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Single-drop microextraction for extraction of some phenolic contents leached from bottle water samples

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Abstract

A single-drop microextraction (SDME) has been developed for the GC-MS determination of phenolic contents in water samples. Factors affecting extraction efficiency were studied including the organic solvent, ionic strength, drop volume, pH of the solution, stirring rate, temperature and time of extraction. Under the selected conditions, i.e., the organic solvent, ionic strength, drop volume, pH of the solution, stirring rate, extraction temperature and extraction time were hexyl acetate, 1% of NaCl, 2.5 µL of drop volume, pH 2, 200 rpm of stirring rate, 30°C of extraction temperature and 10 min of extraction time, respectively. After extraction step, the acceptor drop was withdrawn and directly injected into a GC-MS instrument for analysis. This method is a promising alternative for the sensitive determination of phenolic contents. In addition, the SDME also demonstrated that the method is simple, fast and efficiently for determining of phenolic contents from water.

Introduction

Endocrine disrupting chemicals (EDCs) such as phenolic contents are important pollutants in environment because of their wide use in many industrial processes, such as the manufacture of plastics, dyes, drugs, detergents and pesticides (Saraji *et al*., 2005). The alkylphenols (APs) including, 4-tert-octylphenol (4-t-OP), 4-otylphenol (4-OP) and 4-nonylphenol (4-NP) and chlorophenols (CPs) including 2,4-dichlorophenol (2,4 DCP), 2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP) and bisphenol A (BPA) have been shown to exhibit endocrine disrupting properties in wildlife and laboratory animals (Basheer *et al*., 2004). The APs mainly enter municipal wastewater treatment plants via industrial effluents. Due to their polarity, persistence and water solubility APs can pass through wastewater treatment plant (Braun *et al*., 2003). For example, 4-NP has an estrogenic effect in nature, which can cause the feminization of male fish, resulting in a lock of reproductive success (Helaleh *et al*., 2001). CPs are widely used as wood preservative agents, herbicide, pesticide and disinfectants and used as intermediates in many industrial processes (Sarrion *et al*., 2002). The toxicity of CPs depends on the pH and the total number of chlorine atom in the molecule, PCP being the most toxic of the 19 members of this family (Campillo *et al*., 2006). BPA is one of the most commonly produced industrial chemicals in the world and is a component of polycarbonate plastics and epoxy resins, the use of which include lining food cans, nail polish and food packaging materials (Helaleh *et al*., 2001; Braun *et al*., 2003). They have been discharged directly or indirectly to the environment and contaminated the atmosphere, water and soil. BPA is widely used in tableware as a material in polycarbonate resin and is possibly ingested by humans through food and water. This compound can be leached from the can coating, drinking water bottle and baby milk bottle when heated at a high-temperature. There have been several scientific studies investigating the release of Bisphenol A (Wu *et al*., 2010; Alin, 2012; Li *et al*., 2013; Fan *et al*., 2014; Lane *et al*., 2015) from polycarbonate, because in the past few years it was shown that this compound could have endocrine disrupting or estrogenic properties.

Conventional extraction methods, such as liquid-liquid extraction (LLE), solid-phase extraction (SPE), solid-phase microextraction (SPME) and single-drop microextraction (SDME) are widely used as the extracted of some phenolic contents. Recently, the attractive technique of SDME has been developed as an alternative of SPME. SDME is a fairly new method of sample preparation, initially proposed and developed by Jeannot and Cantwell (Jeannot *et al*., 1996), provides an alternative technique, which integrates sampling, extracting, concentrating and

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sample introduction into a single step (Zhang *et al*., 2008). In practice, two main approaches can be used to perform SDMEs. They are headspace and direct SDME techniques. In direct SDME, a drop of a water-immiscible solvent is suspended directly from the tip of a microsyringe needle immersed in the aqueous sample (Jermak *et al*., 2006).The technique is very inexpensive, and the drop is completely renewable at negligible cost and provides analyte extraction in a few microliters of organic solvents (Saraji *et al*., 2005). This technique was based on the principle of distribution of analytes between a microdrop of extraction solvent at the tip of a microsyringe needle and the aqueous phase. The microdrop is exposed to an aqueous sample where analytes is extracted into the drop. After extraction, the microdrop is retracted back into the microsyringe and injected into the instruments such as GC and HPLC for further analysis (Rahul *et al*., 2006). Therefore, the aim of this research was to separate some phenolic contents leached from bottle water samples by using to the direct-SDME.

