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Simple Extraction of Bisphenol A in Beverages and Water by Membrane-Protected Liquid Phase Microextraction

(Pengekstrakan Mudah Bisfenol A dalam Minuman dan Air menggunakan Pengekstrakan Mikro Fasa Cecair Dilindungi Membran)

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ABSTRACT

Bisphenol A (BPA) is a xenoestrogen that widely used in manufacture of plastics. Concern is mounting up when the application of BPA is widespread. A simple sample pre-treatment technique termed membrane-protected liquid phase microextraction (MP-LPME) combined with high performance liquid chromatography (HPLC)-fluorescence detection (FD) was developed for the determination of BPA in beverages and environmental water samples. The MP-LPME technique utilized a nylon membrane to hold the extractant (1-octanol) and prevent the extractant from dissolution into sample solution during agitation. Under optimal extraction conditions, the MP-LPME-HPLC-FD demonstrated ultra-trace detection of BPA and insignificant matrix effect extraction with good relative recoveries in the range of 87.1-99.7% from spiked beverage and environmental water samples. The membrane can be re-applied in the MP-LPME after the first usage and offered relative recovery of > 94%. The MP-LPME technique is eco-friendly as it consumes only minute amounts of organic solvent which is supportive to green analysis.

Keywords: Bisphenol A; HPLC; liquid phase microextraction; nylon membrane

ABSTRAK

Bisfenol A (BPA) ialah xenoestrogen yang digunakan secara meluas dalam pembuatan plastik. Kebimbangan semakin meningkat apabila aplikasi BPA tersebar luas. Satu teknik pra-rawatan sampel yang mudah dikenali sebagai pengekstrakan mikro fasa cecair yang dilindungi membran (MP-LPME) berpasangan kromatografi cecair prestasi tinggi-pengesanan pendarfluor (HPLC-FD) telah dibangunkan untuk penentuan BPA dalam sampel minuman and air alam sekitar. Teknik MP-LPME menggunakan membran nilon untuk memegang ekstrak (1-oktanol) dan mencegah ekstrak daripada pemelarutan ke dalam larutan sampel semasa pengacauan. Di bawah keadaan pengekstrakan optimum, MP-LPME-HPLC-FD menunjukkan pengesanan ultra surih dan pengekstrakan kesan matriks yang tidak signifikan dengan purata pemulihan semula relatif yang baik dalam linkungan 87.1-99.7% daripada sampel minuman dan air alam sekitar. Membran ini boleh digunakan semula dalam MP-LPME selepas penggunaan pertama dan menawarkan pemulihan semula relatif > 94%. Teknik MP-LPME adalah mesra alam kerana ia hanya menggunakan sedikit pelarut organik dan menyokong analisis hijau.

Kata kunci: Bisfenol A; HPLC; membran nilon; pengekstrakan mikro fasa cecair

INTRODUCTION

Bisphenol A (BPA) is an industrial chemical produced for the manufacture of specific plastics and resins in larger quantities (Ullah et al. 2018). High volumes of BPA are produced annually to fulfil its wide application in making polycarbonate plastics and epoxy resins. Polycarbonate plastic is a clear, highly effective thermoplastic. Polycarbonate plastic is commonly used in consumer items such as water bottles, hard plastics baby bottles and drinking containers due to these properties. Because of its chemical resistance and dimensional stability, epoxy resins are adaptable resins. Hence, it is often used to coat inner lining of metal cans (Jin et al. 2015) or in making thermal paper.

The main route exposes to BPA in human is through a daily diet. Concern of BPA exposure has recently mounted as the majority of adolescents have little BPA measures in their bodies (Cox 2018). The BPA residues in urine of workers who have direct contact with this chemical are 70 times higher than other adults in United States (Oksman 2017). BPA exposure is linked with health issues such as obesity, early puberty, hormone disruption and kidney failure. In this modern era, peoples are changing their consumer habits due to their work demands. Globalization has stimulated a wide variety of food, especially canned food. A survey showed that 98% of Americans has stocked up their kitchen with average of 24 cans (Mohan 2013). Since BPA is a monomer mainly used to manufacture consumer products, therefore contamination is believed to occur in our food, beverage and as well as environmental water. Perhaps most importantly, human exposure to BPA can bring significant adverse health effects such as reproductive disorders, fatal brain development and breast and prostate cancer.

