COMPARATIVE STUDY BETWEEN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND GAS CHROMATOGRAPHY FOR DETERMINATION OF BISPHENOL A IN THE BABY BOTTLES

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Abstract

In this study, bisphenol A (BPA), the migrant from the polycarbonate baby bottles was determined using high performance liquid chromatograph (HPLC) with both detectors, (fluorescence, FLU and UV) and gas chromatograph with mass spectrophotometer detector (GC/MS). The results exhibited a noticable variation in the amounts of BPA measured by HPLC using both FLU and UV detectors at the same condition. However, this variation was more pronounced between the results of HPLC/FLU analyses and the corresponding ones for the same sample at GC/MS. Since, the BPA levels in the HPLC analyses were lower than those in the GC/MS analyses. Meanwhile, the detection limit of BPA was broader for the HPLC method comparing with that for GC/MS method. These differences in measured BPA levels for both two chromatographic methods may result mainly from low sensitivity noticed in HPLC method, particularly, at lower BPA levels and relatively poor resolution at higher BPA levels. This is due to the great varied sensitivity between the two instruments (HPLC)& (GC/MS) and between the two detectors of HPLC (FLU&UV). However, there was no report, which simultaneously evaluated the two methods in real analyses. We found that there were strong variation between BPA levels in the baby bottles samples with the two methods, for this we recommended the GC/MS for The BPA detection method.

Keywords: Bisphenol A-Polycarbonate – Migration- Baby bottles –HPLC–GC/MS.

Introduction

Bisphenol A (BPA, 2,2-bis(4-hydroxyphenyl) propane), is released from polycarbonate. It characterized as estrogenic and an endocrine disrupting chemicals (EDCs) which have been known to interfere with endocrine system by different mechanisms: mimicking, blocking, and triggering actions of hormones, antagonizing the effects of hormones, by altering the synthesis and metabolism of hormones and by modifying hormone receptor levels[(1)]. BPA also is implicated with toxic effect e.g. cancer[(2,3)] and DNA damage[(4)]. BPA is used to manufacture polycarbonate plastic and epoxy resins, which are widely used for a variety of applications such as baby feeding bottles and food-can lining. Although the estrogenic properties of Bisphenol A were reported as early as 1936 by Dodds and Lawson[(5)], while at 1993, when Krishnan[(6)] documented that bisphenol A, (BPA) was released from polycarbonate flasks during autoclaving and had estrogenic activity, the effects of BPA on health has become a controversial issue. In addition, BPA could leach out from many consumer products containers (baby bottles, epoxy resins, 5 liter drinking water bottle and other consumers plastics) under real use condition[(7)] or during

sterilization[8]. A general problem with the interpretation of the results on BPA is background contamination of samples, which may interfere with quantification at low concentration. Therefore, we replaced plastic ware with glassware throughout the entire analytical procedure in order to avoid possible BPA contamination. With the development of the analytical technologies, a number of analytical methods have been developed during the last few years for the determination of Bisphenol A[9] including chromatographic separation such as, high performance liquid chromatography with fluorescence detector (HPLC/FLU)[10,11] and with Ultraviolet detector (HPLC/UV)[12,13] and Gas chromatograph / mass spectrometry GC/MS[14,15]. GC/MS is the commonly used technique for trace analysis of estrogenic phenols[16]. However, the required sample treatment procedures, which involve purification, fractionation, hydrolysis and derivatisation, limit its application for complex sample[17]. HPLC, with detection mode implied the use of multidetector due to the good selectivity that can be achieved by profiting from the differences in the redox behaviour of phenol derivatives, each detector being maintained at different potential value[18]. As that the mass spectrometry assays monitor intensity of several fragments or transitions during the chromatographic separation, so that, they are speedy and specific. However, cost for installing and maintaining the GC/MS instruments is expensive and keeps them from being widely used because that, HPLC is more affordable to install and maintain than GC/MS[19]. On the other hand, the HPLC/FLU assays have been widely used for BPA analysis with suitable sensitivity, There was no report, which simultaneously evaluated these two methods in real analyses rather than reviewing[20]. Therefore, we analyzed the BPA migrated from baby bottles, with both GC/MS and HPLC/FLU and HPLC/UV assays. GC/MS provides higher resolution and lower detection limits than HPLC for the determination of BPA. No derivatisation of BPA is required for this application. Yonekubo[21] et.al, proposed direct analysis by HPLC/MS for the determination of BPA. However, derivatisation processes can make the sample preparation laborious and time consuming, and can increase the possibility of contamination as consequence of undesirable reactions with the matrix

