

Synthesis of a Potential New Internal Standard for the Analytical Determination of Dibutyl Phthalate (DBP) and Monobutyl Phthalate (MBP) in Water Samples

Luis E. Figueroa, Megan C. Brown, Thomas Briscoe, Juliette Chisam, Dalton Lewis, Justin Westover, Erin Brooks, Fakhrideen Albahadily, John Bowen, & Shawna B. Ellis

Department of Chemistry, University of Central Oklahoma, Edmond, OK 73034

Abstract: Dibutyl phthalate (DPB), a common plasticizer, and monobutyl phthalate (MBP), a DBP metabolite, are endocrine disruptors that leach into water sources through various pathways. Some of the most useful methods to find and quantify the concentrations of these pollutants in drinking water include using gas chromatography-mass spectrophotometry (GC-MS) with solid phase micro extraction (SPME), with the application of benzyl benzoate (BB) as an internal standard. However, since BB is also a common water pollutant and could interfere with practical analytical measurements, there is a need for a new internal standard. A new compound, dibutyl 4-chlorophthalate (Cl-DBP), is proposed as a potentially improved internal standard, because it shares characteristics with DBP and MBP, including the high ionizability of both the compounds as well as a common mass spectrometry fragmentation pattern. The synthesis, purification, and analysis of Cl-DBP as a potential internal standard for the quantitation of butyl derived phthalate plasticizers by GC-MS will be presented.

Introduction

Various phthalate esters are currently being used as plasticizers, including dibutyl phthalate (DBP). (Figure 1) More specifically, dibutyl phthalate is used as a plasticizer and softener to increase the flexibility, durability, transparency, and longevity in inks, plastics, adhesives, cosmetics, and countless other products humans come into contact with on a daily basis. Like other phthalates, DBP is physically, not chemically, incorporated into the polymer structures. (GreenFacts 2005) This means the ester can easily migrate from the polymer into the air, soil, food, and water; and in turn can easily absorb into the skin and gastrointestinal tract. The potential health effects of DBP has warranted growing concern and has led to the ban of DBP in products in Europe and Australia. The concerns involving DBP exposure stem

from DBP being a suspected endocrine disruptor. An endocrine disruptor can suppress or overexpress the function of thyroid, androgen, and estrogen hormones; thereby disrupting normal functions of the body.-(Sundar 2016) DBP and one of its isomers has been linked to DNA damage in human mucosal cells and lymphocytes.(Kleinsasser 2001) Additionally,

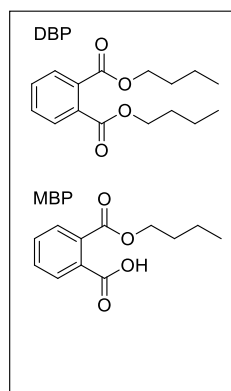


Figure 1. Structure of DBP and MBP

phthalate exposure, including DBP, has been shown to alter physical development in infants and toddlers by blocking hormone activity and reducing function in Leydig cells. (Braun 2013) Furthermore, monobutyl phthalate (MBP), which is a metabolite and hydrolysis product of DBP, is a known endocrine disruptor as well.

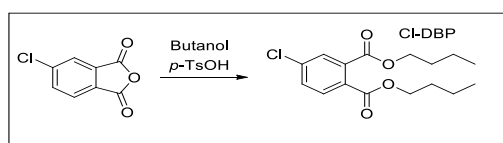
These concerns produce a heightened demand for dependable analytical methods that permit detection and quantitation of the phthalate esters DBP and MBP in water sources. The gas chromatography-mass spectrophotometry (GC-MS) and High-Performance Liquid Chromatography (HPLC) are commonly used methods for the analysis of phthalates. (Peñalver 2000) However, at low concentrations these esters are difficult to quantitate and require additional steps to reach a detectable threshold. Typically, the techniques employed for this are Liquid-Liquid Extraction (LLE) or Solid Phase Extraction (SPE). (Peñalver 2000) While these are employed out of necessity, there are problems with these additional steps, such as requiring large amounts of solvent, being time-consuming and labor-intensive, as well as increasing the potential error of the analytical method. (Liu 2008) On the other hand, solid phase micro extraction (SPME) greatly reduces the use of solvent, time, and labor. The SPME device has a 1-2 cm fused silica fiber with a polymer-coated tip that is shielded inside a hollow needle. The device has a plunger at the top that, once depressed, exposes the fiber for absorption and desorption of the analyte. SPME combines extraction and analysis into one step resulting in a SPME fiber that can be inserted directly into the GC-MS injection chamber. Furthermore, SPME allows determination at low concentrations and reduces the risk of secondary contamination. (Vas 2004)