Materials and Methods

Materials

All standard solutions studied i.e. 2,4,6-TCP (98%), 4-t-OP (97%), 4-OP (99%) and BPA (99%) from Sigma-Aldrich (Milwaukee, WI, USA); 4-NP (98.5%) from Supelco (Bellefonte, PA, USA) were used and prepared as stock standard solutions (1000 µg/mL). Then, working standard solutions of mixture containing each compound at 1 µg/mL were obtained by diluting the stock solution with methanol. Internal standard solution (10 µg/mL) of phenanthrene-d10 was prepared in methanol. All standard solutions were stored in a refrigerator prior to use.

Preparation of synthetic sample solutions

All synthetic samples were prepared as follows: Five bottles samples were purchased from a local supermarket in Chiang Mai province, Thailand. Then, they were added 300 mL of distilled water. All samples were heated at 45ºC in the oven for 72 and 144 hours, respectively. Finally, the synthetic water samples were extracted for the determination of phenolic content by using direct-SDME procedure.

SDME procedure

A 10 µL GC microsyringe (Agilent, USA) was used to perform SDME experiments. A 20 mL of distilled water was spiked with an appropriate amount of phenolic standard solution and adjust pH to 2 with dilute HCl solution. In addition, the increasing ionic strength with 1% NaCl was added. Then the sample was introduced in a 20 mL glass vial equipped with a screw-cap and a silicone septum. After uptake of 2.5 µL of acceptor solution (hexyl acetate) the needle of the syringe was then inserted into the direct of the sample solution. The syringe plunger was depressed and a microdrop of acceptor phase was suspended from the needle tip. After an optimized period of time, the plunger was withdrawn and the microdrop was retracted back into the syringe. The needle was removed from the direct and its content was introduced to a GC-MS for subsequent analysis.

Based upon the SDME procedure, we followed the process of Zhang *et al.* (Zhang *et al*., 2008). Afterwards, 20 mL of donor water sample, 2.5 µL of hexyl acetate, 1% of NaCl, pH 2, 200 rpm of stirring rate, 30ºC of extraction temperature with 10 min of the extraction time were chosen for the analytical application of all target contents in the standard and sample solutions.

GC-MS analysis

All analysis in this study was carried on an Agilent 6890/5973 GC-MS system (Agilent, USA). The GC was fitted with a HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness, Agilent, USA). The carrier gas was high-purity helium (99.999%) with a constant flow of 1.0 mL/min. The injector temperature was kept at 280ºC and all injections were made in the splitless mode. The temperature program applied was as follows: the initial oven temperature was set at 140ºC, held for 1 min, then the temperature was increased to 160ºC, held for 1 min at 20ºC/min and from 160ºC up to 230ºC, held for 0.5 min at 50ºC/min and finally from 230ºC up to 240ºC, held for 3 min at 10ºC/min. Finally, the MS was operated in an electron impact (EI) mode with an ion source temperature of 230ºC and the transfer line was held at 280ºC.

Results and Discussion

Choice of extraction

As immersed SDME was concerned, the extractant has to meet several requirements: to have low solubility in water, to extraction analytes well, to be separated from the chromatographic peaks of the analytes (Ye *et al*., 2007) and to be less toxic. The final choice of solvent should be based on comparison of selectivity, extraction efficiency, incidence of drop loss, rate of drop dissolution (especially for faster stirring rates and extended extraction times). The basis of these considerations, six solvents such as 1-hexanol, 1-heptanol, 1-octanol, butyl acetate,

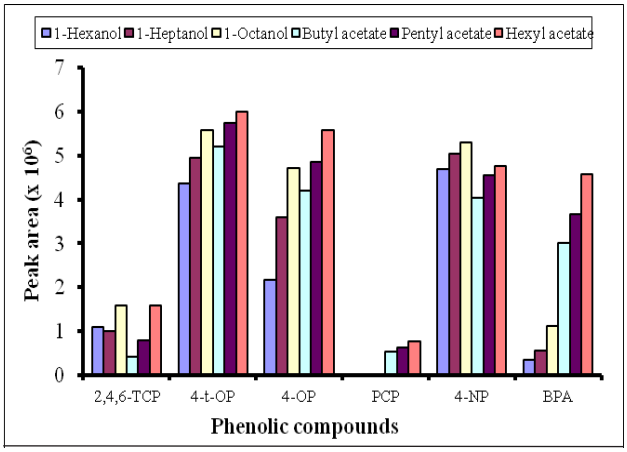

Figure 1. Comparison of the response of SDME analysis using different organic solvents

