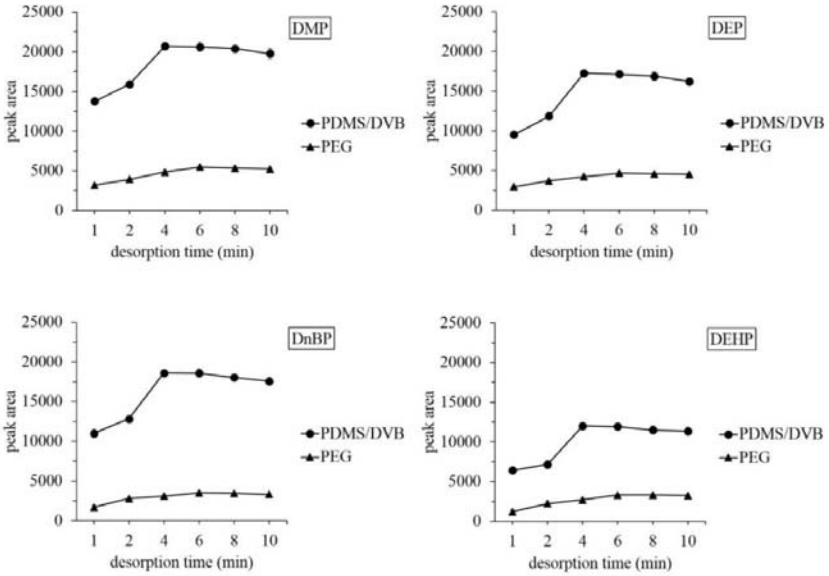

Figure 3. Effect of desorption time on HS-SPME of spiked 200 ng mL^{-1} of four phthalate esters in vegetable oil sample with extraction temperature of 80 °C, extraction time of 30 min and desorption temperature of 200 °C.

According to the results, PDMS/DVB fiber showed better response than PEG fiber. The partially cross-linked phase with porous polymer PDMS/DVB was stable and suitable for extraction of low polar compounds. Therefore, PDMD/DVB fiber was selected for future study. The optimum conditions for oil matrix samples were extraction temperature at 80 °C, extraction time 30 min and desorption time 4 min at 200 °C.

The optimization of the HS-SPME method using PDMS/DVB fiber in oil matrix sample was applied to determination of phthalate esters in soft drink samples. In soft drink samples, the influence of ionic strength on the extraction efficiency of HS-SPME was investigated. Salt is often added to solution to decrease the solubility of the analytes. The addition of 0.1 g mL^{-1} of different salts (NaCl, Na_2SO_4, CH_3COONa, $NaNO_3$ and $(NH_4)_2SO_4$) were evaluated. The results were compared with and without salt addition. NaCl was showed the highest extraction efficiency, as shown in Figure 4A. Moreover, the concentration of NaCl from 0-0.35 g mL^{-1} was investigated. The salting-out effect decreased the solubility of the analytes in water and increased the concentration of analytes in the extraction phase. However, the extraction efficiency of analytes increased with increasing NaCl concentration, except DEHP and DnBP which decreased with NaCl concentration more than 0.5 g mL^{-1} and 0.1 g mL^{-1}, respectively. With a large amount of NaCl concentration, the extraction efficiency of DEHP and DnBP was lower than without salt addition. Therefore, 0.2 g mL^{-1} NaCl was selected. The results are shown in Figure 4B. The optimum conditions for water matrix were extraction temperature at 80 °C, extraction time 30 min, desorption time 4 min at 200 °C and NaCl 0.2 g mL^{-1} addition.

