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# Micro-QuEChERS extraction coupled to GC–MS for a fast determination of Bisphenol A in human urine

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ABSTRACT

Bisphenol A (BPA) is considered an endocrine disruptor and public concern over BPA exposure has been raised. Several studies have assessed human exposure to this plasticizer, confirming its ubiquitous presence and highlighting children as a public of special concern. A simple, efficient, cheap and green analytical procedure is reported within this paper. This paper reports, for the first time, the development of a modified Micro-QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method coupled to gas chromatography-mass spectrometry (GC–MS) as a new strategy for the efficient extraction and determination of Bisphenol A in human urine samples. Several parameters that are known to influence extraction were optimized. Good linearity was achieved at the studied concentration range (1–50 μg/L), with a correlation coefficient ($R^2$) of 0.998. The optimized method proved to be accurate (≥74% recovery), reproducible (< 11% relative standard deviation) and sensitive for BPA determination (detection limit of 0.13 μg/L and quantification limit of 0.43 μg/L). The analytical procedure was applied to the analyses of 12 urine samples collected from children living in the North/Center region of Portugal. BPA was detected in all the analyzed samples in concentrations ranging from 1.5 μg/L to 48.9 μg/L. The proposed methodology is suitable for the determination of BPA in urine samples in the framework of biomonitoring studies and bioanalytical analyses, applying GC–MS detection.

## 1. Introduction

In the last decades, environmental exposure to certain industrial chemical substances has caused apprehension due to their potential toxicity and widespread use [1]. Bisphenol A (BPA), common name for 2,2-bis(4-hydroxyphenyl) propane is one of the major industrial chemicals of emerging concern [1].

Due to its cross-linking properties BPA has been widely used in the manufacture of polycarbonate plastics and epoxy resins, in the production of several products, such as food containers, food and beverage can linings, food packaging materials, CDs, medical equipment, bottles, plastic bags, etc [2]. Bisphenol A has been found to leach and migrate rather easily [2–13]. According to the available studies, BPA exposure from dietary sources is generally considered higher than exposure from non-food sources. Nevertheless, not all sources and routes of exposure contributing to the omnipresent BPA body burden are thoroughly understood [6,14,15].

BPA is considered an endocrine disruptor [2,5,16] and consequently, public concern has been raised by several studies suggesting a link between BPA exposure with several health outcomes, namely obesity [17–25]. However, nowadays these human health effects from low-level exposures to BPA are being debated [4,16,26–32]. In 2015, the European Food and Safety Authority (EFSA) has lowered the Tolerable Daily Intake (TDI) for BPA to a temporary TDI (t-TDI) of 4 μg/kg body weight/day, based on new data and the uncertainties surrounding health effects in regard to mammary gland, reproductive, metabolic, neurobehavioral and immune systems [28]. Moreover, according to several studies, children seem to be a population of special concern as their exposure levels are higher when compared to adolescents and adults [33–35].

Pharmacokinetic studies showed that BPA is rapidly metabolized and conjugated predominantly with the glucuronic acid to the BPA-glucuronide in the gut wall and liver [36–41]. Since most of the BPA taken up orally is excreted in urine within less than 24 h, urine is the preferred matrix for estimating human exposure [38,42–44].

The identification and quantification of BPA is challenging, due to

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the low concentrations at which this compound is typically present in human matrices and due to a possible background contamination. Therefore, biomonitoring should be performed through highly sensitive analytical methods and exposure assessment based on the conjugates [45].

Common sample preparation techniques for BPA human biomonitoring methods include solid phase extraction (SPE) and liquid–liquid extraction (LLE) [5,46,47]. The demand of high-throughput methods in biomonitoring programs has led to the development of automated off-line and on-line SPE methods. These on-line SPE methods tend to reduce significantly the sample volume required and procedure time [48,49]. Generally, liquid chromatography coupled to mass spectrometry is the most common analytical technique for BPA determination, not requiring a derivatization step [38]. However, several studies have reported the use of gas chromatography with a prior derivatization step frequently associated with SPE as the extraction technique [5,38]. Additionally, some authors have addressed the comparison between GC and LC for the determination and quantification of a broad range of compounds, concluding that both techniques are comparable [50–52].

The QuEChERS (quick, effective, cheap, rugged and safe) methodology was initially developed in 2003 for the determination and measurement of pesticides residues in vegetables and fruits, combining the extraction/isolation of pesticides and extract clean-up [53]. This technique comprises extraction using commonly acetonitrile (ACN), as extraction solvent, and purifying the extract by dispersive solid-phase extraction (dSPE) [54]. Since then the QuEChERS technique has become the method of choice for food analysis and has suffered various modifications and improvements over the years. Therefore, nowadays this method is applied to other analytes and matrices [54,55].

This methodology has been applied for BPA determination in food matrices and animal organs, namely in seafood, canned food (seafood, vegetables and fruits), packed food, honey and rat testis [56–65]. In these studies, different compositions of QuEChERS salts were tested such as the original composition (NaCl and MgSO4), citrate buffer (additional citrate buffers) and acetate buffer (additional acetate buffer). Nonetheless, the extraction solvent was always set to the ACN. The QuEChERS has also been used, in few occasions for the determination of pesticides [66–68], diverse environmental contaminants [46], pharmaceuticals [69,70] and lipids [71] in urine samples. For this matrix, different salt compositions were also tested and used, still ACN was once more the chosen extraction solvent, except for the lipids determination [71].

