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ANALYSIS OF DI-(2-ETHYLHEXYL)PHTHALATE IN PLASTIC BOTTLES OF DRINKING WATER WITH CONE-SHAPED MEMBRANE–LIQUID PHASE MICROEXTRACTION

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Abstract. The analysis of di-(2-ethylhexyl) phthalate (DEHP) was done in three kinds of polyethylene terephthalate (PET) plastic bottles of mineral water, namely: new bottle, ten times refilled and sunlight exposed new bottle. The optimal condition of this study is ethyl acetate as an organic solvent, 175 µl of organic solvent and 20 min of extraction time. This method has a limit detection about 0.29 ppm, precision 96.48–110.10 %, accuracy until 1.95 % and enrichment factor until 302.67 times. Based on these results, cone shaped membrane-liquid phase microextraction method (CSM-LPME-HPLC) could be used to analyze DEHP in a PET plastic bottle of drinking water sample under mentioned kinds of circumstances with the concentration of 0.40, 0.53 and 0.76 ppm, respectively.

Keywords: cone shaped membrane, liquid phase microextraction, di-(2-ethylhexyl)phthalate.

1. Introduction

Sample preparation technique is a critical point that must be done before doing an analysis on a particular instrumentation. One of the common preparation techniques is the extraction. Extraction is a technique on withdrawing or separating a component from the mixture using a suitable solvent. Extraction technique promises fast and clean separation process, also very useful for many conditions with organic or inorganic substances, even for both macro and micro analysis. Therefore, the extraction was widely used for chemical analysis in organic chemistry, biochemistry, and inorganic laboratory field as well [1].

Microextraction has been developed for extraction technique that fulfills green chemistry issue. Microextraction is classified into two types, namely liquid-liquid extraction (LLE) and solid-liquid extraction (SLE) [2]. The amount of organic solvent used in microextraction is less than in conventional extraction minimalizing the waste produced. Similarly, the number of samples or substrates is also required to be less and this technique is still suitable for analyzing small substrate concentrations. These facts make microextraction quite popular and can be used for DEHP analysis.

DEHP compound is widely used as a plasticizer which can increase the flexibility and versatility of a polymer [3]. However, this compound has chronic effects while accumulating in body and will cause health problems after several years. In most observed cases, DEHP enters human body through the food consumption and skin adsorption [4]. DEHP compound can be found in plastic bottles with code 1 PET. A plastic with this code is actually safe to be used as disposables stuff. Even more, bad habit of society to reuse scraped bottles without specific treatment opens the chance of releasing DEHP contained in bottles and leaching out it into drinking water and entering our body. To date, the Food and Drug Administration (FDA) still allowed the use of packaging materials containing DEHP in food which is mostly composed in water, even the United State Environmental Protection Agency (US-EPA) has released the maximum limit of DEHP in water to 6 ppb. United State-Occupational Safety and Health Administration (US-OSHA) also released maximum occupational exposure limit as high as 5 mg/m^3 air [5]. Therefore, toxicity issue of DEHP is important for the development of this compound analysis and motivates many researchers to focus their experiment on this field. Microextraction can be one of the attracting alternatives to solve analysis problems of DEHP.

Despite of many advantages, the microextraction technique still has some disadvantages, such as time consuming, multistep operation, and need for organic solvents which are usually toxic in large quantities. Membrane or fiber used in Solid Phase Microextraction (SPME) has a limited lifetime, they are fragile and expensive. Single Drop Microextraction (SDME) requires precision that complicates manual operation and the degradation of stability [6].

Among several microextraction techniques, Liquid Phase Microextraction (LPME) is the simplest one, in which the organic phase as an acceptor is protected by a membrane or fiber. Cone shaped form was developed by LPME method, where the membrane is formed like a cone to protect the organic solvent from the extracted solution. Moreover, this method has several advantages like simplicity, low price and high selectivity. Results of this method can be directly analyzed by Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) [7].

2. Experimental

2.1. Materials

The instruments used in this research are: HPLC Shimadzu LC-5A. Perkin Elmer UV-Vis detector LC 295, 250-4 LiChroCART column of RP-18 5 µm type , microsyringe, 0.45 µm Whatmann filter paper, micropipettes, vial, rod stirrer, hotplate stirrer, magnetic stirrer and glassware used in the laboratory.

