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Identification and Quantification of Bisphenols in Water by Dissipation followed by Silylation using Gas Chromatography-Mass Spectrometry Analysis

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Bisphenols are important endocrine disruptors, which were widely used in the variety of food packing and storage materials which often come into contact with various food products packed in them. The presence of bisphenols in water is harmful for the health of humans as well as aquatic animals and also, they accumulate over a period of time. Hence, the present work aimed to develop a simple and accurate GCMS-SIM method for the quantification of bisphenols in packaged drinking water as well as the water samples collected in river and lakes in Andhra Pradesh state of India. Bisphenols were extracted by simple solvent extraction with acetonitrile and silylated by N,O-*bis* (trimethylsilyl)trifluoro acetamide and analyzed by GC-MS. Various parameters that affect the recovery of the analytes were carefully optimized and the developed method was validated. The recoveries of the analytes were in the range of 80-120 % with quantification limit of 1 ng/L. The calibration curve was linear in the concentration range of 5 ng/L to 10 µg/L. The method was applied for the quantification of bisphenols in packaged drinking water at room temperature and at 50 ºC at various time intervals. The results proved that the water sample kept at room temperature doesn't shows peaks corresponding to bisphenols. The water sample exposed to 50 ºC for 30 days bisphenols content 10, 12, 22 and 8 ng/L respectively for bisphenol G (BPG), bisphenol F (BPF), bisphenol E, (BPE) and bisphenol A (BPA) whereas the same sample at 180 days of exposer shows 60, 51, 61 and 22 ng/L respectively confirms that the leaching of plastic due to temperature increases the bisphenols level. Among the real time samples studied, the bisphenols level was observed to be very high in Kolleru Lake and it is having 17, 14, 8 and 12 ng/L of BPG, BPF, BPE and BPA, respectively confirms that due to high plastic pollution the bisphenols level was high in these samples. Hence, it can be concluded that the method can be suitable for the analysis of bisphenols in drinking water as well as in wastewater samples.

Keywords: Plastic pollution, Bisphenols, Drinking water, River water, Bis silyl trifliuroacetamide, GCMS-SIM.

INTRODUCTION

Bisphenols are a class of aromatic chemicals with two hydroxyphenyl moieties which are commonly used in the processing of plastics [1], such as food, feed packaging and storage materials and are reported to leach into various food products from the packaging materials [2]. Bisphenols are classified as endocrine disrupting chemicals (EDCs) and mimic the estrogens in the human body leading to various irregularities in the female health cycles. Till date, bisphenol A (BPA) is most often used bisphenols, with annual global output of 4.7 million tons in 2012 and an annual production growth of 5.1% from 2014-2019, which is also considered as a xenoestrogen [3].

The European Food Safety Authority (EFSA) has carried out numerous scientific assessments on bisphenol A (BPA) since 2006. Due to new data and refined methodologies, the European Food Safety Authority (EFSA) reduced the tolerable daily intake (TDI) from 50 g/kg body weight per day to a temporary TDI of 4 g/kg bw/day in 2015 [4,5]. In order to protect the consumers health, the usage of BPA in the manufacturing processes and food contact materials was strictly regulated by various international regulatory bodies [6,7]. Hence, the rest of bisphenols have been employed to substitute BPA, which includes bisphenol FL (BPFL), bisphenol C (BPC), bisphenol M (BPM), bisphenol P (BPP), bisphenol PH (BPPH), bisphenol E (BPE), bisphenol F (BPF) and bisphenol AP (BPAP) [8-10].

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However, these analogues also have similar toxicity and endocrine disrupting properties to bisphenol A [11,12]. The presence of bisphenols in fish products was reported previously and the causes for the presence of bisphenols are also evaluated [13,14]. As fish feed is one of the route of entry of the bisphenols in to fish, monitoring of food products and feed products is very important for trace level determination of bisphenols with novel and sensitive analytical methods [15].

