Analysis and Quantification of 2,2',4,4'-Tetrabromodiphenyl Ether using Gas Chromatography Coupled with a Low-Resolution Mass Spectrometry
CHEM 4303
Analytical Separations

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Abstract

Gas chromatography with a mass spectrometer was utilized for analysis.

1 Introduction

Gas chromatography (GC) is a separation technique that analyzes volatile compounds [1]. Consequently, this analysis can lead a lot of useful situations such as the determination of the purity of a compound or even quantifying individual components in mixtures [1]. In GC, the analyte is volatilized and carried through the column by the mobile phase, often called the carrier gas [2]. This carrier gas can either be He or H_2 [2]. These gases are often chosen as the carrier gas as they are chemically inert and would therefore not react with the analytes [1].

Polybrominated diphenyl ethers (PBDEs) are a class of halogenated compounds that are commonly used as flame retardants [3]. These compounds are an environmental health hazard as they have the potential to accumulate in the food chain [4]. In addition, 2,2',4,4'-Tetrabromodiphenyl ether, also known as BDE-47 [5], is a PBDE congener, and has been found to cause neurotoxic effects in adults [4]. Commonly used detection techniques for PBDEs are high-resolution mass spectrometry and low-resolution mass spectrometry (LRMS) [3].

LRMS is commonly done with selected ion monitoring (SIM) [3]. SIM increases the selectivity of mass spectrometry for analytes and reduces its response to everything else [2]. It does this by setting the mass spectrometer to detect just one or even a few m/z values [2]. One of the advantages of SIM is that the signal-to-noise ratio greatly increases as the mass spectrometer spends its entire analysis time measuring that particular ion, or a couple of ions [2].

Internal standards are used for a number of reasons, such as when sample loss is expected during the sample preparation step, or when the response of the quantity of sample detected differs at each analysis [2]. Essentially, an internal standard is a known quantity of a compound that differs from the analyte, and is added to the mixture containing the analyte to be detected [2].

An injection mode of splitless rather than a split is used when attempting to analyze trace chemicals [2]. Furthermore, a splitless injection mode minimizes solvent tailing by opening the split vent after around $1\,\mathrm{min}$ so that the any remaining sample in the injection port can be purged [1].

The main objective of this entire experiment is to detect as well as quantify BDE-47 in a sample of fish oil. This is accomplished by making a method that utilizes a SIM.

2 Chemicals, Methods and Instrumentation

2.1 Chemicals

In the first week, BDE-47, PBB-77 and '2-HCH, all of each were at a concentration of $50\,\mu\mathrm{g/mL}$ in isooctane, were used as standard solutions. In the second week, fish oil (Exact Norwegian Cod Liver Oil), dichloromethane (emd, Lot 5Q160, CAS: 75-09-2) and PBB-77, at a concentration of $10\,\mu\mathrm{g/mL}$ in isooctane, were used as chemicals. Finally, in the last week, BDE-47, PBB-77 and '2-HCH, each at a concentration of $10\,\mu\mathrm{g/mL}$ in isooctane, were used. Through-

out the entirety of the experiment, hexane (Caledon Laboratory Chemicals, CAS no. 110-54-3, LOT: 89001) and isooctane (OmniSolv, CAS: 540-84-1, LOT: 52054) were used.

2.2 Instrumentation

The separation and analysis of the entire experiment was performed on an Agilent 7890A GC, coupled with a 5975C inert XL EI/CI MSD with a triple axis detector. The dimensions of the column used was $30m \times 0.250mm \times 0.25\mu m$, by Agilent Technologies. The stationary phase was (5%-Phenyl)-methylpolysiloxane. Each analysis was performed with the injection mode at splitless, with He as the carrier gas, and the flow rate was set at $1\,\mathrm{mL/min}$. The injection mode used for each analysis was splitless, and the injection volume was $2\,\mu\mathrm{L}$ for each analysis.

2.3 Methods

In the first week of the experiment, a (normalized) full scan spectrum was obtained for BDE-47, PBB-77 and ‘2-HCH. The upper limit of the scan range was set to 30 u more than the molecular weight of the respective analyte that was being analyzed. For each analysis, the oven temperature was initially set to $100\,^{\circ}\mathrm{C}$ for $5\,\mathrm{min}$, then it was increased to $280\,^{\circ}\mathrm{C}$ at a rate of $10\,^{\circ}\mathrm{C/min}$, and it was held at that temperature for $7\,\mathrm{min}$. Furthermore, the temperature of the splitless injection port was set to $260\,^{\circ}\mathrm{C}$, the electron energy was set to $70\,\mathrm{eV}$, and the temperature of the GC-MSD interface was fixed at a temperature of $280\,^{\circ}\mathrm{C}$.

In the second week of the experiment, two samples of fish oil were prepared by adding $2\,\mu\mathrm{g/mL}$ of PBB-77 as the internal standard to the sample, and then having it undergo solid-phase extraction in order to extract lipids. Afterwards, the majority of the solvent was then removed from the sample through rotary evaporation.

In the last week of the experiment, a calibration solution was produced by adding each of BDE-47 and PBB-77, at a volume of $10\,\mu\mathrm{L}$, to a $0.5\,\mathrm{mL}$ of isooctane contained in an auto sampler vial, and it was diluted all the way to $1.0\,\mathrm{mL}$ with the solvent. $50\,\mu\mathrm{L}$ of ‘2-HCH was added to the calibration solution, while $10\,\mu\mathrm{L}$ was added to the fish samples that was prepared last week. Finally, the GC-MS was set to scan only two ions per analyte, the parameters that was selected is shown in table 1. Refer to table ref to see which 2 ions was monitored.

