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EXTRACTION OF BISPHENOL A IN ENVIRONMENTAL WATER AND SOFT DRINK BY C_{18}-IMPREGNATED CELLULOSE TRIACETATE COMPOSITE FILM

(Pengekstrakan Bisfenol A dalam Air Alam Sekitar dan Minuman Ringan Menggunakan Filem Komposit Selulosa Triasetat Berimpregnasikan C_{18})

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Abstract

Bisphenol A (BPA) is an industrial chemical applied in making polycarbonate and epoxy resin that commonly used as food containers, beverage cans and baby bottles. BPA could be discharged into the environment from industrial waste and sewage treatment plant which then causing adverse effects to aquatic organism and consumers. Micro-solid phase extraction (µ-SPE) using C_{18}-impregnated cellulose triacetate (C_{18}-CTA) composite film as the adsorbent films coupled with high performance liquid chromatography–fluorescence detection (HPLC-FD) has been developed for the determination of BPA in environmental water and soft drink samples. Under the optimum extraction conditions, the method showed excellent linearity in the range of 0.1 to 500 ppb with correlation coefficient, r = 0.992. This method provided ultra-trace detection limit of 0.004 ppb, trace quantification limit of 0.01 ppb and acceptable relative recovery average in the range of 80.9 to 102.6% with relative standard deviation ≤ 9.7%. The proposed microextraction method is simple, rapid and eco-friendly as it consumes less amount of non-hazardous organic solvent.

Keywords: bisphenol A, C_{18}, cellulose triacetate, film, micro-solid phase extraction, high performance liquid chromatography

Abstrak

Bisfenol A (BPA) ialah suatu bahan kimia industri yang digunakan dalam pembuatan polikarbonat dan resin epoksi yang biasa digunakan sebagai bekas makanan, tin minuman dan botol bayi. BPA boleh dilepaskan ke dalam alam sekitar daripada sisa industri dan loji rawatan kumbahan yang kemudiannya menyebabkan kesan buruk kepada organisma akuatik dan pengguna. Pengekstrakan mikro fasa pepejal (µ-SPE) menggunakan filem komposit selulosa triasetat berimpregnasi C_{18} (C_{18}-CTA) sebagai filem penjerap berpasangan kromatografi cecair prestasi tinggi-pengesanan pendarfluor (HPLC-FD) telah dibangunkan untuk penentuan BPA dalam sampel air alam sekitar dan minuman ringan. Di bawah keadaan pengekstrakan optimum, kaedah ini menunjukkan kelinearan yang cemerlang daripada kepekatan 0.1 hingga 500 ppb dengan pekali korelasi, r = 0.992. Kaedah ini memberi had pengesanan ultra rendah iaitu 0.004 ppb, had kuantifikasi rendah iaitu 0.01 ppb dan purata pemulihan relatif yang boleh diterima dalam lingkungan 80.9 hingga 102.6% dengan sisihan piawai relatif ≤ 9.7%. Pengekstrakan mikro yang dicadangkan adalah bermanfaat daripada segi operasi mudah, cepat dan mesra alam kerana ia menggunakan sedikit pelarut organik yang tidak berbahaya.

Kata kunci: bisfenol A**,** C_{18}, selulosa triasetat, filem, pengekstrakan mikro fasa pepejal, kromatografi cecair prestasi tinggi

Introduction

Bisphenol A (BPA) is a strong endocrine disrupter with widespread application, mainly as a monomer in making polycarbonate (PC) plastic used as food and drink containers. BPA is also found in epoxy resins where it is used to coat the lining of some metal-based beverage and food cans to prevent corrosion. BPA can be found in the environment mainly from the industrial wastewater and through food that was kept in the PC containers. In January 2010, FDA agreed with National Toxicology Program (NTP)'s scientific assessment of BPA, and thus stopped using the BPA in food supply [1]. Nowadays, many countries regulated BPA since it is widely used in consumer products and the residues of BPA can lead to health problem like diabetes, breast cancer and heart disease.