The classic techniques such as liquid-liquid extraction (LLE) (Elobeid et al. 2012; Kuroda et al. 2003) and solid phase extraction (SPE) (Aurand 2015; He et al. 2007; Lin et al. 2012; Rodriguez-Mozaz et al. 2004) are regularly used to extract BPA. Past research showed that LLE and SPE provide sufficient sensitivity as well as easy to operate. However, both methods involve a tedious and timely procedure. The LLE technique has some drawbacks such as the formation of emulsions that are difficult to break, whereas the SPE technique results in low recoveries of analyte due to loss of targeted analyte when loading into sorbent. Therefore, alternative such as liquid phase microextraction (LPME) has been introduced to compensate the less-ideal-behavior of these classic techniques, as well as to meet the green analysis requirements.

LPME is a solvent-minimized sample pre-treatment compared to LLE. LPME can be categorized into three principal classes, single-drop microextraction (SDME), hollow-fibre protected liquid phase microextration (HF-LPME) (Kawaguchi et al. 2008) and dispersive liquid-liquid microextraction (DLLME) (Cunha & Fernandes 2010; Liu et al. 2013; Loh et al. 2017; Rocha et al. 2016). All these LPME techniques have been demonstrated to offer sensitive and selective detection of BPA in various sample matrices. To-date, the LPME is still of considerable interest mainly because of its high analyte enrichment and simple operation.

This project provided an alternative method for the extraction of BPA in beverages and water samples using membrane-protected LPME coupled with high performance liquid chromatography and fluorescence detection. In comparison with SPE and LLE, the proposed technique offered inexpensive and simple extraction which may simplify the routine analysis.

MATERIALS AND METHODS

REAGENTS AND CHEMICALS

HPLC grade reagents like acetonitrile (ACN), methanol (MeOH) and acetone were purchased from Merck (Darmstadt, Germany). 1-octanol (analytical grade), sodium hydroxide (NaOH) and hydrochloric acid (HCl) were acquired from Sigma-Aldrich (St. Louis, Missouri, United States). Bisphenol A (BPA) reference standard was purchased from Dr. Ehrenstorfer GmbH (Augsburg, German).

PREPARATION OF STANDARD SOLUTION

Standard stock solution of BPA (500 mgL^{-1}) was prepared by dissolving 0.005 g of BPA reference standard in a 10 mL volumetric flask and diluting to volume with methanol. A series of working standard solutions were prepared by further diluting the standard stock solution with methanol. All the solutions were stored in the fridge (4°C) when not in use.

PREPARATION OF SAMPLE

The water (lake water, tap water, river water and sea water) obtained from Kuala Terengganu and beverage samples (isotonic drink and lemon tea) packed in epoxy and purchased from local enterprise shops were applied in relative recovery study. The water and beverage samples were kept in the freezer and fridge, respectively, until analysis. The samples were filtered through Whatman filter paper No. 1 to exclude larger particles prior to extraction. The soft drink samples were subjected to ultrasonic bath for 20 min to degas prior to extraction.

PREPARATION OF NYLON MEMBRANE

Round-shaped nylon membrane filters (I.D. 47 mm, pore 0.2 µm) were purchased from Sartorius Stedim Biotech GmbH (Goettingen, Germany). The membrane filters were cut into half with equal surface area. Each half of the filter paper was overlaid and heat sealed into a cone-shaped membrane with an open distance of 13 mm and height of 20 mm.

