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Original Article

A VALIDATED UPLC-UV METHOD FOR BISPHENOLA (BP-A) LEVELS DETECTION IN IMPORTEDPLASTIC TOYS AND DRINKING BOTTLED WATER IN KUWAIT

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ABSTRACT

Objectives: Bisphenol A (BP-A) is an essential component of polyvinyl chloride, polystyrene, phthalates and polycarbonate plastics linked by ester bonds, and it can leach out of plastics at high temperature, acid and basic medium. BP-A is known to have an endocrine disrupting effect and recent studies have started to link its levels as causative factors in many diseases such as diabetes, obesity, and other endocrine diseases. Kuwait is considered one of the hottest countries in the world, and measurements of BP-A levels due to leakage from plastics have never been reported from this Middle-Eastern country. This study measures the levels of BP-A in four randomly selected plastic toys and two plastic water bottles from two different companies after storage at 45 °C for four days.

Methods: An ultra-pressure liquid chromatography coupled with an ultraviolet detector (UPLC-UV) analytical method was established to investigate BP-A levels in four of randomly chosen plastic toys (plastic tiger-plastic Lego blocks-plastic doll-small dolls) stored at 45 °C for 4 days. BP-A was extracted with 1 L of water and samples were analyzed by UPLC-UV.

Results: The limit of detection (LOD) and the limit of quantification (LOQ) of the established analytical method were equal to 0.4 ppb and 1 ppb, respectively. The analytical method was able to measure accurately and precisely traces of BP-A in all randomly selected toys. BP-A levels was239 ppb in plastic tiger, 30 ppb in plastic Lego, 4 ppb in plastic doll, 3 ppb in small dolls and 59 ppb in drinking bottled water.

Conclusion: The importance of BP-A level in plastics analysis raised due to its health concerns. Heat is a major factor for bisphenol leakage from plastics. However, Kuwait is considered one of the hottest countries, where high level leakage of BP-A from plastic toys and plastic water bottles for drinking water could pose significant health risks. Surprisingly, BP-A was detected in all randomly selected plastic toys and one out of two randomly selected polyethylene terephthalate (PET) drinking bottled water. Therefore, imported mineral water should be filled in a glass container rather than plastics due to high climate temperature. Moreover, Toys manufacturers should use BP-A free plastics or clearly specify storage conditions of their plastic products in order to prevent potential health risks resulting from BP-A leakage.

Keywords: Bisphenol A, Endocrine disruptor, Ultra Pressure Liquid Chromatography, Childhood obesity, Breast cancer, Altered pubertal development, Polyvinyl chloride, polystyrene, Phthalates, Polycarbonate plastics.

INTRODUCTION

Bisphenol A (BP-A, fig. 1) is a monomeric compound synthesized in the 1930s by Dodds and Lawson to mimic estrogenic effect in humans. In the 1950s, scientists employed BP-A in making epoxy resins and polycarbonate plastics due to its desirable chemical and physical characteristics [1]. However, BP-A is a well-known endocrine dis rupter and it is considered one of the highest produced chemicals worldwide. Present reports indicate that BP-A production is more than six billion pounds annually [2]. Scientifically, BP-A is the building block of polyvinyl chloride, polystyrene, phthalates and polycarbonate plastics often used in various consumer products including medical equipment, tableware, plastic water bottles, toys, thermal receipts, pharmaceutical containers and food linings [3]. Additional uses for BP-A include common household and workplace items as the coating of CDs, DVDs, electrical and electronic equipment, automobiles and sports safety equipment [3].

However, BP-A molecules are linked by ester bonds which are susceptible for hydrolysis when exposed to high temperatures, acidic and basic media leading to BP-A leakage from polyvinyl chloride, polystyrene, phthalates and polycarbonate plastics [4]. Recent studies correlates serum and/or urine BP A levels with various diseases, health outcomes and medical conditions such as diabetes [5], cardiovascular diseases [6], carcinogenic risk [7], premature deliveries and breast cancer in females [8, 9], lowered semen quality and sperm DNA damage in males [10, 11] as well as childhood obesity and altered pubertal development [12, 13]. Moreover, BP-A has been detected in varied types of media including air, municipal water, soil, sediments and food items. Thus, people can be exposed to BP-A through different sources. Although oral route is thought to be the main route of BP-A exposure, inhalation and dermal routes are also possible [14, 15]. In addition, plastic toys are considered to be a major source of BP-A at childhood stage as most of toys are made of polyvinyl chloride and polystyrene plastics, nevertheless toys manufacturers never stated leakage risks or an appropriate storage conditions for their plastic products. According to the European Food Safety Authority (EFSA), tolerable daily intake of BP-A should not exceed 50 µg/Kg body weight/day [3], which is the safety standard limit. However, different studies have detected high levels of urinary BP-A in school children, since children are always in contact with plastic toys either by touching and/or sucking. Only few studies measuring BP-A leakage levels from plastics at high temperatures have been reported.