Materials and Methods

- Chemicals and reagents

Bisphenol, 99 % (Sigma- Aldrich, UK); Acetonitrile HPLC grade, water HPLC grade and (MTBE) Methytert-butyl ether (simulant 1) HPLC grade; Ethanol 10% (simulant 2) which are used as milk simulant by Food and Drug Administration.

Samples : Baby Bottles made of Polycarbonate (PC).

- Apparatus and Conditions

- In order to avoid any BPA contamination in the analysis system, plastic wares were excluded throughout the entire analytic procedure, and replaced with glassware. - The Standard of Pure BPA (99%), was first injected before every Batch of Samples,

The two chromatogram of the standard and the Sample are shown in Fig (7).

- **High performance Liquid Chromatography** with using state of-the-art equipment fluorescence and UV detection HPLC-FLU/UV. The HPLC system Agilent 1100 series consisted of dual detectors, Agilent fluorescence (FLU) detector and Ultra Violet (UV) detector (San Jose, Calif., USA); BPA fluorescence was monitored at an excitation wavelength of 234 nm and emission wavelength; 317 nm; with injection volume 10 µl; run length; 30 min; column temperature; 38 ºC; Column: Hypurity Elite Hypersil ODS 5 µm, C18, 250 x 4.6 mm (Alltech - Massachusetts, USA). The analyses were carried out with the gradient mode: flow rate; 1 mL/min for mobile phase A: B, where A= acetonitrile, and B= Water, HPLC grade,

- Gas Chromatography with mass spectrometry (GC-MS)

The quantitative confirmation analysis using a Shimadzu gas chromatograph GC-2010 series connected to a Shimadzu mass spectrometer GCMS-QP2010 (North America, USA), the separation of the compounds was achieved by using a DB5MS capillary column (60 m) (supelco), the carrier gas was helium and maintained at constant flow rate of (0.9 ml / min). A sample volume of 1 µl was injected in Splitless mode at an inlet temperature of 280ºC. The MS transfer line temperature was maintained at 280 ºC, whereas the ion source temperature was 180ºC. The method must include some form of confirmation of the BPA chromatographic peak. It is preferred that the confirmation be based on structural information such as accurate mass, daughter ions or ion ratio obtained from MS techniques. Using a GC/MS methodology, the confirmation ratio for underivatized samples would be calculated using the molecular ion peak (M^+) with a mass-to-charge ratio (m/z) of 228, and the base peak (loss of a methyl group, ($M-CH_3$) with m/z equal to 213.

- Sample preparation for migration studying:

- **The baby bottle**: was filled by 100 or 50 ml of simulant (1,2,3) and shacked at the shaker water bath at desired temp for one hour then was kept at a glass test tube to undergo chromatographic analysis.

(simulant 1, MTBE, simulant 2, ethanol 10%, simulant 3, Acetic Acid 3%).

- **Water Bath**: for heating samples simulants from room temp 22^0(_+2) to 90^0C was attained by water bath BS-06, JEIO Tech (Korea).

Results and discussion

BPA profiles of the two assays were shown in Figures 1-6, and Tables 1-4.

1- Effect of Time of Storage[22] in the concentration of BPA migration were determined and shown in table(1) and Fig.(1,2), for the sample(G) of the baby bottle stored with the simulant 2 inside, for different hour from one hour to 6 days (144 hour), and the results obtained by both GC/MS and HPLC/FLU for the migrated BPA in the simulant 2 (10% ethanol) showed that the migration of bisphenol A was affected by its susceptibility to hydrolyze in the simulant ethanol and the concentration of BPA is mainly decreased due to hydrolysis except some false determination in both instrument[23,24].

