Identification and Quantification of Phthalates in Drinking, River and Lake Water using Solid phase extraction followed by UHPLC-MS

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Abstract

Phthalates are endocrine disruptive compounds that are commonly employed in everyday consumer items. Anti-androgenic action is the possible negative consequences are associated with phthalates and hence there is growing worry regarding human exposure to phthalates. It is critical to have a validated analytical approach that can measure trace quantities of phthalate metabolites in drinking water when investigating environmental exposure to phthalates. In this investigation, we developed and validated an accurate, sensitive and robust LC–MS technique to concurrently detect six phthalates in drinking water samples: dimethyl (DMP), diethyl (DEP), dipentyl (DPrP), diisobutyl (DIBP), dibutyl (DBP) and dioctyl (DOP). These analytes were quantified by using LC–MS system with electrospray ionization.

The validated approach has been utilised effectively in measuring phthalate exposure in drinking water samples and water samples collected at various locations. Packaged drinking water samples separated into two groups, room temperature samples (15 days, 30 days, 60 days, 120 days, 150 days and 180 days) and heated samples at 50 °C with similar interval samples.

The present investigation also applied for the analysis of phthalates content in river and lake water samples collected at various real time locations.

The results proved that the water sample kept at room temperature does not shows peaks corresponding to phthalates in the study. The water sample exposed to 50 °C temperature for 80 days shows Phthalate's content of 87411, 42806, 45690, 40946, 40696 and 24753 ng/L for DMP, DEP, DPrP, DIBP, DBP and DOP respectively confirming that the leaching of plastic due to temperature raises the phthalate's level in water. It can be confirmed that the method can be suitable for the analysis of phthalate in drinking water as well as in wastewater samples.

Keywords: Phthalate's analysis, UHPLC-MS, Plastic pollution, Water bodies contamination.

Introduction

The term phthalate esters are restricted to the ortho form of benzene di carboxylic acid prepared by reaction of phthalic acid with a specific alcohol to form the desired ester[10]. Most of the esters are colourless liquids, have low volatility and are poorly soluble in water but soluble in organic solvents and oils. Two other isomeric forms of benzene di carboxylic acid esters are also available having important industrial applications: the meta form (or isopthalate esters) and the para form (or terephthalate esters). Phthalate acid esters, a class of chemical compounds mainly used as plasticizers for polyvinyl chloride (PVC) or to a lesser extent other resins in different industrial activities, are ubiquitous in the environment and have evoked interest in the past decade due to endocrine disrupting effects and their potential impacts on public health.

Worldwide production of phthalates is approximately 6 million tons per year[3]. As phthalates are not chemically bound to the polymeric matrix in soft plastics, they can enter the environment by losses during manufacturing processes and by leaching or evaporating from final products[12].

Therefore, the occurrence and fate of specific phthalates in natural water environments have been observed and also there are a lot of considerable controversies with respect to the safety of phthalates in water. Six phthalates compounds, including dimethyl (DMP), diethyl (DEP), dipentyl (DPrP), diisobutyl (DIBP), dibutyl (DBP) and dioctyl (DOP), are classified as priority pollutants by the U.S. Environmental Protection Agency (EPA). Though the toxicity of phthalates to humans has not been well documented, for some years, the Ministry of Environmental Protection in China has regulated phthalates as environmental pollutants[7].

In addition, the standard in China concerning analytical controls on drinking waters does not specifically identify any phthalates as organic pollutant indexes to be determined by the new drinking water standard in 2007(Standard for drinking water quality; GB5749-2006), which was forced to be monitored for the drinking water supplies in 2012. Consequently, official data about the presence of these pollutants in the aquatic environment of some cities is not available[2,6,11].

There is always a risk that migration of components from plastics and other packaging materials can occur and although with most solid medicaments, this risk is low, it

must always be considered[4]. The popularization of polymeric packaging materials has resulted in increased concerns over the migration of undesirable components into foods. This has the potential to affect product quality as well as safety[9]. These concerns are generally focused on the levels of residual monomers and plastics additives such as plasticizers and solvents present in polymers intended for direct or close contact with food. Additives are used to aid the production of polymers and to modify the physical properties of the finished material.

