A New Solvent Extraction Method with Gas Chromatography–Mass Spectrometry for Bisphenol A Determination in Canned Foods

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A new reliable simple solvent extraction method for the endocrine disruptor bisphenol A (BPA) in canned food was developed employing an aqueous basic extraction solution of 0.25 M K_2CO_3 /0.10 M NaOH after spiking with BPA- d_{16} as internal standard. The BPA was next extracted into diethyl ether after solution acidification to pH = 4 and filtration. Homogenous acetylation at dry basic conditions (acetic anhydride as derivatization agent and solvent with sodium acetate as catalyst) after diethyl ether evaporation was carried out for 30 min at 110 °C. Detection of the acetylated BPA was carried out by gas chromatography–electrospray ionization/mass spectrometry (GC–EI/MS) in the selected ion monitoring (SIM) mode with pulsed split-less mode. The method was applicable in terms of eliminating the use of solvents like acetonitrile for the extraction step, where relatively long evaporation times may have been needed to evaporate acetonitrile. Also, removing lipids and precipitating most of the proteins at acidic conditions (pH = 4) prior to diethyl ether extraction can replace the often used heptane or hexane or solid sorbents. The method was tested linear with limit of linearity (LOL = 750 $\mu\text{g/L}$) and with coefficient of determination ($R^2 = 0.998$), repeatable with relative standard deviation (RSDr < 7%) with instrument detection limit (IDL) of 0.01 $\mu\text{g/L}$ and limit of quantitation (LOQ) of 0.034 $\mu\text{g/L}$. The method detection limit (MDL) ranged from 0.3 $\mu\text{g/kg}$ to 3.2 $\mu\text{g/kg}$ based on 1 g sample (wet weight). Recovery ranged from 85% to 94% with the relative standard deviations of 2%–13%. BPA concentrations in tested canned foods from outlet stores ranged from <MDL to $57.4 \pm (2.6)$ $\mu\text{g/kg}$ which were below the specific limit for BPA migration in food proposed by the European Union (EU) and within the food safety and quality criteria. The extraction and derivatization steps for BPA were unique and have not been reported in literature.

Keywords: Bisphenol A, canned food, acetylation, alkaline solution, solvent extraction

Introduction

Bisphenol A (BPA) is an organic synthetic compound with the chemical formula $C_{15}H_{16}O_2$ and structural formula as in Figure S1 belonging to the group of diphenylmethane derivatives and bisphenols, with two hydroxyl-phenyl groups [1]. World production is now about six million tons per year as pointed out in the Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Meeting on bisphenol A (BPA) sources and occurrence in Ottawa, Canada [2]. Most of it is used for the production of the polycarbonate used to make water bottles and many other consumer products requiring a clear plastic and resins such as the material used to line food cans to prevent corrosion [3], which ultimately leaches from can lining into food by contact during processing and production, storage conditions, and can lining decay by time [4]. Bisphenol A is an endocrine disruptor—a substance which interferes with the production, secretion, transport, action, function, and elimination of natural hormones [5, 6]. BPA can imitate our body's own hormones in a way that could be hazardous for health [7]. Also, babies and young children are said to be especially sensitive to the effects of BPA [8]; these harmful effects of BPA urged governmental and consumer health agencies to investigate the presence of BPA in food and set related regulations (BPA No-Observable-Adverse-Effect Level [NOAEL], 1 mg/kg bw/day, total daily intake [TDI] of 50 $\mu\text{g/kg/day}$) [2, 9]. Many different analytical methods were proposed and conducted for the detection and determination of BPA in canned food and other matrices employing different extraction, purification, derivatization approaches, and methods, with different instrumental

