Determination Of Di-N-Butyl Phthalate (Dnbp) In Water Samples Of Dal Lake Using DLLME Coupled With HPLC-DAD: Environmental Study.

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Abstract

The present study aims at determining the concentration of di-n-butyl phthalate(DnBP) in water samples from three sites; Hazratbal, Saida kadal and Teilbal of Dal Lake using novel extraction procedure Dispersive liquid-liquid microextraction (DLLME) coupled with (High performance liquid chromatography- Diode array detector) HPLC-DAD. The chromatographic separation was carried out with Zorbax C_{18} column (150× 4.6mm, 5µm) at column temperature of 30˚C and run time of 30 min. The mobile phase used was mixture of methanol and water (70:30 v/v) with flow rate of 1.0ml/min. The Analyte was detected at the wavelength of 230 nm in all the three water samples at the concentration of 25.44µg/L, 40.81µg/L and 22µg/L respectively.

Keywords: di-n-butyl phthalate (DnBP), DLLME, HPLC-DAD

Introduction

The word plastic is derived from plasticos (Greek), which means capable of sculping into different shapes. By dint of the swift advancement in technology and the geometric progression of the global population growth, plastic materials have found resourceful applications in every aspect of modern human life. A number of substances are blended in plastics at various proportions to improve their performance and reduce cost (Tokiwa *et al*., 2009). Phthalates are one of such organic compounds used as plasticizers. Phthalate esters (PAEs), one derivative of phthalic acid (PA), are aromatic colorless liquids with high-molecular weight, high stability, low-volatility and low-solubility in water (Chang *et al*., 2004; Chao *et al*., 2006). Phthalates are family of xenobiotic hazardous compounds amalgamating in plastics to intensify their plasticity, flexibility, longevity, versatility and durability. Besides they are also used as lubricants, solvents, additives, softeners etc. They are present in number of day to day used products such as PVC products, building materials (paint, adhesive, wall

covering), personal-care products (perfume, eye shadow, moisturizer, nail polish, deodorizer, liquid soap, and hair spray), medical devices, detergents and surfactants, packaging, children's toys, printing inks coatings, pharmaceuticals and food products, textiles, household applications such as shower curtains, floor tiles, food containers and wrappers, cleaning materials. Exposure of humans to phthalates can occur via number of routes including ingestion of polluted water and food, inhalation of adulterate air, consumption of certain medications and skin contact with lotions or cosmetics containing phthalates as additives (Wittassek *et al*., 2011).

DnBP is one of the most important phthalate essentially used as plasticizer to ameliorate the flexibility and workability of the products, such as polyvinyl chloride, plastic packaging films, adhesives, lubricants, cellulose materials, cosmetics and insecticides (Gao and Wen, 2016). DnBP leaches out from all these products to the environment and thus become a common contaminant; it has been recognized in environmental strata, inclusive of air, water, and soil (ATSDR, 2001 [drinking water: 0.1-5.0 ppb]). DnBP is not chemically attached to the polymer matrix like other phthalates, directing to its ubiquitous existence in the diverse environmental matrices (Net *et al*., 2015). DnBP is one of the most usual phthalate in the atmosphere and particles we inhale in the indoor environments (Rakkestad *et al*., 2007; Bolling *et al*., 2013). As calculated the exposure from the adulterate indoor air may contribute to more than 20% of the daily internal dose of DnBP (Wormuth *et al*., 2006). In Europe and USA, DnBP has been detected in surface waters (Wypych, 2004). DnBP and its metabolite MBzP were reported to be genotoxic. Both, under Invitro condition, inhibit the action of enzyme superoxide dismutase in a concentration dependent manner by binding to the active site of the enzyme responsible for the binding and deactivation of reactive oxygen species (Prasanth *et al*., 2009). To lessen the adverse impact of phthalate exposure to humans, some regulations have been proposed related to their limited use and maximum concentration levels (Fontelles and Clarke, 2005; NSC, 2008). Owing to health concern, the European Union has issued ban on sale of PVC infant biting toys having phthalates on November 20, 1999 (Biedermann-brem *et al*., 2008).