Moreover, most of these studies were performed by complicated instrumental techniques and/or extraction methods [16-18]. Globally, Kuwait climate is considered as one of the hottest climate in Asian where the temperature may reach up to 54 °C during the summer months [19]. Therefore, level of BP-A leakage from plastic toys is expected to be high due to long contact with high temperature as well as improper storage conditions. Nevertheless, levels of BP-A leakage from plastic toys in Kuwait have never been neither measured nor reported. Thus, this study reports a simple and validated analytical method established to indicate BP-A levels in four randomly selected plastic toys and two drinking bottled water from two different brands stored at 45 °C.

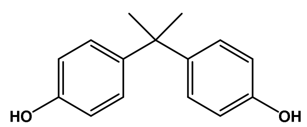

Fig. 1: Chemical structure of bisphenol A (BP-A)

METHODS AND MATERIALS

Chemical and materials

High performance liquid chromatography (HPLC) grade acetonitrile and formic acid were obtained from Sigma Aldrich (Dor-set, UK). HPLC grade water was prepared ''in house'' with a MilliQfilter (Millipore, Watford, UK).

Bisphenol A ≥ 99% was purchased from Sigma Aldrich (Dor-set, UK). A tiger toy, small doll, large doll, 4 Lego blocks and drinking bottled water were purchased from a local supermarket (Al-Mualem). Nylon solvent filters (0.45 um) were obtained from Waters (Milford, USA).

Chromatographic condition

Isocratic elution was carried out with a mobile phase comprised of filtered and degassed 0.1% w/v Formic acid in water (pH 4) and acetonitrile in proportion of 40:60 v/v and pumped at a flow rate of 0.1 ml/min. Column temperature was set at 45 °C and samples, were analyzed at a wavelength of 280 nm and were injected at 10 µm injection volume.

UPLC instrumentation

Waters® Acquity UPLC system with Binary Solvent Manager, Sample Manager and UV detector, Waters® Acquity UPLC BEH C18, 1.7 um, 2.1 x 50 mm analytical column was used for the analysis and method validation. Empower® software was used for data processing and reporting.

LC-MS

A Thermo Scientific LCQ Fleet mass spectrometer was connected to Thermo Scientific Surveyor Liquid Chromatography (LC) plus system via electro spray ionization (ESI) interface. The operating parameters in the negative ion mode were as follows: the sheath gas and auxiliary flow rates were set at 30 and 5 (arbitrary unit), respectively. The capillary voltage was set at 35 V and the ESI needle voltage was controlled at 4.5 kV.

Sample preparation

Bisphenol A was simply extracted by dipping each plastic toy and drinking bottled water in 1 L of HPLC grade water and 1 ml was drawn using Gilson pipette and placed in a 2 ml glass screw thread vials for analysis. Analysis and extraction were done after storing plastic toys and drinking bottled water at 45 °C for four days.

Calibration curve

Stock solution containing 1 ppm of bisphenol A dissolved in HPLC grade water was prepared. Linearity of response was achieved with five concentrations ranging from 0.012 ppm to 0.6 ppm diluted with HPLC grade water.

RESULTS AND DISCUSSION

Linearity of established method was achieved with five concentrations ranging from 12 ppb to 600 ppb as shown in fig. 2. Plastic toys and drinking bottled water were stored at 45 °C for four days and a sample of each plastic item was collected analyzed. Table 1 shows the concentrations obtained by measuring bisphenol Aleakage level from randomly selected tiger toy, small doll, large doll, Lego blocks and drinking bottled water stored at 45 °C for four days.

