Analysis and Quantification of 2,2',4,4'-Tetrabromodiphenyl Ether using Gas Chromatography - Electron Ionization, Coupled with a Low-Resolution Mass Spectrometry in Selected Ion Monitoring Mode
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 $1.47 \times 10^{-5}\,\mu g/g \pm 1.07\%$, while the second sample of fish oil had a BDE-47 concentration of $1.66 \times 10^{-5}\,\mu g/g \pm 13.75\%$.

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 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.

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(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 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/(μg/g)	Std. Dev./(μg/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)].

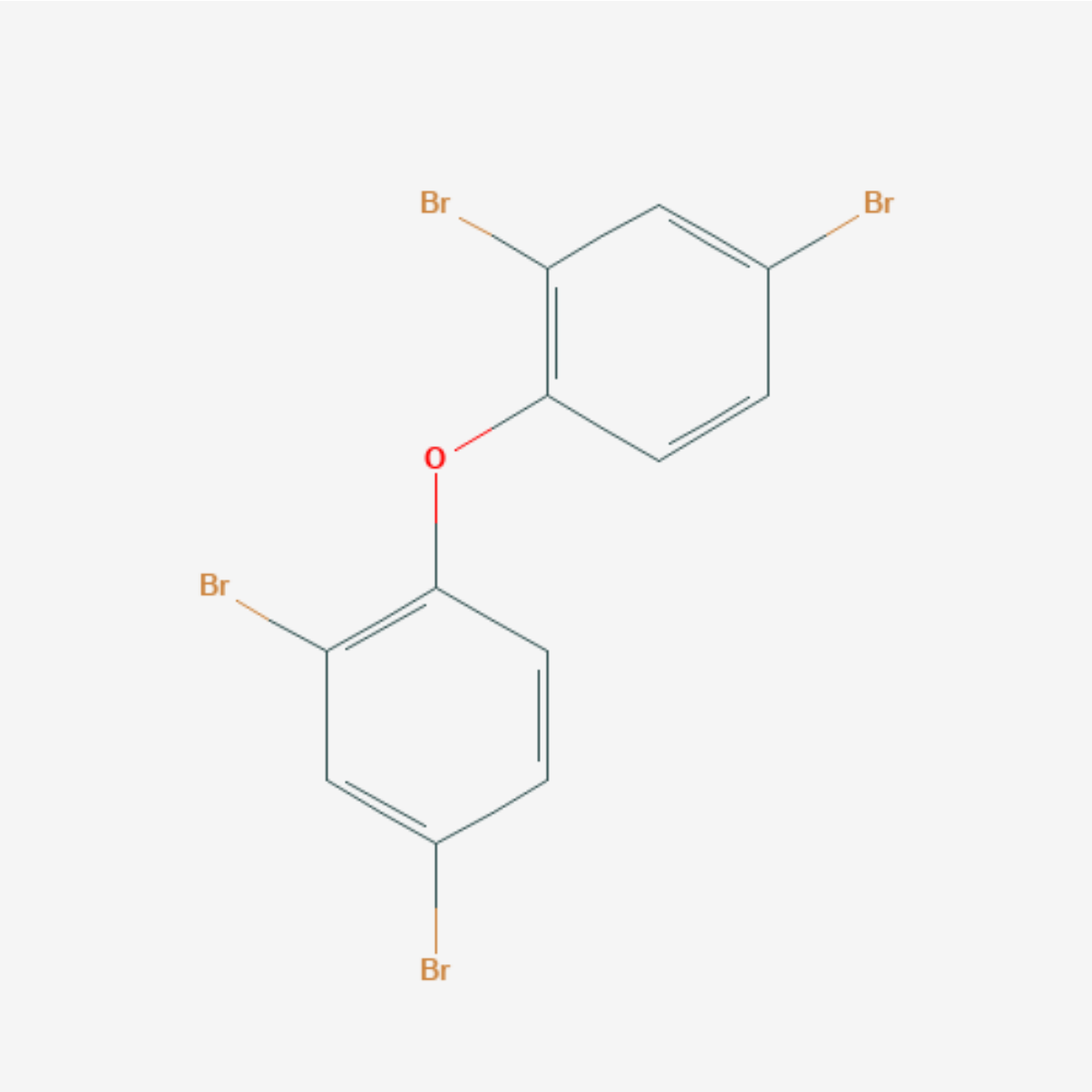

Figure 24: Molecular structure of BDE-47

Table 1 shows the mass of the ions that were chosen to be monitored. These ions were chosen as the peaks of those ions did not overlap with the chosen peaks of the other ions. In addition, the abundance of the peaks was adequately high. Refer to figures 3, 4 and 5 for more clarity.