Several publications were previously reported for the quantitative determination of bisphenols in various food, biological and environmental matrices [16,17]. The analytical techniques like LC-MS, GC-MS have been used for selective, specific and sensitive quantification [18]. GC-MS has been widely employed for the quantification of bisphenols for testing of various food products. However, validated methods for quantitative determination of bisphenols from different kind of fish feeds were not reported. It is necessary to develop an appropriate extraction method prior to their quantification from the complex fish feed samples [19]. Several techniques such as solid phase extraction (SPE), QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe), dispersive liquid-liquid microextraction (DLLME) [20] and solid phase microextraction (SPME), have been successfully applied for the isolation of bisphenols from various food products [21].

As the samples are liquid in nature, QuEChERS [22] would be more suitable technique when compared to other SPE or SPME techniques [23]. However, the experiments revealed a low recovery for the analytes due to the presence on primary secondary amine in QuEChERS tubes which has considerable adsorption tendency towards anionic bisphenols [24]. Hence, in the present work, a simple derivative method using derivatization with N,O-*bis*(trimethylsilyl)trifluoro acetamide was developed and the analytes were quantified by GC-MS in selected ion monitoring mode (SIM) after derivatization with N, O-bis(trimethylsilyl)trifluoro acetamide. The developed method was applied for the quantification of bisphenol residues in packaged drinking water as well the water samples collected in two rivers and two lakes in Andhra Pradesh state of India.

EXPERIMENTAL

The bisphenols analogues in the study such as bisphenol A (BPA), bisphenol F (BPF), bisphenol E (BPE), bisphenol G (BPG), the other chemicals such as BSTFA (bis silyl trifluoro acetamide), acetonitrile were purchased from Sigma-Aldrich, USA. The packaged drinking water samples were purchased from local market and real time samples were collected from the various locations.

Sample collections: A total of 7 samples were collected in triplicates for the analysis and the collected samples were divided in three groups.

Group 1: Packaged drinking water sample kept at room temperature and the sample was analyzed after 15, 30, 60,120,150 and 180 days of incubation.

Group 2: Packaged drinking water sample kept at 50 ºC and the sample was analyzed after 15, 30, 60,120,150 and 180 days of incubation at 50 ºC.

Group 3: Real time samples were collected at the following locations (i) Krishna river at Punnami Ghat, Vijayawada; (ii) Krishna river at Amaravathi, Guntur; (iii) Pulicat lake at Sullurpeta; (iv) Kolleru lake at Kolletikota; and (v) Godavari river at Godavari bridge, Rajahmundry city.

Sample preparation: The packaged drinking water at different intervals were directly used in the study. The real time water samples collected at different locations were filtered through 0.2 µ filters and the filtered samples were used for the study. An accurately measured 10 mL of water sample was cooled to -20 ºC and evaporated under nitrogen purging in overnight for total evaporation of the liquid. The dried residue was reconstituted with 200 µL of acetonitrile and 200 µL of BSTFA (bis silyl trifluroacetamide) solution. The reconstituted solution was heated at 30 ºC for 30 min and then cooled at room temperature. The solution was used for GC-MS analysis.

Preparation of standards: The standard stock solution of bisphenols at a concentration of 10 ppm was prepared using acetonitrile solvent. The prepared solution was stored -20 ºC. The working standard solutions for construction of calibration curve were prepared separately for each bisphenol by mixing selected concentration by serial dilution of the stock solution and derivatization with BSTFA. The linear calibration curve dilutions were prepared in the concentration range of 1 ng/L to 10,000 ng/L separately for each bisphenol in the study. The combined stock solution containing known concentration of each bisphenol was prepared by mixing equal volume of each bisphenol separately. The combined stock solution was used for the simultaneous analysis of bisphenols using GC-MS method as well as method validation study.

GC-MS analysis: In general, bisphenols which has been extensively studied using GC-MS technique and the analysis was performed on selected ion monitoring (SIM) mode. SIM mode is suitable for quantitative analysis of trace components and allows the mass spectrometer to detect specific compounds with very high sensitivity. Hence SIM mode is selected for the identification and quantification of bisphenols in water samples. The GC-MS analysis was carried out with an Agilent 7890 B GC system coupled with an Agilent 5977 A mass selective detector and HP-5MS Column of length 30 m, 0.25 mm internal diameter and 0.25 µm thickness. The injection port was maintained at 230 ºC and helium was used as carrier gas at a flow rate of 2 mL/min. The column oven was programmed from an initiate temperature of 150 ºC held for 5 min and ramped with 20 ºC/min final temperature 300 ºC held for 2 min. The SIM products were selected at 343, 344, 357 and 441 nm. The selected mass operating conditions for the analysis of bisphenols using GC-MS are summarized in Table-1.