A number of oldest techniques of sample preparation and extraction are known but they are time consuming, requiring large amount of organic solvent, relatively expensive and sometimes shows poor batch to batch reproducibility etc. Hence, a lot of research attempts in separation science and related fields have been concentrated on the evolution of new sample preparation techniques, that are more effective, less time consuming and need smaller amount of organic solvent (Raynie, 2004). Among number of latest techniques of sample preparation, Dispersive liquid-liquid micro extraction (DLLME) is innovative environmentally agreeable sample preparation technique, holding evident advantage of simple operation with high enrichment factor, lower requirement of organic solvent, cost effectiveness etc. Contrasting with other sample preparation techniques like Single drop microextraction (SDME) and hollow fiber-based liquid phase microextraction (HF-LPME), DLLME requires very short extraction time. DLLME is based on ternary component solvent system; extraction and disperser solvent are swiftly injected into aqueous sample by syringe. The mixture is benignly shaken and cloudy solution (water/disperser solvent/extraction solvent) is formed in test tube. Finally following centrifugation, fine particles of extraction solvent settle at the bottom of conical test tube. The

resultant settled phase is taken off with microsyringe and then subjected to analysis. DLLME paired with Gas chromatography (GS), High performance liquid chromatography (HPLC) and atomic absorption spectrometry (AAS) have been broadly used in the survey of environmental and food sample. The advantages of DLLME methods are swiftness cost effectiveness, simplicity of operation, the great recovery, high enrichment factor and environment benignity.

High performance liquid chromatography (HPLC) is highly advanced liquid chromatographic technique. In this technique instead of a solvent being permitted to flow through a column under gravity, it is forced through under high pressure of up to 400 atmospheres that results in much faster rates. A very much small particle size for the column packaging is used which provides much greater surface area for interactions between stationary phase and the molecules flowing past it. This permits much better separation of the components of the mixture. The detection methods are highly automated and extremely sensitive, quite efficient, swift and accurate.

Material and methodology

Chemicals and reagants

Di n butyl phthalate (99.5%), Acetonitrile and Methanol were of HPLC grade and were purchased from Sigma Aldrich (Bangaluru, India). Ultra pure water was prepared in the mili-Q Advantage A10 water system product unit (Millipore, Belford, MA, USA). Carbon tetrachloride, acetone, n-hexane, dichloromethane were of analytical grade and were purchased from Himedia (Delhi, India).

All the solvents were filtered through 0.45-µm membrane to eliminate particulate matter before analysis. All the glasswares used in the work were previously washed with the acetone, dichloromethane and n-hexane and were finally dried in an oven at 250˚C.

Preparation of standard solution

The stock solution of di n butyl phthalate (DnBP) was prepared in methanol at the concentration of 1000mg/L and was stored at 4˚C. The standard working solution (20µg/L) was prepared by dilution of stock solution.

Sample collection and preservation

Water samples were collected from three different sites of Dal Lake namely Hazratbal, Saida kadal and Teilbal. Samples were collected randomly into a pre-cleaned, light-preserved glass bottles. Thousand mililitre of each sample was immediately filtered through 0.22µm nylon membrane so as to remove suspended solids. The filters were cleaned with acetone prior to filtration as it was realized that filters may contain considerable amount of phthalates. The filtered samples were stored at dark conditions at 4˚C until further analysis.

Extraction

A novel method, DLLME has been used for the extraction of Analyte from water samples.

An aliquot of 5.00 mL of an aqueous sample was placed in 10mL of glass test tube with conical flask. 0.75mL of Acetonitrile (as dispersive solvent) containing 41µL CCl_4 (as extraction solvent) was injected swiftly into the sample solution by the help of 1.00 mL and after that the mixture was shaken gently. A cloudy solution containing fine droplets of CCl_4 dispersed into aqueous sample got formed and Analyte was extracted into the fine droplets. Centrifugation was done for 5 min at 3000 rpm. The extraction solvent got sedimented at the bottom of the conical test tube (About 25µL). Using 50µL HPLC micro syringe, 20 µL of sedimented phase was removed and then injected into HPLC system for analysis.

HPLC Analysis

The analysis of DnBP in lake water samples was performed by HPLC technique. HPLC analysis was done on Perkin Elmer series 200 HPLC system equipped with quaternary pump, a vacuum degasser, an auto sampler, and a column compartment, coupled to DAD. Separation was done on Zorbax C_{18} reverse phase column (150× 4.6mm, 5µm), at a column temperature of 30˚C and run time of 30 min. The mobile phase was a mixture of methanol water (70:30 v/v) with a flow rate of 1.0ml/min; The Analyte was detected by DAD at 230nm.

