Talanta 178 (2018) 441–448

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of bisphenols with estrogenic activity in plastic packaged baby food samples using solid-liquid extraction and clean-up with dispersive sorbents followed by gas chromatography tandem mass spectrometry analysis☆

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ARTICLE INFO

Keywords:
Endocrine disruptors
Bisphenol
GC-MS/MS
Plastic packaged baby foods

ABSTRACT

Bisphenols (BPs) are a family of chemicals with known endocrine disrupting activity. Bisphenol A (BPA) is the most representative prototype of this group of chemicals. Recently, the use of BPA, a prototype of endocrine disruptors, has been reduced and replaced with structural analogs due to its negative effects on both the environment and consumers. In this work, a new method is presented for the determination of seven BPs, with estrogenic activity in ready-to-eat plastic packaged baby foods. The procedure involves the isolation of the analytes using solid-liquid phase extraction with acetonitrile followed by a clean-up step with a mixture of dispersive-SPE sorbents (C18 and PSA) and magnesium sulphate, to reduce matrix effect from proteins, sugars and lipids. Extraction parameters were optimized using multivariate optimization methods. The compounds were detected and quantified by gas chromatography tandem mass spectrometry (GC–MS/MS). The limits of quantification were between 0.1 and 1.2 ng g^{-1} for the studied analytes. The method was validated using matrix–matched calibration and recovery assays with spiked samples. Recovery rates were between 91% and 110% and % RSD was lower than 13% in all cases. The method has been successfully applied for the determination of these endocrine disrupting chemicals (EDCs) in samples of a novel type of food consumed by preschoolers. This is the first study to analyze EDCs in plastic packaged foods consumed by this target group.

1. Introduction

Global discussion on the potential long-term risks of dietary exposure to EDCs has been ongoing for the last decade. An EDC is a chemical that mimics and antagonizes the biological function of natural hormones [1–4]. Even at low concentrations, chronic exposure to EDCs represents a toxicological concern. This concern increases when these chemicals are present in food for children [5,6]. The list of EDCs is constantly growing as new chemicals, both synthetic and naturally occurring, are found to have endocrine disrupting properties [7]. Bisphenol analogs, which have two phenolic rings banded together through a bridging carbon or other chemical structures, are EDCs, with the prototype being bisphenol A [2,2-bis(4-hydroxyphenyl)propane, BPA]. BPA is a chemical produced in large quantities for use primarily in the production of polycarbonate and epoxy resins. This synthetic material is extensively used in food packaging to protect food from contaminants and to keep the integrity of food and beverages [8]. BPA is found in baby bottles, plastic food containers, epoxy coatings in metal cans, kitchenware, and it is also used in manufacturing toys, medical devices and dental composites and sealants [7,9,10]. Through contact with different types of plastic, such as baby bottles, newborns are exposed to BPA (monomers or additives), which can migrate from the plastic into the foodstuff [11–13]. In addition to being especially susceptible due to their growth and development, exposures are often higher in infants due to body weight and the concern about this issue is of high priority [12,14]. Leaching of plasticizers or additives to the food

☆ In memory of Dª. Teresa Córcoles Fuentes.

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http://dx.doi.org/10.1016/j.talanta.2017.09.067
Received 7 April 2017; Received in revised form 19 September 2017; Accepted 22 September 2017
Available online 23 September 2017
0039-9140/

is affected by time and temperature of storage, heat for sterilization, contact surface, plastic material, type of food and packaging temperatures [15,16]. The European Food Safety Authority (EFSA), in collaboration with the US-EPA, set the tolerable daily intake of BPA at 50 μg kg^{-1} body-weight/day, but it is worth noting that feeding infants with plastic-packed food can result in an intake of 13 μg kg^{-1} body-weight/day [17].

As the concerns about the safety of BPA continue to increase, this compound is being gradually replaced by other bisphenolic compounds in some industrial applications [18]. Bisphenol S [4, 4′-sulfonyldiphenol, BPS], bisphenol F [4, 4′-dihydroxydiphenylmethane, BPF], bisphenol B [2, 2-bis(4-hydroxyphenyl)butane, BPB], bisphenol E [1,1-Bis (4-hydroxyphenyl)ethane, BPE] and bisphenol AF [4,4′-(hexafluoroisopropylidene)diphenol, BPAF] are used as alternatives to BPA in the production of polycarbonate resin [2,19–22]. These BPA analogs are structurally similar to BPA and present similar estrogenic activity. Furthermore, BPS manifests higher estrogenic activity, probably due to its polarity and the presence of sulfur in the structure [22,23] or due to its chemical properties (heat stability and photoresistence) [24]. In this sense, one of the most important aspects of the present research is the determination of free-BPS plastic monomer in these products. This monomer is being used for the synthesis of new plastic for food applications in substitution of BPA. Notably, in the last years, the use to BPA in the production of manufactured products as polycarbonate plastics and epoxy resins for baby food application has drastically decreased. Nowadays, the commercialization of products labeled “BPA free” is constantly growing, but, in parallel, the use to BPA structural analogs such as BPS is increasing in the production of manufactured products but latter compound could not be more safety for human health [25]. Some recent studies have found that BPS is as hormonally active as BPA and, like BPA, it interferes with the endocrine (hormone) system in ways that may produce harmful effects, such as obesity, cancer and neurological disorders [26].

