

Analysis and Quantification of 2,2',4,4'-Tetrabromodiphenyl Ether using Gas Chromatography - Electron Ionization, Coupled with a Low-Resolution Mass Spectrometry in the Selected Ion Monitoring CHEM 4303 Analytical Separations

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Abstract

Gas chromatography - electron ionization mass spectrometry in the selected ion monitoring (GC/EI-MS-SIM) was used to detect and quantify the amount of BDE-47 in fish oil. Initially, the mass spectrum and total ion chromatogram was obtained for the analyte in question, BDE-47, the internal standard PBB-77 and the recovery standard '2-HCH. Afterwards, 2 samples of fish oil was prepared using solid-phase extraction. Finally, the fish samples, blanks, BDE-47, PBB-77 and '2-HCH underwent GC/EI-MS-SIM analysis, in which the ions being monitored were at a m/z of 326 and 486 for BDE-47, 470 and 310 for PBB-77, and 181 and 219 for '2-HCH. Through calculations, it was realized that the average concentration of BDE-47 in the first sample of fish oil was $1.47 \times 10^{-5} \mu\text{g/g} \pm 1.07\%$, while the second sample of fish oil had a BDE-47 concentration of $1.66 \times 10^{-5} \mu\text{g/g} \pm 13.75\%$.

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 H₂ [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 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 $\mu\text{g/mL}$ 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 $\mu\text{g/mL}$ in isooctane, were used as chemicals. Finally, in the last week, BDE-47, PBB-77 and '2-HCH, each at a concentration of 10 $\mu\text{g/mL}$ 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 mL/min. The injection mode used for each analysis was splitless, and the injection volume was 2 μL 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 °C for 5 min, then it was increased to 280 °C at a rate of 10 °C/min, and it was held at that temperature for 7 min. Furthermore, the temperature of the splitless injection port was set to 260 °C, the electron energy was set to 70 eV, and the temperature of the GC-MSD interface was fixed at a temperature of 280 °C.

In the second week of the experiment, two samples of fish oil were prepared by adding 2 $\mu g/mL$ 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 μL , to a 0.5 mL of isooctane contained in an auto sampler vial, and it was diluted all the way to 1.0 mL with the solvent. 50 μL of '2-HCH was added to the calibration solution, while 10 μL 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

All of the GC/EI MS chromatograms are located in the 'Chromatograms' subsection in the 'Appendix' section, and all them are appropriately labelled.

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

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

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($\mu\text{g/mL}$) | 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 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 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 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 |

Table 9 shows the average concentration of BDE-47 in fish oil. The table also shows the relative standard deviation of the concentration as well.

Table 9: The mean, standard deviation, and relative standard deviation of BDE-47 in fish oil in both of the samples

| Fish sample | Mean of BDE-47 in fish oil/($\mu\text{g/g}$) | Std. Dev./($\mu\text{g/g}$) | Relative Std. Dev./% |
|-------------|--|-------------------------------|----------------------|
| 1 | 1.47×10^{-5} | 1.57×10^{-7} | 1.07 |
| 2 | 1.66×10^{-5} | 2.28×10^{-6} | 13.75 |

Table 10 shows the average signal-to-noise ratio of BDE-47 in the fish samples.

Table 10: Average signal-to-noise ratio of BDE-47 in the fish samples

| Fish sample | Signal-to-Noise ratio |
|-------------|-----------------------|
| 1 | 93.65 |
| 2 | 7.27 |

3.2 Discussion

The mass spectra for the compounds BDE-47, PBB-77 and '2-HCH shown in figures 3,4 and 5 respectively. And the identification of its corresponding molecular ion (M^+) is illustrated in figures 24, 25 and 26, except that they have an extra positive charge in the molecule. Note that the figures were taken from the website PubChem [The PubChem Project. <https://pubchem.ncbi.nlm.nih.gov/> (accessed Dec 11, 2018)].