Result and discussion

The suitability of this method for DnBP monitoring was detected by analyzing the water samples collected from three sites of Dal Lake namely Hazratbal, Saida kadal and Teilbal. The result showed that all three water samples were contaminated with DnBP at the concentration of 25.44µg/L, 40.81µg/L and 22µg/L respectively. Chromatogram of standard is shown in **figure (1)** while as chromatograms obtained for water samples (lake water) after DLLME coupled with HPLC under optimum conditions were shown in **figure (2), (3), and (4).**

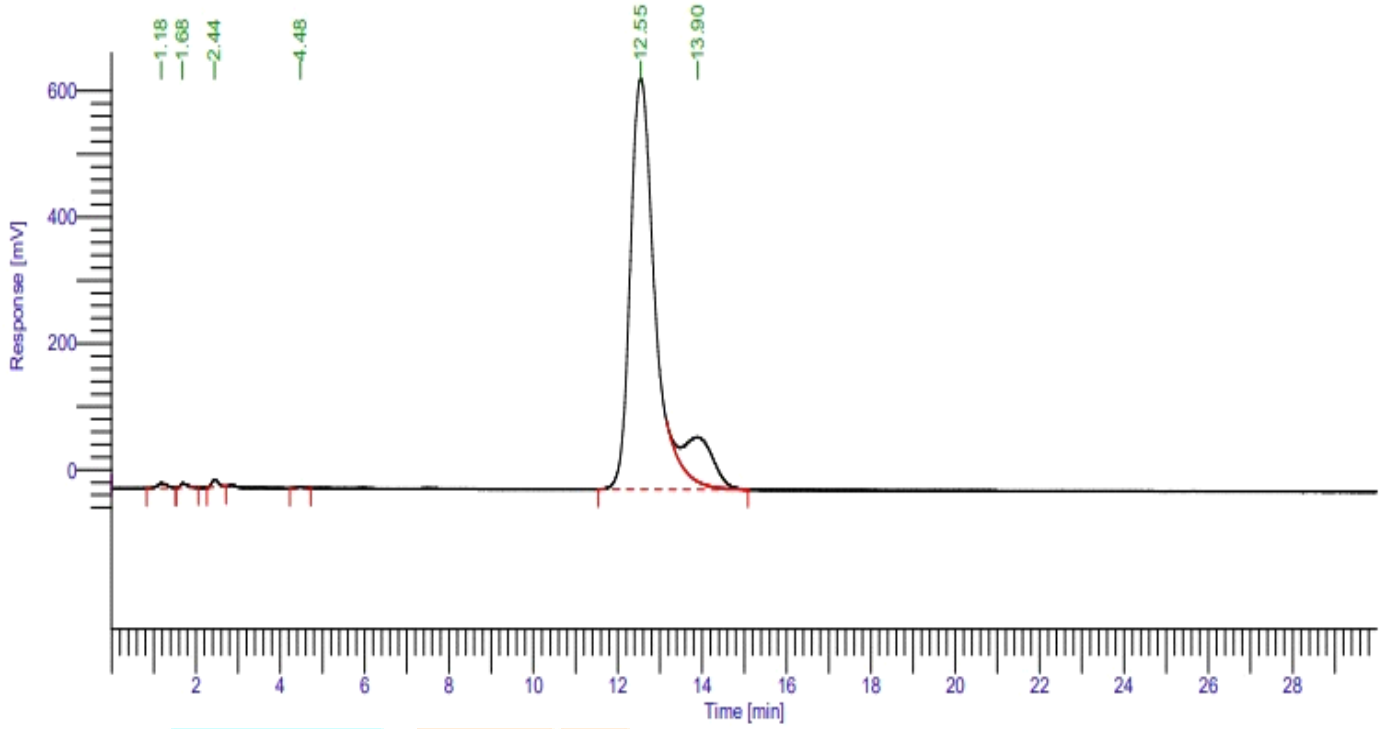

Figure 1 : HPLC chromatogram of standard (DnBP) Analyzed under optimum conditions.