The common methods such as liquid-liquid extraction (LLE) and solid phase extraction (SPE) have been regularly applied to extract BPA in environmental water. Both methods provide adequate sensitivity that fulfil the requirement of laboratory analysis. However, LLE and SPE require relatively large volumes of organic solvent and both are involving evaporation step to pre-concentrate the extract for trace analyses. Besides, large amount of solvent exerted from both LLE and SPE cause environmental pollution, hazard to health and require extra costs for waste treatment. For example, LLE was applied to extract BPA in various brands of drinking bottled water in Riyadh, Saudi Arabia. This research acquired a good recovery which was in the range of 79 to 94% [2]. Besides, SPE was employed to determine the BPA in water and milk and the results achieved ultra-trace detection limit [3, 4]. The great sensitivity shown by SPE is one of the main causes of its popularity, especially when the complicated sample matrices are analysed.

Lately, microextraction techniques have been applied to replace the LLE and SPE in order to meet the green chemistry requirement. These new methods have some advantages such as low cost, minimal solvent consumption, ease of employment and high analytes enrichment compared to both LLE and SPE. Liu and co-researchers demonstrated solid phase microextraction (SPME) coupled with high performance liquid chromatography (HPLC) to determine the BPA in milk and water samples in 2008. This research obtained ultra-trace detection limit and satisfactory relative recoveries which were in the range of 93.1 to 101%, 93.9 to 102% and 100.2 to 104.9% for milk, soybean milk and tap water samples, respectively [5,6]. The SPME is fast, simple, sensitive, and environmentally friendly. De Coensel and co-researchers studied the migration of BPA from baby bottles in 2009. In this research, stir bar sorptive extraction (SBSE) was applied to extract BPA before running through the gas chromatography-mass spectrometry (GC-MS). They also concluded that larger amount of BPA was released at high water temperature [7]. The SBSE simplifies the extraction as it does not require special skill and greatly minimizes the extraction solvent.

Gao and co-researchers demonstrated SDME coupled with HPLC to determine BPA in thermal printing papers in 2013 [8]. Although the SDME is sensitive due to the extractant in a droplet, the slow kinetics of extraction and instability of the drop cause a longer equilibrium time and higher uncertainty. Loh et al. demonstrated dispersive liquid-liquid microextraction based on solidification of floating organic (DLLME-SFO) to extract BPA from water and soft drink samples [9]. The DLLME-SFO successfully extracted the bisphenol A from samples within a minute applying dispersive concept and simplified the collection of extractant by cooling the extractant with low melting point.

This study embeds the C_{18} adsorbent within the cellulose triacetate film to function as an adsorbent film in the µ-SPE technique. The technique simplifies the procedures and stands as a potential on-site extraction tool to minimize the analytes degradation throughout the transportation from sampling sites to laboratory for analysis.

Materials and Methods

Chemicals and materials

HPLC grade reagents like methanol (MeOH) and acetonitrile (ACN), ethanol, isopropanol and chloroform were obtained from Merck (Darmstadt, Germany). Cellulose triacetate (CTA) and octadecylsilane (C_{18}) were obtained from Sigma (St. Louis, Missouri, United States). Bisphenol A (BPA) reference standard was purchased from Dr. Ehrenstorfer GmbH (Augsburg, German).

Preparation of standard solutions

A standard stock solution of BPA (500 ppm) was prepared by dissolving 0.005 g of bisphenol A (BPA) reference standard in a 10 mL of volumetric flask and then diluted to volume with methanol. A series of working standard solutions were prepared by diluting the stock solution with methanol. All of the solutions were stored at 0˚C in darkness when not in use.

Preparation of samples

Three type of environmental water samples were collected from areas around Universiti Malaysia Terengganu which included river water, lake water, and sea water. In addition, soft drink samples that supplied in epoxy can were purchased from retailed shop in Kuala Terengganu. The samples were kept in freezer prior to extraction.

