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Determination of di-n-butyl Phthalate in Environmental Samples

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**Abstract:** A devised methodology presented here allows the determination of di-n-butyl phthalate in environmental samples (water and landfill leachate) using solid-phase extraction (SPE) and gas chromatography. It is developed based on the use of a gas chromatograph with an FID detector. Preliminary testing has also provided extraction parameters and conditions for chromatographic determination, with calibration applied by reference to an internal standard. The linearity of the calibration curve has been tested in DBP concentrations ranging from 0 to 7.5 mg/L, with the data obtained showing that, throughout this range, the detector readings as a function of the DBP concentrations remain linear (R2 coefficient >0.99). The average levels of recovery of DBP from aqueous solutions of phthalates are in the range of 97-109%, while the corresponding figures for leachates are 85-101%. The values of the coefficients of variation associated with the results obtained do not exceed 5%. The results, therefore, indicate that the applied extraction method is effective as regards DBP extraction from both water and landfill leachate, while numerous other substances present in the leachate from landfill sites apparently do not affect the correct determination of di-n-butyl phthalate by the method developed.

**Keywords:** di-n-butyl phthalate, aqueous samples, landfill leachate,solid phase extraction, method validation

1. Introduction

Recent years have brought increasing concern over the environmental risks posed by organic pollutants, especially the so-called endocrine-disrupting compounds (EDCs) (Popenda & Włodarczyk-Makuła 2018, Kida & Koszelnik 2015, Kudlek & Dudziak 2018, Pochwat 2018, Pochwat & Słyś 2018, Ziemowicz et al. 2018, Kumar et al. 2020). Di-n-butyl phthalate (DBP), a known EDC, is the pollutant that occurs most frequently in the natural environment. Di-n-butyl phthalate is mainly used in the production of plastics as a plasticiser for polyvinyl chloride and as a softening additive in the production of sealants, adhesives, and printing inks. It processes rubber or as a solvent in producing oils, pigments, paints, and dyes. DBP has also been used in the cosmetics industry (Chiou et al. 2006, Rivera-Utrilla et al. 2012, Dobrzyńska et al. 2010). Release from plastics is expected to be the main source of phthalate environmental pollution due to their non-covalent binding to polymers, especially from microplastics due to the increased specific surface area (Kida et al. 2022, Jang et al. 2016, Net et al. 2015). Di-n-butyl phthalate is, next to di-2-ethylhexyl phthalate (DEHP), the most common environmental pollutant, and since it is soluble in water and stable in environmental conditions, it is often detected in aquatic ecosystems, especially in surface water, groundwater, raw and treated wastewater, landfill leachate, and even in drinking water (Rivera-Utrilla et al. 2012, Kida & Koszelnik 2015, Piątek et al. 2016). Its main metabolite is mono-butyl phthalate (Lee & Veeramachaneni, 2005). DBP concentrations in the United States and European surface waters range from 0.01 to 622.9 μg/L (Wypych 2004). The main sources of introduction of phthalates into the aquatic ecosystem are atmospheric rainfall, treated wastewater from industrial and municipal treatment plants, and landfill leachate (Roslev et al. 2007). The accumulation of phthalates in natural waters causes further distribution in other water systems (Rivera-Utrilla et al. 2012). In Poland and Germany, research by Luks-Betlej et al. (2001) confirmed the presence of DBP phthalate in drinking water in the concentration range of 0-380 ng/L. These values do not exceed permissible levels. However, due to the relatively easy penetration of these compounds from various surfaces of everyday materials into food products and air, efforts should first be made to minimise their presence in water.

Di-n-butyl phthalate is included in the REACH list (Registration, Evaluation, Authorisation, and Restriction of Chemicals) as a high-risk substance, in this case, suspected to have a negative effect on the human endocrine system. The harmfulness of this relationship lies in the fact that, due to its chemical structure, this compound can disrupt the proper functioning of the system above. Chronic contact with this pollutant can among other things lead to problems with fertility, reproductive system cancer, abnormal development of a foetus, metabolic disorders, obesity, and diabetes. The European Union and the US Environmental Protection Agency have each identified di-n-butyl phthalate as a priority substance, mainly due to its toxicity and resistance to degradation (Smol et al. 2017, Duty et al. 2003, US EPA, 2011).

