Endocrine disrupting Bisphenol A detection in different water samples in Iraq

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Abstract. Poly-carbonate plastics containing endocrine disruptor BPA (Bisphenol A), it have been used in the production process of water bottles, food products containers to storage, infants feeding milk bottles and raw material for some medical supplies. Trace quantity of bisphenol has been measured in water-samples that kept in poly-carbonate containers. It has been recognized that bisphenol a can be generated from poly-carbonate containers and then transfer into stored food. Such transfer is accelerated by leaving the containers in sunlight for long time. In this study a simple and rapid method was established to quantify BPA using high performance liquid chromatography (HPLC) in water samples in Al-Diwaniah city, Iraq. The chromatographic HPLC separation includes, extraction then detection of BPA using C18 as a HPLC column, followed by quantitative determination BPA using pre-measured known concentration as a standard. The analysis method shows a recovery value around 85% ± 2.9. A suitable sensitivity value was obtained with an instrumental limit of detection and quantification (LOD and LOQ) ranged from 33 to 72 µgL-1. The detection of BPA was effectively achieved using homemade and commercially supplied samples. The obtained numbers were inside the allowable concentration

1. Introduction
Recently, numerous studies have been done to get more attention in the environment of a large group of numerous types of chemical materials, which may have an effect on the human health due to their possible undesirable effect on the endocrine system in human and wildlife, these compounds were known as endocrine disruptors.

These compounds are dioxins, alkyl phenols, organo-chlorine pesticides and bisphenol. Among these compound bisphenol A consider to be the greatest worry phenolic from controlling and scientific institution due to large range of use as a monomer in industry to produce the polycarbonate plastic such as bottles for baby milk, bottles for water, and millions of household products. The rising concern due to the fact that the estrogenic performance in living oraminism as endocrine disrupting chemicals. BPA have a potential that can imitate the horomnic activity that produced naturally and can affect their normal function of endocrine system of human body, therefore resulting in distorted sex ratio, higher level of externalizing behavior, less fertility effect, and sex characteristic.
The United States environmental protection agency (EPA) reports the concentrations of BPA in US drinking water are typically below 1 micro gram per litter. Around 90 percent of population around the world have been exposed to certain amount of bisphenol a during their life, either by eating or drinking food or beverage that include trace quantity of bisphenol a.

Plastic containers in general are the main source of bisphenol a, with recycle code three and seven these plastic containers are more likely to have bisphenol a in their structure. Bottled water consuming in Iraq is the common way to get a good hydration thus it is considering to be the major drinking water source. The aim of this work was to quantitatively determine the presence of bisphenol a in 10 of bottled drinking water brands from several sources in Iraq.

2. Materials and Methods

2.1. Chemicals

Bisphenol A were purchased from sigma, DCM (Dichloromethane) and methanol was obtained Fluka.

2.2. Sample collection

Bottled water samples (10 samples) have been collected and obtained from the local market in Diwaniah city-Iraq, these samples were from local production companies or imported from different foreign countries.

2.3. UV-Visible absorption measurements

UV absorption spectra were obtained by using a double-beamed spectrophotometer type UV-1650 obtained from Japan, this instrument use xenon type lamp, with fixed band pass during the analysis. The solutions were measured using 4 mille Litter quartz type cuvette with a 1 centimetre cuvette path length absorption.

2.4. Chromatographic detection of Bisphenol A

High performance liquid chromatography sample analysis was performed using HPLC system which consist of a controlling system model SCL10VP. This system equipped with gradient pump typeLC10AVP, an (UV) detector model SPD-10A-V-P, and a non-line de-gasser model D-GUR-A.

2.5. Reagents

To reduce any possible contamination of glassware, all glassware have been cleaned and washed with de-ionized water before and after use, then dipped in a 7Molar HNO3 solution for twelve hours before any further use, then washed again with distilled water for three times.

2.6. Preparation of Standard solutions

Stock solutions of Bisphenol a (200 mg L-1) have been prepared by dissolving 2 milligrams of standards solutions in 10 milliliters of methanol. The stock solutions were kept in black and dark container at 6°C prior to use, stock solutions were tested and examined using Uv spectrophotometer to be sure no any possibility of degradation by light for the solutions, and they used within period of twelve months of preparation. Bisphenol A working solutions were prepared each day by serial dilution of the 200 ppm solution.