Preparation of C_{18}-impregnated cellulose triacetate composite film

Approximately, 0.04 g of cellulose triacetate (CTA) was weighed in a vial and 2 mL of chloroform was added into the same vial. The solution was then left for at least 5 hours at room temperature to make sure the dissolution was complete. Then, C_{18} (0.01 g) was weighed into a petri dish (5 cm diameter). The resulting CTA solution was added into the petri dish containing C_{18}. The solution was sonicated for 2 minutes to ensure even distribution of C_{18} in the CTA solution. The mixture was allowed to cool and dry in the fume hood at room temperature for 2 hours. C_{18} impregnated cellulose triacetate (C_{18}-CTA) composite film was then formed. The film was cut into small pieces (5mm) to function as an adsorbent film.

Micro-solid phase extraction procedure

The C_{18}-CTA composite film was conditioned by immersing the film in methanol for 30 seconds to wet and activate the sorbent surface. The sample was adjusted to pH 7 using either 0.1 M sodium hydroxide (NaOH) or 0.1 M hydrochloric acid (HCl). Sample (40 mL) was added into a sample vial and the films (7 pieces) were placed into the vial. The sample solution was then sonicated for 5 minutes. After 5 minutes of extraction, the films were removed from the sample solution and transferred to a safe-lock vial. The analytes were desorbed from the film using 50 µL of acetonitrile (ACN) under ultra-sonication for 10 min. The extract was filtered *via* 0.45 µm nylon syringe filter and injected into HPLC-fluorescence detection (FD) for quantification.

High performance liquid chromatography chromatographic conditions

All analyses were performed using HPLC (Shimazu, Kyoto, Japan) coupled with FD (Shimazu, Kyoto, Japan). The chromatographic determination of BPA was carried out on a reversed phase C_{18} column (4.6 x 250 mm, 5 µm). The determination was performed using isocratic mobile phase, acetonitrile-deionized water (60:40) (v/v) at column temperature of 30˚C. The flow rate, injection volume and detection wavelength were fixed at 1.0 mL/ min, 10 µL and 275/305 nm of excitation/emission wavelengths, respectively.

Optimization and validation of µ-SPE HPLC-FD

Several extraction parameters were thoroughly investigated to improve the extraction efficiency of the µ-SPE using C_{18}-impregnated CTA composite film as an adsorbent film. The parameters included amount of C_{18}-CTA films, sample pH, extraction time, volume of desorption solvent, desorption time, salt addition and type of desorption solvent. The µ-SPE coupled with HPLC-FD was then assessed for linearity, relative recovery, limit of detection (LOD), limit of quantification (LOQ) and precision before sample analysis. The LOD and LOQ were determined using signal-to-noise ratio. The peak-to-peak noise around the analyte retention time is measured by using this method. A signal-to-noise ratio (S/N) of three is used for measuring LOD and signal-to-noise ratio of ten is applied for measuring LOQ.

Results and Discussion

Optimization of micro-solid phase extraction procedure

Seven parameters which included amount of C_{18}-CTA composite film, extraction time, type of desorption solvent, volume of desorption solvent, desorption time, sample pH and salt addition were examined to increase the analyte enrichment when the µ-SPE technique was applied to extract BPA. The optimization was carried out using deionized water spiked with 20 ppb of BPA. Triplicate extractions were performed for each parameter to ensure the consistency of the results. The relative standard deviation (%RSD) was controlled in less than 10% to ensure precision and repeatability of the result [10].

The C_{18} which was immobilized within CTA acts as an adsorbent to extract the target analyte, BPA. The amount of C_{18}-CTA composite films vary from 4 to 8 pieces were investigated when extracting the BPA from 40 mL sample solution to determine the adsorption capacity of the C_{18}-CTA composite film. Figure 1(a) shows that the extraction efficiency increased when the amount of C_{18}-CTA composite film was increased from 4 pieces to 8 pieces. In this study, t-test (paired two sample for means) was used to determine the significant difference between 7 and 8 pieces. The result showed that the p-value for this statistics was 0.09 ($p > 0.05$). This mean that there was evidence that there was no significant difference between 7 pieces and 8 pieces. Although the 8 pieces of C_{18}-CTA film have the highest peak area among others, but there was no significant difference ($p > 0.05$) between 7 and 8 pieces. Therefore, 7 pieces of C_{18}-CTA film was applied in the subsequent experiments because the slightly higher peak area obtained by 8 pieces was due to random error.