Due to the common occurrence of di-n-butyl phthalate in the aquatic environment and its negative impact on the environment, as well as living organisms, research has been undertaken to verify the suitability of SPE extraction and the use of a gas chromatograph in determining DBP concentrations in aqueous solutions and landfill leachates.

2. Materials and Methods

2.1. Reagents and materials

Standard (1 g/mL) solutions of di-n-butyl phthalate and benzyl benzoate were obtained from Sigma Aldrich (Saint Louis, USA). Ultra-pure water was obtained from Purix CNX-100, while SPE cartridges and C18 sorbents were purchased from SiliCycle (Quebec City, Canada). Analytical grade dichloromethane, methanol, and acetone were also purchased from POCH (Gliwice, Poland).All glassware was submerged in NaOH solution for 24 h, rinsed with ultra-pure water, and dried for 5 h. A further rinse with acetone was applied before heating at 280°C for 5 h.

2.2. Preparation of model solutions

Model solutions for di-n-butyl phthalate were produced in ultrapure water by adding specific amounts of a standard solution of DBP and mixing using an electromagnetic stirrer. Landfill leachate samples were collected and analysed in September 2017 from a landfill located at Kozodrza, SE Poland (50o06′38N, 21o37′10E). This site (of about 200,000 m2) has been in operation since 1990, mainly accepting solid municipal waste from the areas of the Podkarpackie Voivodeship (with its approximately 2,000,000 inhabitants). The site located in Kozodrza is a non-hazardous and inert landfill with a separate section for hazardous asbestos-containing waste. The total capacity of the landfill exceeds 25,000 tons and can receive more than 10 t/day. The sample of landfill leachate used in the tests was raw, representing direct runoff from the landfill quarters. The leachate was subjected to physicochemical analysis, and the di-n-butyl phthalate content was determined. Then, to obtain various concentrations of DBP, specific doses of phthalate were introduced into the leachate. The solutions were prepared daily, just before use.

2.3. Development and validation of an analytical method for determining di-n-butyl phthalate in aqueous solutions and landfill leachates

2.3.1. Operating conditions of the gas chromatograph

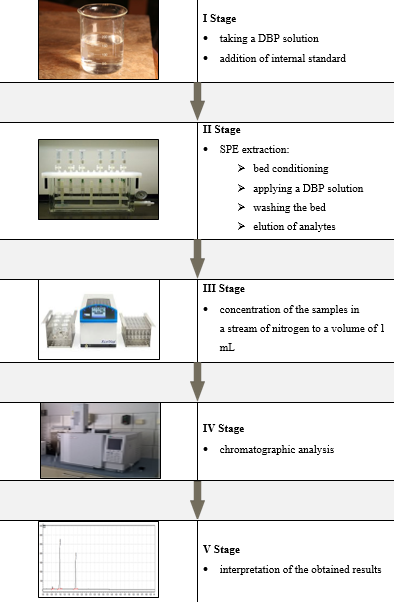
The investigation of di-n-butyl phthalate concentrations in water and landfill leachate was carried out at the Laboratory of the Department of Environmental Engineering and Chemistry (Faculty of Civil Engineering, Environmental Engineering and Architecture, Rzeszów University of Technology) using a Shimadzu gas chromatograph equipped with an FID flame ionisation detector. The operating parameters of the chromatographic system were selected by reference to the literature and the author's own research. A ZB-5MSplus Zebron Capillary G column (analytical column) of 30 m × 0.25 mm × 0.25 μm was applied, with hydrogen and synthetic air as the carrier gases. The temperature programme was the initial temperature of 150°C held for 1 min, a ramp temperature increase by 20°C/min to 300°C and then held for 10 min, with the total time being 16.5 min, the inlet temperature 300°C, injection volume 0.5 μL and detector temperature 320°C.

