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LABORATORY EXPERIMENT

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GC−MS Quantitation and Identification of Bisphenol-A Isolated from Water

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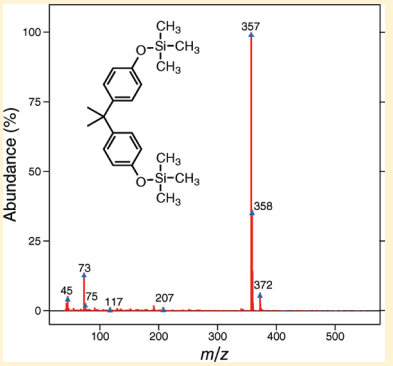
Supporting Information

ABSTRACT: Isolation and identification of organic compounds is a necessary skill chemistry students must be able to do with proficiency. In this upper-level undergraduate laboratory, students isolate bisphenol-A (BPA; 4-4′-isopropylidenediphenol) from water using solid-phase extraction (SPE) followed by derivatization with analysis by GC−MS. The students learn the proper steps and techniques of SPE that include conditioning, equilibration, washing, and eluting. The students are then asked to identify the BPA peak from the GC−MS analysis of a mixture of standards based upon the molecular ion, keeping in mind the change in molecular weight upon derivatization. Interpretation of GC−MS data showed predictable fragmentation and highlights the formation of benzylic carbocations. Quantification was done by an external calibration curve and the sample was quantified and a percent recovery is calculated.

KEYWORDS: Upper-Division Undergraduate, Analytical Chemistry, Environmental Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Applications of Chemistry, Consumer Chemistry, Gas Chromatography, Mass Spectrometry



One of the biggest challenges in trace organic analysis is concentrating any given sample enough to reliably identify and quantitate above the method detection limit. There are several ways to do this depending on the sample matrix. For aqueous samples, liquid−liquid extraction can be used to partition an analyte to the organic solvent layer. Another example is solid-phase extraction (SPE) where the aqueous layer is passed through a solid phase to selectively extract the analyte of interest. SPE offers a variety of advantages over liquid−liquid extraction because (i) different solid phases can be used to selectively partition analytes from the aqueous phase, (ii) large sample volumes can be passed through the SPE column, and (iii) organic solvent use is minimized. Similar experiments published in this *Journal* have used SPE to isolate and GC−MS to quantitate and characterize trace organic components.[1−4]

Trace organic analysis is especially important for compounds that pose human health and environmental risks. One compound that has received considerable research and public interest is bisphenol-A (4-4′-isopropylidenediphenol) (Figure 1) due to its endocrine disrupting properties.[5] Bisphenol-A (BPA) is a monomer used in polycarbonate plastic products and other products such as epoxy resins, adhesives, optical coatings, and so forth.[6] Recently, it has been shown that BPA is leached from a wide variety of products such as bottles, plastic food wrappers, and resin-lined food cans.[7,8] One method that is suitable for the detection and quantitation of trace organic compounds is gas chromatography−mass spectrometry (GC−MS).

GC−MS is ideal for undergraduate level experiments because it is used in many fields of chemistry in both academic and industrial settings due to its low cost, ease of use, and minimum space requirements.[9−12] The basic steps involve injection of the sample in a solvent into the GC injector where it is vaporized and swept through a column where separations occur by selective partitioning between gas mobile phase and the column stationary phase. Detection is done by mass spectrometry with the added bonus of structural information being obtained through characteristic mass fragments. These mass fragments can be pieced together to identify the original molecule.

The polarity of an organic compound must be considered before GC−MS analysis because peak broadening and adsorption onto the injector linear are possible. Bisphenol-A has two polar phenolic groups that need to be derivatized prior to injection onto the GC and one of the common derivatizing agents for phenols or alcohols is BSTFA [*N*,*O*-bis(trimethylsilyl)-trifluoroacetamide].[13] The BPA reacts with BSTFA to give a trimethylsilyl ether (Figure 1). When derivatizing for GC−MS, it is important for the students to realize that as protecting groups are added to the molecule, the molecular weight changes. The reaction can be considered as nucleophilic attack on the silicon atom producing a bimolecular transition state. The transition state will then proceed to give the trimethylsilyl (TMS) ether product.