The calibration and tuning of the GC-MS instrument were performed daily before the starting of the analysis. The quantification of bisphenols was performed using the characteristic retention time of each analyte, a min of two *m/z* values, from which one was the quantitative ion and the rest were the confirmation/reference ions; and additionally, the ratio of quantitative ions to confirmation ions for each standard. The calibration curve was constructed by using MS response in the tested concentration range against the concentration of analyte prepared.

TABLE-1
MASS OPERATING CONDITIONS FOR THE ANALYSIS OF BISPHENOLS USING GC-MS METHOD

Analyte	RT (min)	SIM ions	Dwell time	RSD (%)	Source	Split mode	Flow rate (mL/min)
BPA	11.01	343	100	6.4	EI	10:1	2
BPG	11.20	344	100	3.8	EI	10:1	2
BPF	11.35	357	100	4.7	EI	10:1	2
BPE	12.01	441	100	3.3	EI	10:1	2

The regression equation of the calibration curve was used for the quantitative analysis of bisphenols in the samples.

Validation of GCMS- SIM method: For the validation of the method, the latest version of the IUPAC guidelines for single-laboratory validation of methods of analysis was followed. A validation was performed using the matrix-matched method by spiking the deionized water samples with the bisphenols to obtain a concentration of 1 ng/L to 10,000 ng/L. The obtained calibration curves were used for the quantification of bisphenols in the collected water samples. The other method validation parameters such as recovery, sensitivity, detection limit, quantification limit and precision were carried in the developed method and statistical analysis was carried in excel software.

The method validation parameters such as selectivity, trueness, precision and applicability of the method were evaluated for the developed method. The selectivity of the method was evaluated by analyzing the bisphenols free blank sample and bisphenol spiked sample. The chromatographic and mass spectral results observed for blank and spiked sample were compared and the selected of the method was evaluated. The accuracy is the trueness of the method and was confirmed by spiking/recovery method. Known and selected concentrations in the linearity range were spiked to the blank samples and were analyzed in the developed method. The % accuracy was calculated by comparing the chromatographic results observed with the calibration results for each bisphenol separately. The repeatability and reproducibility of the developed method was confirmed based on the results observed in precision study. Known and fixed concentration of bisphenols was spiked to the blank solution and were analysed 6 times in the developed method. The peak area response observed in each analyzed for each bisphenols were summarized and the % relative standard deviation (RSD) of the peak area response of each bisphenol was calculated and the % RSD of less than 2 was considered as the precise. The matrix variation was tested by the spiking/ recovery of deionized water, raw and treated water.

The sensitivity of the developed GC-MS method was confirmed based on the detection limit (DL) and quantification limit (QL) of each analyte. The minimum concentration of an analyte that can produce the % RSD of less than 5 in precision study and the recovery in the range of 70 and 130% was considered as the quantification limit of the method. The detection limit of the method was determined based on QL/3 and a signal noise ratio of 3:1.

Screening of bisphenols in packaged drinking water and surface water collected at various locations: The packaged drinking water and various real time samples collected and prepared for determination of bisphenols were analyzed in the developed GC-MS method in SIM mode. The qualitative identification of each bisphenol in sample was done by comparing the chromatograms observed in the sample with the standard. The SIM product of the sample also compared with the standard for qualitative determination of bisphenols in the samples studied. The response of the individual analyte was compared with the corresponding standard calibration curve and the quantity of each bisphenol in the sample was calculated.

RESULTS AND DISCUSSION

Bisphenols are classified as endocrine disrupting chemicals that are used in the processing of plastic materials. Bisphenols mimic the estrogens in the human body leading to various irregularities in the female health cycles. In India, haphazard dumping of plastic waste into water bodies such as rivers, lakes, canyons, *etc*. causes its pollution and the water these water bodies were reused for different purposes. Unfortunately, there is little information on the concentration of many of the pollutants present in such bodies of water. Hence the present work intended to analyze the bisphenols in packaged drinking water as well as the river and lake waters in Andhra Pradesh state of India.