The materials used in this study are: DEHP standard solution (99 %), Nylon 66 membrane filter with a pore of 0.2 µm, acetone, acetonitrile, methanol, *n*-hexane, chloroform and ethyl acetate. Each material has a purity degree according to pro-analysis. DI-water samples are coded bottled water replenished as much as 10 times and stored in a place exposed to direct sunlight.

2.2. Cone Shaped Membrane – Liquid Phase Microextraction (CSM-LPME)

Study on CSM-LPME was done by determining various parameters, namely, the stirring speed of 600 rpm and a volume of 15 ml sample solution. Extraction process was carried out using 0.2 µm Nylon membrane of Whatmann to accommodate and protect the organic solvent. Before use, the nylon membrane was stuffed and then sealed on each side using a flame to form a cone. After cone-shaping the membrane was rinsed with an organic solvent for cleaning and removing impurities and saturating the membrane pores with an organic solvent.

CSM-LPME extraction process was performed by inserting an organic solvent into the cone-shaped membrane and then placed on top vial containing 15 ml of the sample solution. Further, the extraction was carried out using a magnetic stirrer with a speed of 600 rpm (Fig. 1).

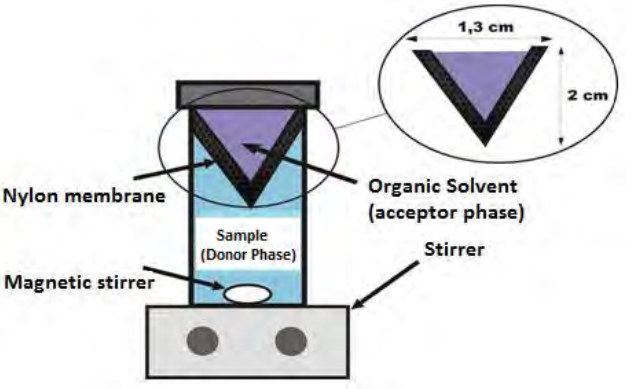

Fig. 1. CSM-LPME set-up [11]

3. Results and Discussion

HPLC instruments used in this research have stationary phase column LiChroCART 250-4 type RP-18 5 µm with Perkin Elmer UV-Vis detector LC 295 with a wavelength of 226 nm. RP-18 column which acts as the stationary phase is non-polar. This column serves as a reverse phase that conjugates stationary phase columns with analyte. HPLC column is composed of a stationary phase made of silica gel which is a polar phase. The maximum wavelength of DEHP used is 226 nm.

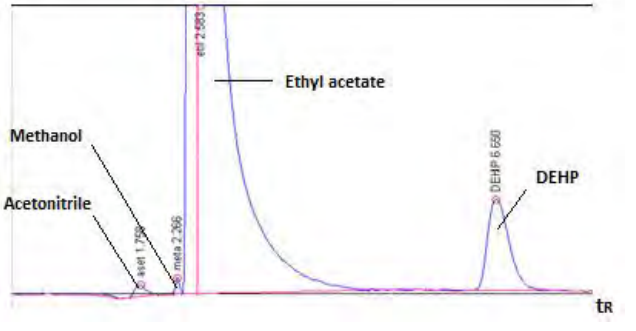

Fig. 2. Chromatograms of DEHP, ethyl acetate, methanol and acetonitrile

Following the procedure proposed by Aignasse *et al.* [8], DEHP investigation on HPLC uses eluent combined with acetonitrile and methanol with a ratio of 9:1 (v/v). DEHP standard solution was also used to confirm the eluent composition ratio in HPLC and obtain chromatogram with good separated peaks. In this study, the flow rate was 1 ml/min, because at this flow rate the chromatogram peaks of each compound are separated with good and relatively short analysis time (8 min).

Acetonitrile peak appeared at a retention time (t_R) of 1 min; 2 min for methanol; 3 min for ethyl acetate and 6 min for DEHP (Fig. 2).

3.1. Optimization of Organic Solvents Type

In this study the effect of organic solvent type was investigated. Three organic solvents were studied, namely *n*-hexane, ethylacetate and chloroform. Selection of the organic solvent type is based on like dissolves like principle. Organic solvents should have a non-polar nature. In addition, the physical properties of the organic solvents are also considered, including water solubility, boiling point, dipole moment, volatility, and toxicity [9, 10].