2.3.2. Calibration of the method for di-n-butyl phthalate determination

Quantification was performed using internal calibration. Benzyl benzoate served as the internal standard by which DBP was quantified. With basic solutions of di-n-butyl phthalate and benzyl benzoate at a concentration of 1 g/mL, working solutions of volume 10 mL and concentration 1 mg/mL were prepared. Standard solutions containing 0, 0.05, 0.25, 0.5, 2.5, 5 and 7.5 mg/mL of DBP and 0.5 mg/mL of benzyl benzoate (BB) were prepared from these solutions. Methanol was used in dilutions. Standard solutions were stored at 4°C. The calibration curve was determined on the basis of 6 repetitions at each concentration, with determinations performed by reference to the relationship between DBP concentration and the ratio of the di-n-butyl phthalate's peak area to the internal standard's surface.

2.3.3. SPE extraction

To develop the method for extracting the test substance, aqueous solutions containing 0, 0.05, 0.25, 0.5, 2.5, 5 and 7.5 mg/L of DBP phthalate and 0.5 mg/L of benzyl benzoate were prepared by introducing appropriate amounts of standard solutions into distilled water (or leachate). The extraction efficiency of di-n-butyl phthalate was calculated based on the difference in concentrations in the samples of the standard solutions and the samples of the model solutions subjected to the extraction process. The main stages of the isolation and analysis of liquid samples in DBP are shown graphically in Fig. 1.



**Fig. 1.** Main stages to the isolation and analysis of liquid samples in DBP (author's own work)

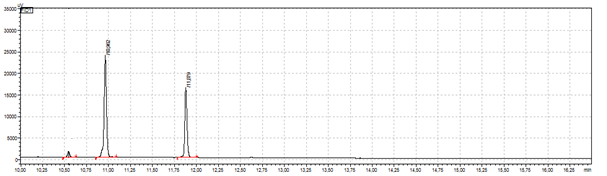
Di-n-butyl phthalate was isolated from a 20 mL model solution sample by solid phase extraction (SPE). The latter involved Phenomenex Strata C-18 extraction columns and the method developed by Fatoki and Noma (2002). The column bed was conditioned with 5 ml of methanol and rinsed with 5 mL of distilled water. The sample solution was then passed through the columns at a flow rate of about 1 mL/min. Di-n-butyl phthalate and the internal standard were eluted with a 1:1 mixture of methanol and dichloromethane and a total volume of 6 mL, and the collected fraction was concentrated to 1 mL under a nitrogen atmosphere before being subjected to chromatographic analysis.

2.3.4. Validation of the method for di-n-butyl phthalate determination

Validation aims to determine the parameters that characterise a given analytical method. In this case, such validation was based on results with 6 replicates for calibration solutions of various concentrations, according to the procedures described in Babu et al. (2016) and Cheng et al. (2016). The parameters determined were selectivity, linearity, repeatability, and recovery. The linearity of the method was determined by reference to the course of the calibration curve. At the same time, selectivity was related to model systems (distilled water, leachates) containing di-n-butyl phthalate. Determining one component against the others in a real composite sample is possible without the accompanying substances interfering. The method is selective when the analyte generates the signal in the composite mixture alone. The method's repeatability was, in turn, estimated based on the coefficient of variation (CV), expressed as a percentage. The accuracy of the method was evaluated by determining recovery.

3. Results and Discussion

A method for determining di-n-butyl phthalate using a gas chromatograph with an FID detector was developed. The conditions for chromatographically determining di-n-butyl phthalate were established based on preliminary tests. Chromatographic separation conditions were chosen to obtain a peak of di-n-butyl phthalate separated from the internal standard peak. The chromatogram of the standard DBP solution and benzyl benzoate (BB), i.e. the internal standard, is shown in Fig. 2.



**Fig. 2.** Example of a chromatogram of a standard solution of di-n-butyl phthalate made using a gas chromatograph with an FID detector, where: RT = 10.962 – retention time for BB, RT = 11.879 – retention time for DBP

The calibration function, which is then used to calculate the unknown concentration of the analyte in the tested material, was determined based on measurements made with the appropriate standard solutions. Chromatographic analyses were performed on prepared standard solutions containing different concentrations of DBP (0, 0.05, 0.25, 0.5, 2.5, 5 and 7.5 mg/L) and an equal amount of internal standard to generate the calibration curve described. Six determinations were made for each concentration. The peak areas were then read in line with integrator indications, and the ratio of the DBP peak area to the BB peak area was calculated. The results are presented in Table 1. For a DBP concentration equal to 0 mg/L, no peaks were observed in the chromatograms, and therefore, all the ratios obtained were equal to 0 and were not included in the tables.