The volatility and thermal stability presented by bisphenols make it suitable for detection by gas chromatography. Prior to the analysis, the individual bisphenols in single or in combination were analyzed in the GC-MS method. The resultant chromatogram shows peaks corresponds to bisphenols and the chromatogram shows peaks corresponds to impurities or other compounds in the sample. The samples were analyzed after derivatization with BSTFA shows signals corresponds to bisphenols in the study and rest of the analytes did not give signals confirms that after derivatization pure signals corresponds to bisphenols were only identified in the method. Furthermore, the limits of detection of BSTFA derivatives are mostly lower than those for underivatized analytes. The chromatograms observed in the analysis along with corresponding mass spectra of bisphenols in the method are given in Fig. 1.

The linearity was observed in the concentration range of 1 ng/L to 10,000 ng/L for BPA, BPF, BPE and BPG in the developed method. An accurate fit calibration curve with high correlation was observed bisphenols in the study. Table-2 give the results observed for bisphenols in the developed method whereas Fig. 2 shows the calibration curve observed in the developed method.

Recovery experiment was performed by spiking bisphenols at all concentration levels of the lowest concentration range before extraction. The recovery rate was determined using the average area value obtained in five repeat measurements. Satisfactory recoveries were obtained for all anaytes ranging from

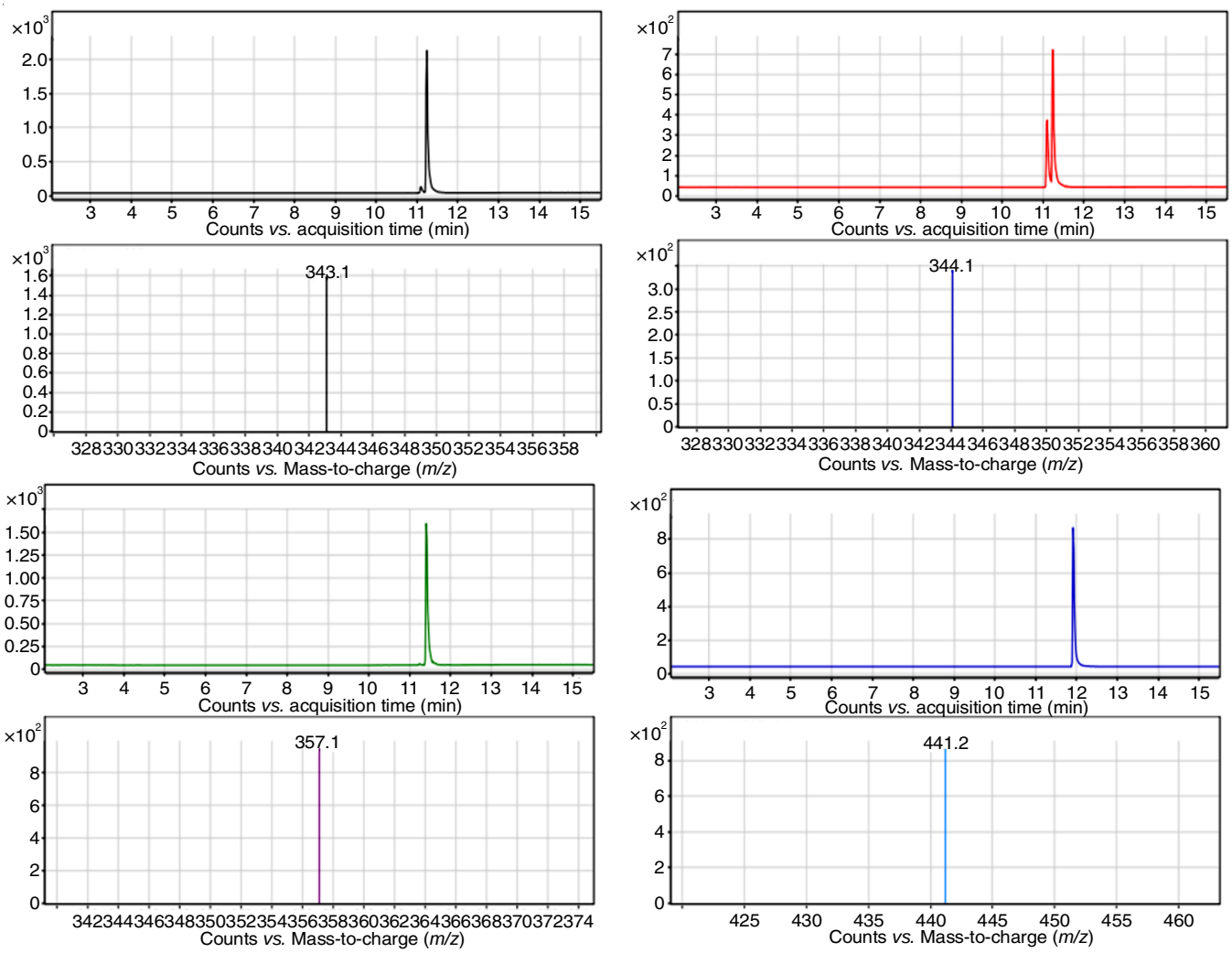

Fig. 1. GC Chromatograms and MS-SIM mass spectra obtained for standard bisphenols in the developed method