For instance, plasticizers, added to give a plastic the desired flexibility, have been identified as a potential threat to health. The World Health Organization has published opinions on a number of commonly used plasticizers with comments on toxicity[5]. The use of plasticized PVC as cling film has been targeted as a potential problem in terms of migration. Studies have been conducted on the migration of the plasticizer di-(2-ethylhexyl) adipate from PVC films into food during home use and microwave cooking and in retail food packaging. The level of migration increased with both the length of contact time and temperature of exposure, with the highest levels observed where there was a direct contact between the film and food and where the latter had a high fat content on the contact surface. Use of a thinner PVC film was suggested as means of reducing the migration of this plasticizer[8].

Sample analysis for phthalates usually involves isolation and clean up followed by separation, identification and quantification. Many different techniques have been used for analysis including gas chromatography (GC) with electron capture detection, flame ionization detection or mass spectrometry (MS)), liquid chromatography with ultraviolet or mass detection and thin-layer chromatography. These techniques are often used in conjunction with liquid–liquid extraction (LLE), solid-phase extraction (SPE) solid-phase microextraction (SPME)[1]. However, some of the reported methods suffer from low recovery, insufficient sensitivity or high injection volumes.

Other extractions methods available were environmentally damaging and hence there is a need to develop a simple, cost effective and environment friendly method for the analysis of phthalates. In view of this, the present study aimed to develop a UHPLC-MS method for the analysis of phthalates in bottled water with exposure to heat. The molecular structures of phthalates in the study were given in fig. 1.

Material and Methods

Experimental materials: Standards drinking water bottles were purchased from local market. Acetonitrile (ACN) was purchased from Sigma Aldrich (Bengaluru). Homogenizer (NETZSCH lambda vita) was purchased from Millipore water (MILLI-Q® HX 7000 SD). Solid phase extraction cartridges (SPE) were purchased from Waters (Miford, MA, USA) and filter paper (0.22µ) was purchased from Millipore.