2- Effect of Rinse and Washing by detergent[25] and the BPA concentration result obtained by both GC/MS and HPLC/FLU are shown in table (2) and Fig. (3,4), for the same sample (G) of the baby bottle, and with the same simulant 2 after washing by normal soap detergent, it showed that the migration of bisphenol A were affected by rinsing the baby bottle by tap water before used and the concentration of BPA is mainly decreased due to the PC degradation process originated by the contact between detergent and PC after several using of the baby bottles, except some false determination in both HPLC and GC/MS Chromatograms.

Note: In the tables: 2nd used, means that filled by simulant after 1st time washed by soap, and also, 3rd used, that filled by simulant after 2nd time washed by soap, and for, 4th used, filled by simulant after 3rd time washed by soap detergent.

3- Effect of rinse of the baby bottle before used: was shown in table (3) and Fig. (5), as mentioned before that the migration of bisphenol A were affected by rinsing the baby bottle by tap water before used and the concentration of BPA is mainly decreased by rinsing. The comparison between the two detector for the same sample show that the results obtained by the Fluorescence detector (FLU), is more accurate than UV detector.

4- Effect of rinse of the baby bottle after second used : It shown in the table (4) and Fig. (6), the result shown that, the migration of bisphenol A were affected by rinsing the baby bottle by tap water before used and the concentration of BPA is mainly decreased by rinsing and after the second using. The comparison between the two detector for the same sample show that the results obtained by Fluorescence (FLU) detector is more accurate than UV detector.

5- Comparison of BPA levels between the two detector of HPLC and GC/MS: Each baby bottle sample was analyzed with the two methods. The frequencies of detection were higher in the GC/MS method than those in the HPLC/FLU and UV method. In addition we compared between the HPLC/FLU and HPLC/UV detectors. The BPA levels in the GC/MC method were somewhat broad compared to those in the HPLC method. That is the GC/MS method obtained higher values for the samples, which were low detectable in the HPLC method. Thus, the detection rate of total BPA in all samples was higher in the GC/MS method than that in HPLC analysis, However, after the further matched pair analysis, we found that the BPA levels in the HPLC were lower than those in the GC/MS. Thus as mentioned above low levels of BPA may be overestimated in the GC/MS, however some outliers, which showed higher levels of BPA in the HPLC method, reflect the drawback of the HPLC, i.e. poor resolution for other compounds, which have similar characteristics with BPA in the HPLC/UV more than fluorescence (FLU), and also for HPLCL/FLU more than GC/MS.