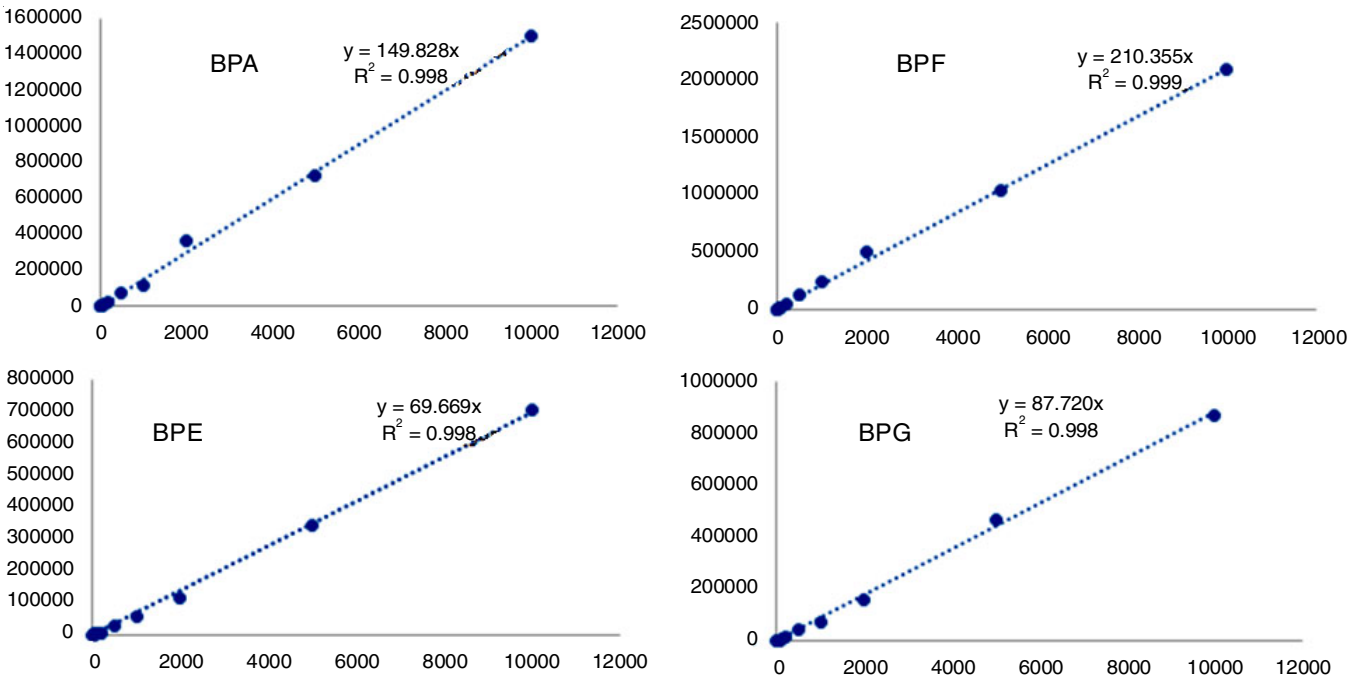

Fig. 2. Linear calibration curves observed for bisphenols in the method

TABLE-2
LINEARITY RESULTS OBSERVED FOR BISPHENOLS IN THE DEVELOPED GCMS-SIM METHOD

Conc. (ng/mL)	Peak area			
	BPA	BPE	BPF	BPG
1	265	220	93	20
10	506	773	154	38
20	1001	1787	402	85
50	4237	4800	915	200
100	9454	10221	2238	2566
200	19426	42440	5355	8537
500	72664	117836	25506	37965
1000	119659	241903	58275	70649
2000	361667	496834	111833	152245
5000	723580	1042911	340524	464837
10000	1502076	2089100	707946	870994

80-110%. Regarding the water samples used for the recovery testing there was no indication of significant interaction due to contaminating compounds. The results confirmed that the developed method was accurate with high recoveries for the analysis of bisphenols.

Precision was determined in terms of repeatability and reproducibility expressed as percent relative standard deviation (RSD%) repeatability and reproducibility was determined by intra-day and inter-day experiments analyzing spiked bisphenols in water samples at all calibration ranges in triplicate. Experiments showed good a precision for all analytical with values of below 15%. Hence, it can be confirmed that method is precise with enough repeatability. The sensitivity of the method was determined in terms of the detection limit (DL) and quantification limit (QL) of bisphenols by spiking bisphenols at the respective lowest calibration range. The QL was observed to be 1 ng/L for bisphenols in the study confirms that the method was sensitive and can quantify bisphenols in the study up to a very lowest concentration of 1 ng/L.

Analysis of bisphenols content in packaged drinking water samples: The developed method was applied for the determination of bisphenols content in packaged drinking water at various treatment conditions and the results observed are given in Table-3. The packaged drinking water sample stored in various time intervals from 0 day to 180 days at room temperature doesn't shows the bisphenols confirms that there is no leaching of plastic was observed when the sample was stored at room temperature. Hence no bisphenols