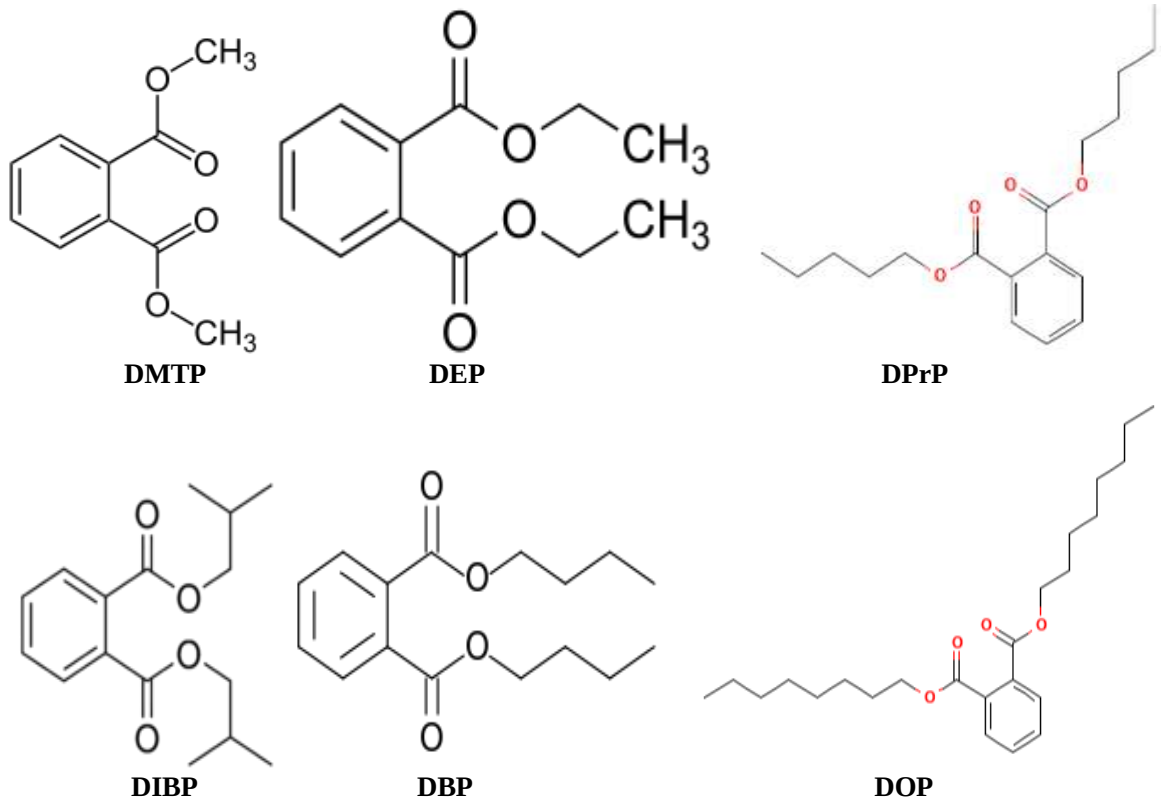

Fig. 1: Molecular structure of phthalates in the study

Sample collection: A total of 7 samples were collected in triplicate 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 analysed after 15, 30, 60,120,150 and 180 days of incubation.
Group 2: Packaged drinking water sample kept at 50 ^{o}C temperature and the sample was analysed after 15, 30, 60,120,150 and 180 days of incubation.
Group 3: Real time sample collected at the following locations: a) Krishna River at Punnami Ghat, Vijayawada andhra Pradesh, b) Krishna River at Amaravathi, Guntur andhra Pradesh, c) Pulicat Lake at Sullurpeta andhra Pradesh, d) Kolleru Lake at Kolletikota, Krishna district andhra Pradesh and e) Godavari River at Godavari bridge, Rajahmundry andhra Pradesh

Sample preparation: An accurately pipetted 10 mL of drinking water sample was taken into C18 solid-phase glass cartridge. After that, wash with 5 mL of 80 % methanol, then reconstitute with 10 mL of methanol and dichloromethane solution in the ratio of 50:50 (v/v). The sample was collected into a glass tube and evaporated in lyophilization. After lyophilisation, reconstitute it with 300 µL of acetonitrile, vortex for 5 min and analyse by UHPLC-MS.

Preparation of Standards: The stock solutions of individual standards were prepared with concentration of 1 mg/mL with acetonitrile as diluents. A total of selected concentration of mixed standard solution was prepared by taking stock solution of each analyte in acetonitrile and all standards were stored in a glass volumetric flask and kept in -20 ^{0}C further analysis.

Preparation blank samples: A blank sample was used to detect possible contamination during analysis to avoid carryover. Plastic materials were not used in sample collection, preservation, preparation and analysis. Standards and samples were prepared in glass tube to prevent possible contamination from air and degradation from temperature and light. All glassware were cleaned with acetone and kept in oven for further analysis.

UHPLC-MS analysis: The separation of phthalates was carried out using acuity UHPLC WATERS coupled with SQ-DETECTOR with MS consisting of a binary solvent manager, degasser, thermostat auto sampler and column oven. The chromatographic separation of phthalates was achieved using Acquity BEH C_{18} column (50 × 2.1 mm, 1.7 µm, Water, MA, USA). The column and auto sampler temperature were maintained at 35^{0}C.The mobile phase A is 0.05% of formic acid in water and mobile phase B is 0.05% of formic acid in acetonitrile. The gradient program started from 10% mobile phase B with an initial hold of 0.3 min to a increase to 40% mobile phase B from 3.0min and then decreased to 50% mobile phase B in 5 min to 8min. The mass identification and quantification of phthalates were performed using binary consisting of an Electro spray ionization source (ESI)., source voltage 3.48 kv, source temperature 150 ^{0}C, Rf lenses 2.5, source gas flow desolation 750L/Hr, analyser lon resolution 15, ion energy 0.5ev.