MEMBRANE-PROTECTED LIQUID PHASE MICROEXTRACTION

The membrane-protected liquid phase microextraction (MP-LPME) technique was modified from Sanagi et al. (2007). The outer layer of the pre-sealed membrane was immersed into 1-octanol to impregnate the pores of the membrane wall. Then, the membrane was placed in the sample vial containing 40 mL of sample solution with pH pre-modified to pH6. The 1-octanol (50 µL) was added into the membrane and the sample solution was agitated at 700 rpm for 30 min. After 30 min, 50 µL of the extract was withdrawn and transferred into a 1.5 mL sample vial prior to quantification using high performance liquid chromatography coupled with fluorescence detection (HPLC-FD). Figure 1 shows the schematic of MP-LPME.

CHROMATOGRAPHIC CONDITIONS

Analyte measurements were performed using HPLC coupled with FD (Shimadzu, Kyoto, Japan). The HPLC conditions were adopted from Loh et al. (2017). The chromatographic separation of BPA was carried out on a reversed phase C18 column (4.6 mm × 150 mm, 5 µm) (Supelco, Harrison Road, Bellefonte, USA). The partition was performed with isocratic elution utilizing acetonitrile-water (50:50) (v/v) at column temperature of 30°C. The flowrate, injection volume and detection wavelength were fixed at 1.0 mL/min, 10 µL and 275/305 nm of excitation/emission wavelengths, respectively.

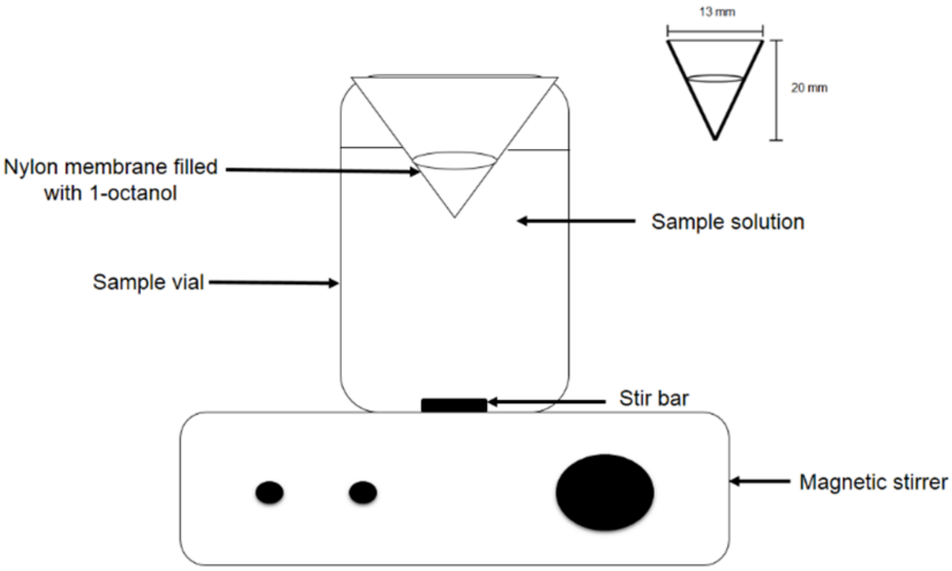

FIGURE 1. Schematic of membrane-protected liquid phase microextraction

Optimization of MP-LPME

In this study, several parameters were thoroughly examined and optimized to enhance the extraction efficiency of the MP-LPME coupled with HPLC-FD for the determination of BPA from environmental water and beverage samples. The parameters included volume of 1-octanol, agitation speed, extraction time and sample pH. The method was then evaluated for linearity, relative recovery, limit of detection (LOD) and limit of quantification (LOQ) before sample analysis.

Results and Discussion

Optimization of Membrane-Protected Liquid Phase Microextraction

Several parameters that influenced the extraction efficiency of BPA in environmental water and beverage samples using membrane-protected liquid phase microextraction (MP-LPME) were thoroughly investigated. These included volumes of 1-octanol, agitation speed, extraction time and sample pH. The optimization was performed using deionized water samples spiked with 50 μgL^{-1} of BPA. Triplicate extractions were carried out for each variable to ensure the consistency of results.