Extraction time is one of the important factor in the extraction procedures. The microextraction technique is time-dependent since the target analyte mass transfer is the time-dependent process [11]. In this study, the extraction time in the range of 2 to 15 minutes was investigated with other optimized parameters remained unchanged. Figure 1(b) indicates the peak area of BPA increased from 2 to 5 minutes and then dropped from 5 to 15 minutes. The result shows that 5 min was effective to enrich the BPA. Thus, 5 minutes was chosen as optimum extraction time.

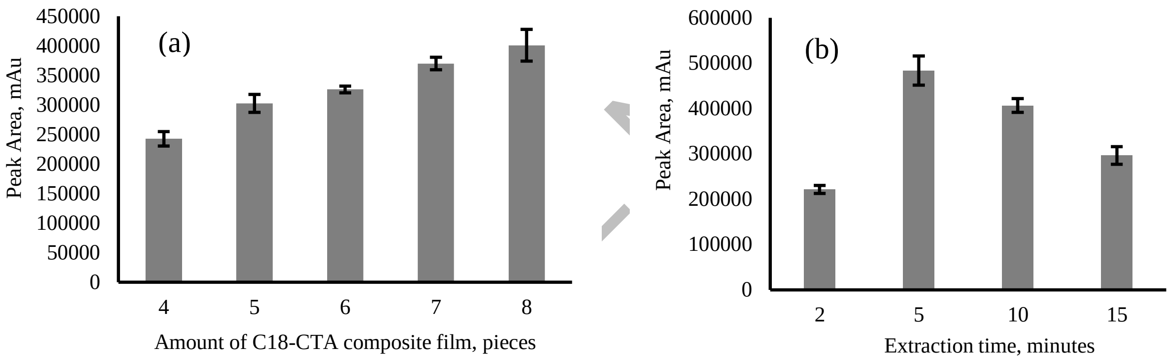

Figure 1. Effect of amounts of composite film (a) and extraction time (b) on µ-SPE for the extraction of BPA from spike deionized water samples. Each error bar indicates the standard deviation of triplicate extractions

The desorption solvent was applied to desorb the target analyte from the adsorbent. The affinity of analyte to the desorption solvent must be stronger as compared to the adsorbent to succeed the desorption process. The acetonitrile (ACN), isopropanol (IPA), ethanol (EtOH) and methanol (MeOH) were investigated as desorption solvents. As summarized in Figure 2(a), the highest peak area was obtained while using the ACN as desorption solvent which exhibited higher extraction efficiency. The higher the value of the polarity index, the more polar the molecule. Although ACN has higher polarity index among IPA, EtOH and MeOH. BPA is a polar compound and it tended to dissolve in polar solvent which depended on the concept of “like dissolve like”. Consequently, ACN was chosen as the optimum desorption solvent.

Generally, the volume of desorption solvent should be kept low to increase the analyte enrichment factor because high volume of desorption solvent tends to dilute the analytes [12]. The volume of desorption solvent in the range of 50 to 200 µL was investigated. Figure 2(b) shows that the peak area dropped significantly from 50 µL to 200 µL. A suitable desorption solvent can effectively desorb the target analyte from the C_{18}-CTA films with a minimum volume [13]. The highest peak area was obtained when 50 µL of desorption volume was used. The desorption solvent which was less than 50 µL was not examined in this study because the volume was insufficient to submerge the films. Besides, the peak area decreased from 100 to 200 µL was due to the dilution of analytes. Therefore, 50 µL desorption solvent was chosen as the optimum volume of desorption solvent in the subsequent experiments.