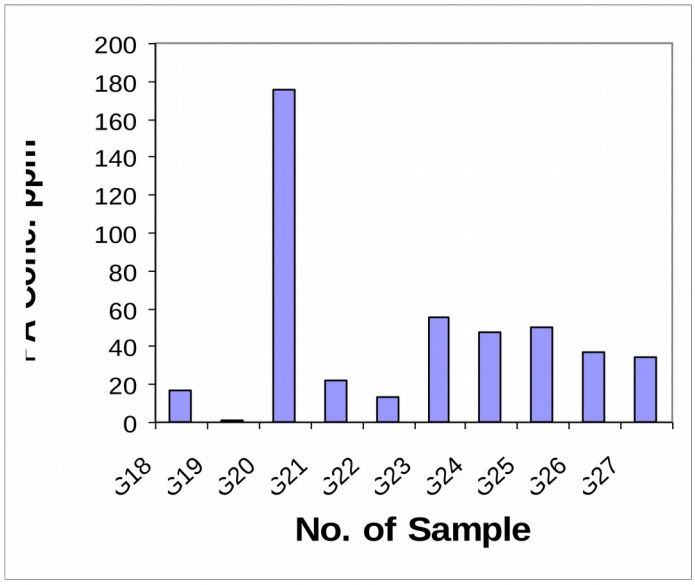


Fig (1) Effect of Time of Storage by GC/MS

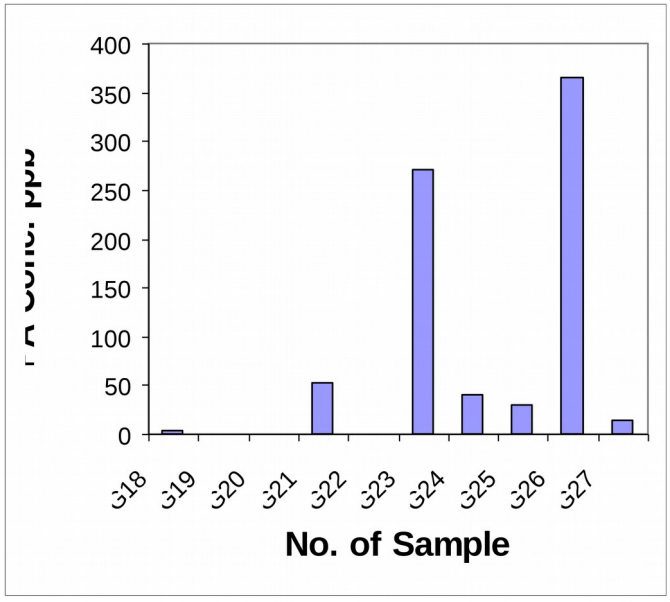


Fig (2) Effect of Time of Storage by HPLC/FLU

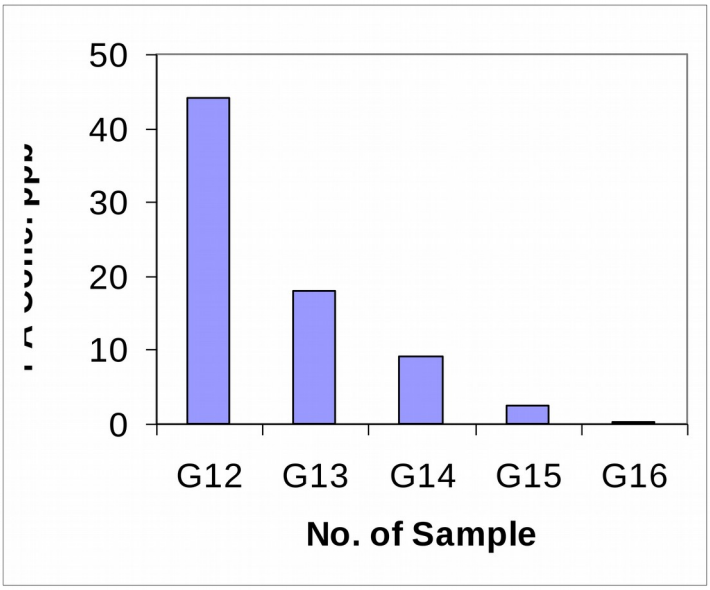


Fig (3) Effect of rinse after washing by detergent by HPLC/FLU

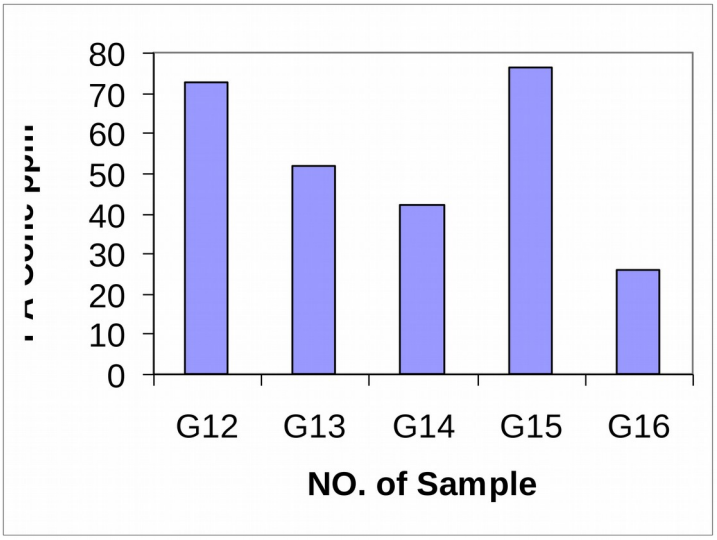


Fig (4) Effect of rinse after washing by detergent by GC/MS

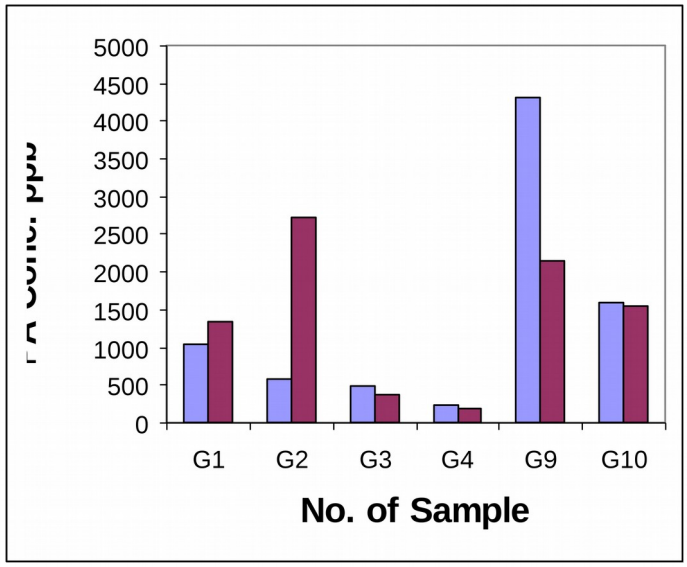