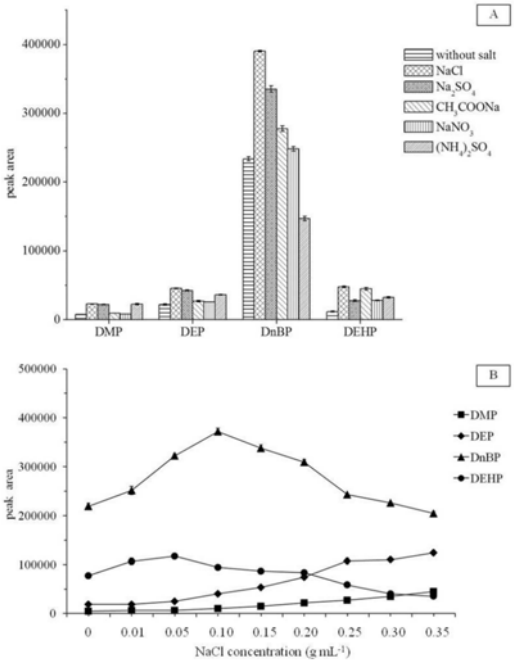

Figure 4. Effect of salt addition on HS-SPME in water matrix spiked sample using PDMS/DVB fiber with extraction temperature of 80 °C, extraction time of 30 min and desorption time 4 min at 200 °C. (A) effect of salt type, (B) effect of NaCl concentration.

3.1 Method Validation and Application to Real Samples

The HS-SPME method using PDMS/DVB fiber for analysis of four phthalate esters in oil matrix sample was validated. The performance data of the proposed method are summarized in Table 1. Linearity was observed with the correlation coefficient in the ranges of 0.9989-0.9998. The limit of detection (LOD) was measured by identifying a concentration giving a signal-to-noise ratio (S/N) of 3 while ranging from 2.6-3.3 ng mL^{-1}. The limit of quantification (LOQ) (S/N = 10) was found in the range of 8.6-11.1 ng mL^{-1}. The repeatability and reproducibility were studied by replicate injections of a standard mixture of 2 μg mL^{-1} five times (n = 5) over three days (n = 3×5). The results were obtained in relative standard deviation (RSD) in the range of 2.3-4.9% and 2.8-4.0%, respectively. The enrichment factor (EF) was used to evaluate the extraction efficiency, defined as the concentration ratio of the analytes with preconcentration by the HS-SPME method and without HS-SPME method. The result showed a range of 11-17.

Table 1. Validation data of the HS-SPME method using PDMS/DVB fiber for analysis of four phthalate esters in vegetable oil and soft drink samples.

	Linear equation	Linear range (ng mL^{-1})	R^2	EF	LOD (ng nL^{-1})	LOQ (ng mL^{-1})	% RSD	
							Interday (n = 5)	Intraday (n = 3 × 5)
DMP	y = 11042.0x+794.6	8.6-3000	0.9998	14	2.6	8.6	2.3	4.0
	(y = 30275x+7841.9)	(4.9-3000)	(0.9989)	(37)	(1.5)	(4.9)	(3.9)	(4.9)
DEP	y = 9839.1x+329.7	9.6-3000	0.9989	17	2.9	9.6	2.9	3.6
	(y = 96573x+24789)	(1.6-3000)	(0.9987)	(167)	(0.5)	(1.6)	(2.7)	(2.4)
DnBP	y = 10218.0x+617.6	9.3-3000	0.9996	14	2.8	9.3	2.6	3.0
	(y = 653646x+41548)	(0.2-3000)	(0.9992)	(848)	(0.1)	(0.2)	(4.7)	(4.0)
DEHP	y = 8513.6x+229.4	11.1-3000	0.9997	11	3.3	11.1	4.9	2.8
	(y = 188865x+4314.3)	(0.8-3000)	(0.9991)	(241)	(0.2)	(0.8)	(1.6)	(2.0)

( ) The values in parentheses represent the results obtained using HS-SPME method for soft drink samples.

