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A fast, low-cost and eco-friendly method for routine determination of Bisphenol-A in landfill leachate employing vortex assisted liquid-liquid extraction

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ABSTRACT

Landfills are sites designed to receive and final disposal of a broad variety of urban solid wastes (USW). The decomposition and biodegradation processes generate a leachate of high complexity and toxicity, containing persistent and recalcitrant contaminants that are not usually monitored. Bisphenol-A (BPA) is a synthetic compound applied mostly on the production of polycarbonate plastics, epoxy resins, and is an endocrine disruptor. BPA negatively affects biological receptors, resulting in harmful effects to nervous and reproductive system as well metabolic and immune function. The presence of BPA in USW urges the development of feasible analytical methods to support the effluent treatment plants and reduce the risks of contamination. The main goal of this work was to develop an efficient, eco-friendly, fast and simple method for routine analysis of BPA in the leachate from landfill. A vortex assisted liquid-liquid extraction (VALLME) using 1-octanol as solvent was performed. BPA recoveries at spiking levels of 2.5, 6.5 and 12.5 μ g L⁻¹ were between 60 to 104% with relative standard deviation (RSD) lower than 26%. The linearity of the method was evaluated and the correlation coefficient was (r) 0.9985. The limit of quantification (LOQ) was 2.5 μ g L⁻¹ with a preconcentration factor of 20. The method has advantages such as low consumption of extraction solvent (150 µL), low cost, easy and fast determination.

1. Introduction

Bisphenol-A (BPA) is a synthetic compound of wide applicability used in the synthesis of materials as detergents, polycarbonates, thermal paper, epoxy resin, and food packaging [1]. It is classified as endocrine disrupter for having a structure similar to the steroid hormone 17- β -estradiol, what confers ability to imitate the estrogen activity [1-3]. BPA effects were discussed in neurochemistry alterations, prostate cancer, breast cancer, hormonal alterations, infertility, and ovaries problems [1, 4, 5]. The usage of BPA in packs of plastic, thermal papers, coating of food cans, pharmaceuticals, and general industry represents a direct source for human and environment contamination [6,7]. In countries where the classification and recycling

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of urban solid wastes (USW) is not efficient, the final disposal of USW occurs mainly in landfill sites. These sites are projected to work in a long and slowly process of degradation. Although, degradation processes as photolysis, hydrolysis and biological still occurs and generates a leachate of high complexity and toxicity, where the presence of BPA threatens the environment safety [8, 9]. A reliable and efficient method is necessary to evaluate the trace levels of BPA in the complex matrix of leachate. For that, a pre-concentration and/or clean-up step have been used to prepare the sample prior to extraction and analytical determination of BPA, allowing the quantification of BPA at trace levels in water and plastic materials as toys. [10, 11]. In general, the sample preparation has been performed by methods based on liquid or solid phase extraction. In the solid phase extraction (SPE), the analyte is extracted from the sample by passing the sample through a column/cartridge packed with polar (e.g. alumina) or nonpolar sorbent (e.g. C18). Modifications as solid phase microextraction (SPME) have also been observed for BPA extraction. On the other hand, liquid-liquid extraction (LLE) methods are based on the usage of solvents to extract and separate the analyte from the matrix, usually in an aqueous and organic phase [7, 11, 13, 14]. SPE and LLE have been used and adapted for years and their application is well dependent to the matrix composition, sample properties and concentration. However, there is a need for nontedious and environmentally friendly methods, employing lower volumes of non-toxic solvents and significant recoveries. Vortex-assisted liquidliquid microextraction (VALLME) is a technique based on emulsification procedure where the extraction applies reduced volumes of a lowdensity solvent into water associated with high energy of vortex mixing [15]. The fine droplets can rapidly extract target analytes from water due to shorter diffusion distance and larger interfacial area. After centrifugation, the floating extractant phase restores its initial single-drop shape for the following instrumental analysis. This technique has showed to be eco-friendly for BPA extraction in water samples [16]. Gas chromatography (GC) and high-pressure liquid chromatography (HPLC) are the main analytical instruments used for BPA detection. Either are based on the difference of interaction between the sample in mobile phase (liquid for HPLC, and gas for GC) and stationary phase (packed column). Then, the analyte interacts with the detector that generates an electronic signal, translated into a peak. Different GC and HPLC setups have already been described. The mass spectrometry (MS), ultraviolet (UV) and fluorescence are detectors frequently used in HPLC, while in GC the MS have been more applied for the detection of BPA [3, 5, 10-15]. In this work, the usage of HPLC with diode-array detector has been chosen due advantages as the simultaneous acquisition in different wavelengths, improving the separation of peaks, an important factor to be considered when working with complex matrices as leachate [11, 14, 15]. The challenges of controlling the destiny of USW to landfills and aware society of a responsible waste discard is aggravated by the analytical limitations of detection and quantification methods for BPA in complex matrices. On this way, a feasible and lowcost method could collaborate with better health policies and increase the alert to BPA exposure. The aim of this study was to develop a fast, robust, low-cost and accuracy method for routine analysis of BPA in the complex matrix of landfill leachate, employing VALLME and HPLC-DAD.