detection methods [1, 9]. All developed and adapted methods were aiming to be simple, reaching low limits of detection (LODs), accurate, precise, and cover adequate range of BPA concentrations. Liquid chromatography methods (liquid chromatography [LC], high-performance liquid chromatography [HPLC], or ultra-performance liquid chromatography [UPLC]) coupled to different detectors including ultraviolet (UV), fluorescence (FL), electrochemical detection (ECD), mass spectrometry (MS), and tandem mass spectrometry (MS/MS) were developed, validated, and adapted to determine BPA in different food matrices with different extraction approaches as summarized by Cao [1, 9] and Voutsas [10]. Recently, Rozaini et al. [11] used rapid ultrasound-assisted emulsification microsolid-phase extraction based on molecularly imprinted polymer for HPLC–diode array detection (DAD) determination of bisphenol A in aqueous matrices. In 2017, Cunha and coworkers [12] developed QuEChERS-based extraction and liquid chromatography–tandem mass spectrometry method for simultaneous quantification of bisphenol A and tetrabromobisphenol A in seafood: fish, bivalves, and seaweeds. Pasquale and coworkers [13] determined BPA, bisphenol B (BPB), bisphenol F (BPF), bisphenol A diglycidyl ether (BADGE), and bisphenol F diglycidyl ether (BFDGE) in canned energy drinks by molecularly imprinted polymer cleaning up and UPLC with fluorescence detection. Other groups used and developed methods based on solid-phase microextraction, QuEChERS-like liquid–liquid partition in the presence of salts, magnetic solid-phase extraction, and vortex-assisted supramolecular microextraction employing HPLC and LC–MS [14–19]. Gas chromatography (GC) coupled to MS, flame ionization detector (FID), or ECD for BPA detection using different extraction procedures till 2013 are also well summarized by Cao [1, 9] and Voutsas [10]. Using GC for BPA and other semi- and non-volatile compounds

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determination mandates a derivatization step [20]; this derivatization step involves chemical modification of the chemical compound to produce a new compound with properties suitable for GC analysis (less polarity, higher volatility, higher stability, and higher peak efficiency and detectability) [21]. This is achieved by employing different derivatization approaches like alkylation [22], silylation [23], and acylation [24] where product stability and percent yield depends on reaction conditions and reagents used [20, 21]. Recently, Cesen and coworkers [25] determined bisphenols and related compounds in honey and their migration from selected food contact materials using Oasis HLB cartridges, silylated and analyzed by GC–MS. In 2015, Cao and coworkers [26] reported levels and temporal trend of bisphenol A in composite food samples from Canadian total diet study 2008–2012 using the same procedure used previously in 2008 by Cao [27]. Where BPA was extracted with acetonitrile after spiking sample with BPA- d_{16} , followed by a buffering step to pH = 7 and cleaning on SPE₁₈ cartridge. This was followed by an acetylation step at room temperature with emulsion of 1.0 M K_2CO_3 , acetonitrile, acetic anhydride, and extraction with isooctane (provided a combination of derivatization and extraction of the reaction product). Cunha et al. (2013) [28] assisted bisphenol A and bisphenol B in canned vegetables and fruits by gas chromatography–mass spectrometry after QuEChERS and dispersive liquid–liquid microextraction (DLLME), where the DLLME procedure involved the use of tetrachloroethylene as extractive solvent while the own acetonitrile extract obtained from QuEChERS was used as dispersive solvent and acetic anhydride as derivatizing reagent (heterogeneous reaction conditions). Fontana et al. (2011) [29] developed an ultrasound-assisted emulsification microextraction in situ derivatization (USAEMEISD) with acetic anhydride for the one-step derivatization, extraction, and pre-concentration of BPA in beverage samples prior to its determination by GC–MS. In this study, we have introduced a new simultaneous solvent extraction and cleaning procedure (excluding organic solvents like acetonitrile or methanol for BPA extraction, and hexane, or heptane for proteins and lipids removal) which is different from the aforementioned procedures. Also, acetylation reaction (derivatization) conditions also excluded the use of dispersive solvents like acetonitrile in the absence of water. The extraction and derivatization steps for BPA were unique and have not been reported in literature. Canned food is initially extracted into alkaline medium (BPA is highly soluble in this medium) and filtered, and then filtrate was followed by pH adjustment to 4 to precipitate proteins. This guarantees that extract is free from all lipophilic proteins and lipids with eliminating the need of solvents like acetonitrile, hexane, and heptane for the extraction and cleanup steps. This was followed by diethyl ether selective extraction to BPA leaving behind water soluble compounds including carbohydrates, followed by further acetylation step (diethyl ether is evaporated in few min to dryness) for 30 min at 110 °C. The acetylated BPA is monitored quantitatively in the GC–EI/MS in the selected ion monitoring (SIM) mode. To our knowledge, this solvent extraction procedure accompanied with a further acetylation step in dry conditions and detection using GC–EI/MS in SIM mode accompanied with the pulsed split-less mode has not been reported in literature.