was detected in these samples. Whereas the water sample treated at 50 ºC shows the peaks corresponding to the bisphenols and the mass spectral SIM analysis confirms the identification of bisphenols in these samples. The water sample stored up to 15 days at 50 ºC doesn't show any bisphenols confirms that the plastic leaching was very less and hence was not identified. The bisphenols was detected in all samples stored more than 15 days and the quantity was increased with increase in incubation time. The packaged drinking water sample stored at 50 ºC observed to be leaching with plastic and hence shows the bisphenol content. The quantity of BPG was found to be more in the samples than other bisphenols in the study. The BPE was not detected in major number of samples and very low quantity was estimated in the detracted samples. Bisphenol A (BPA) was not detected

TABLE-3
SILYLATED BISPHENOLS ESTIMATED IN THE SAMPLES STUDIED USING THE DEVELOPED GCMS-SIM METHOD

Sample	Amount estimated (ng/L)			
	BPG	BPF	BPE	BPA
Package drinking water sample stored at room temperature				
0 Day	ND	ND	ND	ND
15 Days	ND	ND	ND	ND
30 Days	ND	ND	ND	ND
60 Days	ND	ND	ND	ND
120 Days	ND	ND	ND	ND
150 Days	ND	ND	ND	ND
180 Days	ND	ND	ND	ND
Package drinking water sample stored at 50 °C				
0 Day	ND	ND	ND	ND
15 Days	ND	ND	ND	ND
30 Days	10	12	22	8
60 Days	12	18	31	12
120 Days	50	22	38	16
150 Days	55	35	41	18
180 Days	60	51	61	22
Realtime water sample collected at various locations				
Krishna river (a)	8	4	ND	7
Krishna river (b)	5	ND	ND	ND
Godavari river	7	5	ND	ND
Pulicat lake	11	8	9	10
Kolleru lake	17	14	8	12

Values given in table are the average of three measurements; ND = not detected.

in two real time samples. At maximum exposer time of 180 days, the quantity of bisphenols was found to be 60 ng/L, 51 ng/L, 61 ng/L and 22 ng/L, respectively for BPG, BPF, BPE and BPA. The leaching of plastic was increased with increase in expose time and hence the quantity of bisphenols in water was increased with increase in exposer time. The water sample exposed to 50 ºC doesn't leach plastic up to 15 days and hence bisphenols were not detected in these samples.