The extensive use of bisphenols (BPs) in foodstuff and the risks associated with this use require the development and implementation of new efficient, sensitive and consistent analytical methods to control human exposure. Mass spectrometric methods, in particular gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS), are currently used for separation, identification and quantification of BPs because of their selectivity, sensitivity and reliability [27]. LC-MS with electrospray is also an adequate technique for determination of BPs [28,29] and GC-MS has been used by some authors to analyze BPs in foodstuff samples [4,12,16,30–33]. For GC analysis, a derivatization step of bisphenols is recommended in order to increase the volatility of the compounds and the sensitivity of the method. The most used derivatization reactions of BPs are silylation [4,24,32] and acetylation [31–33].

In addition, due to the complexity of food matrices, sample treatment involves numerous steps (generally an extraction procedure, followed by clean-up and pre-concentration) prior to analysis. The preferred extraction procedures for BPs from food matrices are liquid-liquid or solid liquid extraction [4,28,34,35]. Depending on the matrix phase (solid or liquid), these methods usually involve the use of acetonitrile, methanol, ethanol, ethyl acetate and dichloromethane as extraction solvents, followed by a clean-up step using reverse SPE columns filled with C_{18} stationary phases [4,6,10]. Additionally, recent sample treatment methods, such as QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) [16,33,35,36], have been applied to remove interfering substances, such as proteins, sugar or lipids, to reduce the total analysis time, and to increase method sensitivity. In some instances, sample treatment involves an acetonitrile extraction, liquid-liquid partitioning with $MgSO_4$ and finally clean-up by solid phase extraction (SPE) with primary secondary amine (PSA) [37]. In addition, to being a solvent-saving method easy to implement, the QuEChERS approach has the advantage of being flexible and selective.

The objective of present work was to develop and validate a sensitive and accurate GC-MS/MS method for the determination of seven bisphenols (BPA, BPS, BPF, BPAF, BPE, BPB and BPP) in baby food samples from different brands. Sample treatment is based on a liquid-liquid extraction with a solvent mixture (acetonitrile/water), and partitioning with $MgSO_4$ combined with NaCl, followed by a clean-up step using dispersive solid phase extraction (d-SPE) with PSA sorbent. After validation, the method was applied to 15 different plastic packaged baby food samples with excellent results.

2. Materials and methods

2.1. Chemicals and reagents

All reagents were of analytical grade. BPA (≥ 99%), BPF, BPS, BPAF (≥ 99%), BPE (≥ 98%), BPB (≥ 98%), BPP (99%) and deuterium-labeled bisphenol A (BPA-d_{16}) were supplied by Sigma-Aldrich (Madrid, Spain). Fig. S1 (Supplementary Material) shows the chemical structures of target analytes. Stock solutions of BPA, BPS, BPF, BPAF, BPE, BPB and BPP (100 mg L^{-1}) were prepared in ethanol, and internal standard (BPA-d_{16}) was also prepared (10 mg L^{-1}) in ethanol. Working standard solution containing all seven analytes at 10 mg L^{-1} each was prepared in ethanol from the stock solutions and stored in amber glass vials at −20 °C until use. This solution was used to spike the baby food matrix for preparing calibration standards and for method validation.

Ethyl acetate and N,O-bis(trimethylsilyl)trifluoro-acetamide with trimethylchlorosilane (BSTFA/1%TMCS) were purchased from Sigma-Aldrich (Madrid, Spain). Acetonitrile and methanol (LC-MS grade) and acetone and ethanol (HPLC grade) were obtained from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q Gradient water purification system from Millipore (Bedford, MA, USA). Sodium chloride and magnesium sulphate were purchased from Panreac (Barcelona, Spain). PSA bonded silica and octadecyl-functionalized silica (C18) were purchased from Scharlab (Barcelona, Spain).

2.2. Instrumentation and software

Determination of the seven bisphenols was performed on a triple quadrupole GC-MS/MS system Agilent 7890 A (Agilent Technologies, Palo Alto, CA, USA) coupled to a detector Quattro Micro GC Waters (USA) triple quadrupole mass spectrometer with inert electron-impact ion source. SIM and full SCAN data acquisitions were performed simultaneously. A ZB-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 μm) from Phenomenex (Torrance, CA, USA) was used. Other laboratory equipment used included a vortex-mixer (IKA, Staufen, Germany), a Mettler-Toledo AND GX400 analytical balance (Columbus, OH, USA), a Hettich Universal 32 centrifuge (Tuttlingen, Germany), a Spectrafuge™ 24D centrifuge from Labnet International, Inc. (New Jersey, USA), a sample concentrator SBHC0NC (Stuart, Staffordshire, UK), and a ScanVac CoolSafe™ freeze dryer (Lynge, Denmark). For sonication of the vials in the reconstitution step, an Ultrasons-HD ultrasonic bath from Selecta (Barcelona, Spain) was used. MassLynx 4.1 software (Waters, Manchester, UK) was used for instrument control and for data acquisition and analysis. Statistical calculations were performed using Statgraphics plus 5.0 (Statpoint Technologies, VA, USA) [38].

2.3. Sample collection, storage and treatment

Fifteen ready-to-eat baby food products, including powder milk, cereals with milk, juices, yoghurt and homogenized of fruit, meat and fish; packaged in plastic containers were purchased from local supermarkets in Granada, Spain, and stored at 4 °C until laboratory analysis.

For analysis, a 2 g portion of each food sample was weighted into a 10 mL glass tube containing 5 μL of a methanol solution (10 mg L^{-1}) with the surrogate BPA-d_{16} (final concentration of surrogate was 50 ng mL^{-1}). The sample was homogenized with 2 mL of water and