2. Material and Methods 2.1. Chemical and samples

The bisphenol A (BPA) standard and analytical grade acetonitrile (J.T Baker, Mallinckrodt, NJ, USA) were purchased from Sigma-Aldrich (São Paulo, Brazil). BPA standard solutions were prepared in methanol and stored at 4 °C until use. Ultrapure water was prepared in a Direct-Q UV3 (Millipore, France), and used as mobile phase as well as acetonitrile.

The leachate used in this study was collected according to standard methods (SM:2005) from a

landfill located in the city of Camaquã, located at the south of Brazil (30° 49' 41.9''S 51° 47' 39.3''W). The samples were stabilized with H_2SO_4 (1 mol L⁻¹) and kept in the dark at 4 °C until the analytical procedures. Then, leachate samples were filtered out with a Micropore system using cellulose acetate membranes of 0.45 µm (Sartorius Biolab Products, Goettingen, Germany), assisted with vacuum from a vacuum-pump Tecnal TE-0581 (Tecnal, São Paulo, Brazil), and kept in a pre-washed amber bottle with acetone aqueous solution 1 mol L⁻¹.

2.2. Apparatus

The BPA determination was carried out in a Waters high performance liquid chromatography coupled to a diode array detector 2996 (Waters, Milford, MA, USA), equipped with a quaternary pump model 600, Empower PDA software was employed on the data acquisition. The separation was realised in a silica-based, reversed-phased analytical column C18 5 μ m ODS2 150 mm x 4.6 (Waters, Milford, MA, USA). The analytes were eluted with a mixture of ultrapure 60% H₂O and 40% analytical grade ACN as mobile phase in isocratic mode and at a flow-rate of 1 mL min⁻¹. The injection was made manually using a syringe with 20 μ L of VALLME extracted sample.

2.3. Vortex-assisted liquid-liquid microextraction procedure

VALLME extractions were carried out in 10 mL glass tubes with conical bottom with 10 mL of filtered leachate and 150 µL of 1-octanol in quadruplicate (Sigma-Aldrich, São Paulo, Brazil). The tubes were vortexed (Certomat MV, B. Braun Biotech International) at 4500 rpm for 5 min, and centrifuged at 2000 rpm for 5 min (Quimis, São Paulo, Brazil). The supernatant was taken out after phase separation with a 250 µL syringe and transferred to 2 mL tube (Eppendorf 5804 R, Eppendorf, São Paulo, Brazil). The 2 mL tube was centrifuged once more at 2000 rpm for 2 min and the supernatant was collected with a syringe, transferred to a new tube and the volume filled up to 0.5 mL with methanol. The parameters related to VALLME and HPLC-DAD were adjusted before the validation process. The lowest volume of solvent and sample that resulted in a detectable and reliable signal of BPA were chosen. Vortex and centrifugation were based on the visual formation and stability of the organic layer. Then, a second centrifugation was realised to remove possible contaminants carried by the pipetting. Five hundred microliters of methanol were used to ensure the complete resuspension of organic phase (Fig.1).