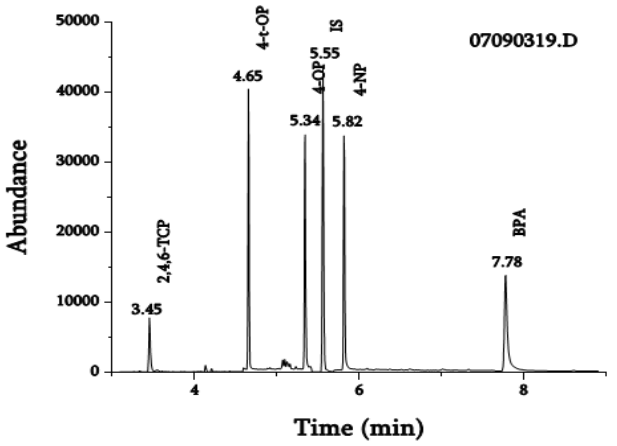

Figure 2. Total ion chromatogram of mixed phenolic contents standard at 4.0 µg/L and IS 5.0 µg/L under the optimum conditions of SDME

pentyl acetate and hexyl acetate were tested to obtain the best for the extraction of some phenolic contents. For the group of alcohol peaks, it partly masked the analyte peaks. While the extraction in the part of acetate group had efficiency of hexyl acetate better than butyl acetate and pentyl acetate are shown in Figure 1. Therefore, it was suggested that hexyl acetate was employed for further experiments.

Salting-out effect

The effect of sodium chloride concentration (ranging from 0 to 4%) was investigated. The results showed that an initial increase in the extraction efficiency with an increase in salt concentration, with a maximum being reached at 1%, followed by a decrease in extraction efficiency with further increase in salt concentration. Extraction is usually enhanced with increasing salt concentration and increased polarity of the compound (salting-out effect) (Wardencki *et al*., 2007). In SDME, generally, there is an unexpected decrease in extraction efficiency with increased ionic strength (1% NaCl, w/v) for the majority of analytes, which is more pronounced for the less polar analytes. When salt was added to the solution, water molecules could form hydration spheres around the ionic salt molecules. These hydration spheres reduce the amount of water available to dissolve analyte molecules. It is plausible that the salts drove additional analytes into the organic extractant (Ye *et al*., 2007).

Drop volume

In this experiment, the results showed the influence of the drop volume on the extraction efficiency of some phenolic contents ranging from 1.0 to 3.0 µL. As it was expected, an increase in the drop volume (up to 2.5 µL) resulted in a sharp enhancement in the extraction efficiency of the system. However, at larger volume (i.e. >3 µL), the drop revealed a great tendency to fall down from the tip of the microsyringe. On the other hand, the larger volumes, after insertion into the chromatographic column, might cause peak tailing. Thus, a 2.5 µL drop volume was chosen for all subsequent extractions.

pH of the sample

In this work, a wide range of sample pH from 2 to 8 was evaluated. Based on results evident that the extraction efficiency at pH > 2 were lower than for all analytes. This may have resulted from the hydrolysis of some phenolic contents under strongly acidic or basic aqueous environments (Ye *et al*., 2007). Thus, sample solution was adjusted to pH value of 2 in the following experiments.

Stirring rate

To evaluate the effect of sample stirring, aqueous sample (spiked at 0.1 mg/L with all analytes and internal standard solution) were extracted in extraction solvent for 10 min at different stirring rate (0-400 rpm). As expected, the results revealed that stirring dramatically enhanced extraction efficiency. According to the amount of extracted analytes reached a maximum at 200 rpm. However, if higher stirring rate were not used because of spattering, which damaged the drop (disruption and dislodgement). Based on these observations stirring of the sample at 200 rpm was found to be optimum, yielding thus acceptable results for all target analytes.