Analysis of bisphenols content in real time water samples: The developed method also applied for the determination of bisphenols content in five real time samples collected from three rivers and two lakes in Andhra Pradesh state of India. Bisphenol G (BPG) was detected in all the five samples studied. The samples collected in floating river at Krishna river at Amaravathi having less bisphenol content than the water sample collected at stagnated location of the same river collected at Punnami Ghat, Vijayawada. The high plastic pollution and accumulation of plastic in stagnated water at this location may be the reason for increased detection of bisphenols. The water sample at Godavari river shows peaks corresponds to BPG and BPF confirms that these two bisphenols were identified in the sample and quantification results confirm that at a quantity of 7 and 5 ng/L was observed. BPA and BPE were not identified in this sample water.

The water sample collected at Pulicat and Kolleru lake contain high content of bisphenols and among these two samples, bisphenols content was high in Kolleru Lake. All the bisphenols studied were detected in lake samples. The quantity of BPG was very high in these samples whereas the quantity of BPE was observed to be significantly less. The water sample collected

from Kolleru Lake contains 17 ng/L, 14 ng/L, 8 ng/L and 12 ng/L for BPG, BPF, BPE and BPA respectively. Among the samples studied, the quantity of bisphenols was observed to be very high in lake samples than the river samples. The pervasive plastic pollution may be the reason for the detection of bisphenols in both stagnated as well as floating water. The results obtained for the analysis of bisphenols in various real times samples are shown in Fig. 3, while the GCMS-SIM chromatograms obtained for the GCMS analysis of bisphenols in water sample collected at Kolleru Lake are shown in Fig. 4.

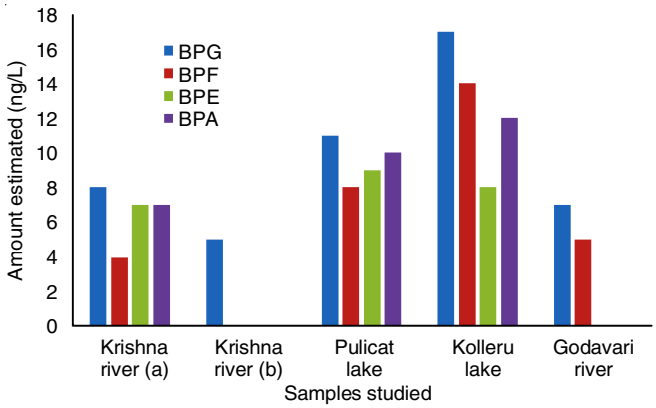

Fig. 3. Comparison graph showing the silylated bisphenols content estimated in real time samples collected from various locations

Similar studies have reported BPA levels in plastic water bottles in India [25,26] as well as in several countries [27]. Based on the results achieved it can be confirmed that the levels of bisphenols in the collected samples in the present study was significantly less than the findings reported in India as well as globally. Hence, it can be confirm that developed GCMS-SIM method was suitable for the identification and quantification of bisphenols in water as well as waste water samples.

Conclusion

In summary, four bisphenols (BPG, BPF, BPE and BPA) in the study were detected in both heat-treated packaged drinking water sample as well as the water sample collected in various locations in Andhra Pradesh state of India. Bisphenol A (BPA) is the predominant compound in all the samples studied and the quantity of BPA was estimated to be very high in the all the samples. The concentrations of bisphenols were varied in samples from various locations suggesting diverse contamination sources of bisphenol analogues. The packaged drinking water treated at 50 ºC shows 60, 51, 61 and 22 ng/L of BPG, BPF, BPE and BPA, respectively confirmed the leaching of plastic from bottle to water due to heat exposer. The water sample collected at Kolleru lake shows 17, 14, 8 and 12 ng/L of BPG, BPF, BPE and BPA, respectively confirms that due to high plastic pollution the bisphenols level was high in these samples. Hence, the developed GCMS-SIM method for quanti-