Experimental

Chemicals and Solvents. BPA (purity >99%), BPA- d_{16} (98 atom % D), acetic anhydride (min. assay 99%), and sodium sulfate (AR, anhydrous min. assay 99.9%) were purchased from Sigma-Aldrich (USA). Dichloromethane (GC grade, min. assay 99.9%), hexane (min. assay 99.5%), and ethyl ether anhydrous (min. assay 99.9%) were purchased from TEDIA (USA). Sodium acetate (min. assay 99.9%) was purchased from

Gainland Chemical Company, UK, sodium hydroxide pellets (ACS grade, min. assay 98.5% from Scharlu), potassium carbonate (ACS grade, min. assay 99% from BDH chemicals/UK), and water (HPLC grade from Fisher Scientific/USA). Sodium sulfate was heated at 300 °C in a muffle furnace (Carbolite model ELF 11/6B/UK).

Sampling. Thirty food cans with different brand names and contents were bought from food outlet stores in Amman-Jordan representing most used items at shelves. Labeling detailed information about each food can was recorded including carbohydrate/sugar, protein content, fat, sodium, and calcium content, in addition to net weight and expiry date. The pH of each food sample was measured, and cans were classified as 2-piece can or 3-piece can and which piece surface is lacquered with the grey-white coating responsible for the BPA migration to food as described in Table 1. Purchased food cans were stored at laboratory shelves, and then each can was opened where all the food contents were homogenized with Warning two-speed laboratory blender (120 Vac model, USA). A 1–10 g portion when necessary was taken for the extraction step, where 50% of the samples were portioned in duplicate. The rest of the homogenized food was returned to the same can with lid and wrapped with aluminum foil and kept in freezer at –20 °C.

Sample Preparation. All glassware was previously heated to 220 °C to eliminate any possible contamination with BPA and rinsed with ethanol or acetone if necessary. Also, the stainless steel blender container, after homogenizing each sample using Warning two-speed laboratory blender (120 Vac model, USA) was washed well with detergent, rinsed well with tap water and deionized water, and then rinsed with ethanol and dried with air dryer. A 1–10 g portion (wet weight) of each pre-homogenized sample was spiked with 400 μ L of 100 μ g/L BPA- d_{16} and addition of 20.0 mL of 0.25 M K_2CO_3 /0.1 NaOH followed by blending and mixing for 2 min and left to stand for 5 min. The aqueous alkaline extract was filtered using suction filtration employing a fritted glass funnel, and then, the solid sample residue was washed with 15.0 mL (0.25 M K_2CO_3 /0.1 NaOH). The pH of the pooled filtrate was adjusted to 4 using 1.2 M HCl and transferred into a 250.0 mL separatory funnel where there it was extracted with two portions of 20.0 mL diethyl ether. The ether upper layer was removed and transferred to a beaker containing sodium sulfate. The dry ether layer was decanted into a second dry beaker and left on a hot plate (30 °C) to evaporate to dryness. The walls of the dry beaker were then rinsed with 5.0 mL diethyl ether, and the contents were transferred into a 10 mL reaction vial (Reacti-vial Thermo, USA) where diethyl ether was evaporated using a gently stream of nitrogen at 40 °C. Three milliliters (3 mL) of acetic anhydride and 0.5 g sodium acetate (excess amounts to make acetylating conditions basic) were added to the reaction vial, and then, it was sealed tightly with screw cap and heated on thermo heating block (Reacti-Vap-Evaporator model, Thermo Fisher Scientific, USA) at 110 °C for 30 min. After cooling down, reaction was quenched with water (to destroy excess acetic anhydride and remove excess catalyst), and the acetylated BPA and BPA- d_{16} were extracted with two portions of 2.0 mL (50%:50%) hexane–dichloromethane and passed through sodium sulfate columns into 10 mL conical bottom centrifuge tubes. The hexane–dichloromethane mixture was evaporated into to dryness under using a stream of nitrogen, and then, 200 μ L hexane–dichloromethane was added to the tube followed by agitation on vortex mixer (KMC-1300 V from Vision Scientific Co. Ltd.) for 30 s and then transferred into GC vial with 250 μ L insert for GC–EI/MS analysis. Method blank is prepared by taking 1–10 g of 0.25 M K_2CO_3 /0.1 NaOH and applying the above mentioned procedure.