While the direct detection of phthalates in water is possible, their quantitation will require that an internal standard be added to the sample before SPME extraction. This requires the internal standard be chemically similar to the analyte (DBP) in order to maintain the appropriate sensitivity in the instrumental response, yet it needs to be chemically unique

enough to avoid potential overlap of the compounds during separation. Additionally, the standard employed must not interact or alter the analyte in a manner that could alter the outcome of the analysis. It should compensate for any variations that may occur during the process, such as sample preparation, quantitative errors, and variations in the GC separations since ideally whatever affects the analyte will equally affect the internal standard. Benzyl benzoate is used almost universally as the internal standard for the analytical determination of phthalate in water because it meets these criteria. (Ziembowicz, 2018) However, benzyl benzoate is used as a solvent, a chemical synthesis component, a perfume fixative, a food flavoring, a plasticizer, and in human and veterinary external medicine as a miticide and can be released into the environment through various waste streams. (O'Neil 2013, Larranaga 2016) This means that, in practice, water samples that are already contaminated with benzyl benzoate would cause an erroneously low measurement of DBP using this method. Therefore, it is necessary to design and test a new internal standard that can be used in this way, and that is not already present in the environment.

Materials and Methods

4-chlorodibutyl phthalate (Cl-DBP), was designed to be the new internal standard because of its similarity in structure to DBP. Cl-DBP was prepared by dissolving commercially available 4-chlorophthalic anhydride (0.5g, 1.6 mmol) in butanol (5mL, 54.6 mmol) with the addition of a catalytic amount of *p*-toluenesulfonic acid (0.104g, 0.6 mmol) (Scheme 1). The reaction mixture was heated to 65°C for four hours under an inert atmosphere. After evaporating the remaining ethanol, the crude product was purified by flash chromatography (silica) using 2 :3 ether:petroleum ether as the mobile phase.



Scheme 1. Synthesis of dibutyl-4-chlorophthalate

The procedure yielded 0.116 g (22.7%) of the desired compound. The identity of the pure compound was confirmed by ^1H NMR (Figure 2) and ^{13}C NMR (Figure 3) using a Bruker 300 MHz instrument. ^1H NMR (300 MHz, CDCl_3): δ 7.70 (d, $J=8\text{Hz}$, 1H), 7.65 (dd, $J=2\text{Hz}$, 1H), 7.49 (d, $J=8\text{Hz}$, $J=2\text{Hz}$, 1H), 4.31 (t, $J=9\text{Hz}$, 2H), 4.30 (t, $J=7\text{Hz}$, 2H), 1.71 (m, 4H), 1.42 (m, 4H), 0.96 (t, 6H). ^{13}C NMR (300 MHz, CDCl_3): δ 166.66, 166.57, 137.42, 134.40, 130.81, 130.48, 130.13, 128.85, 65.96, 65.78, 30.52, 19.16, 13.72.

The GC-MS used was an Agilent 7890A Gas Chromatogram, 5975C Mass Selective Detector (MSD). For this procedure, a 15 meter VF-5MS column was used with helium as the carrier gas. The mass spectrometer was set to SCAN/Single Ion Measurement (SIM) mode to collect both the ion count for a full range of mass/charge (m/z) ratios, which generally this relates to the mass of the ion with a charge of one (SCAN) and SIM mass spectra, which is just the ion count for only specific m/z ratios. After data collection, the raw results were processed using ChemStation

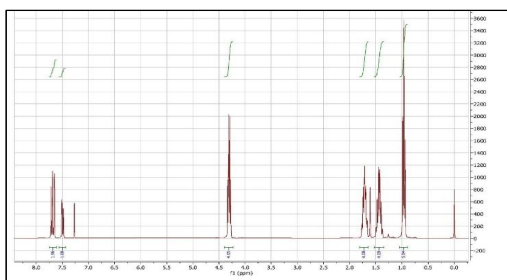


Figure 2. ^1H NMR of Cl-DBP

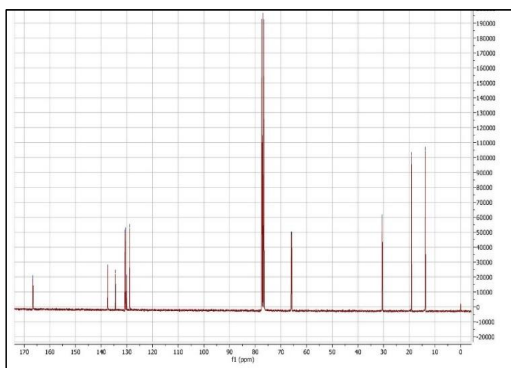


Figure 3. ^{13}C NMR of Cl-DBP

software. The temperature program for the GC-MS procedure is a variation of the one used in the Peñalver study and is as follows.⁶ The injection chamber temperature was 270°C and column temperature employed started at 60°C and increased at a rate of 70°C per minute up to 200°C . After reaching 200°C , the rate of the temperature increase slowed to 15°C per minute until 225°C and was held there for four minutes. The resulting total run time was 7.667 minutes. (Peñalver 2000)