LEARNING OUTCOMES

The students should learn the following upon performing this experiment: (i) the proper procedure for SPE, (ii) purpose of derivatization, (iii) mass spectral interpretation, and (iv) clean

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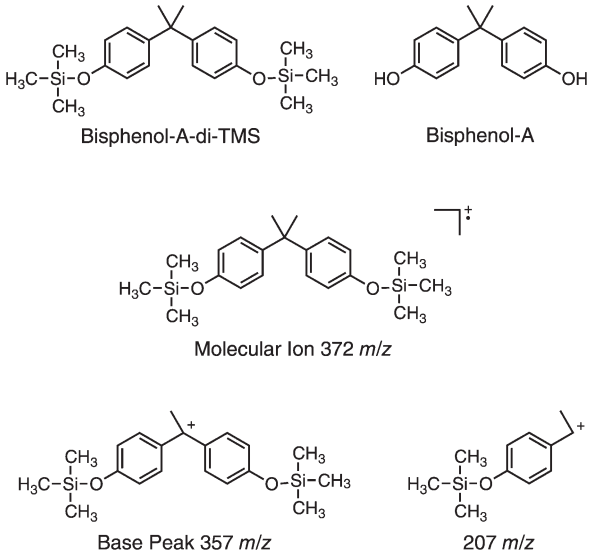

Figure 1. The structures of bisphenol-A underivatized, derivatized, and major mass spectral fragments.

techniques for trace organic analysis and quantitation using a standard calibration curve.

EXTRACTION OF BPA

This experiment has been integrated into our laboratory curriculum for two years and, from our experience, takes 3 h for the extraction and derivatization and 30 min for the GC−MS analysis, which includes an introduction to the instrument. Students obtain BPA fortified water and check the pH to make sure it is between 5.5 and 6.5 or adjust it accordingly. This is to make sure the BPA is fully protonated,[14] thus, maximizing hydrophobic interactions with the reverse-phase C_{18} column. Using the vacuum apparatus shown in Figure 2, the SPE column is conditioned with methanol and equilibrated with Milli-Q water prior to passing a known volume of the fortified water through it. The column is then washed with 5% methanol/water and dried by pulling air through it under vacuum on the vacuum apparatus. Finally, the analyte is eluted with 100% methanol into a clean vial and blown to dryness under a gentle stream of nitrogen gas. The BPA is then derivatized by adding 70 μL of BSTFA and 1% TMCS (trimethylchlorosilane) and 10 μL of pyridine to the vial and placed on a heating block or oven at 70 °C for 15 min. Once complete, the reaction mixture is blown to dryness under N_2 gas and then brought up to a final volume of 1 mL in hexane. Analysis is performed by GC−MS using a DB-5 column (J & W Scientific DB-5 with 0.25 μm phase, 0.25 mm i.d., 30 m).

HAZARDS

Protective clothing and eyewear should be used at all times when handling the chemicals. All of the procedures involved in this experiment should be done in a fume hood away from open flames. Bisphenol-A is a skin sensitizer, irritant, and reproductive hazard. Pyridine is an irritant as well. Methanol is extremely flammable and toxic by inhalation. Hexane is a flammable liquid and vapor, is harmful or fatal if swallowed, and causes irritation to skin, eyes, and respiratory tract. BSTFA is flammable and may cause irritation to skin, eyes, and respiratory tract and may be harmful if swallowed or inhaled.

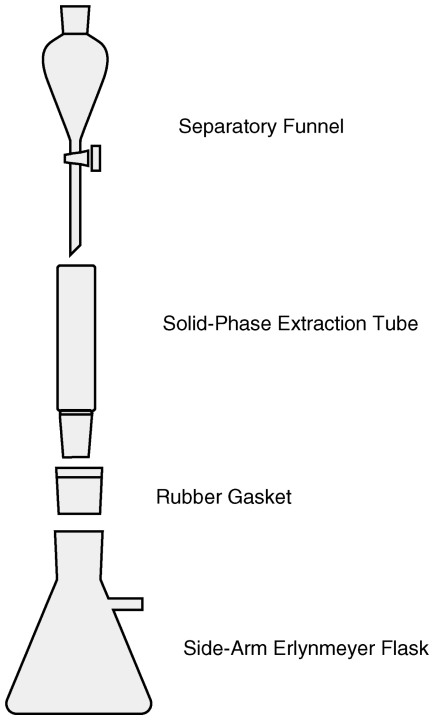

Figure 2. Solid-phase extraction schematic used in this experiment.