Table 1. Method detection limits and percent recoveries of BPA in different food matrices

Food type	BPA spiking concentration (µg/kg)	MDL (µg/kg)	BPA spiking concentration (µg/kg)	Percent average recovery ± RSD*
Homemade chick peas	0.40	0.30	90 60 30	94 ± 6 93 ± 3 91 ± 4
Homemade Medames Fava Beans	0.40	0.40	90 60 30	91 ± 4 93 ± 3 90 ± 5
Condensed milk from farm	5.00	3.18	60 30 5	91 ± 9 90 ± 11 88 ± 13
Sausages from Butchery	3.00	1.20	10 5 3	89 ± 4 88 ± 5 87 ± 3
Sliced beets	1.00	0.80	10 5 1	89 ± 6 90 ± 3 88 ± 5
Chili made with organic black beans and spice	0.40	0.40	10 5 1	94 ± 6 92 ± 4 90 ± 4
Rice and beans	0.40	0.38	10 5 1	93 ± 6 91 ± 4 90 ± 6
Sardines	5.00	2.52	30 10 5	86 ± 9 88 ± 11 84 ± 8
Homemade green peas and carrots in tomato sauce	3.00	0.92	90 60 30	92 ± 7 90 ± 4 89 ± 5
Corn	1.00	0.50	30 10 5	90 ± 5 92 ± 2 91 ± 3
Sliced Pears light syrup	1.00	0.44	10 3 1	94 ± 3 94 ± 5 93 ± 6

* RSD: relative standard deviation.

Chromatographic Conditions and MS Detection. In the GC–MS (Agilent 6890 series II gas chromatograph with auto-sampler injector series 7683 and Agilent 5973 N [MSD]), an optima Delta-3 capillary column (25 m × 0.20 mm × 0.20 µm) with helium as a carrier gas were used. A 2 µL injection volume with pulsed split-less mode for 0.08 min at a constant flow of 20 mL/min was employed with column flow rate at 1 mL/min. The injector temperature was set at 285 °C, auxiliary (transfer line) at 285 °C, and the oven temperature program was as follows: 120 °C (held for 2 min), ramp at 10 °C/min to 275 °C (held for 3 min). The mass spectrometer source was operated in the electron impact ionization mode (EI) at 70 eV and 230 °C whereas the mass analyzer was operated in the SIM mode at 150 °C for better sensitivity, selectivity, and matrix effect eliminating. Calibration of the mass spectrometer was performed by autotuning mode in Chemstation software using perfluorotributylamine (PFTBA) with tuning masses (69/219/502). The monitored ions (*m/z*) for the diacetyl derivative of both BPA-d₁₆ and BPA were 224/242/284 and 213/228/312 as obtained from GC–MS spectra in the scan mode of a standard mixture of diacetylated BPA-d₁₆ and BPA, respectively, whereas retention times were 17.06 ± 0.02 min and 17.19 ± 0.03 min, respectively, as indicated in Figure 1.

Linear Calibration Curve Standards. Stock solutions of BPA (10.0 mg/L) and BPA-d₁₆ (10.0 mg/L) were prepared by weighing accurately the exact amount of each using microbalance (MXA5, RADWAG, Poland) and dissolved in 0.25 M K₂CO₃/0.1 M NaOH solution, where appropriate dilutions were made to end up with the working standards. Diacetylated BPA ten calibration standards (1–750 µg/L) were obtained by serial dilutions of the BPA stock solution and working standard (1.0 mg/L) with 0.25 M K₂CO₃/0.1 M NaOH solution. Each individual standard was subjected to the above mentioned procedure.

Linear Range. For the calculation of the performance data, a calibration was carried out with ten concentration levels of PBA in the range from 1 to 500 µg/L and up to 750 µg/L in addition to the blank, where a plot of concentration of BPA vs. BPA/BPA-d₁₆ area ratio from mass detector was plotted. From the resulting calibration curve, the regression coefficient was calculated, characterizing the linearity of the calibration function. The coefficient of determination was 0.998, indicating a very good linearity of the calibration function in this concentration range.

