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Bisphenol A in aquatic environments

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Abstract. The present study describes the application of chromatographic techniques for the identification, separation and quantitative determination of Bisphenol A (BPA) used in water samples. BPA was separated and quantified by reversed-phase high performance liquid chromatography with diode-array detection (HPLC-DAD). Analytical separation was performed on C_{18} column, mobile phase containing acetonitrile: water (60:40, v/v) at a flow rate of 1.5 mL/min isocratic method and ultraviolet detection at 272 nm. A linear response (r=0.9956) was observed in the range of 20-200 μg/mL. The method presents repeatability. A rapid, simple and low cost method was developed to determine BPA in water samples and the results suggest that this method could be used as an alternative method for routine analysis of BPA in quality control.

1. Introduction

Bisphenol A (BPA) is the monomer [2,2-bis(4-hydroxphenyl) propane] (cAS No.80-05-7), a common industrial chemical component in many products, which is widely used in thermal paper industry and as a intermediate (binding, plasticizing, hardening) of synthetic plastic (vinyl-chloride) due to its mechanical and extreme-temperature resistance [1]. Bisphenol A does not occur naturally but has become ubiquitous in the environment as a result of its high production, consumption, and subsequent environmental introduction [2-5].

Polycarbonates are used in food-grade plastics such as reusable beverage bottles, infant feeding bottles, cutlery (plates and mugs), microwave ovenware and storage tanks including water dispensing tanks, in while epoxy resins are used in protective linear for food and beverage cans and vats [6].

BPA is also used in a variety of non-food applications: epoxy resin paints, wood fillers, PVc medical devices, adhesives, surface coatings, printing inks, carbonless and thermal paper, flame retardants, the production of brake fluid, resin-based composites and sealants used in dentistry [7].

There is extensive evidence that many consumer products contain and release BPA. There is also detailed evidence that many of these products leach BPA under normal conditions of use. BPA has been detected in baby bottles, epoxy resins, and other consumer plastics. BPA has also been detected in a wide range of foods stored in cans with epoxy resins. The BPA can be detected in environmental samples, including air, dust and water [8].

Due to the high production volumes and disposal of products made from BPA, polycarbonate plastic and epoxy resins, BPA has entered terrestrial and aquatic environments. In the presence of oxygen, diverse taxa of fungi, bacteria, algae, and even higher plants metabolize BPA. Another reports indicated that abiotic processes mediate BPA transformation and mineralization in the absence of oxygen, indicating that BPA is susceptible to degradation under anoxic conditions [9].

Small amounts of BPA can potentially leach out from food containers into foodstuffs and beverages and therefore be ingested. BPA is permitted for use in food contact plastics in the European Union with a specific migration limit of 0.6 mg/kg food [10].

Based on calculations with a EU environmental exposure and risk assessment model (EUSES), for the general population the main route of human exposure to BPA is the oral route (Ec, 2003; 2008) [11].

Food constitutes the primary route for human exposure to BPA, one of the highest volume chemicals produced worldwide. The estrogenic properties of BPA, its wide dispersive use and the recent literature describing low-dose BPA effects in animals, have raised concerns about its possible adverse effects on human health [12].

At this time, only a few small studies have explored the associations between BPA levels and human health issues. These limited data indicate that additional studies are warranted on human health and BPA exposure. BPA is one of the most prevalent and best studied endocrine disruptors. currently, there is limited evidence to suggest that BPA levels vary between women and men and/or with several diseases and endocrine-related syndromes [13].

There is a concern that BPA has potential endocrine-disrupting properties, which may adversely impact neurological, physical and behavioural development [14, 15].

Following exposure, BPA is mainly metabolized in humans to BPA-glucuronide through the hepatic glucuronide transfer and excreted from the body [16].

considering the large emission estimates to surface water, their high tendency to spread in aquatic systems and the fact that BPA is moderately toxic to aquatic organisms, it is recommended to perform an additional studies on these compound with emphasis on collection and verification of emission factors, the concentration in food and furthermore to generate new toxicity data.

There are methods which have been developed over the years for the determination of phenolic compounds in water and waste water. HPLc is one of the major separation methods used frequently that can also allow for quantitation of BPA in water samples.