Method validation: The developed UHPLC MS method was validated in various parameters such as linearity, specificity, accuracy, precision, matrix effect and sensitivity.

Solid phase extraction: Solid-phase extraction (SPE) is a widely used sample preparation technique for the isolation of selected analyses. The principal goals of SPE are to enrich and purify samples and transfer them from the sample matrix to a different solvent or to the gas phase. There is no doubt that SPE is a popular sample preparation technique in many areas including environmental, pharmaceutical, clinical, food and industrial chemistry. SPE has been used to expertly enrich and purify phthalates from water.

The C18 SPE column was pre-treated by washing with 5 mL of methanol and 5 mL of purified water before each SPE procedure. The sample water was passed through the pre-conditioned C18 SPE column at the optimum flow rate. After, the phthalates retained on the SPE column were eluted with an optimal volume of 10 mL of acetonitrile and the resulting eluent was blown to nearly dry with a gentle nitrogen flow at 30°C. The dried sample was re-dissolved in 300µl of acetonitrile.

Analysis of phthalates in water samples: The phthalates in the packaged drinking water were studied at treatment conditions and the water samples were collected at various locations using the developed UHPLC MS method. The SPE was performed for all the samples in the study and the reconstituted sample was analysed using the UHPLC method. The phthalates present in the water samples were identified by comparing the retention times of the peaks identified in the samples with the standard retention time and the quantification was performed by comparing the peak area of the sample peak with the corresponding standard calibration curve. The results were expressed in terms of ng/mL. The chromatogram that does not shows any peak corresponds to the phthalates confirms that the sample contains phthalates less than the detection limit of the developed method.

Results and Discussion

The developed UPLC MS method can effectively separate all the six different phthalates in the study. No overlapping of the compounds was observed in the chromatogram and clear mass fragmentation pattern was obtained for all the compounds in the study. The standard chromatogram observed for phthalates in the developed method along with its mass fragmentation spectra is given in fig. 2.

Linearity: Linearity is the ability to produce results that are directly proportional to the analyte concentration in the

sample. The linearity was taken as ten-point calibration curve of phthalates in drinking water in the concentration range of 5-5000 ng/L using linear least square method. The coefficient of determination R^2 was found to be in the range of 0.996-0.999 for all phthalate compounds. The linear regression data for the linearity plot shows an excellent linear relationship throughout the linearity range. Fig. 3 shows the calibration curves observed for standard phthalates in the developed method.