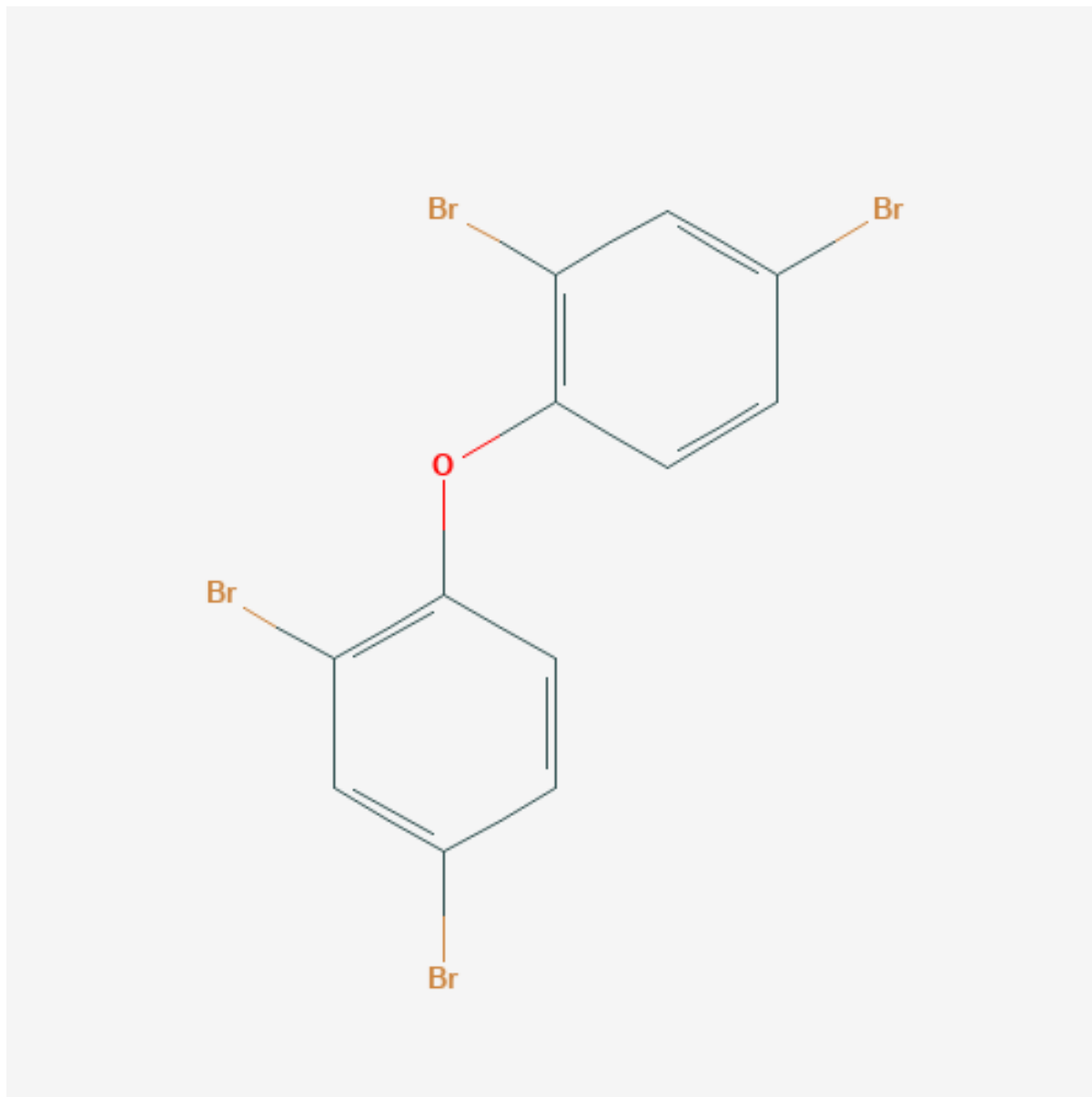


Figure 24: Molecular structure of BDE-47

As it can be seen in figures

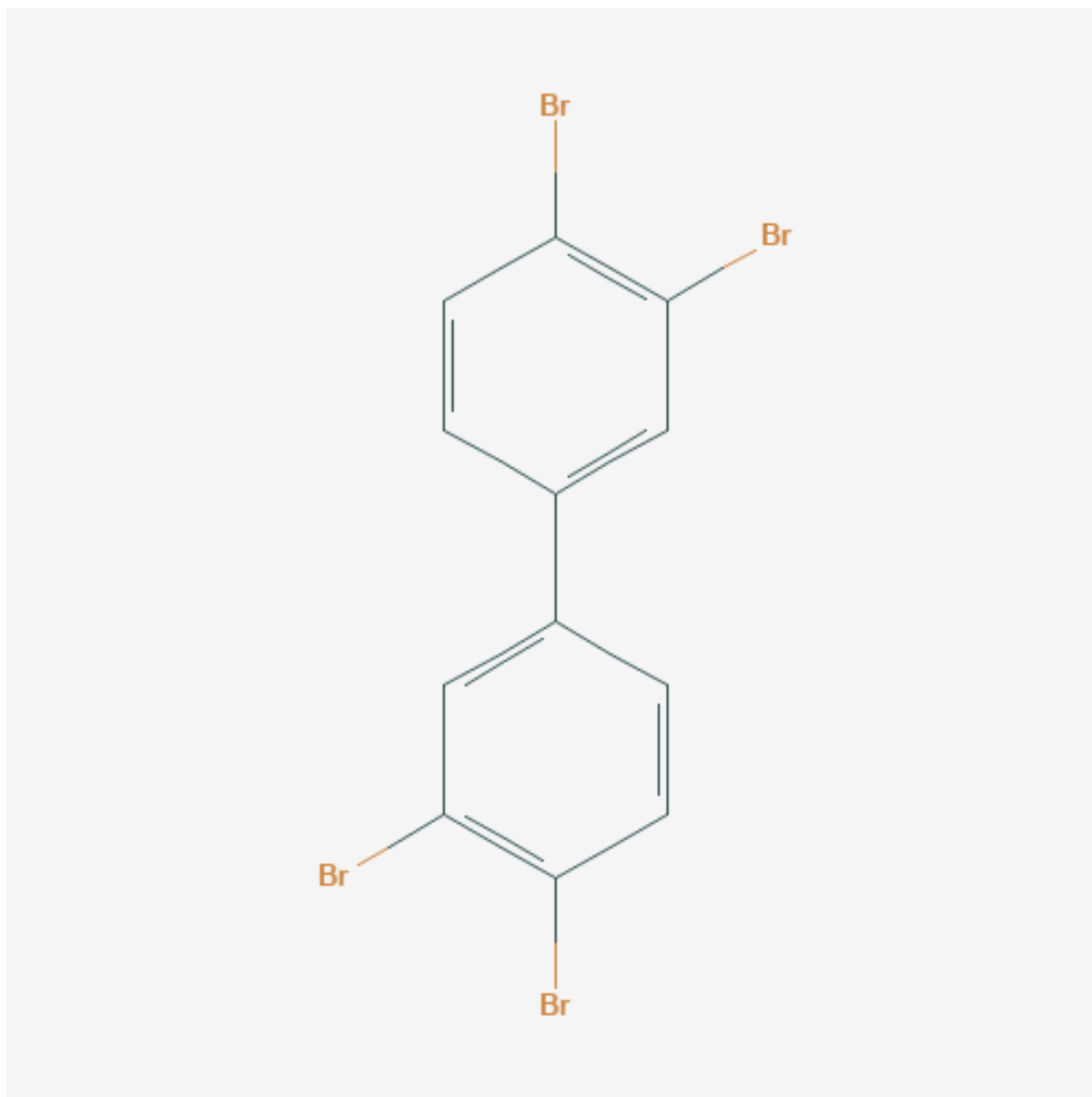


Figure 25: Molecular structure of PBB-77

4 References

References

- (1) Vitha, M. F., *Chromatography: Principles and Instrumentation*; Wiley: Hoboken, New Jersey, 2017.
- (2) Harris, D. C., *Quantitative chemical analysis*, 8th ed; W.H. Freeman and Co: New York, 2010.
- (3) Björklund, J.; Tollbäck, P.; Östman, C. *Journal of Mass Spectrometry* **2003**, 38, 394–400.
- (4) Thomsen, C.; Småstuen Haug, L.; Leknes, H.; Lundanes, E.; Becher, G.; Lindström, G. *Chemosphere* **2002**, 46, 641–648.

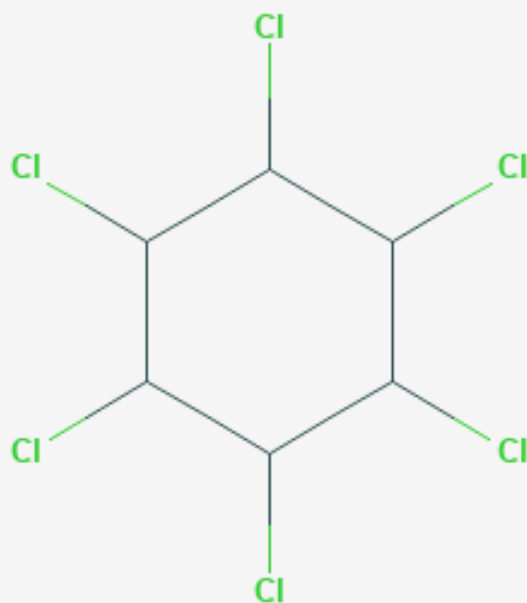


Figure 26: Molecular structure of '2-HCH

- (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

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

Equation 1 was taken from [2].

Calculating the relative response factor of BDE-47

Mass of BDE-47 in the calibration solution = volume \times concentration

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

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

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

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

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

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

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

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

5.2 Chromatograms

There are 23 figures of GC/EI chromatograms.