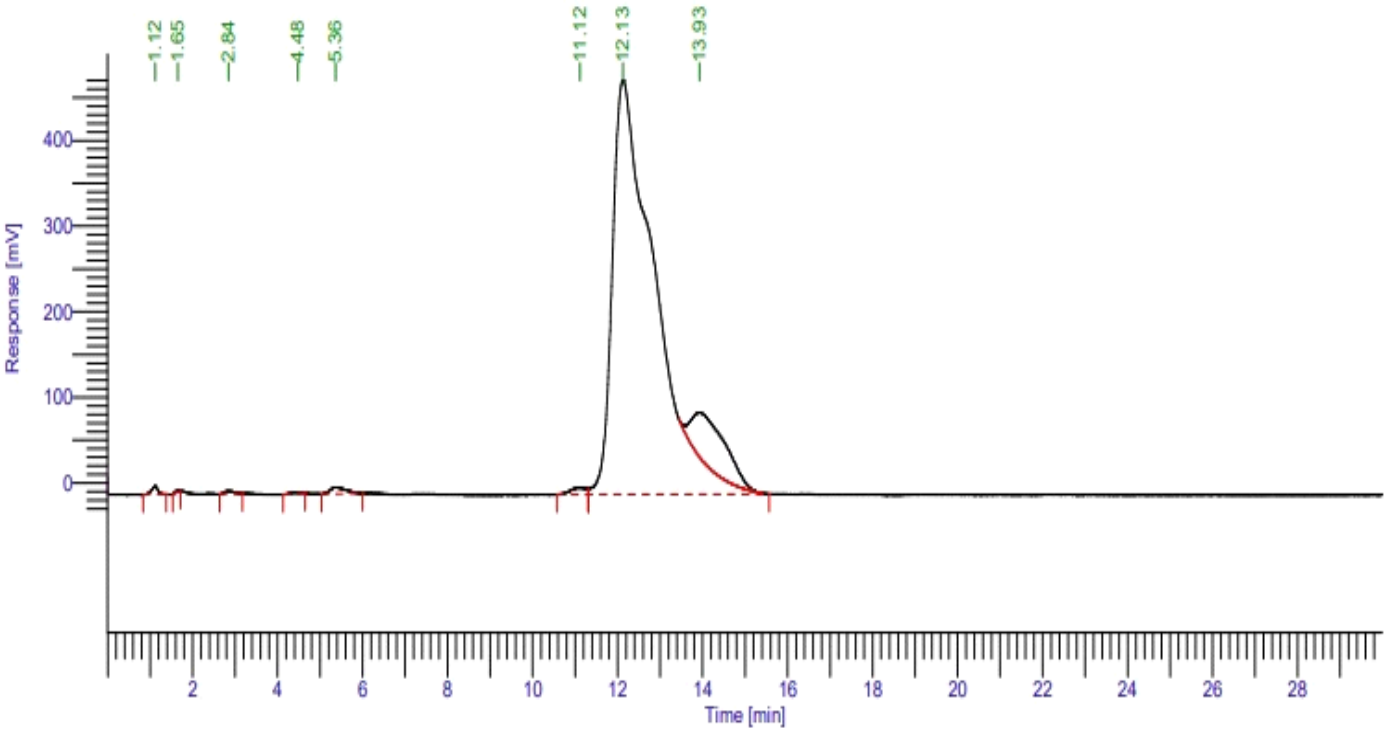

Figure 2: HPLC chromatogram of water sample (Hazratbal) of Dal Lake analyzed under optimum conditions by proposed extraction procedure DLLME coupled with HPLC-DAD.