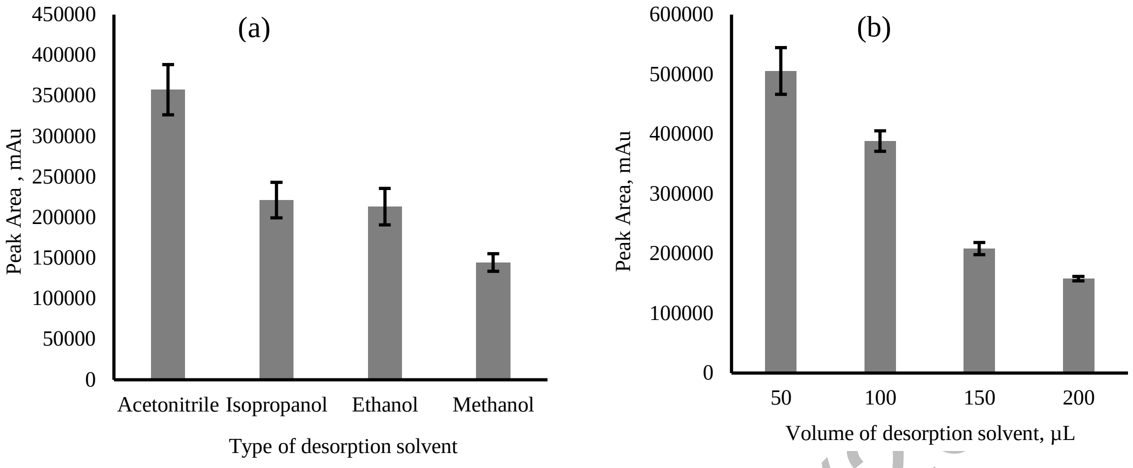

Figure 2. Effect of type of desorption solvent (a) and volume of desorption solvent (b) on µ-SPE for the extraction of BPA from spike deionized water samples. Each error bar indicates the standard deviation of triplicate extractions

Desorption time in the range of 2 to 20 minutes was investigated with other experimental conditions remained unchanged in order to achieve the maximum recovery of BPA. The microextraction technique is time-dependent and the analyte transfer from the C_{18}-CTA composite films to the solvent is also a time-dependent process [11]. Figure 3(a) indicates the peak area increased from 2 to 10 minutes and then dropped significantly from 10 to 20 minutes. Since 10 minutes of desorption time have the highest peak area, thus 10 minutes was applied for next experiment.

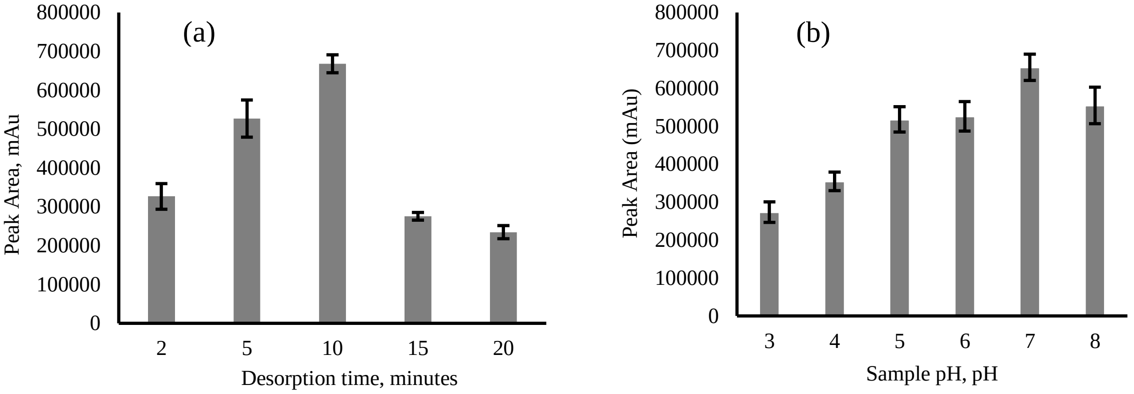

Figure 3. Effect of desorption time (a) and sample pH (b) on µ-SPE for the extraction of BPA from spike deionized water samples. Each error bar indicates the standard deviation of triplicate extractions