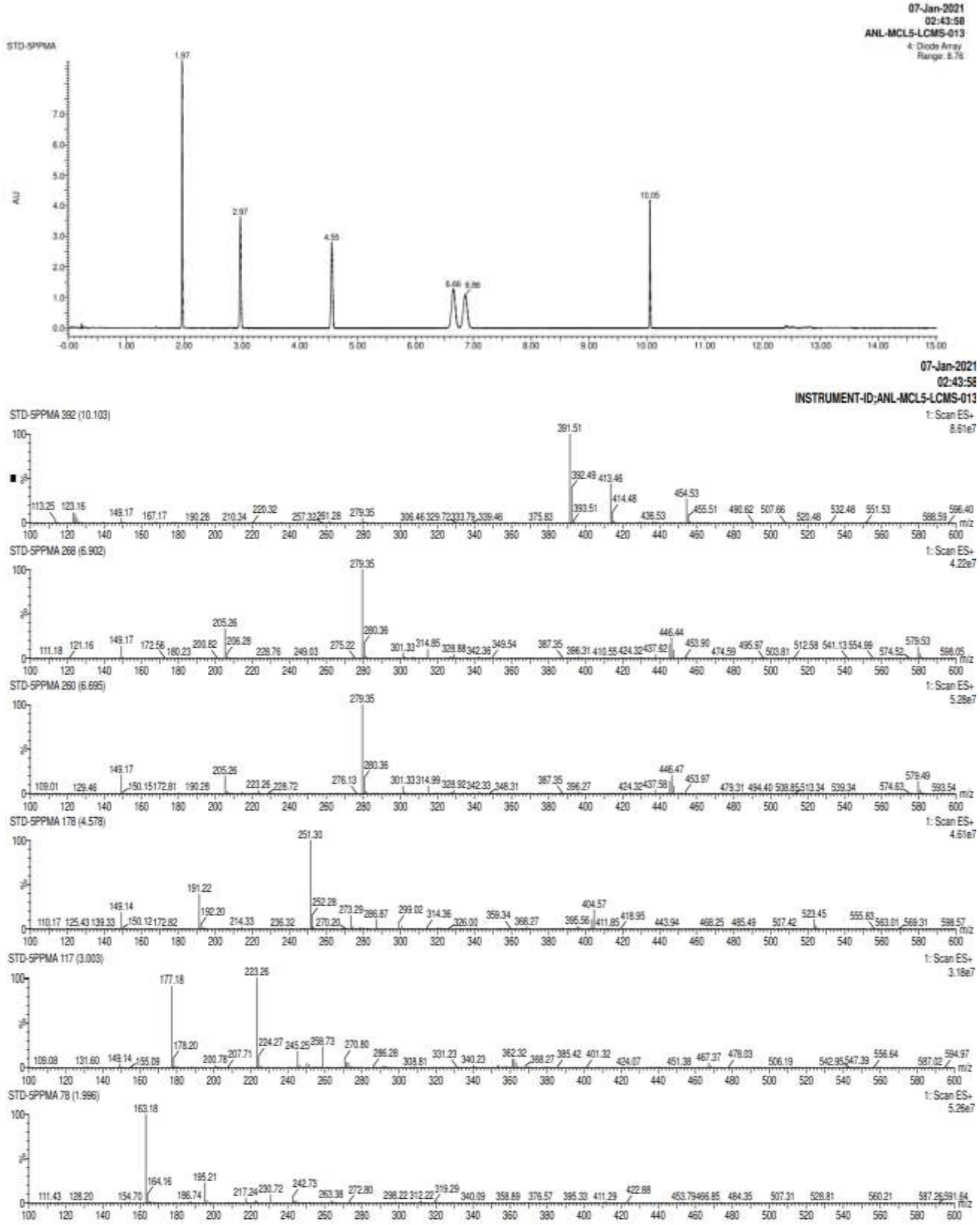

Fig. 2: Standard UHPLC chromatogram and its mass spectra observed in the developed method