2.7. Sample preparation for Bisphenol A determination

Ten brands (labelled from 1 to 10) of commonly purchased bottled water were. In each case, the bottled water was immediately extracted and analyzed. 10 water bottles for each brand were analyzed All bottles were stored for a couple of days at room temperature prior to extraction.

A one litter sample of water was transferred to a separating funnel for liquid-liquid extraction. Liquid-liquid extraction was done with 10 ml dichloromethane for three times. The extract
was concentrated using a stream of air then re-dissolved in 05 ml methanol followed by injection in HPLC.

3. Results and Discussion

3.1. Uv Spectrum

Ultraviolet spectrum has been obtained for bisphenol a (Figure 1) using 1 ppm concentration and the relationship obtained from graphing intensity versus wavelength show that 233 nm show the highest intensity.

![Figure 1. chemical structure of bisphenol A](image)

3.2. HPLC analysis

For High performance liquid chromatography separation, a generally used C8 chromatographic column has been selected as a moderate reverse phase type stationary phase column, it has length equal to 15 centimetre, and a diameter of 5 millimetre while the average particle size was 5mirometer. the C-8 column was employed before to achieve the analysis after work that published earlier.

The chromatographic separation via HPLC was done using a 80 percent methanol as a mobile phase with a flow rate of 1 ml/min and a constant column temperature at room temperature which is 25 Celsius. In figure [2] the typical HPLC chromatogram for injection of 50 ppb standard solution of bisphenol a in methanol, using 233 nm as absorption wavelength inside the UV detector has been shown.

![Figure 2. 50 ppb bisphenol a standard solution injected into HPLC-Uv](image)

In this work, 3 chromatographic HPLC runs for the standard solution obtained from 3 separate and different injections of 20µL in volume which provided 6.1 ± 0.3 minutes retention time for bisphenol a. To generate a calibration curve (figure 3), 5 different concentrations of bisphenol a have been made using distilled water and injected directly into HPLC.
3.3. Determination of Bisphenol A via HPLC

Bisphenol a consider to be the essential chemical to make epoxy resins (ERs) and plastic type polycarbonate. In recent articles, the concentration of bisphenol a in water bottles samples made from polyethylene plastic to be around 1 ppb, while several other studies did not show any level of bisphenol a in plastic containers. The water samples were prepared for analysis and injected to HPLC-Uv detector. Figure [4] show an example of injection of methanolic extract of water samples for analysis.

Table 1 show the concentrations of bisphenols in different water samples that obtained from HPLC analysis. The values obtained showed a variation in bisphenol a concentration among the samples with no significant difference among the different brands, in general the results show a higher content of the compounds in several brands than the others. The results obtained show high agreement with the results obtained from other articles studies. The reason behind this could be due to the movement of plasticizers from the plastic bottle material to the contained beverage as container quality vary based on the raw material and the production technology to produce the container. Another source of contamination with bisphenol a is the photolytic formation/degradation of several compounds during storing the bottles. Several articles show that the concentration levels increase as the storing time increase and as the temperature increase. In the current study, did not test samples to determine the concentration in different storage time and temperature.
Table 1 Obtained results using HPLC-Uv

| Sample name | BPA conc. ppb |
|-------------|--------------|
| Lujain      | 35 ± 3       |
| Waha        | 39 ± 4       |
| Lo’loa      | 42 ± 7       |
| Diwnaiah    | 71 ± 9       |
| Kafeel      | 45 ± 2       |
| ZamZam      | 49 ± 5       |
| Zulal       | 61 ± 4       |
| Baghdad     | 78 ± 8       |
| Rayan       | 67 ± 4       |
| Borak       | 55 ± 2       |

4. Conclusion
In this article an effective straightforward procedure have been applied for the quantified determination of BPA in several water samples using HPLC-Uv. The results obtained show that using high performance liquid chromatography with ultraviolet detector is good technique for quantification of BPA. The examinations and the results achieved in this article bring the scientists a slight closer to determine the actual content of BPA by reducing the causes of the error such as the partial extraction process.

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