MASS SPECTRAL INTERPRETATION

Before each lab period, samples for a BPA calibration curve spanning a concentration range of 10−250 ng/μL are analyzed by GC−MS. An additional mixture is analyzed as well before class that contains phthalates (dimethyl-, diethyl-, dibutyl phthalates) and BPA (Figure 3A). The student pairs are then taken to the instrument with the sample they have prepared for analysis. The student is given an overview of operation and layout of the GC−MS prior to injection. The student's sample is then injected, and while it is running, they use the instrument's software to find the BPA peak in the phthalate mix. This gives the students experience using the instrument's software for data interpretation because the molecular weight of the BPA has increased from 228 to 372 by the addition of the TMS groups. This reinforces the idea that derivatization changes the molecular weight and must be accounted for when interpreting MS. Upon finding the BPA in the standard mix, the students use the retention time and mass spectra (Figure 3B) to identify BPA in their unknown sample. Once they have identified it, the instrument software is used to integrate the peak to obtain the area. Data from the standard BPA samples are provided to the students so they can generate a calibration curve to determine the concentration of the analyte in the sample. Based upon previous experience, the students recover 0.07 ± 0.01 mg of the 0.1 mg BPA, which is equal to a 70% ± 10% recovery. As a refresher for mass spectrometry, the students should be briefed as to what information is contained in the mass spectrum. The ordinate displays the percent abundance of the ions normalized to the most abundant ion. The abscissa is the mass-to-charge ratio (m/z), which is the mass divided by the charge. In this experiment, an electron ionization source was used and forms singly charged molecules. Therefore, the m/z data is considered to be the mass of the molecule and fragments. Another piece of information that can be obtained from the mass spectrum is the relative abundance of

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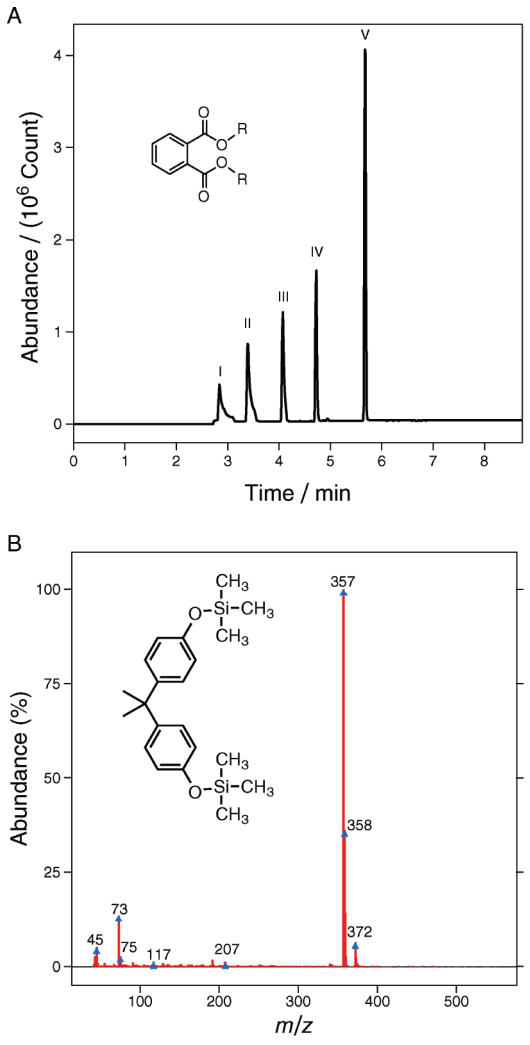

Figure 3. (A) Chromatogram of standard mixture showing phthalates and bisphenol-A: structure I, R = CH_3; structure II, R = CH_2CH_3; structure III, R = $CH_2CH_2CH_3$; structure IV, R = $CH_2CH_2CH_2CH_3$; and V is bisphenol-A. (B) Mass spectra of di-TMS- bisphenol-A.

the m/z peaks relative to the molecular ion and other fragments. A higher relative abundance of a fragment is indicative of formation ease and thus stability.

The mass spectra of the di-TMS-BPA gave a low-abundance molecular ion (8%) (Figure 3B). A proposed structure for base peak (357 m/z) is shown in Figure 1 and results from the neutral loss of 15. If a geminal methyl group was lost, formation of a dibenzylic carbocation results. The dibenzylic carbocation is very stable and therefore leads to the formation of the base peak. From introductory organic chemistry, the student can be reminded that carbocation stability follows the trend of methyl < primary < secondary < tertiary < benzylic.[15] Another fragment the students can be asked to identify is at 207 m/z (2.5%), which is postulated to be the result of the neutral loss of $C_9H_{13}SiO$ (Figure 1), forming another benzylic carbocation. In addition to the di-TMS-BPA, the phthalates also provides the students with more hands-on use of the MS software to identify peaks based on molecular weight and characteristic fragments, such as the 149 m/z ion from structure II, III, and IV fragmentation and cyclization.[16]

CONCLUSIONS

Extraction, analysis, and identification by mass spectrometry of an analyte provide critical hands on experience for students, especially when combined with a lecture course such as organic spectroscopy. Challenging the students to identify fragments generated from mass spectrometry will provide preparation for the rigors of upper-level chemistry courses and in industry. The students also have experience with quantification using a calibration curve.

ASSOCIATED CONTENT

Supporting Information

Equipment, chemicals, notes for the instructor; typical student data. This material is available via the Internet at http://pubs.acs.org.

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