**Table 1.** Obtained results of peak area ratios DBP and BB (ADBP/ABB)

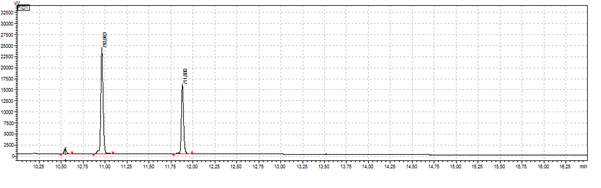
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Number | DBP concentration [mg/L] | | | | | |
| 0.05 | 0.25 | 0.5 | 2.5 | 5.0 | 7.5 |
| ADBP/ABB | | | | | |
| 1 | 0,072 | 0.416 | 0.778 | 3.931 | 6.954 | 10.806 |
| 2 | 0.067 | 0.380 | 0.787 | 4.203 | 7.243 | 10.387 |
| 3 | 0.068 | 0.406 | 0.790 | 3.986 | 6.930 | 10.339 |
| 4 | 0.069 | 0.399 | 0.800 | 4.116 | 7.149 | 11.000 |
| 5 | 0.076 | 0.404 | 0.738 | 3.940 | 7.260 | 10.719 |
| 6 | 0.076 | 0.391 | 0.734 | 3.808 | 7.392 | 10.430 |
| Statistics | | | | | | |
| Mean | 0.071 | 0.399 | 0.771 | 3.997 | 7.15 | 10.613 |
| SD | 0.004 | 0.013 | 0.028 | 0.141 | 0.182 | 0.267 |
| CV [%] | 5.58 | 3.14 | 3.65 | 3.54 | 2.55 | 2.52 |

A plot of the ratio of the peak area of the test substance to the internal standard was drawn from the concentration of the test substance in standard solutions (Fig. 3). The linearity of the calibration curve was tested across the range of concentrations of DBP phthalate envisaged in the procedure with individual methods, that is for 0-7.5 mg/L. The individual points on the curve are presented as the arithmetic mean of the surface ratios obtained during the analysis. The results for the examined concentration range show that the detector readings as a function of the DBP concentration are linear (the R2 coefficient is 0.9984).

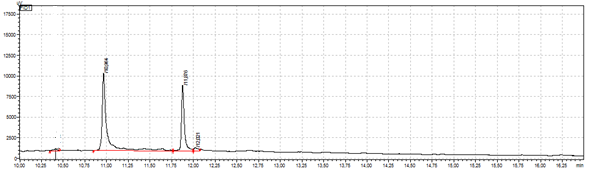
**Fig. 3.** Calibration curve for DBP (0-7.5 mg/L)

The coefficient of variation (CV) was calculated for 6 series of samples with different concentrations of DBP (Table 1) to estimate the method's repeatability). The coefficients of variation obtained for consecutive concentration levels, i.e. 0.05, 0.25, 0.5, 2.5, 5 and 7.5 mg/L, were: 5.58, 3.14, 3.65, 3.54, 2.55 and 2.52%. According to the AOAC guidelines (Association of Official Analytical Chemists), the coefficient of variation for quantities determined at the level of 1 ppm should not exceed 16%. However, for quantities determined at the level of 1 ppb, CV may not be greater than 45% (Matuszak 2012). The coefficients obtained indicate very good precision, so the proposed method is acceptable across the tested range of concentrations. It means the chromatographic analysis method developed is reproducible.

An important element in the development of a method is the determination of selectivity, i.e., the ability to determine a specific substance in the presence of other sample matrix components. The selectivity of the method presented here was assessed by comparing the chromatograms obtained from the analysis of the standard solution, and the analysis of samples after SPE (Solid Phase Extraction) involving both distilled water (Fig. 4) and landfill leachates (Fig. 5). The results confirm that the numerous substances present in landfill leachate do not interfere correct determination of di-n-butyl phthalate using the method developed.