Volume of 1-Octanol

1-octanol was chosen as the acceptor phase because it has low volatility (Lauritsen 1990) which minimized the uncertainty due to solvent lost by evaporation. Volumes of 1-octanol ranged 50 to 150 μL were investigated for the extraction of BPA using MP-LPME. Generally, smaller volume of extraction solvent enhances analyte enrichment (Sanagi et al. 2012). However, the quantity of extraction solvent should be small enough yet feasible to extract the targeted analyte (Pawliszyn 2012). Figure 2(a) shows that the highest analyte enrichment was obtained with 50 μL of 1-octanol. The decrease in peak area was observed when 1-octanol exceeded 50 μL was applied. This was due to the larger volume of extraction solvent may dilute the BPA extracted.

Agitation Speed

Agitation speed is one of the important variable to study separation process because it facilitates analyte diffusion to acceptor phase (Yiantzi et al. 2010). In this study, agitation speed ranging from 125 to 900 rpm was optimized to obtain the highest extraction efficiency. Figure 2(b) indicates that BPA response shown by peak area gradually increased from 125 rpm and achieved optimum at 700 rpm. There was significant decrease in peak area after 700 rpm due to the occurrence of a strong vortex flow (Chong et al. 2018). Higher agitation speed generated vortex which reduced the contact between the analyte and the membrane and hence resulted in decreased peak area (Niu et al. 2012). Therefore, 700 rpm was applied in subsequent experiments.

Extraction Time

Mass transfer is time-dependence and the mass transfer rate slows down when it closes to the equilibrium (Psillakis & Kalogerakis 2003). MP-LPME is an equilibrium dependence extraction technique. In this study, extraction time in the range of 10 to 40 min was examined and other parameters were kept constant. Figure 3(a) shows that the peak area increased gradually from 10 to 30 min. This was because BPA required a longer time for diffusion from the sample solution to the 1-octanol (Saraji et al. 2011). The peak area plateaued out after 30 min because the system

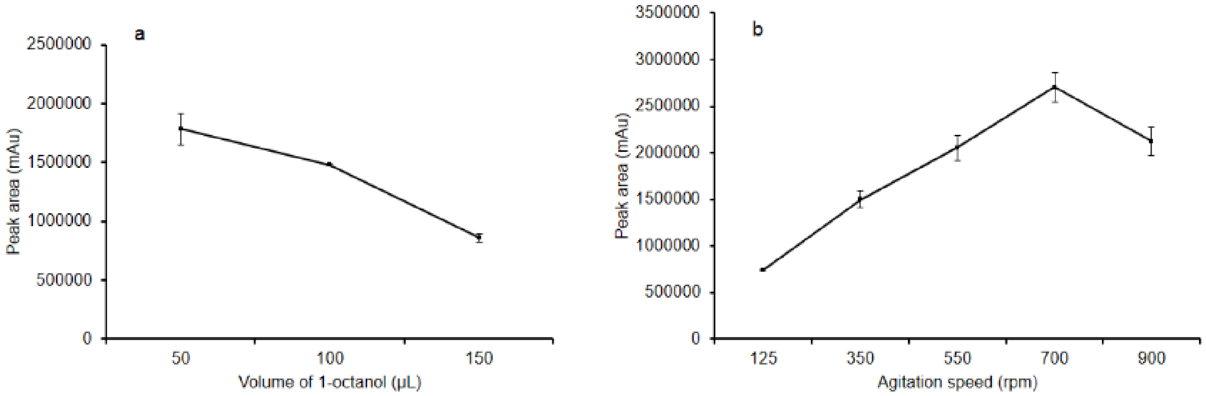

FIGURE 2. Effect of volume of 1-octanol (a) and agitation speed (b) for the extraction of BPA from spiked deionized water samples using MP-LPME. Each error bar indicates the standard deviation of triplicate extractions