The SPME fiber used was an $85\ \mu\text{m}$ polyacrylate SPME fiber. Before using it for absorption, the SPME fiber was conditioned in the GC-MS at a temperature of 270°C for 12 minutes in order to rid the fiber of any residual phthalates. The 4-chlorodibutyl phthalate samples were prepared by dispensing $1\ \mu\text{L}$ of product solution into a GC vial and diluting to 0.5mL using methanol, and the DBP and MBP samples were prepared in DI water at 0.77 ppm and 1.23 ppm, respectively. To increase the polarity of the solution and promote adsorption on the SPME fiber, approximately 1% by weight of sodium chloride was added to the water solution. Samples were then exposed to the SPME fiber for an absorption time of 15 minutes at the chosen standard stir rate. (Ormsby 2016) The SPME needle was then inserted into the GC-MS inlet, exposed for desorption for 1 minute, and then removed. (Peñalver 2000)

Results and Discussion

Utilizing the temperature program described earlier, the GC-MS analysis of the Cl-DBP sample yielded a chromatogram that showed ionizable compounds that eluted at approximately four minutes with an ion abundance of over 2×10^7 (peak 1) and a second peak as the result of ionizable compounds that eluted from the column at approximately 5.5 minutes and had an ion abundance of 1×10^6 (peak 2). (Figure 4). Analysis of ions that resulted in peak 1 showed two m/z ratios, or masses in this case as the charge was one. The first mass was $312.8\ \text{g/mol}$, which correlates to the Cl-DBP as expected and a base peak of 183.2, which correlates to 4-chlorophthalic anhydride.

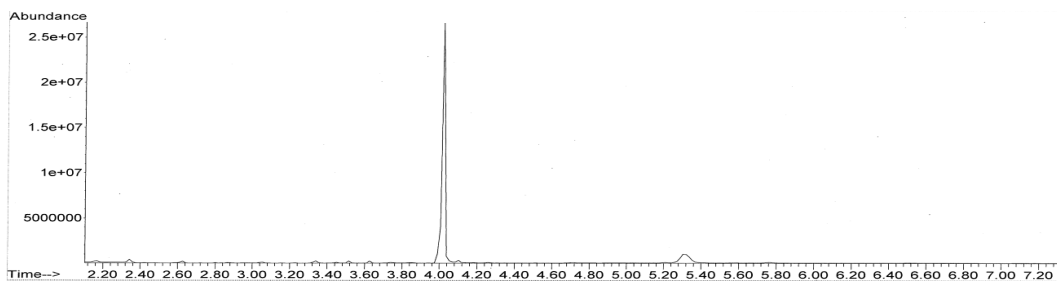


Figure 4. GC-MS of Cl-DBP using SPME Fiber

(Figure 5) The presence of the second mass can be explained as a fragmentation peak of the 4-DBP which results from the ion generation process within the detector.

After establishing the purity of the Cl-DBP sample, it was necessary to investigate its retention time in relation to DBP and MBP under the standard conditions described earlier. The GC-MS analysis revealed four peaks that resulted from ionizable compounds that eluted from the column at 3.22, 3.49, 3.79, and 4.356 minutes. (Figure 6) Identifications of the compounds that give rise to each peak in the chromatograph were assigned by analyzing the m/z for the ions that eluted at the appropriate retention time. Analysis of the ions present with a retention time of 3.49 min. showed parent peak with a molar of 223.0 amu as expected for MBP and a base peak in of 148.9 amu which corresponds to phthalic anhydride (Figure 7). As described earlier, the anhydride peak found within this ion set was the result of a fragment of the parent, MBP. The ions corresponding to a retention time of 3.79 min yielded parent peak with a ionic mass of 278.1 amu, as expected for DBP and, a base peak of 148.9 amu. (Figure 8) Again the ion with a m/z of 148.9 corresponds to phthalic anhydride which would be a common fragment of either MBP or DBP. Analysis of the

final peak at 4.356 to elute in this experiment demonstrated the highest total ion count and provided a parent peak with a ionic mass of 312.1 amu as expected for Cl-DBP. (Figure 9) The base peak among these ions is 183.0 amu which corresponds to 4-chlorophthalic anhydride which was observed in the GC-MS of the purified compound. Therefore, the retention times for Cl-DBP, DBP, and MBP under these conditions were 4.356, 3.787, and 3.493, respectively. Additionally, baseline separation was achieved for each of the compounds by GC in less than five minutes.