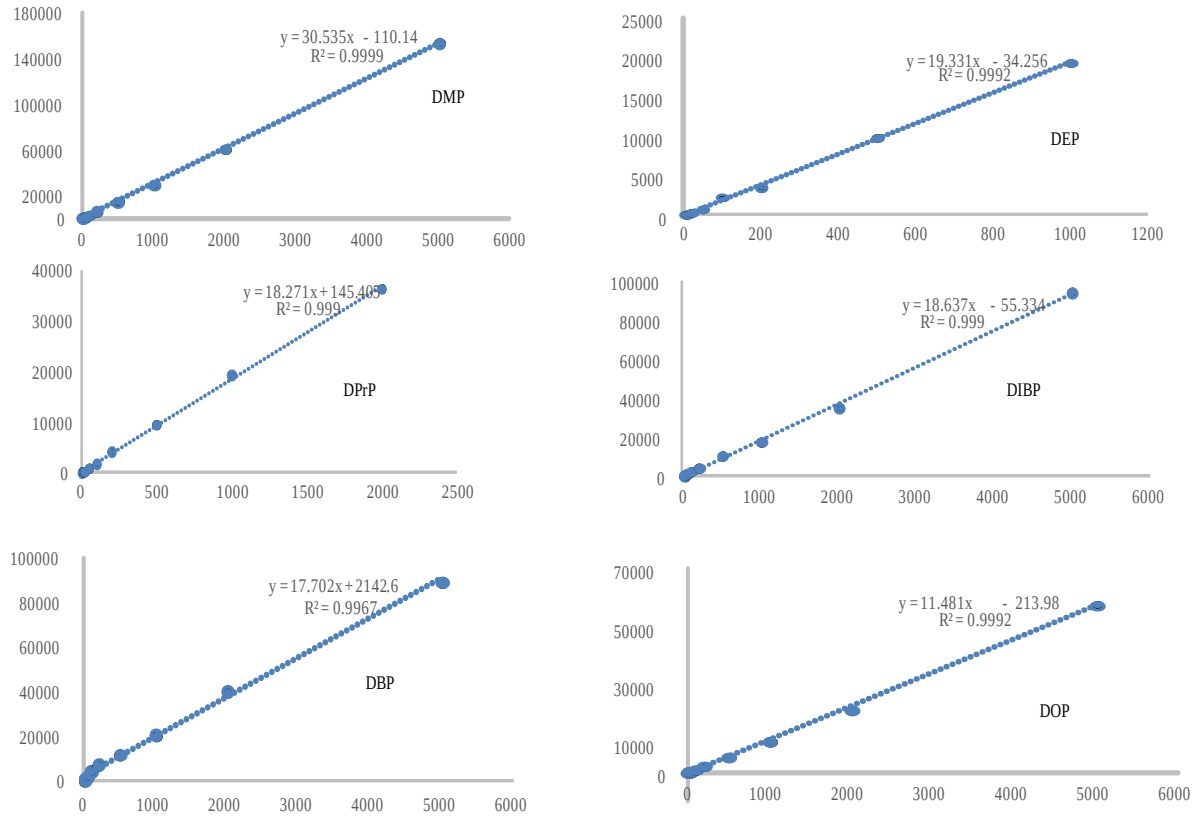

Fig. 3: Recovery linearity of phthalates

Table 1
Recovery results

Name	RT (min)	% RSD	% recovery obtained at a studied concentration in ng/L		
			5	200	2000
DMP	1.92	10.1	82	84	80.3
DEP	2.94	9.9	84.6	80.5	80.3
DPrP	4.51	8.4	86.9	86.7	80.2
DIBP	6.62	8.2	97.3	81.3	85.6
DBP	6.80	4.3	97.3	81.6	92.7
DOP	10.04	7.3	90.9	81.1	80.9

Method specificity: The method specificity was performed in the real river water samples like with and without spiking of the compounds. The samples without spiking were designated as blank water samples whereas those spiked with phthalates at their LOD level were identified as spiked drinking water samples (n=5). In the blank sample, no peaks were observed at the specific retention time showing the absence of analytes in the blank water sample. The spiked water samples displayed peaks indicating that the method is highly specific for the phthalate compounds.

Precision: The precision is the ability of the assay to reliably reproduce the results when sub samples were taken from the same specimen. The precision of the measurement was determined by performing five replicates at each concentration 50, 200,1000 and 5000 ng/L in the water sample for inter and intra-day repeatability and is represented as percent relative standard deviation (% RSD). The precision was found to be in the range of 1.2-9.6% and 2.0-14% for intra and inter-day respectively.

Recovery: For recovery study, three concentrations 5, 200 and 5000 ng/L were taken one at limit of quantification level, second one at middle and third one is the highest level of the linearity. Recovery was calculated based on equation and was found to be in the range of 80-114.7%. Table 1 gives the recovery results observed in the developed method.

Sensitivity: The method sensitivity was assessed based on its Limit of quantification (LOQ) and Limit of detection (LOD) concentrations for analysis of phthalates. LOD is the lowest concentration of the analyte with signal to noise ratio less than 3. The LOD was observed to be 1.515 ng/L and LOQ was calculated as 5 ng/L. The results confirm that the method was very sensitive and can detect the phthalates up to a very low concentration.

Matrix effect: Matrix effect is a co- dependent phenomenon and can affect the ionization efficiency of the analytes and it is evaluated to measure the impact of matrix interferences on the analysis of phthaltes and to understand the ion intensity enhancement or suppression. Matrix effect can affect the quantification of phthalates unless they are diminished or compensated. The results confirm that no matrix effect was identified in the developed method confirming that there is no interference of impurities or other compounds for the analysis of phthalates in the developed method.