Fig. 1. Vortex-assisted liquid-liquid microextraction (VALLME) procedure.

2.4. Analytical performance

The proposed method was validated by analysing parameters as analytical curve, linearity, limit of detection (LOD), limit of quantification (LOQ), recovery and precision (intermediate precision and repeatability), according to Brazilian legislation (INMETRO - DOQ-CGCRE - 008, 2011) which stablish the procedures and standards for analytical determinations. Accuracy was evaluated using recovery experiments with extraction of 3 different BPA standard concentrations: 2.5, 6.25 and 12.5 $\mu g L^{-1}$. The precision in terms of repeatability was obtained by carrying out the extraction and analysis of fortified samples. Each spike level was extracted in three replicates and each extract injected three times in the HLPC-DAD equipment. Different days were used for the same spike levels of repeatability to evaluate the intermediate precision of the method. Limit of detection (LOD) and the limit of quantification (LOQ) were obtained using the ratio signal/noise 3:1 and 10:1, respectively.

3. Results and discussion

3.1. Preliminary analysis

Landfill sites receives a broad variety of solid waste from different sources. The wide application of BPA and indiscriminate holding of plastic materials at these sites represents a direct source for BPA to contaminate soil, water, and environment, posteriorly reaching human by direct contact or indirect through contaminated food. Due to the matrix complexity and low concentrations, a method to identify and quantify estrogens need to be specific and selective [17]. A standard solution of BPA was diluted in methanol (mobile phase) and added to leachate sample to verify the presence of interferences in the absorption spectrum of BPA, required to avoid false positive results. The retention time for BPA standard in methanol was at 5.8 min of run and detection wavelength at 227 nm. BPA fortification was detected in leachate sample at same retention time as in methanol. The chromatogram of landfill leachate with no fortification is presented in Figure 2, and it can be observed the absence of interference at BPA retention time (5.8 min) and 227 nm, confirming the method selectivity.





3.2. Validation of analytical procedure

The determination of analyte concentration in different matrices is usually made by a calibration curve, preparing concentrations of stock standard solution and relating to absorbance units obtained for each concentration, generating an equation used to quantify the analyte in real samples. Furthermore, this procedure is also applied to measure the correlation between 2 factors, a necessary factor in the process of validation. The maximum value for correlation coefficient (r) is 1, ensuring the relation between absorbance and concentration. The calibration curve was prepared with 5 concentrations of standard BPA in methanol, ranging from 0.05 to 2.5 mg L^{-1} (n= 3). The curve showed a high linear correlation (r) of 0.9985, overcoming the requirements stablished by the Brazilian legislation (ANVISA: 0.99 and INMETRO:

0.90) and allowing the use to determine BPA in leachate sample.

LOD and LOQ represents the limits of detection and quantification of a method. LOD is defined as the smaller concentration of analyte that can be detected without guarantee or reliability. LOQ is the lowest concentration where the analyte can be determined with precision. Appling the ratio signal-noise to obtain the LOD (3:1) and LOQ (10:1), the values found were 0.8 and 2.5 μ g L⁻¹, respectively. These values are in agreement with other microextraction techniques proposed for BPA detection as showed in Table 1. Recoveries were determined in 2.5, 6.25 and 12.5 μ g L⁻¹ levels (Fig. 3), obtaining an RSD from 60 to 104% and RSD from 11 to 26%. Intermediate precision was determined in different days of analyses and the recoveries obtained were between 81% and 97% with RSD lower than 16%.





Fig. 3. Evaluation of method accuracy, repeatability, and intermediate precision by recoveries (%) of different BPA fortifications (μg L⁻¹)

Stock (µg L ⁻¹)	Samples (%recovery)						
	1	2	3	4	5		
2.5	69.89	73.87	38.87	75.93	40.28		
6.25	103.46	110.89	105.49	95.13	95.73		
12.5	97.00	93.24	114.55	117.02	80.66		

Table 1. Recoveries (%) in landfill leachate fortified with 2.5, 6.25, and 12.5 μg L⁻¹ of BPA stock. Results are based on the recoveries obtained for the respective stock in methanol.