Recovery testing was carried out by spiking 100 and 500 ng mL^{-1} of all standard solution in vegetable oil samples and 100 ng mL^{-1} of all standard solution in soft drink samples. The recoveries were found in the range of 84.5-102.1 % with RSD less than 10 %, indicating the feasibility of the HS-SPME method for the determination phthalate esters in vegetable oil and soft drink samples. DMP, DEP, DnBP and DEHP were found in vegetable oil samples in the range of 6.4-25.1, 9.7-43.1, 13.3-29.8 and 81.1-97.3 ng mL^{-1}, respectively. For soft drink samples, DMP was not found in all samples, DEP, DnBP and DEHP were found in the range of 12.2-29.0, 4.7-38.4 and 4.0-20.1 ng mL^{-1}, respectively, as shown in Table 2.

Table 2. Recovery of the four phthalate ester in vegetable oil and soft drink samples.

Sample (package)	Standard Add (ng mL^{-1})	DMP		DEP		DnBP		DEHP	
		Total found (ng mL^{-1})	Recovery ±RSD% (n=5)	Total found (ng mL^{-1})	Recovery ±RSD% (n=5)	Total found (ng mL^{-1})	Recovery ±RSD% (n=5)	Total found (ng mL^{-1})	Recovery ±RSD% (n=5)
palm oil	0	25.1	-	43.1	-	13.3	-	93.6	-
	100	122.9	97.8±6.7	143.9	100.8±8.5	109.0	95.7±5.0	190.6	97.0±9.5
	500	495.2	94.0±3.6	492.5	89.9±3.6	491.8	95.7±6.4	587.4	98.8±5.5
soybean	0	nd	-	9.7	-	29.8	-	92.6	-
oil	100	95.4	95.4±5.8	102.9	93.2±9.5	127.6	97.8±7.6	189.5	96.9±5.0
	500	441.0	88.2±5.3	462.2	90.5±2.4	491.7	92.4±6.2	603.1	102.1±9.3
sunflower	0	nd	-	nd	-	nd	-	97.3	-
oil	100	100.1	100.1±9.9	94.5	94.5±7.2	87.6	87.6±8.6	195.0	97.7±9.6
	500	452.9	90.6±5.3	472.2	94.4±5.1	483.2	96.6±6.0	572.6	95.1±7.2
Rice bran	0	6.4	-	nd	-	nd	-	81.1	-
oil	100	94.9	88.5±9.3	96.8	96.8±3.8	90.6	90.6±5.9	177.1	96.0±10.0
	500	485.7	95.9±4.4	460.0	92.0±8.3	481.3	96.3±5.5	585.9	101.0±4.6
soft drink	0	nd	-	29.0	-	38.4	-	20.1	-
	100	98.4.0	98.4±5.0	124.9	95.9±8.9	137.5	99.1±6.6	116.7	96.6±6.3
soft drink 2	0	nd	-	12.2	-	17.7	-	13.2	-
	100	96.	96.2±7.8	107.3	95.1±5.8	114.4	96.7±8.2	110.5	97.4±3.5
soft drink 3	0	nd	-	nd	-	4.7	-	4.0	-
	100	94.8	94.8±7.4	84.5	84.5±5.9	99.5	94.8±8.9	97.0	93.0±5.9

nd=not detected, less than LOD

3.2 Confirmation the Results with Gas Chromatography- mass Spectrometry

The mass spectra of the compounds content in oil sample was compared with the standard phthalate esters by gas chromatograph-mass spectrometer. The results showed good agreement with the standard mass spectra, as shown in Figure 5.