Peak of *n*-hexane tends to overlap DEHP peak. Peaks of chloroform and ethyl acetate are completely separated from DEHP peak, but ethyl acetate gives the larger chromatogram area than chloroform. Ethyl acetate gives the largest chromatogram area and has a dielectric constant approaching DEHP. So ethyl acetate can be used as an organic solvent for further optimization.

3.2. Optimization of Organic Solvent Volume

The volume of ethyl acetate was varied as 100, 125, 150, 175 and 200 µl. Based on Fig. 3, the extraction with an organic solvent of 175 µl volume provides the largest area on the chromatogram. The higher volume of organic solvent is used, the greater area of the chromatogram is generated which means that more DEHP can be extracted. However, if the volume exceeds 200 µl, the chromatogram area dramatically decreases due to the cone shaped membrane which is completely filled by an organic solvent and allows the solvent to evaporate during the extraction process. Therefore, the optimum volume of organic solvent used for further optimization is 175 µl.

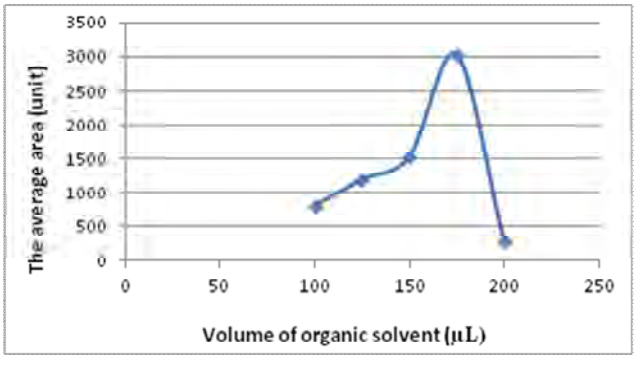

Fig. 3. Optimization of organic solvent volume using CSM-LPME

3.3. Optimization of Extraction Time

To find the optimal extraction time, the process was carried out for 10, 15, 20, 25 and 30 min. DEHP extraction for 20 min gave the largest area of the chromatogram peak (Fig. 4). The chromatogram area increases with the increase in extraction time. It is caused by prolonged contact time resulting in a mass transfer of analytes into an organic solvent in order to reach the point of equilibrium [11]. If the extraction time is too short, the organic solvent cannot be completely extracted from the sample solution due to short contact time between the organic solvent and the sample. If it is too long, there is a possibility that organic solvents will be saturated with the analyte [12].

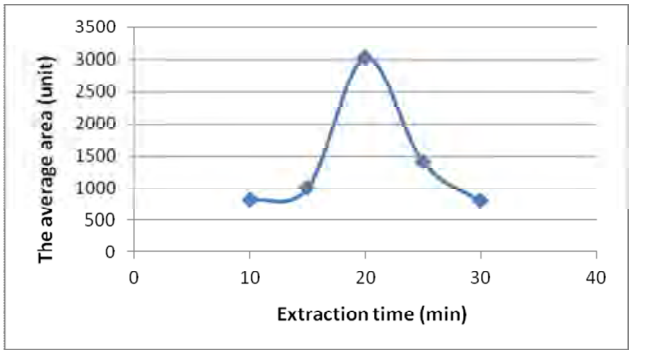

Fig. 4. Optimization of extraction time using CSM-LPME

3.4. Standard Curves of DEHP using CSM-LPME

Standard curve of DEHP obtained from CSM-LPME was used to calculate validation parameters, including recovery, accuracy, and detection limit. Fig. 5 proves that it is proportional relationship between the concentration of DEHP standard solution and the outer area of the generated chromatogram, where the higher the concentration of DEHP standard solution, the higher the area of the chromatogram. Linear regression equation generated by the standard curve is $y = 534.07x - 211.98$ with a correlation coefficient $R^2 = 0.997$.

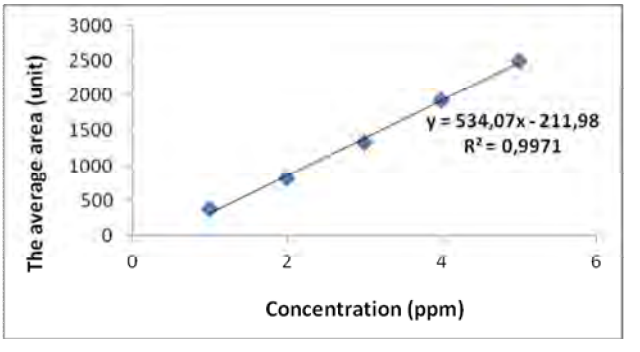

Fig. 5. DEHP concentration *vs.* average area using CSM-LPME

The results show a limit detection of the process of 0.29 ppm. The obtained recovery value is within 96.48–110.10 %, and the coefficient of variation is up to 1.95 %.