The highest recoveries and consequently accuracy, repeatability and intermediate precision were obtained with 12.5 μ g L⁻¹ of BPA. These results can also be observed in Table 1, where are presented the corresponding recoveries of 2.5, 6.25, and 12.5 μ g L⁻¹ BPA fortifications in landfill leachate.

The specificity of the method is also observed in the chromatograms (Fig. 4) of BPA in methanol (4a) and landfill fortified leachate (4b).

In the Table 2 are presented the results of BPA determination in diverse matrices less complex than landfill leachate. Therefore, the obtained results

in our research agree with the literature for BPA extraction from liquid samples: SPE [7] with serial processes of homogenization-vortex-sonicationcentrifugation-evaporation-resuspension of sample [18], Micro-QuEChERS-GC/MS [19], and DLLME [20]. Correia-Sá et al [19] obtained recoveries of BPA from 70 to 120% and RSD from 3 to 11% applying Micro-QuEChERS-GC/ MS in human urine, a simpler matrix than landfill leachate. Laganà et al [17] obtained 99-103% of recovery for BPA fortifications in river and sewage treatment (influent and effluent) samples.



The signals were generated with 12.5 μ g L⁻¹ of BPA

Matrix	Recoveries (%)	LOQ	LOD	RSD (%)	References
Landfill leachate	60-104	2.5 μg L ⁻¹	0.8 μg L ⁻¹	11-26	**
Urine	0-120	0.43 µg L ⁻¹	0.13 μg L ⁻¹	3-11	[19]
Sewage effluent/ influent	88.6-96.2	Effluent: 0.98 ng L^{-1} Influent: 3.84 ng L^{-1}	*	1.5-15	[21]
Blood serum	101-106	0.028 ng mL ⁻¹	0.009 ng mL ⁻¹	3.9-5.8	[22]
River water	84.7-95.7	0.01 ng mL ⁻¹	0.003 ng mL ⁻¹	5.3-9.6	[23]
Effluent wastewater, bottled and surface water	89-113	6; 24 and 7 ng L^{-1}	20; 7; 22 ng L ⁻¹	<17	[24]
Wastewater effluent and estuarine water	89-94	11-20 ng L-1	*	2-13	[25]

 Table 2. Recovery (%), limit of quantification (LOQ), limit of detection (LOD) and relative standard deviation (RSD%) of BPA determination methods obtained by different authors in diverse matrices and complexities.

As showed in Figure 4, BPA can be determined in landfill leachate with no significant matrix interference and reliable results, followed by easily detection. The method is also eco-friendly, requiring less than 1 mL of solvent per analysis and not time demanding.

The protocol in this method is ideal for routine and quick analysis, versatile and can be used in a broad spectrum of matrix with high extraction rates, easy cleanup step, low RSD, feasibility, low consumption of solvents, and non-expensive.

4. Conclusions

On this article we developed an analytical procedure for BPA determination in landfill leachate. A low volume of 1-octanol (150 µL) was used as extraction solvent in the vortex-assisted liquid-liquid microextraction (VALLME) in a simple procedure that takes around 20 min to be executed. The proposed method was validated by adding standard concentrations of BPA in leachate and quantifying the recoveries, with a full analysis of standard deviation, accuracy, repeatability, intermediate precision, LOD, and LOO. BPA recoveries were between 60 to 104% with relative standard deviation (RSD) lower than 26%, and linearity of 0.9985. The limit of quantification (LOQ) was 2.5 μ g L⁻¹ with a preconcentration factor of 20. Thereby, the proposed methodology is eco-friendly, requiring a low volume of sample and extraction solvent. The method present technical features that adequate for BPA routine analysis and also the potential to be applied in BPA quantification in simpler matrices.

5. Declaration of interest

The authors declare no conflict of interest.

6. Acknowledgements

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