Because BPA is a high production volume chemical, is necessary to examine his global distribution in tributaries discharges, surface waters, sewage sludge, biosolids, sediments, soils, air, wildlife, and humans. Future research efforts are to understand environmental exposure of BPA and often industrial chemicals in surface waters, effluent dominated and dependent systems which often represent worse-case scenarios in urbanizing inland and coastal waters.

2. Reagents

Bisphenol A (CAS RN 80-05-7; purity grade > 99%) was purchased from Sigma-Aldrich (St. Louis, MO). The organic solvents, acetonitrile HPLC grade water and methanol were purchased from Merck (Merck, Darmstadt, Germany) and used for all washes, dilutions, and sample preparations.

Seawater samples were collected from coastal seawater of Black Sea in new 250 mL glass bottles fitted with Teflon® faced polyethylene lined caps. All samples were kept in dark.

Within four days, the samples were filtered through nitrocellulose filters with 0.45-mm pore size membrane filters (Millipore), and were kept refrigerated at 4 ºC in the dark prior to analyses. The extracts were analysed within 2 weeks after collection. Two replicates of each sample were carried out.

Water working solutions were prepared by spiking into samples water with standard solution of BPA.

3. Chromatographic conditions (reversed-phase HPLC-DAD)

Experiments were performed on a HPLC system Agilent technology model 1200 (Germany) consisting of a pump, a column oven, an auto sampler and a photodiode array detector (DAD) was. The system was controlled by an HP ChemStation, which also performed data acquisition from the diode array detector and quantitative measurements.

Separation of compounds was achieved using a Zorbax Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 µm) from Agilent Germany. Isocratic mixture of acetonitrile-Milli-Q grade water (60:40 v/v) was used as the mobile phase. The mobile phase mixture was filtered through a 0.47 µm nylon membrane filter and degassed ultrasonically before use. The flow-rate was 1.5 mL/min and the injection volume was 10 µL. The detector was set to scan from 200 to 800 nm. The bisphenol-A was detected with UV detector at a wavelength of 272 nm, which was the wavelength used for quantification, with a total run time of 10 minutes. The analytical column was thermostatted at 39 °C.

3.1 Standard preparation.

Stock Solution Preparation

The stock solution of BPA (50 mg/mL) was prepared by dissolving 2.5 g of BPA in 50 mL methanol.

Working Standard Solution Preparation

Ten-point working standard solutions were prepared for BPA at concentrations of 20, 25, 30, 40, 50, 60, 70, 80, 90, 100 and 200 µg/mL.

4. Results and discussion

4.1 Optimization of conditions and chromatograms:

During the development stage, the individual or different mixture use of acetonitrile and water as the mobile phase, column temperature, flow rate and injection volume were tested. In the optimized conditions, isocratic mixture of acetonitrile and water (60:40 v/v) was used as the mobile phase, the mobile phase flow rate and maximum absorption wavelength was set at 1.5 mL/min and 272 nm, respectively. The injection volume was set at 10 µL. Acceptable retention times 6.717 min was determined for the peak of BPA using a reversed-phase C18 analytical column.

4.2 Specificity and selectivity

The selectivity of the method was also tested by observing potential interferences with BPA peak arising from the water components solution. No interfering peaks and ghost peak were observed at the retention times of BPA in the blank water and spiked water samples. A chromatogram is shown in Figure 1. A representative chromatogram corresponding to a blank sample without BPA, a blank sample with BPA standard solution shows also the overlay of typical chromatogram obtained from the analysis of the working water solutions (Figure 2). Peak purities for BPA were further confirmed by means of a photo-DAD system.

4.3 Linearity

An appropriate calibration model is necessary for a reliable quantification. The relationship between the concentration of BPA and the area was investigated. The least-squares fit method was employed to achieve the linearity.

The linearity was studied by preparing standard solution at different concentration levels. Ten-level calibration series with analysed at each concentration level were measured. The calibration curves obtained by plotting the peak area against the concentrations of BPA were highly linear over the range 20-200 µg/mL.