Extraction temperature

The effect of temperature can be studied by exposing solvent drops for a specified time in sample at different temperatures (25-40ºC). Generally, an increase the temperature results in an enhancement of extraction efficiency. Nevertheless, a high temperature can cause solvent drop damage and

Table 1. The synthetic water leached from bottle samples were heated at 45ºC for 72 h.

Analyte	Correlation coefficient (r^2)	Concentration (µg/L)				
		Sample C1	Sample C2	Sample C3	Sample C4	Sample C5
2,4,6-TCP	1	ND	ND	ND	ND	ND
4-t-OP	1	ND	ND	ND	ND	ND
4-OP	1	ND	ND	ND	ND	ND
4-NP	0.9999	ND	ND	ND	ND	ND
BPA	0.9999	1.24	0.72	1.07	ND	ND

C1-3 = baby milk bottle and C4-5 = drink water bottle

Table 2. The synthetic water leached from bottle samples were heated at 45ºC for 144 h.

Analyte	Correlation coefficient (r^2)	Concentration (µg/L)				
		Sample C1	Sample C2	Sample C3	Sample C4	Sample C5
2,4,6-TCP	0.9996	ND	ND	ND	ND	ND
4-t-OP	0.9981	ND	ND	ND	ND	ND
4-OP	0.9979	ND	ND	ND	ND	ND
4-NP	0.9986	ND	ND	ND	ND	ND
BPA	0.9989	3.50	1.96	2.11	ND	0.30

ND = not detected

drop loss. To simplify the method, most chemists in practice perform extraction experiments at room temperature (30ºC).

Extraction time

A sufficient extraction time is necessary to attain equilibrium of analytes between the aqueous and organic drop, but a longer extraction time of microextraction to reach complete equilibrium may result in drop dissolution and have a high incidence of drop loss (Zhang *et al.*, 2008). To extract the maximum amount of analytes the effect of sampling time in the yield of the microextraction was optimized. Based on this fact, extraction time was optimized in the range 5-40 min. The extraction time profiles show that the equilibrium curve were attained in 20 min for all phenolic contents. Although an extraction time of 20 min provided higher sensitivity, a 10 min extraction time was chosen for subsequent experiments as a compromise between extraction efficiency and analysis time (Saraji *et al.*, 2005).

Application of the direct-SDME to water samples

Under optimum conditions as mentioned above, the Figure 2 showed the total ion chromatogram of five phenolic standards, which was well separated. The synthetic sample solutions were extracted direct-SDME and analyzed by GC-MS. From the results

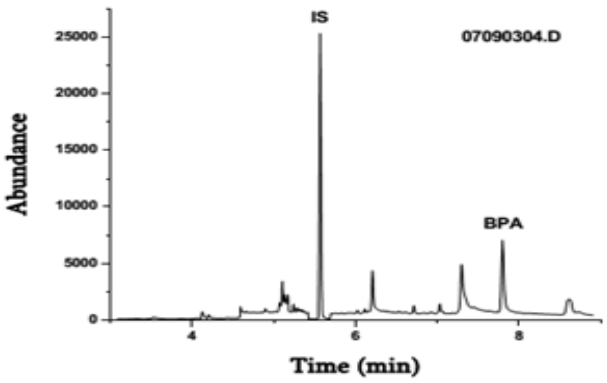

Figure 3 Total ion chromatogram of the sample under the optimum conditions of SDME

(Table 1-2), BPA was the highest contents (0.30-3.50 µg/L) in this sample whereas the 2,4,6-TCP, 4-t-OP, 4-OP and 4-NP were not observed (Figure 3).

Conclusion

In brief, some phenolic contents leached from bottle water samples were extracted by using the single-drop microextraction from this study. The result showed the organic solvent, ionic strength, drop volume, pH of the solution, stirring rate, extraction temperature and extraction time were hexyl acetate, 1% of NaCl, 2.5 µL of drop volume, pH 2, 200 rpm of stirring rate, 30ºC of extraction temperature and 10 min of extraction time, respectively. After extraction, all samples were analysis by GC-MS. It was found that the samples contained only BPA between 0.30-3.50 µg/L. Herein, the SDME is considered as being simple, rapid as well as efficient to use in this study.

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