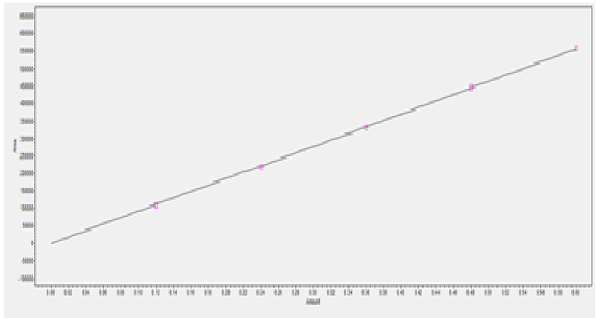

Fig. 2: Linearity graph of BP-A

Table 1: Analysis of BP-A levels in six randomly selected plastic products (four toys and two drinking bottled water from two different brands) stored at 45 °C for four days

Plastic item	Bisphenol A levels (ppb) after 12 h exposure at 45 °C	Bisphenol A levels (ppb) after 4 days exposure at 45 °C
Tiger toy	97.1 ppb	239 ppb
Small doll	Detection below LOQ	3 ppb
Big doll	Detection below LOQ	4 ppb
Lego blocks	1.3 ppb	30 ppb
drinking bottled water 1	50 ppb	59 ppb
drinking bottled water 2	ND	ND

Abbreviations

ND: No detection, ppb: Part per billion (ug/l), LOQ: Limit of quantification

Fig. 3 shows the chromatogram obtained for BP-A levels in tiger toy after storage at 45 °C for four days, while fig. 4 shows the chromatogram obtained for bisphenol A levels in drinking bottled water after storage at 45 °C for four days.

Accuracy of the method for BP-A was evaluated by preparing a stock solution containing 20 ppb of BP-A in water. Accuracy was performed in triplicate using five concentration levels of LOQ, 50%, 100%, 130% and 150%. The percentage recoveries for each concentration were calculated as shown in table 2.

Abbreviations

% recovery: mean±%Relative Standard Deviation for three determinations LOQ: limit of quantification. All samples were analyzed by LC-MS system to confirm the identity of the peak obtained as shown in fig. 5.

The exposure to high levels of BP-A could be correlated to various medical conditions namely diabetes [5], cardiovascular diseases [6], carcinogenic risk [7], premature deliveries and breast cancer in females [8,9], lowered semen quality and sperm DNA damage in males [10, 11] as well as childhood obesity and altered pubertal development [12, 13].

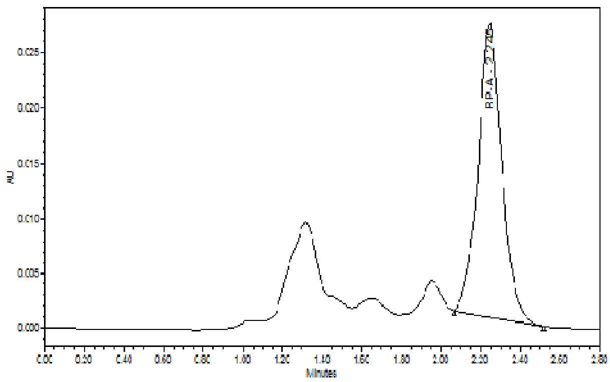

Fig. 3: UPLC-UV analysis of BP-A levels in a tiger toy stored at 45 °C for four days

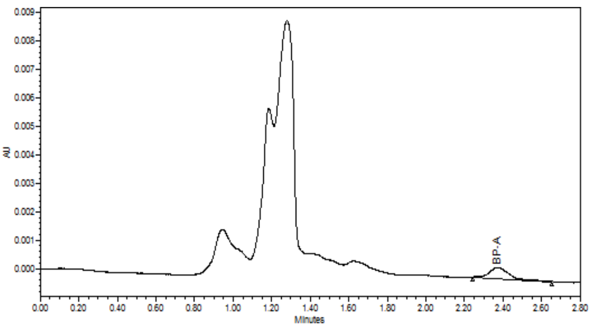

Fig. 4: UPLC-UV analysis of BP-A levels in a drinking bottled water stored at 45 °C for four days

Table 2: Results from the evaluation of established method accuracy.

Spiked level	% recovery of BP-A
LOD	103.5±2.5%
50%	98.2±0.9%
100%	100.6±0.7%
130%	101.1±1.8%
150%	99.5±1.1%

It is important to ensure that humans exposure is reduced, hence the need to develop efficient analytical methods. In the present study, BP-A was analyzed with the developed chromatographic conditions and eluted at 2.45 minutes. Other peaks were eluted prior to the peak of interest as shown in fig. 3 and 4, which are believed due to migration of different plasticizers from polycarbonate plastics, which need further study to be identified and quantified.