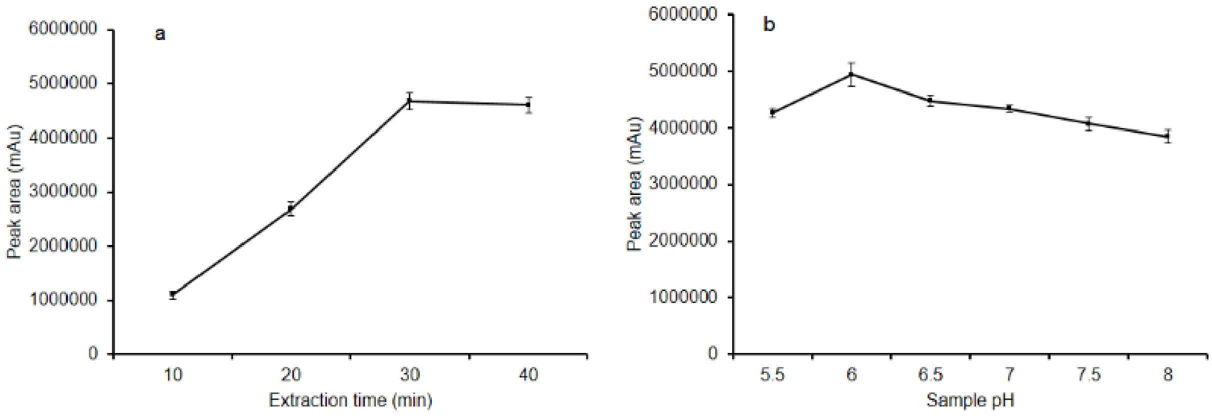

FIGURE 3. Effect of extraction time (a) and sample pH (b) for the extraction of BPA from spiked deionized water samples using MP-LPME. Each error bar indicates the standard deviation of triplicate extractions

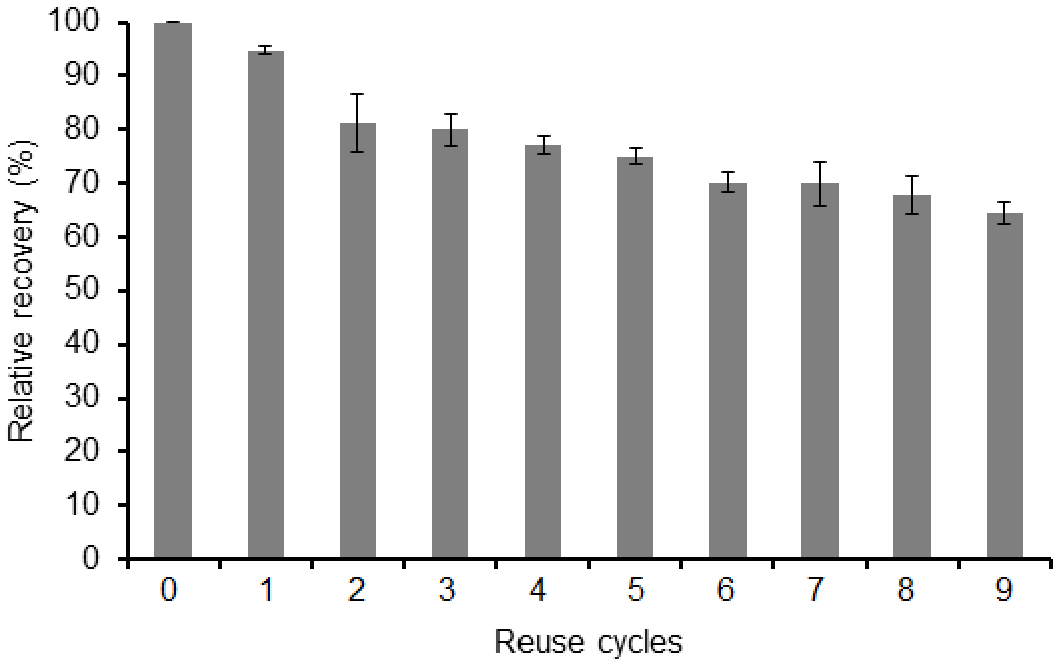

FIGURE 4. Recycle study of nylon membrane as an acceptor phase holder in MP-LPME. Each error bar indicates the standard deviation of triplicate extractions

has achieved equilibrium and no further increment in peak area was observed.