In summary, an efficient synthetic procedure and method of purification has been introduced for the preparation of 4-chlorodibutyl phthalate in high purity. GC-MS analysis has been presented to establish that Cl-DBP has chemical characteristics in line with butyl phthalate plasticizers. These characteristics include the high ionizability of both the samples as well as a common fragmentation pattern. Additionally, a GC protocol has been established that allows for the baseline separation of the potential internal standard and its possible target analytes. While future studies remain in order to establish and compare the standard curve of the ionization of both the Cl-DBP and the target analytes versus concentration, it seems that Cl-DBP may serve as a more suitable internal standard for the determination of the concentration of DBP and MBP in water samples compared to current literature examples.

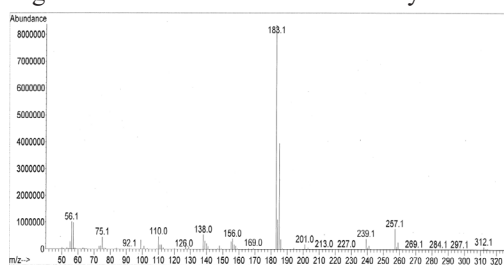


Figure 5. MS of ions eluting at 4.01 Minutes of GC-MS analysis of Cl-DBP

Acknowledgments

The author thanks NSF, OK-LSAMP, UCO CURE-STEM, and the SWOSU NMR facility for support of this research.

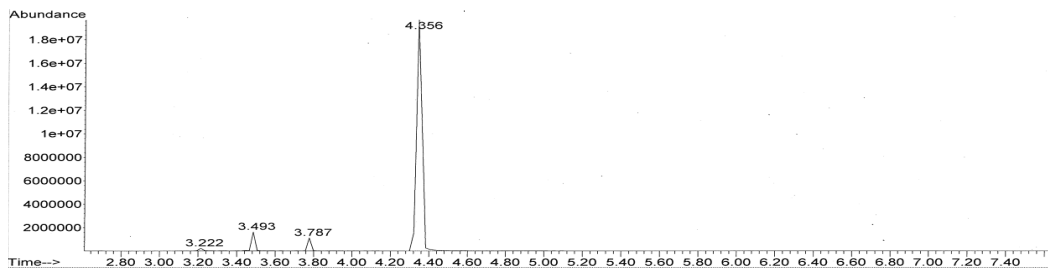


Figure 6. GC-MS of a mixture of MBP, DBP, and CI-DBP

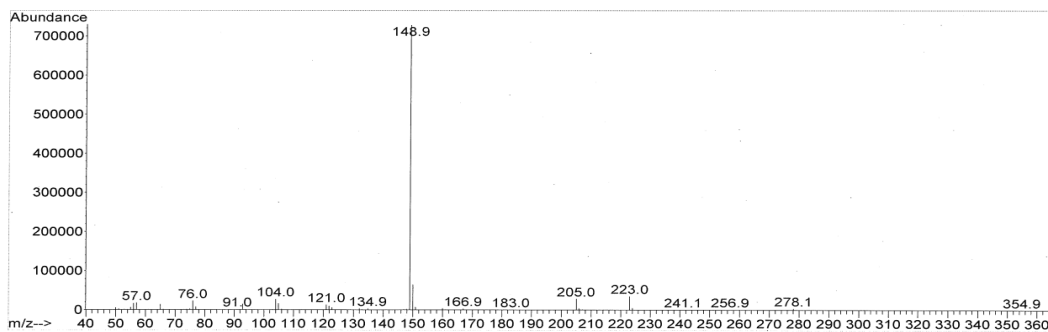


Figure 7. MS of ions eluting at 3.49 min. during GC-MS analysis of CI-DBP, DBP, MBP mixture