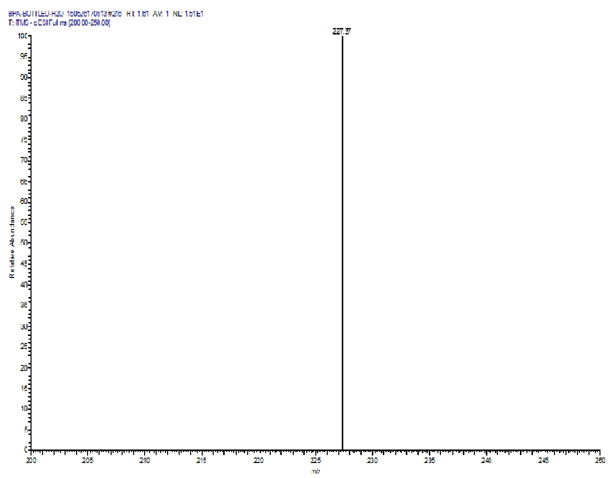

Fig. 5: A negative mode-ESI mass spectrum of BP-A

The LOD and LOQ for BP-A was determined at a signal-to-noise ratio of 3:1 and 10:1, respectively. The established method showed to have a limit of quantification (LOQ) equal to 1 ppb and a limit of detection (LOD) of 0.4 ppb for BP-A. Blanks were analyzed between samples injections to ensure that the system was free from any BP-A contamination. Linearity of detector response was achieved with r^2 value of 0.9999 by analyzing five diluted concentrations (12, 240, 360, 480 and 600 ppb) of stock solution.

The extracted BP-A levels were quantified using the calibration curve generated during standard analysis. The precision of BP-A area and retention time was estimated by injecting a sample containing 20 ppb for 10 times. The relative standard deviations for BP-A area and retention time were±1.7% and±0.4%, respectively. In the current study, BP-A was found in all randomly selected toys and one out of two randomly selected drinking bottled water. Although the detected amounts are below the daily tolerated dose stated by the EFSA, the EFSA criteria have been questioned as epidemiological and animal investigations continue to demonstrate the harmful effects of BP-A daily intake dose below the current reference daily tolerated dose [7, 20]. Moreover, 1.3-1.8 ng/ml of urinary BP-A concentration range was found to have externalizing and internalizing behavioral effects in 2 years-old children [21]. Although the highest estimated BP-A level exposure in childhood believed to be due to polycarbonate plastic bottles, canned foods with polycarbonate coating, consumption of beverages lined with polycarbonate bottles and micro waved foodstuff [22, 23], toys are persistent and additive pathways of bisphenol exposure [24].

Moreover, drinking bottled water selected in the current study were made from polyethylene terephthalate (PET) resin of the polyester family, which is considered to be safe and bisphenol detection is unattainable [25]. Although Shao and Han did not detect BP-A in drinking water stored in different plastic containers made from polyethylene terephthalate (PET) [25], Toyo'ka and Oshige indicated an average concentration range of 3 to 10 ng/l of BP-A in drinking water bottles made from polyethylene terephthalate (PET) [26]. Therefore, the raw material and the technology used in plastic bottles production may contribution greatly to the BP-A level present in drinking water [27]. Surprisingly, in the present study, 0.59 ppb of BP-A was detected by the established method in one of randomly selected drinking bottled water which may be indicative of poor quality of the raw materials and the technology used in plastic bottles production for the brand. Therefore, multiple sources of BP-A exposure can increase levels of this potentially toxic compound in children's body, which may lead to serious health complications. Moreover, all extracted samples were analyzed by LC-MS system to confirm the identity of obtained peak as shown in fig. 5. BP-A was indicated in all analyzed samples by LC-MS technique. Various types of impurities were extracted along with PB-A as it was extracted from different types of toys and drinking bottled water. The Selectivity factor (α) was ranging between 1.5 to 3.1 with RSD% of±0.5% and±0.7% respectively. Moreover, resolution (Rs) was indicated to be ranging from 1.7 to 0.9 with RSD% of 0.4% and 0.9% respectively. Our study agrees with results from other studies [27] which indicated that BP-A leakage increases with long storage time and high temperature. In Kuwait and probably elsewhere in the world there are no strict rules and regulations for storing imported and/or locally manufactured toys and plastic drinking bottled water.

CONCLUSION

The importance of BP-A level in plastics analysis was raised due to its potential health concerns. Heat is a major factor for bisphenol leakage out of plastics. However, Kuwait is considered one of the top hottest countries, BP-A leakage out of plastic toys and mineral water plastic bottles neither discussed nor measured. Surprisingly, BP-A was detected in all randomly selected plastic toys and one out of two randomly selected polyethylene terephthalate (PET) drinking bottled water. Therefore, imported drinking bottled water should be filled in a glass container rather than plastics due to high climate temperature. Moreover, Toys manufacturers should consider using BP-A free plastics in their manufacturing to avert health risks of BP-A leakage or clearly specify storage conditions of their plastic products.

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CONFLICTS OF INTERESTS

Author has no conflicts of interest to declare.

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