Fig (5) Effect of rinse after washing by detergent by HPLC/FLU and HPLC/UV

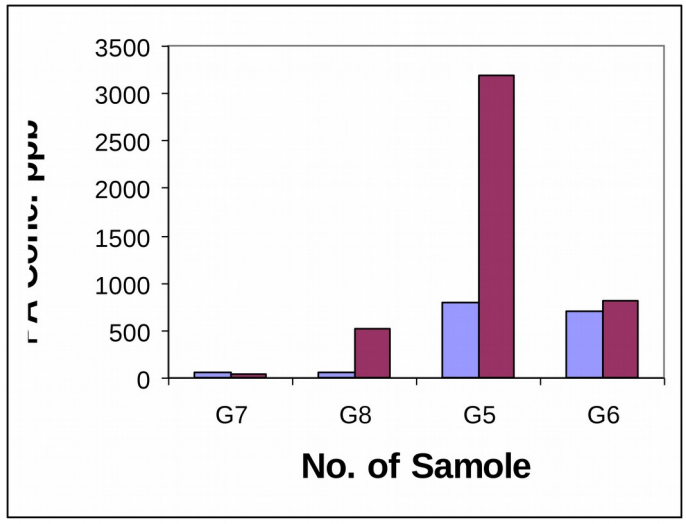
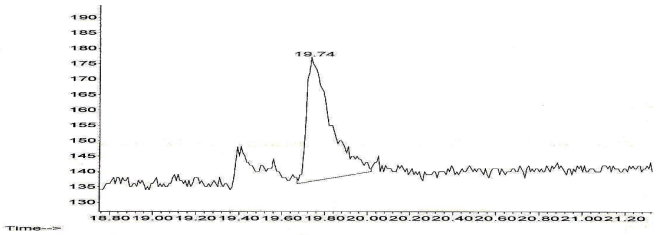
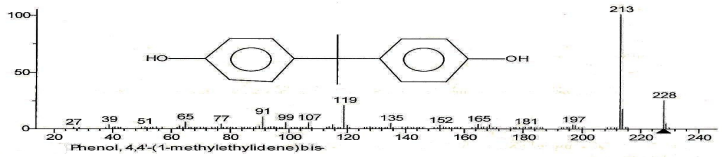
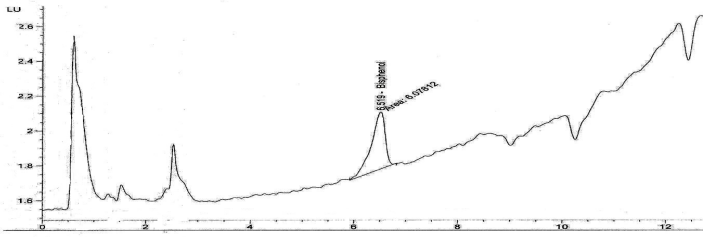


Fig (6) Effect of rinse of the baby bottle after second used by HPLC/FLU and HPLC/UV





Total ion chromatogram of Bisphenol A standard and NIST 2005 MS spectra.