The calibration curve is described by a linear regression equation and it was found to be $y = 5.9486 \cdot x + 37.411$, where: y is the peak area, x is BPA concentration (µg/mL), The correlation coefficient (r) of the standard curve was found 0.9956, ($r < r_{cr} = 0.6319$ for 8 degrees of freedom and $\alpha = 0.05$) (Figure 3). The performance parameters of linear regression equation are presented in Table 1.

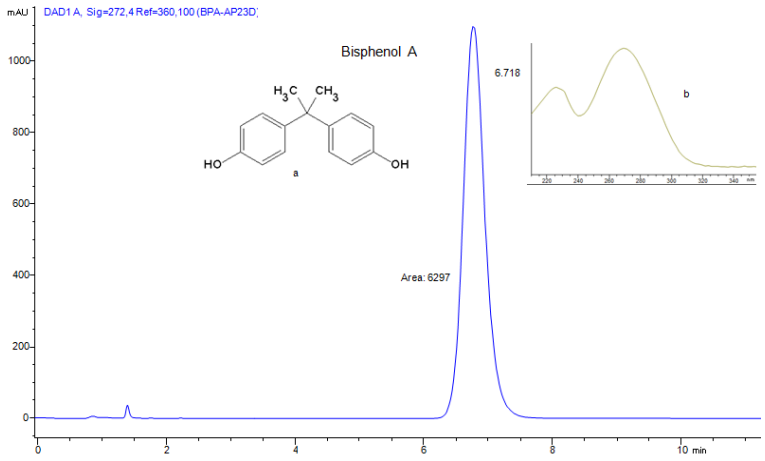

Figure 1. The chromatograms of BPA in standard solution:
a- UV spectra of BPA, b- chemical structure of BPA

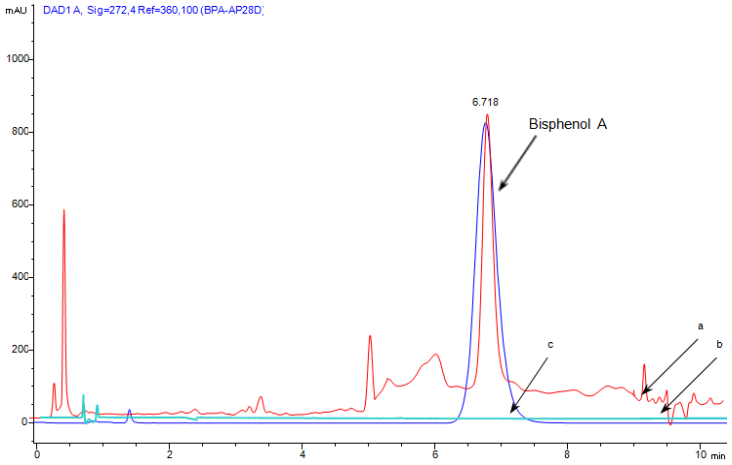

Figure 2. The chromatograms of BPA in water samples (a),
BPA in standard solution (b) and in blank (c)

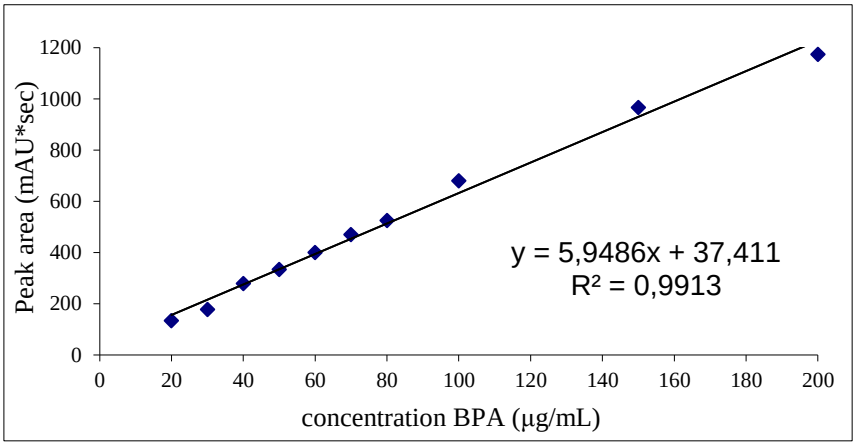

Figure 3. The linear regression curve for the determination of BPA etalon curve

In order to validate the model, linear regression was performed.