Precision: The precision is the ability of the assay to reliably reproduce the results when sub samples were taken from the same specimen. The precision of the measurement was determined by performing six replicates at each concentration 50,200, 1000 and 5000 ng/L in the water sample for inter and intra-day repeatability represented as percent relative standard deviation (%RSD). The precision was found to be in the range of 1.2-9.6% and 2.0-14% for intra and inter-day respectively.

Real sample analysis

Drinking water at room temperature: Drinking water is divided into two sets, drinking water at room temperature and drinking water exposed to heat at 50^0C at day's intervals like 0 days, 30 days, 60 days, 120 days, 150 days and 180 days. In-room temperature drinking water sample was not contaminated with plastic and not showing any phthalates among six samples (table 2).

Drinking water exposed to heat: Another set of drinking water samples was heated at 50 ^{0}C for zero, 15, 30, 60, 120, 150 and 180 days respectively. It is not polluted with plastic after zero days of exposure to be heated at 50 °C. Only one chemical discovered in water after 15 days is DMP phthalate, which shows up at 1.7 µg/L. Some phthalates, DBP and DOP, degrade with heat after 30 days, yielding 2 µg/L and 0.14 µg/L respectively. At 60 days, DBP, DPrP and DOP are showing 0.673µg/L, 0.662 µg/L and 0.664 µg/L. Among the six phthalates, DPrP and DBP are more concentrated in water with concentrations of 10 µg/L and 10 µg/L respectively. Almost all phthalates are polluted with plastic after 180 days. Among the six chemicals, five exhibit greater concentration. According to the data, water subjected to heat may cause plastic breakdown in water samples. Table 2 gives the results observed for the analysis of phthalates in packaged drinking water sample.

Analysis of phthalates in water samples collected at various locations: The analysis of phthalates in the water samples collected at various locations was carried using the developed UHPLC-MS method. The result as shown in table 2 confirms that high quantities of phthalates were identified in the water samples studied. Among the samples studied, the water sample collected at Kolleru Lake shows high quantity of phthalates (Fig. 4).

The quantity was observed to be 1197, 1008, 1090, 1243, 809 and 1441 ng/L for DMP, DEP, DPrP, DIBP, DBP and DOP respectively. Among the samples studies, less quantity of phthalates was detected in water sample collected in Krishna River at Amaravathi andhra Pradesh. The water sample collected at rives shows significantly less quantity of phthalates than the water samples collected in lakes. The samples collected in all the locations show positive for the phthalates and this may be due to the accumulation of plastic in the water bodies leaching the phthalates into the water bodies.