External Standard Report

Sorted By : Signal
Calib. Data Modified : 11/10/2009 10:24:48 ?
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FlD1 A, Ex=235, Em=317

RetTime [min]	Type	Area LU	Amt/Area *s	Amount [mg/ml]	Grp	Name
6.519	MM	6.07812	1.54320e-5	9.37973e-5		Bisphenol

Totals : 9.37973e-5

Results obtained with enhanced integrator!

*** End of Report ***

mubarak city-central lab

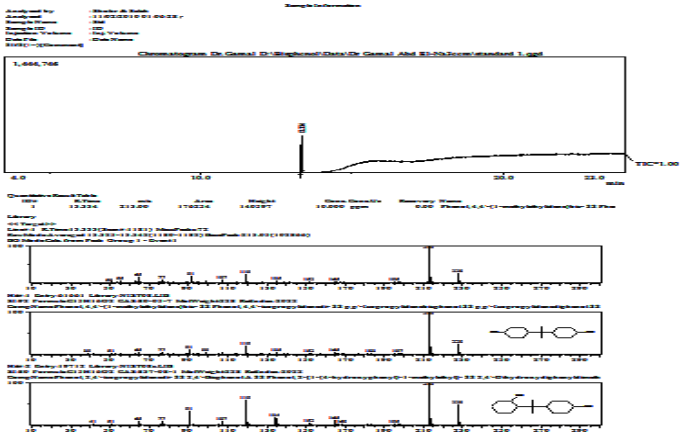


Fig (7)

Table 1 : Effect of Time of Storage in the concentration of BPA and the result obtained by both GC/MS and HPLC/FLU (migrated BPA) in stimulant 2 (10% ethanol)

No. of Sample	Conc. ppm GC/MS	.Conc ppb GC/MS	.Conc mg/ml HPLC/FLU	.Conc ppm HPLC/FLU	.Conc ppb HPLC/FLU	Time Hour
G18	16.93	16930	$3.6x10^{-6}$	$3.6x10^{-3}$	3.6	1
G19	0.646	646	0.0000	0.00	000	2
G20	175.6	175600	$8.3x10^{-7}$	$8.3x10^{-4}$	0.83	3
G21	21.6	21600	$5.2x10^{-5}$	$5.2x10^{-2}$	52.0	12
G22	12.9	12900	$5.6x10^{-7}$	$5.6x10^{-4}$	0.56	24
G23	55.22	55220	$2.7x10^{-4}$	$2.7x10^{-1}$	270	36
G24	47.02	47020	$3.97x10^{-5}$	$3.97x10^{-2}$	39.7	48
G25	49.66	49660	$2.96x10^{-5}$	$2.96x10^{-2}$	29.6	72
G26	36.93	36930	$3.65x10^{-4}$	$3.65x10^{-1}$	365	96
G27	34.12	34120	$1.38x10^{-5}$	$1.38x10^{-2}$	13.8	144

Table 2 : Effect of Rinse and Washing by detergent and the BPA Conc. result obtained by both GC/MS and HPLC/FLU

No. of Sample	Conc. ppm GC/MS	.Conc ppb GC/MS	.Conc mg/ml HPLC/FLU	.Conc ppm HPLC/FLU	.Conc ppb HPLC/FLU	Effect
G12	72.43	72430	$4.4x10^{-5}$	$4.4x10^{-2}$	44	Without Rinse
G13	51.57	51570	$1.8x10^{-5}$	$1.8x10^{-2}$	18	With Rinse
G14	42.14	42140	$9x10^{-6}$	$9x10^{-3}$	9	2nd used 1st soap
G15	76.07	76070	$2.5x10^{-6}$	$2.5x10^{-3}$	2.5	3rd used 2ndsoap
G16	25.7	25700	$3.2x10^{-7}$	$3.2x10^{-4}$	0.32	4th used 3rd soap