SAMPLE pH

Logarithmic acid dissociation constant (pK_a) of BPA is around 9.59 to 11.30 and it is a weak acidic compound (Rykowska & Wasiak 2006). Analyte in its molecular form can be easily extracted using organic solvent (Dulski 2010). The BPA exists in different forms when it is in different sample pH. In this experiment, pH of the sample solutions was modified in the range from pH5.5 to pH8 using 0.1 M HCl and 0.1 M NaOH. Figure 3(b) shows that the highest extraction efficiency was obtained when sample was modified to pH6. When sample pH was 2, values more acidic than the pKa values, BPA existed in molecular form. The decrease in peak area after pH6 was probably because BPA was in transition to ionic form (Li et al. 2005). Hence, the sample was modified to pH6 prior to extraction for the following experiments.

VALIDATION OF MP-LPME COUPLED HPLC-FD

The validation was performed to ensure the reliability, accuracy and preciseness of the MP-LPME technique coupled with HPLC-FD in the analysis of BPA in beverages and environmental water samples. Calibration was carried out using deionized water samples spiked with BPA in the concentration range of 0.1 to 50 μgL^{-1}. Good linearity was obtained in the designated range with coefficient of determination (R^2) of 0.9976.

LOD and LOQ were calculated to determine the limitations of the analytical method. LOD was calculated on the basis of three signal-to-noise ratios, whereas LOQ was calculated on the basis of ten signal-to-noise ratios (Vial & Jardy 1999). The LOD and LOQ were recorded as 0.01 and 0.1 μgL^{-1}, respectively. The results suggested that MP-LPME was capable to perform ultra-trace quantification and detection of BPA in beverages and environmental water samples.

Relative recovery was studied by spiking each of the lake water, tap water, river water, sea water, isotonic drink and lemon tea samples at 1 and 10 μgL^{-1} of BPA, respectively. Both blank and spiked samples were subjected to extraction using optimized MP-LPME. Table 1 shows that good relative recovery range from 82.2 to 99.7% with good repeatability presented using relative standard deviation (RSD) of <8% were obtained. This concluded that matrix effect was negligible when MP-LPME was applied in various sample matrices.

TABLE 1. Relative recovery study using MP-LPME for the extraction of BPA in beverages and environmental water samples

Sample	Average of relative recovery ± RSD, % (n=3)	
	Spiked at 1 μgL^{-1}	Spiked at 10 μgL^{-1}
Isotonic drink	87.4 ± 3.4	87.1 ± 4.7
Lemon tea	87.4 ± 5.9	97.4 ± 2.4
Lake water	90.6 ± 4.0	94.2 ± 1.6
Tap water	98.1 ± 5.3	82.2 ± 0.7
River water	97.3 ± 5.6	97.4 ± 1.0
Sea water	95.8 ± 7.9	99.7 ± 3.1

RECYCLED OF NYLON MEMBRANE

Nylon membrane used to hold the organic solvent was recycled or reused to study its feasibility to be re-applied in microextraction after its first usage. The relative recovery of BPA using recycled membrane was conducted using deionized water sample spiked with 50 μgL^{-1} of BPA. The used membrane was sonicated in acetone for 2 min in an ultrasonic bath before reapplied in MP-LPME. As shown in Figure 4, the relative recovery of BPA was decreasing with the recycle number of nylon membrane. However, the results also indicated the relative recovery of BPA achieved 94.7% at second use (written as first cycle in Figure 4) of nylon membrane with RSD 0.6%. The results is supportive to the maximum of 2 times use of nylon membrane in MP-LPME. The third use of nylon membrane caused a drastic drop in relative recovery of BPA which was 81.2%. On the economic front, the MP-LPME technique is cost-effective.

CONCLUSION

The optimized and validated MP-LPME coupled HPLC-FD was successfully performed to determine ultra-trace of BPA in beverages and environmental water samples. The method is sensitive, easy to operate and minimizes the consumption of organic solvent which meets the requirement of green analytical chemistry. The application of nylon membrane in microextraction has been an added value since the membrane is commonly applied to filter HPLC mobile phase only.

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