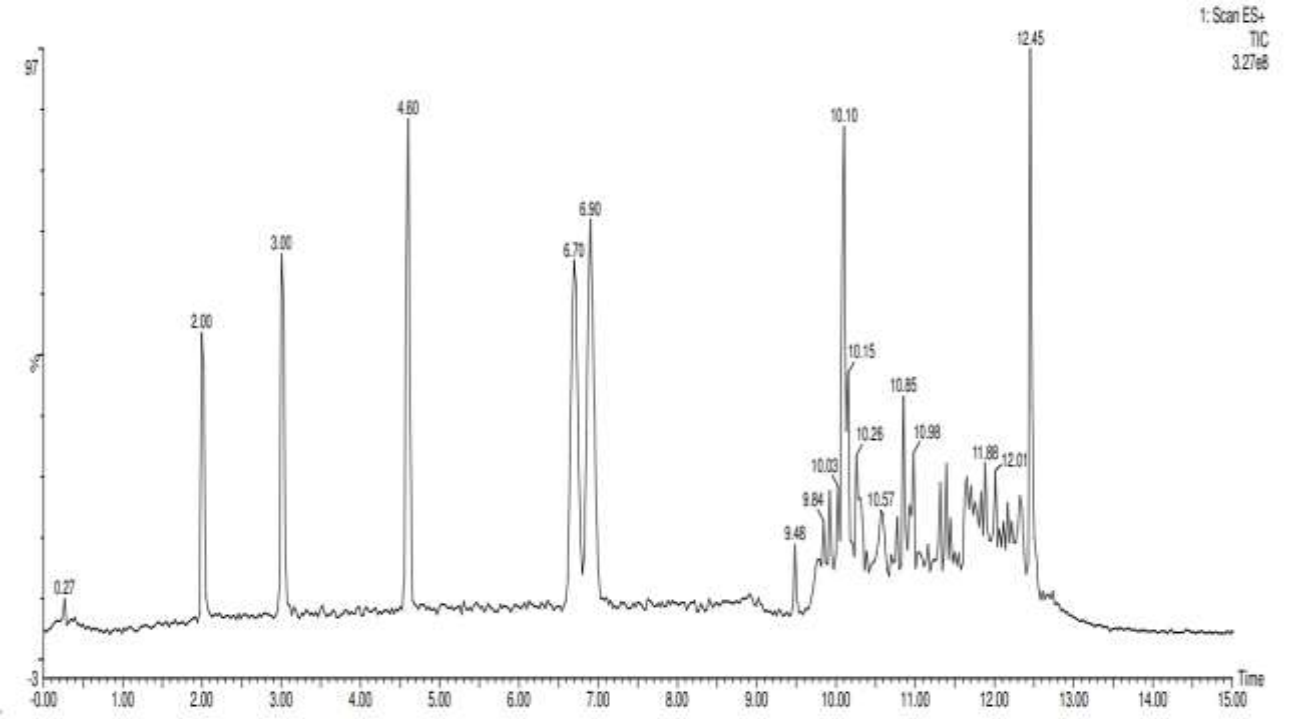

Fig. 4: chromatogram identified for phthalates in water sample collected at Kolleru Lake

Table 2
Phthalates estimated in various samples in the study using the developed UHPLC – MS method

S.N.	Sample	Amount estimated in ng/L					
		DMP	**DEP**	**DPrP**	**DIBP**	**DBP**	**DOP**
Package drinking water sample stored at room temperature							
1	0 Day	ND	ND	ND	ND	ND	ND
2	15 Days	ND	ND	ND	ND	ND	ND
3	30 Days	ND	ND	ND	ND	ND	ND
4	60 Days	ND	ND	ND	ND	ND	ND
5	120 Days	ND	ND	ND	ND	ND	ND
6	150 Days	ND	ND	ND	ND	ND	ND
7	180 Days	ND	ND	ND	ND	ND	ND
Package drinking water sample stored at 50 °C temperature							
8	0 Day	ND	ND	ND	ND	ND	ND
9	15 Days	ND	ND	ND	ND	1749	ND
10	30 Days	ND	ND	ND	ND	2141	114
11	60 Days	2235	673	662	ND	3514	604
12	120 Days	4103	2023	2278	1858	3185	1137
13	150 Days	4103	2023	2278	1858	3185	1137
14	180 Days	87411	42806	45690	40946	40696	24753
Realtime water sample collected at various locations							
15	Krishna River (a)	185	213	590	208	499	230
16	Krishna River (b)	456	ND	ND	321	545	ND
17	Godavari River	318	600	701	ND	ND	416
18	Pulicat Lake	731	209	500	944	917	805
19	Kolleru Lake	1197	1008	1090	1243	809	1441

Conclusion

The method developed was sensitive and efficient for the identification and quantification of phthalates in water samples. This study was designed to investigate the presence of six phthalates in packaged drinking water at different treatment conditions and water samples collected in two rivers and two lakes in Andhra Pradesh. The packaged drinking water at room temperature does not show any phthalates whereas the same bottle kept at 50 ^{o}C shows phthalates in it and the quantity of phthalates increased with increase in the temperature exposure time.

The water samples collected at rivers and lakes show phthalates at high quantity. The leaching of plastic due to temperature and the plastic pollution in the rivers and lakes cause the entry of phthalates into the water. Hence the present method can confirm to be suitable for the identification and quantification of phthalates in water and wastewater samples.

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(Received 12th December 2021, accepted 16th January 2022)