Table 1. Parameters for the response function linearity

Parameter	Value
Observations	10
Linear range (μg/mL)	20.0– 200.0
Slope	37.411
Intercept	5.9486
Regression coefficient r	0.9956
Coefficient of determination r^2	0.9913
Standard error of the regression line	0.0078
Sample variance	114224
Mean of peak area	513.30
Standard deviation (SD)	337.97

The results are statistically significant (*Significance F* $=1.55 \cdot 10^{9} < 0.05$, p-value=0.023<0.05 for intercept and p-value$=1.25 \cdot 10^{7}$ concentration coefficient)

Table 2. Analyses of variance

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1019116	1019116.056	915.2321	1.55E-09
Residual	8	8908.044	1113.505556		
Total	9	1028024			

df is the degree of freedom, SS is sum of squares according to the relation global sum of squares = sum of squares regression + residual sum of square, MS - The average of squares sums: SS divided to the number of degrees of freedom, F-statistic Fischer value, Regression is the variation source, the variation explained by regression, Residual is the residual variation, Total is total variance.

Table 3. The estimated values for the model's coefficients

	coefficients	*Standard*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	37.41111	15.94192	4.97504351	0.02368	6.26903	81.09125
Concentration	5.948611	0.19663	30.2528035	1.25E-07	5.495181	6.402041

comprises the estimated values of the coefficients, t Stat is the t statistic for the verification of hypothesis, P value is the bilateral critical probability of the t test, Lower 95%, Upper 95% are the upper and lower limits of the confidence interval.

The detection of the compound was carried out by UV at 276 nm. The detection's repeatability was demonstrated by analysing the 6 samples having the same concentration (50 μg/mL), independently prepared and taking into consideration that the value of the obtained relative standard deviation, RDS = 0.2726%, so it is lower than 2%, as it can be seen in Table 4, it can be stated that this data confirm the BPA s detection repeatability.

4.4 Analysis of environmental water samples

The developed method was further used to analyse the ten water samples from seawater were analysed (Figure 2). The recoveries were obtained by spiked real water samples with 70 μg/mL individual BPA. The results are listed in Table 5.

Table 4. The statistical parameters which characterize the precision of the BPA determination method (6 samples)

Parameter	Value
Mean concentration ($\bar{x}$) (μg/mL)	25.73
Standard error of detection repeatability (μg/mL)	0.0033
Standard deviation of mean of detection repeatability (SD_x) (μg/mL)	0.0006
RSD (%)	0.2726

Table 5. Results for the determination of BPA in water samples spiked with 70 μg/mL BPA

Replicate number	Spiked concentration in real water samples (μg/mL)	Calculated concentration (μg/mL)	Peak area (mAU*sec)	Recovery (%)	Mean ±SD recovery (%)
1	70	70.81	458.68	101.16	99.68±1.002
2		69.36	450.06	99.09	
3		70.76	458.36	101.09	
4		69.51	450.91	99.30	
5		69.40	450.26	99.14	
6		69.42	450.37	99.17	
7		69.64	451.68	99.48	
8		69.03	448.06	98.62	
9		69.14	448.74	98.78	
10		70.73	458.17	101.04	

The results indicated that the concentration of BPA in water samples does not increased, the recovery studies ware appreciable. Differences of estimated concentration and actual concentration varied from 98.62 to 101.16

Conclusions

A novel method was developed for separation and quantification of BPA from water samples, presenting robust capacity for this pollution. This method enabled selective and sensitive analysis of the BPA at low concentrations in complex water environment. The method was demonstrated to be readily applicable for the routine analysis of the BPA in water samples.

Consumption of BPA a common industrial chemical component in many products, has steadily grown over the last years.

We need to continually analyse BPA levels in marine water and other sources of nearness such as marine sand because a source of endocrine disruptor bisphenol is probably originated from a surprising source: hard plastic trash discarded in the sea water but also epoxy plastic paint used to seal the hulls of the ships. When sufficient data were available, probabilistic hazard assessments were performed to understand global environmental quality concerns.

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