Instrumental Detection Limit and Limit of Quantitation. The instrument detection limit (IDL) was obtained by diluting the standard mixture solution until the ratio of signal to noise (*S/N*) is equal to 3, while the limit of quantitation (LOQ) was calculated as (*S/N*) ratio equal to 10. The calculated IDL was 0.01 µg/L, and LOQ was 0.034 µg/L. LOQ was verified to be within the linearity dynamic range.

Method Detection Limit, Repeatability, and Recovery. The method detection limit (MDL) was calculated using the following equation:

$$\text{MDL} = t(n - 1, 1 - \alpha = 0.99) \times \text{SD}$$

where SD is the standard deviation of seven replicates of a definite spiked sample concentration in µg/kg, *t* is the degrees of freedom, *n* is the number of replicates, and α is the confidence level ($t = 3.143$ for $n = 7$ at 99%). Homemade samples (not canned) were tested to be void of BPA including homemade chick peas, Medames Fava Beans, farm condensed milk, sausages from Butchery, corn from farmers market, and home-made green peas and carrots, which were spiked with four concentration levels (0.4 µg/kg, 1.0 µg/kg, 3.0 µg/kg, and 5.0 µg/kg) of BPA, then extracted and acetylated as in the abovementioned procedure for GC–EI/MS in the SIM mode

Figure 1. GC-EI/MS total ion chromatogram (TIC) of a standard mixture of diacetylated BPA- d_{16} and BPA respectively in the scan mode (a) mass-spectra of the diacetylated derivatives of BPA- d_{16} (b) and BPA (c)

analysis. For other matrices, canned food samples were spiked with the appropriate level of BPA. Also, average recoveries were calculated ($n = 3$) for the above void BPA samples by spiking them with appropriate BPA concentration (three concentration levels) close to the concentration found in real canned foods as in indicated in Table 1. The repeatability and intermediate precision were evaluated at three concentration levels (1 $\mu\text{g/kg}$, 30 $\mu\text{g/kg}$, and 90 $\mu\text{g/kg}$) ($n = 6$). The calculated MDL values ranged from 0.3 $\mu\text{g/kg}$ (homemade check peas) to 3.2 $\mu\text{g/kg}$ (farm condensed milk), whereas the recovery ranged from 85% (sardines) to 94% (Sliced Pears in light

syrup) with the relative standard deviations (RSDs) of 2%–13% as indicated in Table 1.

Results and Discussion

The obtained BPA quantitative GC-EI/MS results for the tested canned foods are summarized in Tables 2 and 3 indicated with the food type and manufacturing country with final units in $\mu\text{g/kg}$. Samples were injected in triplicate with 50% of the samples prepared in duplicate in addition to method blanks. The levels of PBA in the samples were ranged from <MDL to

Table 2. BPA concentrations in different types of canned food with their labeling detailed information