Table 3 Effect of Rinse before used with Comparison of BPA Conc. in ppb Between HPLC/FL and HPLC/UV

No. of Sample	Conc. mg/ml /HPLC FLU	Conc. ppm /HPLC FLU	.Conc /ppb HPLC FLU	Conc. mg/ml HPLC UV/	.Conc ppm HPLC UV/	.Conc ppb HPLC UV/	Effect
G1	1.04 x 10^{-3}	1.04	1040	1.33 x 10^{-3}	1.33	1330	Without Rinse
G2	x 10^{-4} 5.68	x 10^{-1} 5.68	568	2.72 x 10^{-3}	2.72	2720	With Rinse
G3	4.86 x 10^{-4}	4.86 x 10^{-1}	486	3.76 x 10^{-4}	4.86 x 10^{-1}	376	Without Rinse
G4	2.3 x 10^{-4}	2.3 x 10^{-1}	230	1.87 x 10^{-4}	1.87 x 10^{-1}	187	With Rinse
G9	4.31 x 10^{-3}	4.31	4310	2.14 x 10^{-3}	2.14	2140	Without Rinse
G10	1.58 x 10^{-3}	1.58	1580	1.54 x 10^{-3}	1.54	1540	With Rinse

Table 4 Effect of rinse of the baby bottle after second used and comparison between the FLU and UV detector of HPLC in ppb

Sample No of	.Conc mg/ml HPLC FLU/	.Conc /ppm HPLC FLU	.Conc ppb HPLC FLU/	.Conc mg/ml HPLC UV/	.Conc ppm HPLC UV/	.Conc ppb HPLC UV/	Effect
G7	5.39 x 10^{-5}	5.39 x 10^{-2}	53.9	3.32 x 10^{-5}	3.32 x 10^{-2}	33.20	Without Rinse .2nd used
G8	4.74 x 10^{-5}	4.74 x 10^{-2}	47.4	5.2 x 10^{-4}	5.2 x 10^{-1}	520	With Rinse .2nd used
G5	x 7.87 10^{-4}	x 10^{-1} 7.87	787	x 10^{-3} 3.19	3.19	3190	Without Rinse .2nd used
G6	x 6.96 10^{-4}	x 10^{-1} 6.96	696	x 10^{-4} 8.15	x 10^{-1} 8.15	815	With Rinse .2nd used

6. Conclusion:

The risk assessment of environmental toxicants. particularly, end point of BPA on health are not clearly understood, yet. Thus , continuous detection of BPA with different methods of analysis is a unique method for protection of unknown health risk especially for infants. So we followed the HPLC on both detectors FLU and UV detector and found that method has several drawbacks, e.g. long running time and low sensitivity or false positives. On the other hand, the GC/MS method becomes popular to overcome the drawbacks of the HPLC method, i.e. short running time and accuracy of identification. In this study, we performed the comparison between the two methods. At first, the analyzed BPA levels with the HPLC/FLU method are confirmed with the GC/MS method. However, there is some possibility of false positives in both methods: The GC/MS assay may have some false positives, because the GC/MS analyzed BPA levels were various and mostly bigger accuracy than HPLC assays. The case of HPLC/FLU, also may have false positives in some samples, which were low with GC/MS and provide some higher levels than GC/MS

dose. Therefore, we should carefully consider the merits and drawbacks of the two methods. Even though there was a strong variations between BPA levels, which were analyzed with the two methods, the BPA levels in the HPLC were lower than those in the GC/MS. Thus to avoid error in the determination of BPA risk assessment, we recommend severe guidelines for identification of BPA with the HPLC method and confirmation of BPA identification with GC/MS method, particularly in high levels of BPA, which were obtained with the HPLC method. This paper proposes avoiding the tedious and critical step performing, by direct analysis of the MTBE extracts by GC/MS and HPLC/FLD and UV. detector. So, a comparison between the Three methods has been performed, the obtaining results will allow demonstrate that the elimination of the derivatisation step is feasible and the used of GC/MS is more accurate.

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