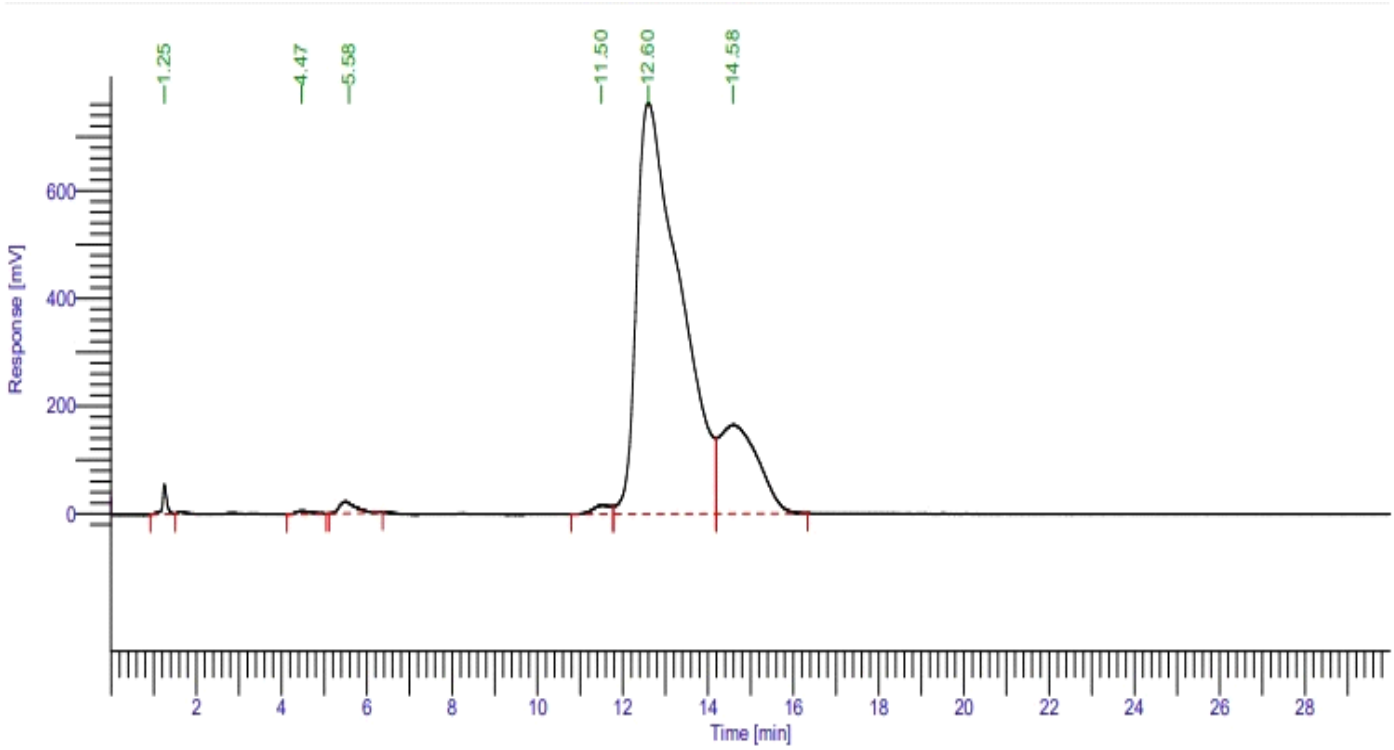

Figure 3 : HPLC chromatogram of water sample (Saida kadal) of Dal Lake analyzed under optimum conditions by proposed extraction procedure DLLME coupled with HPLC-DAD.