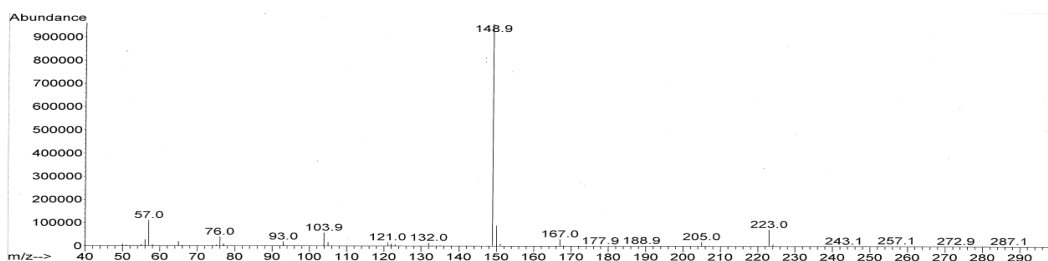


Figure 8. MS of ions eluting at 3.79 min. during GC-MS analysis of CI-DBP, DBP, MBP mixture

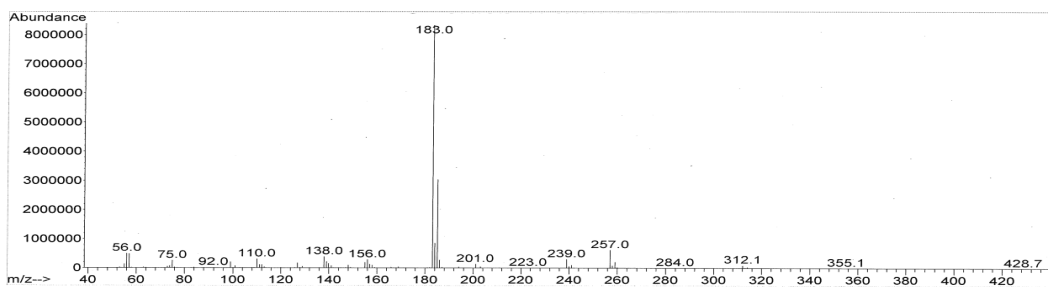


Figure 9. MS of ions eluting at 4.36 min. during GC-MS analysis of CI-DBP, DBP, MBP mixture

References

- Braun, J. M.; Sathyanarayana, S.; Hauser, R. 2013. Phthalate Exposure and Children's Health. *Current opinion in pediatrics*, **25(2): 247-254**.
- GreenFacts Facts on Health and the Environment. Phthalate Di-butyl phthalate. 2005. Available from: <http://www.greenfacts.org/en/dbp-dibutyl-phthalate/> (Accessed August 15, 2019)
- Harris, D. C. 2007. *Quantitative Chemical Analysis*; W.H. Freeman and Co.: New York (NY)

- Kleinsasser, N. H.; Wallner, B. C.; Kastenbauer, E. R.; Weissacher, H.; Harréus, U. A. Genotoxicity Of Di-Butyl-Phthalate and Di-Iso-Butyl-Phthalate in Human Lymphocytes and Mucosal Cells. *Teratog. Carcinog. Mutagen. Teratogenesis, Carcinogenesis, and Mutagenesis*. **2001**, *21*, 189–196.
- Larranaga MD ed; 2016. Hawley's Condensed Chemical Dictionary 16th ed., Hoboken (NJ) John Wiley & Sons, Inc., p. 159
- Liu, W. 2008. Determination of Sub-ppb Level of Phthalates in Water by Auto-SPME and GC-MS. Available from <https://www.agilent.com/cs/library/applications/5989-7726EN.pdf> (Accessed August 15, 2019)
- O'Neil MJ, ed; 2013. The Merck Index. 15th ed., Cambridge (UK) Royal Society of Chemistry, p. 199
- Peñalver, A.; Pocrull, E.; Borrull, F.; Marcé, R. 2000. Determination Of Phthalate Esters in Water Samples by Solid-Phase Microextraction and Gas Chromatography with Mass Spectrometric Detection. *Journal of Chromatography A*. **872**, 191–201.
- Sundar, R. 2016 Phthalates and Phthalate Alternatives: Effects on Proliferative and Estrogenic Target Genes in Ishikawa Cells. *Young Scientist Journal*. *Young Scientist Journal*. Available from: <https://www.youngscientistjournal.org/article/phthalates-and-phthalate-alternatives-effects-on-proliferative-and-estrogenic-target-genes-in-ishikawa-cells> (Accessed August 15, 2019)
- Vas, G.; Vékey, K. 2004. Solid-Phase Microextraction: a Powerful Sample Preparation Tool Prior to Mass Spectrometric Analysis. *Journal of Mass Spectrometry J. Mass Spectrom.* **39**, 233–254.
- Ziembowicz, Sabina & Kida, Małgorzata & Koszelnik, Piotr. 2018. Development of an analytical method for dibutyl phthalate (DBP) determination in water samples using gas chromatography. *E3S Web of Conferences*. **44**. 00200.

Submitted August 15, 2019 Accepted September 24, 2019