The present work displays a novel methodology based on QuEChERS technique coupled to GC–MS for BPA determination in human urine samples. As far as the authors know this is the first time this method has been applied with this aim. The developed method, based on miniaturized QuEChERS method presents several advantages when compared with more traditional extraction techniques (namely manually-operated SPE) as it is less laborious, time consuming, cheaper and greener (less solvent consumption). The present work is therefore relevant for human biomonitoring studies and biomedical analysis in GC–MS field.

## 2. Material and methods

### 2.1. Reagents and chemicals

GC-grade Hexane and Methanol, Acetonitrile (ACN) HPLC-grade (purity ≥99.9%) were purchased from Merck (Darmstadt, Germany). Commercially available bisphenol A (BPA, 2,2-(4,4-dihidroxydiphenyl) propane) (99% purity), and the isotope labeled internal standard $^{13}C_{12}$-BPA (99%) were provided by Sigma (St. Louis, MO, USA). The derivatization reagent was BSTFA + TMCS (99:1) (GC/GC–MS) from Supelco (Bellefonte, PA, USA). Three different types of QuEChERS and two dSPE were tested: QuEChERS A ((4 g magnesium sulfate ($MgSO_4$) and 1 g sodium chloride (NaCl)); QuEChERS B (6 g $MgSO_4$ and 1.5 g anhydrous sodium acetate ($CH_3COONa$)); and QuEChERS C (6 g $MgSO_4$, 1.5 g NaCl, 1.5 g of sodium citrate dihydrate ($Na3Cit{\cdot}2H_2O$) and 0.750 g sodium citrate sesquihydrate ($Na_2HCit.1.5H_2O$)). The two tested dSPE had the following composition: dSPE 1) was composed of 25 mg of C18 ((octadecyl sorbent (C18)) and 150 mg of $MgSO_4$; and dSPE 2) was composed by 50 mg of primary and secondary amine (PSA) exchange material, 50 mg of C18 and 150 mg $MgSO_4$. The QuEChERS and the dSPE were supplied by Agilent technologies (Bond Elut Sample preparation solutions) (Lake Forest, CA, USA). For enzymatic hydrolysis was used the enzyme β-glucuronidase/arylsulfatase from *Helix pomatia* (EC 3.2.1.31/EC3.1.6.1; 5.5/2.6 U/mL) purchased from Roche Diagnostics (Indianapolis, USA).

### 2.2. Standard solutions

Stock solutions (100 mg/L) were prepared in methanol. Working solutions were prepared daily from these stocks by appropriate dilution. The solutions were stored at −20 °C. Two five-concentration-level calibration curves within the 1–50 μg/L concentration range in methanol and urine (matrix-matching calibration, spiked after extraction) were prepared. Deuterated internal standard ($^{13}C_{12}$BPA) was employed to compensate for possible matrix effects and analyte losses.

### 2.3. Instrumentation

Chromatographic analysis was carried out in a TRACE GC Ultra gas chromatograph Polaris Q coupled with ion trap mass spectrometer (Thermo Fisher Scientific) operated in the electron impact ionization (EI) mode at 70 eV and controlled by Xcalibur 1.3. The helium carrier gas (Linde Sógas purity ≥99.999%) was maintained at a constant flow of 1 mL/min. Injection (2 μL) was carried in splitless mode. A Phenomenex column ZB-XLB (30 m x 0.25 mm I.D, 0.25 μm film thickness) was used. The GC oven temperature was programmed from an initial temperature of 90 °C (1 min hold), ramped at 15 °C/min up to 250 °C (1 min hold), increased to 255 °C at 20 °C/min (5 min hold) and finally to 270 °C at 10 °C/min (1 min hold). This program resulted in a total run time of 23.42 min. The other optimized parameters included a transfer line temperature of 250 °C and an ion source of 250 °C. The BPA was quantified in the selected ion monitoring (SIM). The selected ions for BPA were 357 and 372, for $^{13}C_{12}$BPA were 364 and 383, and the retention time of the compounds was 12.85 min. The identification was confirmed by retention times and ion ratios. To homogenize the samples a VWR vortex mixer (Radnor, Delaware, USA) was used. For centrifugation, a Thermo Scientific™ Heraeus™ Megafuge™ 16 Centrifuge (Germany) was applied.

### 2.4. Urine samples

Urine samples were obtained from voluntary children that were normal and obese/overweight. The study was approved by the ethics committee of the Centro Hospitalar S. João/FMUP (Medicine Faculty of Oporto University ref. 163.13) and all the parents provided written consent. All samples were stored in glass material at −20 °C until analysis. Urinary creatinine concentration was measured through a modified Jaffe method [72] with an Olympus AU5400® (Beckman–Coulter®, Porto, Portugal) at São João Hospital, Department of Clinical Pathology.

### 2.5. Micro-QuEChERS optimization

Different composition of QuEChERS (Original, AOAC and EN methods) salts as well as two dSPE (dSPE 1 and dSPE 2) were tested through preliminary recovery studies. Additionally, several parameters that can affect the extraction efficiency were analyzed. During method optimization, the following parameters were evaluated: urine (1.0 and 1.5 mL) and ACN (1.0, 1.5 and 3 mL) volumes; ACN acidification (1%