Product name/country	Average BPA $\pm$ (SD) ($\mu\text{g/kg}$)	pH ^a	Sugar ^b	Protein ^b	Sodium ^b	Lipids ^b	2- or 3-piece/lacquered part
Luna cream/KSA	28.1 $\pm$ (2.1)	5.50	3.0	1.5		21.0	2-piece/all lacquered with light grey-white
Sliced Pears light syrup/China	<MDL	3.80	^c	^c	^c	^c	Glass jar with metallic lid/lacquered white from inside
Hummus tahini/Al-Rawabi/Jordan	12.6 $\pm$ (1.7)	4.50	^c	7.3	0.32	1.8	3-piece/all pieces coated white
Cucumber in brine/Zadona/Palestine	6.6 $\pm$ (0.4)	4.60	3.0	2.0	0.99	0.6	3-piece/all pieces coated white
Whole artichoke hearts/Lariqueza Espanola/Spain	16.2 $\pm$ (1.2)	4.50	1.0	3.0	0.400	0.0	3-piece/all pieces coated white
Artichoke hearts/Spain	13.4 $\pm$ (0.9)	4.50	8.0	1.6	0.402	0.12	3-piece/all pieces coated white
Hearts of palm/Ecuador	10.3 $\pm$ (1.1)	4.50	5.0	2.0	0.290	0.0	3-piece/all pieces coated white
Luna Chick peas/Jordan	57.4 $\pm$ (2.6)	5.55	0.6	6.6	0.295	1.9	2-piece/all grey white and lid white
Green peas and carrots/Al-rawabi/Jordan	16.1 $\pm$ (1.8)	5.50	22.5	7.3	0.32	1.8	3-piece/all grey white and lid white lacquering
Green peas/Luna/Jordan	42.2 $\pm$ (2.2)	5.70	11.0	5.2	0.760	0.0	2-piece/all grey white and lid white lacquering
Peaches halves in syrup/South Africa	<MDL	3.95	26/per 140 mL serving	1	0.000	0.0	2-piece (unlined tin plated steel)
Beets sliced/USA	n.d.	5.10	8.0/120 g serving	<1 g	0.250	^c	2-piece/all yellowish-lacquering
Cheddar cheese/Bahrain	2.2 $\pm$ (0.8)	—	1.0	16.5	1.73	24.5	2-piece easy pull lid/excluding lid lining is white
Zawan cocktail sausages chicken/Holland	<MDL	6.00	4.5	11.0	^c	12.5	No grey or white lining (may be tin plated steel)
Plain Fava beans/UAE	30.7 $\pm$ (1.6)	5.70	15.0	7.0	0.430	1.0	3-piece/all pieces coated with white lining

^aMeasured value.^bg/100 g wet weight.^cStands for not indicated.

57.4 $\pm$ (2.6) $\mu\text{g/kg}$ with some samples labeled as n.d. (not detected). Two of the samples that indicated with n.d. were rice and beans, and chili made with organic black beans, where their labeling indicated BPA-free lining. These two samples were used as quality control (QC) standards after spiking with 1 $\mu\text{g/kg}$ and 5 $\mu\text{g/kg}$ of BPA as indicated in Table 1. Both samples showed relative errors of -8% which are within the food analysis criteria. Also, all spiked samples in Table 1 showed recovery values from 85% (sardines) to 94% (Sliced Pears in light syrup) with relative standard deviations (RSDs) of 2%–13% affirming the trueness of the method according to the guidelines for performance criteria and validation procedures of analytical methods used in controls of food contact materials [30]. The obtained recovery values in this study especially for food samples with high fat content like sardines (85 $\pm$ 5) were lower than those reported by Kawamura and coworkers in 2014 (recovery = 97.1 $\pm$ 4.4) [22] employing methanol as extracting solvent and hexane for cleanup step and fat removal, but higher than reported recoveries by Cunha and coworkers in 2013 (recovery = 77% at 5 $\mu\text{g/kg}$ spiking level) [28] employing heptane for fat removal, acetonitrile, and solid sorbent for BPA extraction. The samples with BPA concentrations <MDL were in glass containers or cans that are void from any BPA lining like tin, gold plated, or steel plating. This indicated also that raw food (before canning) contamination with BPA was minor and could be coming from different sources. Regarding the meat tuna in salted water (tin plated can produced in Thailand) and light meat tuna chunks in vegetable oil (Thailand), the BPA concentrations were 2.6 $\mu\text{g/kg}$ and 2.7 $\mu\text{g/kg}$ respectively which are a little bit higher than the MDL (2.5 $\mu\text{g/kg}$) which could be attributed to minor cross contamination from other source (production line, sea). Other samples ranged from 2.2 $\pm$ (0.8) $\mu\text{g/kg}$ (cheddar cheese) to 52.3 $\pm$ (2.5) $\mu\text{g/kg}$ (chick