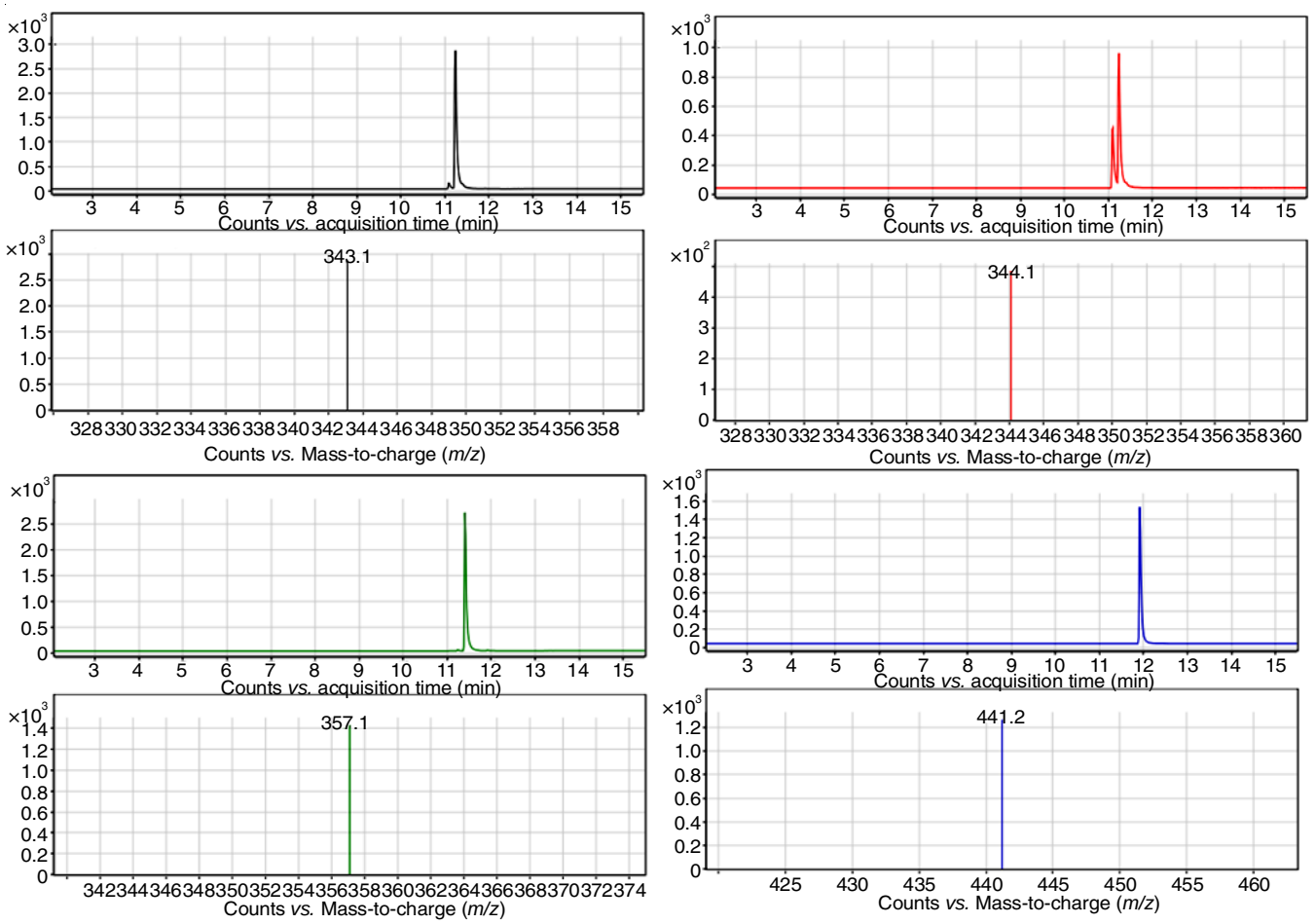

Fig. 4. GC Chromatograms and MS-SIM mass spectra obtained for bisphenols in water sample collected at Kolleru lake

tative determination of bisphenols in water samples had good performance with respect to selectivity, sensitivity, accuracy and repeatability. The results achieved for the quantification of bisphenols in the samples confirmed that there is no bisphenols content was detected in the packaged drinking water at room temperature. The water samples collected in various natural sources shows the presence of bisphenols and hence it is advised to use proper purification methods before consuming water from any natural water bodies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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