At different value of pH, the BPA will be present at different forms [12]. In general, the BPA in molecular form is easier to be extracted using organic solvent [14]. Therefore, the sample pH was fixed to assure the BPA was in molecular form. The BPA is a weak acid compound with pK_a value in the range of 9.6 to 10.2 [15].Various pH of sample solution ranging from 3 to 8 were investigated in this study where the pH was adjusted using either 0.1 M hydrochloric acid or 0.1 M sodium hydroxide. As shown in Figure 3(b), the best extraction efficiency was achieved at pH 7. When the pH of the sample less than the pK_a value of BPA, the BPA existed in molecular form. On the other hand, the BPA existed in ionic form which was more soluble in water when the sample pH over the pK_a value of BPA. The BPA existed in molecular form at pH 3 to 7. Besides, the decreasing peak area was obtained at pH 8. The BPA was protonated when the sample pH was made alkaline. Therefore, pH 7 was used as the optimum sample pH for the following analysis.

The addition of salt was applied to reduce the solubility of the analyte by increasing the ionic strength of sample because the salting-out effect could improve the analyte enrichment [13]. To study the effect of salt addition, this experiment was performed by spiking different concentrations of sodium chloride, NaCl into the sample solution which ranging from 0 to 7.5%, while other experimental conditions were kept constant. The sample without the addition of NaCl gave the highest peak area as compared to other samples with NaCl. Therefore, the factor of salt addition was not considered in the µ-SPE technique due to the salting-in effect.

Validation of micro-solid phase extraction procedure

The validation was carried out to verify the applicable of optimized µ-SPE technique using C_{18}-CTA composite films as the adsorbent films coupled with HPLC-FD in the analysis of BPA in environmental water and soft drink samples. Calibration was performed using deionized water spiked with target analyte, BPA in the range of 0.1 to 500 ppb. The results showed good linearity in the specified concentration with the correlation coefficient, r = 0.992.

The limit of detection (LOD) and limit of quantification (LOQ) are important characteristics applied in the method validation. The LOD and LOQ were 0.004 and 0.01 ppb, calculated based on the signal-to-noise ratio (S/N) of 3 and 10, respectively. The LOQ calculated was at levels lower than the maximum residue limit (MRL) of 0.25 ppb established by the European Union [16]. This revealed that the µ-SPE technique developed in this study was able to perform ultra-trace quantification of BPA in environmental water and soft drink samples.

Relative recovery was conducted by spiking the sea water, lake water, river water and soft drink samples each at 1 and 10 ppb of BPA, respectively. The sample blanks and spiked samples were then extracted using the optimized µ-SPE technique. All samples were free from BPA and Table 1 shows that the good relative recovery average ranging from 80.9% to 102.6% were obtained. Good repeatability was indicated by relative standard deviation (RSDs) of ≤ 9.7%. Thus, the matrix effect was insignificant and µ-SPE techniques can be employed in the analysis of these environmental water and soft drink samples.

Table 1. Relative recovery study using µ-SPE for the extraction of BPA in environmental water and soft drink samples

Sample	**Average of relative recovery ± relative standard deviation, % (n=3)**	
	Spiked at 1 ppb	**Spiked at 10 ppb**
Sea water	84.1 ± 2.4	81.2 ± 1.0
Lake water	90.3 ± 0.8	84.0 ± 2.9
River water	80.9 ± 2.4	92.5 ± 2.3
Isotonic soft drink	102.6 ± 2.7	87.6 ± 9.7
Carbonated orange juice	98.8 ± 8.8	98.7 ± 7.2

Conclusion

The µ-SPE technique has minimized the consumption of organic solvent and also amount of adsorbent throughout the extraction process. This technique applied cellulose triacetate (CTA) as a medium to hold the C_{18} which acts as adsorbent for the extraction of BPA. In addition, the optimization and validation results indicated that this technique gives sensitive extraction within a short analysis time. The µ-SPE technique is simple and easy to operate since it has low requirement of technical skill as compared to other microextraction technique. This technique is fulfilling the basis green analytical chemistry.

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