peas) where most of these canned foods had in common; the 3-piece can with white color lacquered heavily from inside (most of them produced in Jordan, United Arab Emirates, and Kingdom of Saudi Arabia). The most striking feature of the analysis is that all Medames Fava Beans and chick peas or hummus cans are those with the highest BPA concentrations reaching maximum value of 57.4 $\pm$ (2.6) $\mu\text{g/kg}$ and minimum value of 12.6 $\pm$ (1.7) $\mu\text{g/kg}$ with an average value of 38.2 $\pm$ (3.1) $\mu\text{g/kg}$. These relatively high results were expected since these food cans were totally lacquered heavily from inside with the white lining (epoxy resins that leaches BPA) with adequate solution salinity, in addition to the manufacture production step that involves adding hot fava beans or chick peas after passing frying machine to the cans ending with sterilization step (heating under steam), which give ideal conditions for BPA to leach from resin. They could be consumed in daily basis with an average consumption of 250 g per person (traditional meal) in Middle East and Africa region (MENA). Canned green peas samples also showed BPA concentrations of 42.2 $\pm$ (2.2) $\mu\text{g/kg}$ (2 pieces/all grey white and lid white lacquering that are epoxy resins that could leach BPA). Still, in this research, one of the aims was to investigate the concentrations of BPA in different food types without further going in correlations to food type and food ingredients. According to a small-scale consumer questioner and market survey on canned foods in Jordan, the most sold and consumed items are fava beans, chick peas or hummus, tuna and sardines, corn and baby corn, sliced black olives, and finally condensed milk. Still, no knowledge is available about the true quantities consumed daily. The measured obtained maximum BPA values using the developed method are lower than those values reported in other countries like Canada [9], New Zealand [31], Belgium [32], Portugal [28], Turkey [19], US [33], UK [34], and

Table 3. Continue BPA concentration in different types of canned food with their labeling detailed information

Product name	Average BPA± (SD) (µg/kg)	pH ^a	Sugar ^b	Protein ^b	Sodium ^b	Lipids ^b	2- or 3-piece/lacquered part
Chick peas/Red rose/Jordan	52.3 ± (2.5)	5.68	^c	^c	^c	^c	2-piece/all lacquered with white lining
Medames Fava Beans/UAE	35.8 ± (2.2)	5.58	17.38/per 150 g serving	8.3	0.499	2.3	2-piece/all lacquered with white lining
Medames Fava large Beans/UAE	32.9 ± (1.8)	5.58	18.45/per 150 g serving	8.3	0.610	2.0	2-piece/all lacquered with white lining
Sliced black olives/Spain	12.5 ± (0.1)	6.56	2.0/30 g serving	0.0	0.262	3.0	2-piece with pull open top lid (excluding lid white lining from inside)
Spiced sardines in vegetable oil/Morocco	<MDL	6.35	0.00	22.3	0.24	8.3	2-piece tin plated steel with easy open lid
Sweetened Condensed milk/Germany	n.d.	6.30	56.5	7.1	0.300	8.0	2-piece tin plated steel with pull open lid
meat tuna in salted water/Thailand	2.6 ± (0.8)	6.40	0.00	27	1.0	0.5	2-piece easy open lid enameled tin plated lining
Chili made with organic black beans and spices/USA	n.d.	5.60	36/per 250 serving	10	0.48	2.0	Labeled BPA free lining
Rice and beans/USA	n.d.	6.12	21/per 130 serving	4.0	0.200	1.0	Labeled BPA free lining
Whole baby corn in brine/Thailand	15.1 ± (1.3)	5.00	8 g/per 115 g serving	3 g/per 115 g serving	270 mg/per 115 g serving	0.0	2-piece easy open lid grey-white lining
Light meat tuna chunks in vegetable oil/Thailand	2.7 ± (0.8)	5.7	0.0	25	400 mg/100 g	5.7	2-piece drawn and ironed steel
Red kidney beans/Italy	n.d.	5.80	5.3 g/100 g serving	4.4 g/100 g serving	300 mg/100 g serving	0.3 g	2-piece tin plated steel with pull open lid
Chicken luncheon meat/Holland	n.d.	—	7 g/ 100 g	12 g/100 g	—	15 g/100 g	2-piece tin plated steel with pull open lid
Papaya in syrup/Thailand	<MDL	4.00	11.3 g/100 g	<1 g	^c	0.00	3-piece can, interior coating gold

^aMeasured value.^bg per 100 g wet weight.^cStands for not indicated.