3.5. Enrichment Factor

An important part of microextraction is preconcentration, usually called as an enrichment factor (*EF*). *EF* of the analytes is related to the sample volume (V_{aq}) and the enrichment efficiency (*EE*), which also relates to the distribution constant ($K_{org/aq}$), defined by the below-mentioned equations:

$$EE = \frac{n_{org}}{n_o}, \quad EE = \frac{K_{org/aq} . V_{org}}{K_{org/aq} . V_{org} + V_{aq}} \tag{1}$$

$$EF = \frac{C_{org}}{C_o}, \quad EF = \frac{n_{org} . V_{aq}}{n_o . V_{org}} \tag{2}$$

$$EF = \frac{EE . V_{aq}}{V_{org}} \tag{3}$$

where C_{org} and n_{org} are the concentration and the mass of extracted analyte, respectively; C_o and n_o are the concentration and the mass of analyte originally present in the sample, respectively; V_{org} is the volume of the acceptor phase [11, 13].

Enrichment factor is the value that states the amount of concentration during the extraction process. Enrichment factor theoretical value (EF_{th}) or theoretical concentration in this study was 300 and the true value of enrichment factor (EF_{tr}) or the actual concentration was 302.67.

3.6. Sample Analysis

Three kinds of polyethylene terephthalate (PET) plastic bottles of mineral water were used in the study, namely: new bottle, ten times refilled and sunlight exposed new bottle. For replenishment, PET plastic bottles were fully filled with tap water, sealed and allowed to stand for 1 h. Duration of the samples stored under direct sunlight was 2 h. After all treatments were completed, the samples were directly used for the extraction of CSM-LPME with the parameters that have been optimized previously.

DEHP concentration in water samples of new PET plastic bottles was 0.42 ppm. This amount exceeds the threshold of DEHP in drinking water (0.60 ppb) [14] as well as the concentration of DEHP in water samples PET plastic bottles that were 10 times refilled (0.53 ppm). DEHP concentration in new PET bottles exposed to direct sunlight was 0.76 ppm.

4. Conclusions

The CSM-LPME extraction method can be succesfully used as a sample preparation technique for the analysis of DEHP. The determined optimal parameters are: solvent is ethyl acetate, volume of organic solvent is 175 µl and extraction time is 20 min. DEHP analysis using CSM-LPME shows following results for the bottles coded 1: 0.42 ppm for new plastic bottles, 0.53 ppm for plastic bottles which were 10 times refilled and 0.76 ppm for plastic bottles kept under direct sunlight. This method can extract analyte until 302.67 times of the enrichment.

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АНАЛІЗ ДІ-(2-ЕТИЛГЕКСИЛ) ФТАЛАТУ У ПЛАСТИКОВИХ ПЛЯШКАХ ДЛЯ ПИТНОЇ ВОДИ ЗА ДОПОМОГОЮ РІДКОФАЗОВОЇ МІКРОЕКСТРАКЦІЇ З КОНІЧНОЮ МЕМБРАНОЮ

Анотація. *Проведено аналіз ді-(2-етилгексил) фталату (ДЕГФ) для трьох видів пластикових пляшок для мінеральної води, а саме: нових пляшок, десять разів заповнених пляшок і нових пляшок після дії сонячного світла. Визначено оптимальні умови екстракції: етилацетат як органічний розчинник, його кількість 175 мкл, час екстракції 20 хв. Встановлено, що межа виявлення методу становить близько 0,29 м.д., сходимість 96,48–110,10 %, точність до 1,95 % і коефіцієнт збагачення до 302,67. Показано, що метод рідкофазової мікроекстракції з конічною мембраною може бути використаний для аналізу вмісту ДЕГФ у вказаних типах пластикових пляшок для питної води з концентрацією 0,40; 0,53 і 0,76 м.д., відповідно.*

Ключові слова: *конічна мембрана, рідкофазова мікроекстракція, ді-(2-етилгексил)фталат.*