3 Results and Discussion

3.1 Results

Table 1 shows the parameters that was set in the GC/EI-MS and the mass of the ions that was monitored.

Table 2 tabulates the molecular weight of BDE-47, PBB-77, and ‘2-HCH.

Table 1: Selected Ion Monitoring Parameters

Compound	Ions monitored (m/z)	Time window (min)
‘2-HCH	181, 219	0.5 - 19
BDE-47	326, 486	19 - 22.5
PBB-77	470, 310	22.5 - 24

Table 2: Molecular weight of BDE-47, PBB-77 and ‘2-HCH

Compound	Molecular weight (u)
BDE-47	486
PBB-77	470
‘2-HCH	291

Table 3 shows mass of fish oil that was weighed out for this experiment.

Table 3: Mass of fish oil weighed out

Sample number	Mass (g)
1	1.1234
2	0.9062

Table 4 tabulates the volume, concentration and mass of BDE-47, PBB-77, and ‘2-HCH that was added into the calibration solution, while table 5 shows the response and relative response factors of the aforementioned analytes to the internal standard, which was PBB-77, in the calibration solutions.

Table 4: Table of data showing the volume and concentration of each analyte in the calibration solution

Component	Volume/mL	Concentration(μg/mL)	Mass/μg	Area
BDE-47	0.01	0.0952	9.52×10^{-4}	367089
PBB-77	0.01	0.0952	9.52×10^{-4}	230205
‘2-HCH	0.05	0.476	2.38×10^{-2}	260358

Table 5: Table of data showing the response and relative response factors of each analyte to the internal standard (PBB-77) in the calibration solution

Component	Mass/μg	Area	Response factor/μu	Relative Response Factor
BDE-47	9.52×10^{-4}	367089	2.59×10^{-9}	6.27×10^{-1}
PBB-77	9.52×10^{-4}	230205	4.14×10^{-9}	1.00
‘2-HCH	2.38×10^{-2}	260358	9.14×10^{-8}	2.21×10^{1}

Table 6 shows how much mass of BDE-47 was contained in each sample of fish.

Table 7 shows the mass of PBB-77 and ‘2-HCH that was added to the calibration solution as well as areas of their respective peaks.

Table 6: Table of data showing how much mass of BDE-47 is in each fish sample

Fish sample	Area of BDE-47	Area of PBB-77	Mass of BDE-47 in sample/μg
1 (first run)	13799	100528	1.64×10^{-5}
1 (second run)	14383	103212	1.66×10^{-5}
2 (first run)	12800	112480	1.36×10^{-5}
2 (second run)	13938	100772	1.65×10^{-5}

Table 7: Mass of PBB-77 and ‘2-HCH that was added to the calibration solution

Mass of PBB-77 /μg	Mass of ‘2-HCH/μg
1.90×10^{-4}	4.76×10^{-3}

Table 8 shows the recovery rate of the internal standard, PBB-77, in each fish sample.

Table 8: Recovery rate of PBB-77 in each fish sample

Fish sample	Mass of PBB-77 recovered/μg	Actual Recovery Rate/%
1 (first run)	4.90×10^{-5}	25.72
1 (second run)	4.99×10^{-5}	26.22
2 (first run)	8.38×10^{-5}	44.04
2 (second run)	7.60×10^{-5}	39.90

3.2 Discussion

4 References

References

(1) Vitha, M. F., *Chromatography: Principles and Instrumentation*; Wiley: Hoboken, New Jersey, 2017.

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(4) Thomsen, C.; Småstuen Haug, L.; Leknes, H.; Lundanes, E.; Becher, G.; Lindström, G. *Chemosphere* **2002**, *46*, 641–648.

(5) Erratico, C. A.; Moffatt, S. C.; Bandiera, S. M. *Toxicological Sciences* **2011**, *123*, 37–47.

5 Appendix

5.1 Calculations

Calculating the final concentration of BDE-47 in the calibration solution

$$\begin{aligned} \textit{initial concentration} \times \frac{\textit{initial volume}}{\textit{final volume}} &= \textit{final concentration} \\ 10\mu\text{g/mL} \times \frac{0.01\text{mL}}{1.05\text{mL}} &= 0.0952\mu\text{g/mL} \end{aligned} \tag{1}$$

Equation 1 was taken from [2].

Calculating the relative response factor of BDE-47

$$\textit{Mass of BDE-47 in the calibration solution} = \textit{volume} \times \textit{concentration}$$

$$0.01\text{mL} \times 0.0952\mu\text{g/mL} = 9.52 \times 10^{-4}\,\mu\text{g}$$

$$\textit{Response Factor} = \frac{\textit{Mass}}{\textit{area}}$$

$$\frac{9.52 \times 10^{-4}\,\mu\text{g}}{367089} = 2.59 \times 10^{-9}\,\mu\text{g}$$

$$\textit{Relative response factor} = \frac{\textit{Response factor}_{BDE-47}}{\textit{Response factor}_{PBB-77}}$$

$$\frac{2.59 \times 10^{-9}}{4.14 \times 10^{-9}} = 6.27 \times 10^{-1}$$

Actual recovery rate of internal standard, PBB-77, for the first run of fish sample 1

$$\textit{Actual Recovery Rate} = \frac{\textit{Mass}_{IS_{recovered}}}{\textit{Mass}_{IS_{added}}}$$

$$\frac{4.90 \times 10^{-5}}{2.21 \times 10^{1}} = 25.72\%$$

5.2 Chromatograms

There are 23 figures of GC/EI chromatograms.