**Fig. 4.** Chromatogram obtained with the extraction of DBP from aqueous solutions of phthalates (RT = 10.962 – retention time for BB, RT = 11.879 – retention time for DBP)



**Fig. 5.** Chromatogram obtained extraction of DBP from landfill leachate (RT = 10.962 – retention time for BB,   
RT = 11.879 – retention time for DBP)

Suppose it is necessary to separate the analyte from the environmental matrix. In that case, it is necessary to develop a method for isolating substances from a given system and determine the analyte recovery rate. Therefore, a recovery test was carried out by introducing different amounts of the DBP standard and a fixed amount of the internal standard into water or landfill leachate. The enriched samples contained the test substance at the same concentrations as those used to calibrate the determination method (described above). SPE extraction was used to isolate DBP from aqueous solutions (aqueous solutions or leachate). The conditions for conducting this extraction were selected based on literature (Fatoki & Noma 2001). Six analyses were carried out for 6 concentrations. The results obtained are shown in Tables 2 and 3.

**Table 2.** Results for the efficiency of extraction of DBP phthalate from aqueous solutions of phthalates by means of SPE extraction

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Number | DBP concentration [mg/L] | | | | | |
| 0.05 | 0.25 | 0.5 | 2.5 | 5.0 | 7.5 |
| Recovery [%] | | | | | |
| 1 | 103 | 103 | 107 | 100 | 95 | 102 |
| 2 | 112 | 105 | 103 | 99 | 99 | 99 |
| 3 | 109 | 100 | 105 | 104 | 103 | 94 |
| 4 | 110 | 110 | 106 | 103 | 97 | 94 |
| 5 | 105 | 108 | 101 | 105 | 100 | 97 |
| 6 | 114 | 106 | 107 | 106 | 96 | 98 |
| Statistics | | | | | | |
| Mean [%] | 109 | 105 | 105 | 103 | 98 | 97 |
| SD [%] | 4.17 | 3.56 | 2.40 | 2.79 | 2.94 | 3.08 |
| CV [%] | 3.83 | 3.38 | 2.29 | 2.71 | 2.99 | 3.16 |

**Table 3.** Results for the efficiency of extraction of DBP phthalate from landfill leachate by SPE extraction

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Number | DBP concentration [mg/L] | | | | | |
| 0.05 | 0.25 | 0.5 | 2.5 | 5.0 | 7.5 |
| Recovery [%] | | | | | |
| 1 | 88 | 90 | 99 | 103 | 94 | 108 |
| 2 | 90 | 92 | 94 | 101 | 101 | 95 |
| 3 | 83 | 88 | 95 | 99 | 97 | 102 |
| 4 | 85 | 90 | 94 | 95 | 89 | 105 |
| 5 | 83 | 87 | 93 | 98 | 99 | 100 |
| 6 | 82 | 86 | 96 | 99 | 100 | 97 |

**Table 3.** cont.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | DBP concentration [mg/L] | | | | | |
| 0.05 | 0.25 | 0.5 | 2.5 | 5.0 | 7.5 |
| Statistics | | | | | | |
| Mean [%] | 85 | 89 | 95 | 99 | 97 | 101 |
| SD [%] | 3.19 | 2.23 | 2.14 | 2.71 | 4.50 | 4.87 |
| CV [%] | 3.74 | 2.51 | 2.25 | 2.74 | 4.66 | 4.82 |

The concentration of di-n-butyl phthalate was also tested in the collected landfill leachate. The presence of DBP was confirmed at 67 µg/L. However, much higher concentrations of DBP in landfill leachate are often found in the literature. For example, leachates from the landfill in Łubna were characterised by a concentration of DBP of 348 µg/L (Dmochowska & Dmochowski 2011, Dmochowski et al. 2015).

4. Conclusion

An analytical method for determining di-n-butyl phthalate in water and landfill-leachate samples using solid phase extraction and GC-FID analysis has been developed and validated. Following the optimisation of extraction and separation parameters, the method was validated by evaluating selectivity, linearity, repeatability, and recovery.

A calibration curve was constructed across the concentration range of interest (0-7.5 mg/L), using benzyl benzoate as an internal standard. Calibration curve regression coefficients (R2) assumed values above 0.99. The SPE extraction recovered 97 to 109% on average in the case of water samples and 85-101% for leachates, with coefficients of variation below 5%. The applied extraction and chromatographic methods are effective for extractions of water and landfill leachate, although the latter contain many other substances. The concentration of di-n-butyl phthalate in the analysed landfill leachate was 67 µg/L.

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