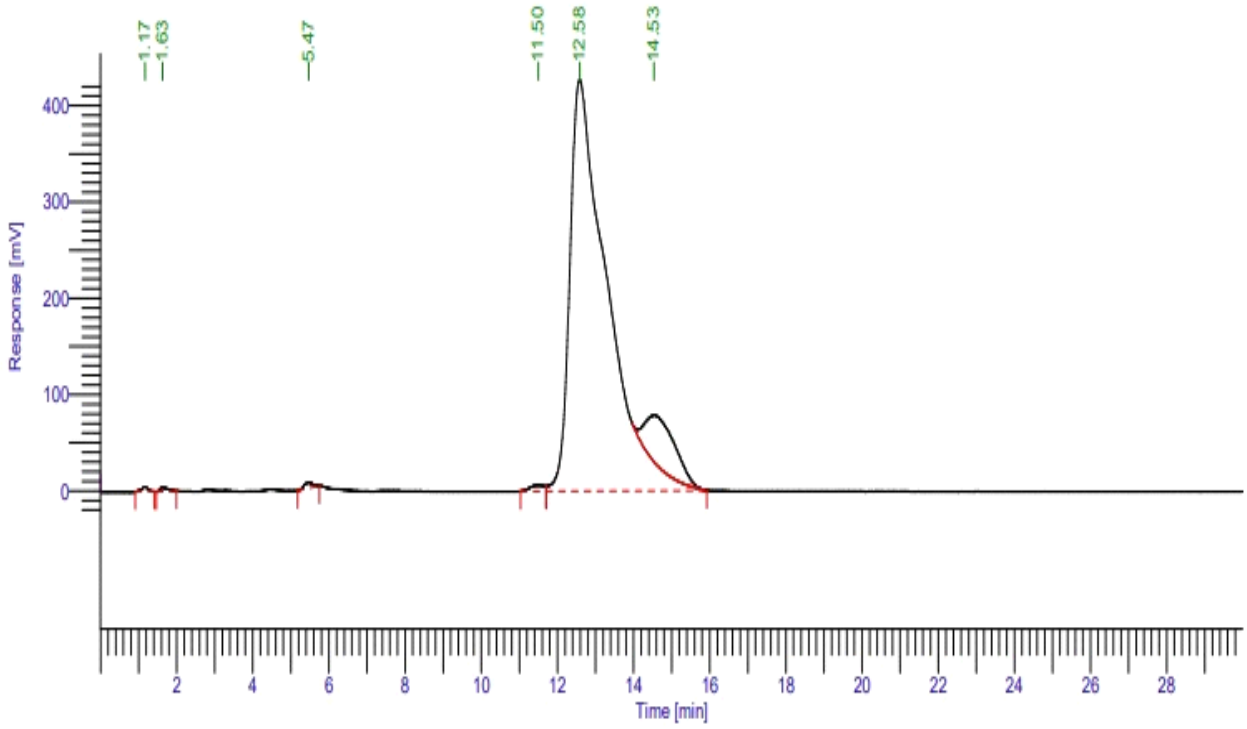

Figure 4 : HPLC chromatogram of water sample (Teilbal) of Dal Lake analyzed under optimum conditions by proposed extraction procedure DLLME coupled with HPLC-DAD

As Phthalate (DnBP) is ubiquitous contaminant and potentially toxic, its existence in environmental matrices should be observed to provide data for evaluation of human exposure. In the current study, to get accurate qualitative and quantitative data in a rational time, the analytical conditions were optimized. DLLME was selected for extraction of target phthalate (DnBP) with high recovery and purity. A low flow rate and splitless injection was used to obtain desired sensitivity. Additionally reverse phase column and column temperature programme were used to provide baseline resolution of DnBP which was eluted almost within 12 min. The use of reversed-phase mode of gradient elution in HPLC provided clear chromatograms. Diode array detector was used to detect the phthalate and to provide sufficient sensitivity for its identification and /or confirmation. DnBP was detected in water samples by comparing its retention time with that of standard and wavelength which is 230nm.

A number of studies were conducted to detect the presence of phthalates in various environmental matrices using HPLC and other related techniques. Jing *et al*. (2006) described an effectual HPLC method using UV detection for the determination of phthalates in drinking water samples. All 19 phthalates listed in key environmental regulatory documents—EU Directive 2005/84/EC; USEPA Methods 606 and 8061A; the Chinese HJ/T 72-2001; and the Standardization Administration of China (SAC) GB/T 20388-2006 and GB/T 21911-2008—are well separated on the Acclaim C30 column (3 μm, 3.0 × 150 mm), and the separation time is <25 min. The study concluded that HPLC with UV detection is an excellent method of determining phthalates in drinking water. Fatoki and Noma. (2001) applied solid phase extraction procedure coupled with capillary gas liquid chromatography (GLC) for the determination of phthalate ester plasticizers in water samples of river and seas that receive effluents from industries using phthalates. The outcome of the study showed that water samples were grossly contaminated as many phthalate esters (DMP, DEP, DBP, DEHP) were present at the level that raise concern. Ma *et al*. (2010) demonstrated the use of Dispersive SPE procedure coupled with HPLC-MS for determination of PAEs in fruit jellies. Five types of PAEs [(dimethyl phthalate (DMP), diethyl phthalate (DEP), dipropyl phthalate (DPP), benzyl butyl phthalate (BBP), and dicyclohexyl phthalate (DCHP)] were analyzed. Zaater *et al*. (2013) used HPLC coupled with UV detection for analysis of phthalates in commercial branded bottled mineral water of Jordon. The result showed that bottled water was polluted with DnBP, DEHP, DnOP, with total phthalate concentration ranging between 8.1 and 19.8μg/L. The detected amount is much lower than the globally tolerable daily intakes (0.5-1mg/L). Nevertheless even these small quantities of phthalates may get accumulated in vulnerable individuals like babies and/or pregnant ladies, which may be a health risk. Liang *et al*. (2008) outlined the use of DLLME coupled with HPLC-VWD for the analysis of three PAEs in water samples. Under optimum conditions the limits of detection were 1.8, 0.88 and 0.64 ng/mL for mineral water, tap water and lake water samples respectively. All these studies showed that HPLC is an excellent method of detecting the phthalates with higher sensitivity in different environmental matrices.

Conclusion

An easy, sensitive and precise procedure was developed and applied for the analysis of DnBP in water samples of Dal Lake. DnBP was detected in all the three water samples but the detected amounts were below then globally tolerable daily intakes 0.5-1mg/L for any individual phthalate. (Leitz *et al*., 2009; Serodio and Nogueira , 2006; Holadova *et al*., 2007; Agilent technology, 2012). Nevertheless these small amounts of phthalates may accumulate in vulnerable persons such as babies /or pregnant ladies, that may be a health risk. The advantages of DLLME coupled with HPLC-DAD in detection of DnBP in water samples of lake compared with other methods includes its ease, accuracy, high recovery and cost effectiveness. The method is appropriate and provides precise and definitive results.

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