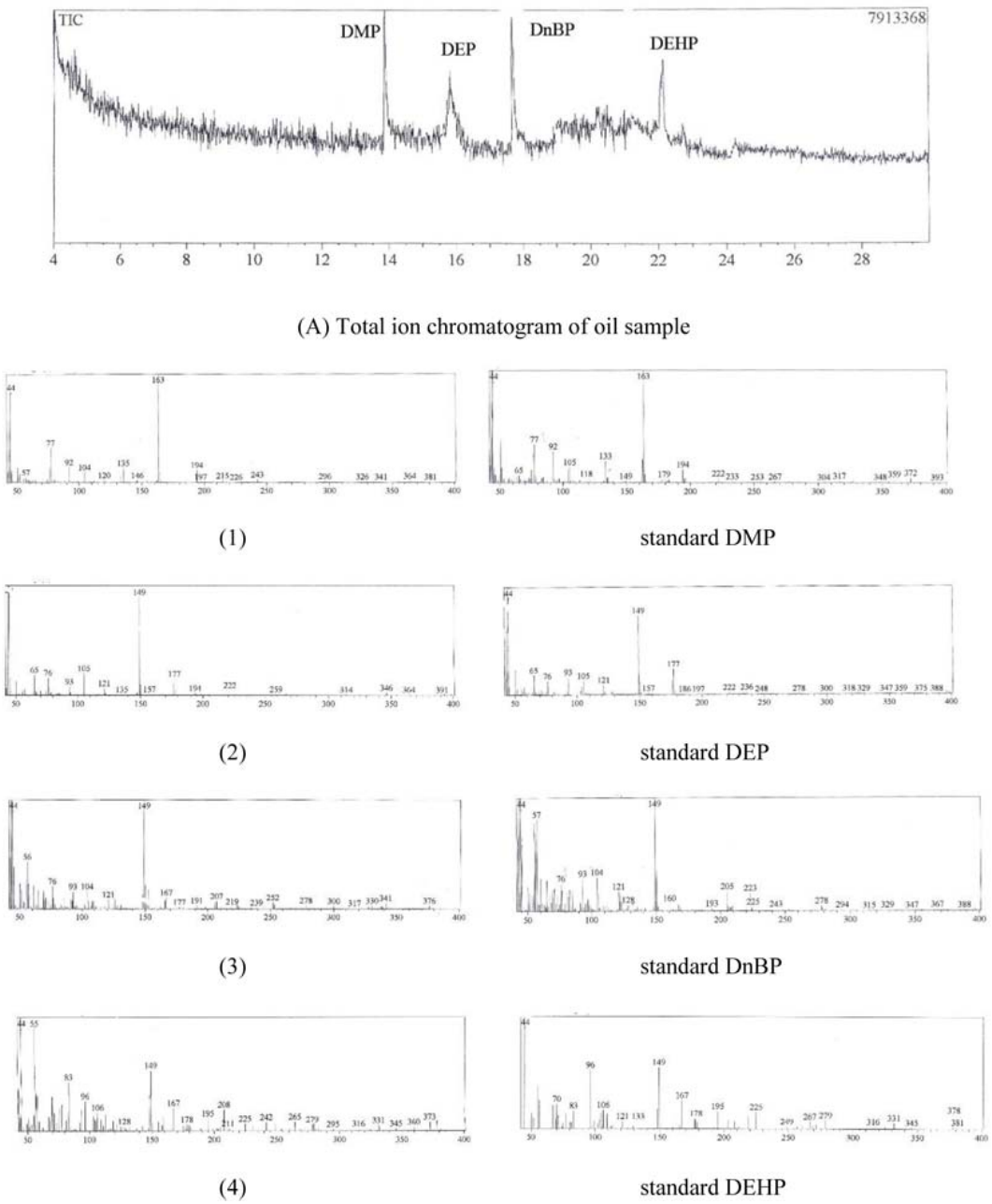

Figure 5. Total ion chromatograman of phthalate esters in sample (A) and mass spectra of compound 1,2,3,4, correspond to standard DMP, DEP, DnBP and DEHP, respectively.

4. CONCLUSIONS

The HS-SPME method using PDMS/DVB fiber were developed and validated for analysis of DMP, DEP, DnBP and DEHP in vegetable oil and soft drink samples. The method exhibits good sensitivity and selectivity to four phthalate esters to be determined at ng mL^{-1} levels in all samples. The contents of four phthalate esters in vegetable oil and soft drink samples were found in the safe levels. The method avoids the use of solvents in the extraction steps

and minimizing sample manipulation and contamination. Therefore, this method is an environmentally friendly sample pretreatment technique to extract a very wide range of analytes.

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