France [35], but higher than that reported maximum BPA value in domestic canned food in Japan [22] as indicated in Table S₁. Also, the obtained results are much below the specific migration limit of 600 mg/kg set by EU commission (Commission Regulation EU 10/2011) [36]. With regard to sensitivity and linearity of the method, the lowest MDL obtained was 0.3 µg/kg based on 1 g portion of the sample which is comparable with previously reported methods [26, 28] that employ the acetylation procedure at different conditions as shown in Table S₁. The linearity of the method was in between low and high linear calibration ranges reported in literature where some are shown in Table S₁. The developed method showed excellent validation for selectivity where the internal standard (BPA-d₁₆) and target analyte (BPA) peaks were well resolved at retention times 17.06 ± 0.02 min and 17.19 ± 0.03 min, respectively, in calibration standards and canned food samples with no interfering peaks. The aforementioned compounds were also confirmed with their finger print mass fragments (224/242/284) and (213/228/312), respectively, and employed method blank for subtraction as indicated for different samples in Figure 2 based on area ratio. Since varying trace or minor amounts of BPA were sometimes detected as expected in method blanks since BPA exists everywhere, one blank sample was carried out and analyzed for each batch of real samples as shown in Figure 2 and Figure S₂ where minor amounts of BPA in method blank in different batch were detected. The solvent extraction procedure was applicable in removing lipids, proteins, or any major interferences as noticed in Figure 2, where the method blank, spiked standard, and canned food sample shared the same background in the pre- and post-regions of BPA-d₁₆ and BPA peaks in the GC chromatograms. The developed method showed good repeatability (RSDr < 7%, *n* = 6) and good intermediate precision (RSDw < 11, *n* = 6) not exceeding the acceptable criteria for precision (% relative standard

deviation [RSD] = 22.6% based on 100 µg/kg concentration [30]. Also, canned food results had standard deviations (SDs) ranging from 0.42 µg/kg to 2.6 µg/kg which correspond to RSDs of 6.3% and 4.5%, respectively.

Conclusion

The introduced analytical method (solvent extraction, acetylation, detection) developed in this study demonstrated to be reliable in terms of the low MDL (0.3 µg/kg) for BPA compared with other methods and closeness to other reported values. Also, the method showed excellent linearity (0.034–750 µg/L), accuracy expressed in recovery values (85%–94%), trueness as demonstrated by relative error of –8% for QC standards after spiking with 1 µg/kg and 5 µg/kg of BPA, repeatability (RSDr < 7%, *n* = 6), and good intermediate precision (RSDw < 11, *n* = 6), according to the guidelines for performance criteria and validation procedures of analytical methods used in controls of food contact materials. It also demonstrated simplicity, relative short time of analysis (18 min run time), and cheapness and availability of chemicals, especially for acetic anhydride which is a cheap and available derivatization reagent with boiling point at 139.8 °C that is convenient for use with mass detectors and a useful alternative for silylation. In addition to the possibility of application to a wide range of food types, the BPA concentrations ranged from <MDL to 57.4 ± (2.6) µg/kg with the highest content found in chick peas or hummus cans in addition to green peas (42.2 ± (2.2) µg/kg). The method provides an alternative procedure for BPA extraction utilizing alkaline solution of (0.25 M K₂CO₃/0.10 M NaOH) instead of using the most common used organic solvents like acetonitrile and methanol. Also, it provides a cleanup step by lowering the pH to 4 followed by diethyl ether extraction similar to using

Figure 2. GC–EI/MS in the SIM mode chromatogram of (a) method blank, (b) 250 µg/L, calibration standard, and (c) artichoke hearts sample, where peaks 1 and 2 correspond to diacetylated derivatives of BPA- d_{16} and BPA, respectively

organic solvents like cyclohexane or solid-phase extraction (SPE). The detected levels of BPA found in this study were much below the specific migration limit of 600 mg/kg set by EU Commission (Commission Regulation EU 10/2011). Still, in the future, a more comprehensive food survey of BPA with detailed information about most consumed and purchased items as well as their daily consumption amounts, employing independent analytical methods in all types of canned foods, is needed in the Jordanian market to build an exposure risk assessment model that includes daily intake from different canned food items in units of µg/kg/day. This will help to draw a general conclusion and raise consumer awareness about BPA concentrations in canned food, frequent monitoring of BPA in canned food, and urging

legislation agencies in Jordan to obligate food industry to use alternatives to BPA can linings. To our knowledge, this is the first study of its kind to be conducted in Jordan.

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