The analysis of hexanes as blanks (figures 6 - 11) regarded that the contribution of BDE-47

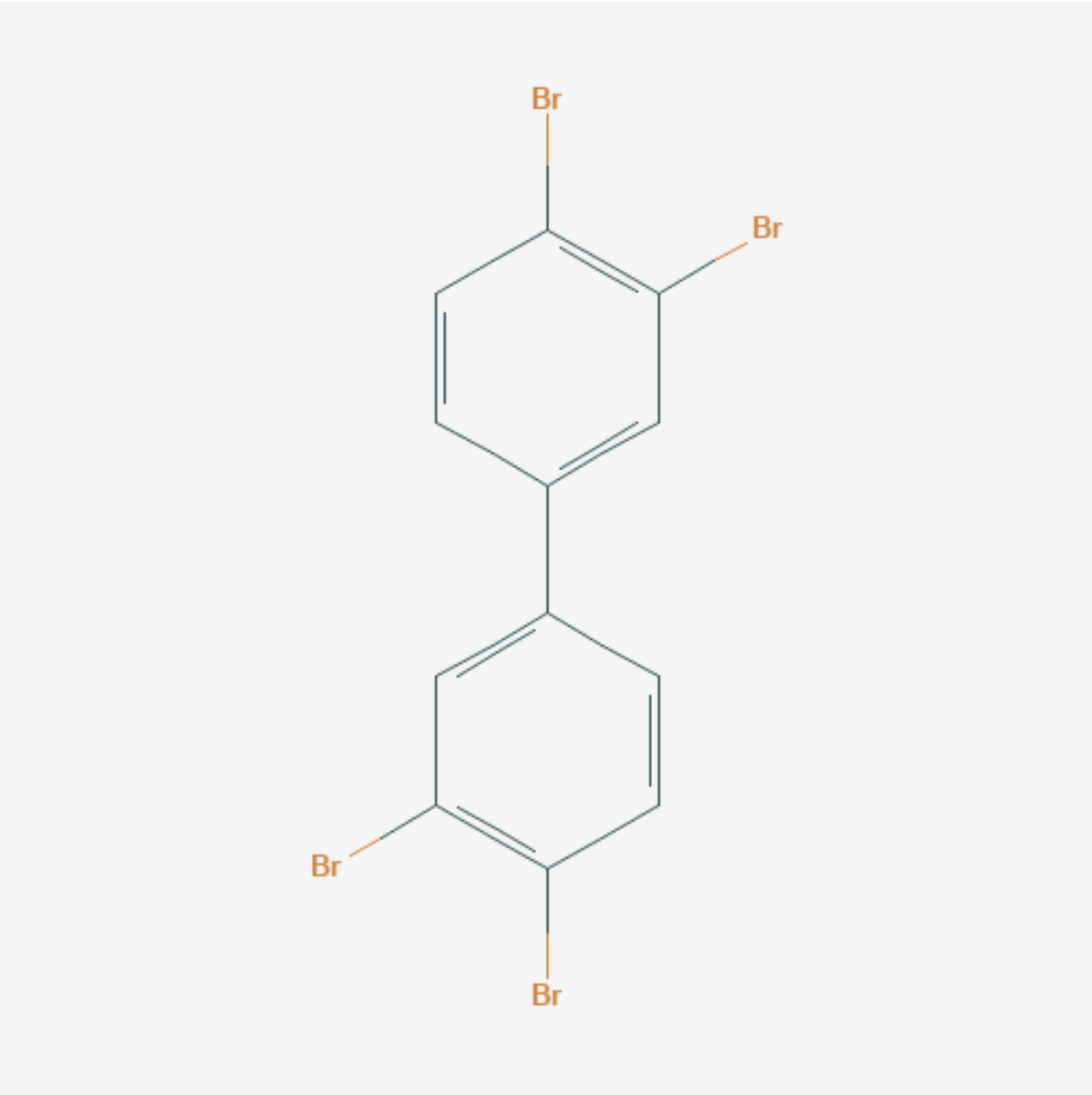

Figure 25: Molecular structure of PBB-77

is negligible, considering that at a retention time of around $22.35\,\mathrm{min}$, the abundance of the peak caused by BDE-47 is extremely low. So one can safely assume that the concentration of BDE-47 calculated was largely contributed to its presence in the oil of the fish.

As it can clearly be seen in table 9, the average concentration of BDE-47 in the very first sample of fish oil was $1.47 \times 10^{-5}\,\mu\mathrm{g/g}\ \pm 1.07\%$, and in the second sample, the concentration of BDE-47 was $1.66 \times 10^{-5}\,\mu\mathrm{g/g}\ \pm 13.75\%$. Also, as evidenced in table 10, the average signal-to-noise (S/N) ratio during the analysis of the first fish oil sample was 93.65, and it was 7.27 in the second analysis. This can be concluded that since the value of the S/N ratio for the second sample it not quite high, compared to the first sample, this experiment can be repeated multiple times until a reasonable value is obtained. This statement goes double when the standard deviation of the concentration of BDE-47 is also taken into account; its value is much higher than the first sample of fish oil.

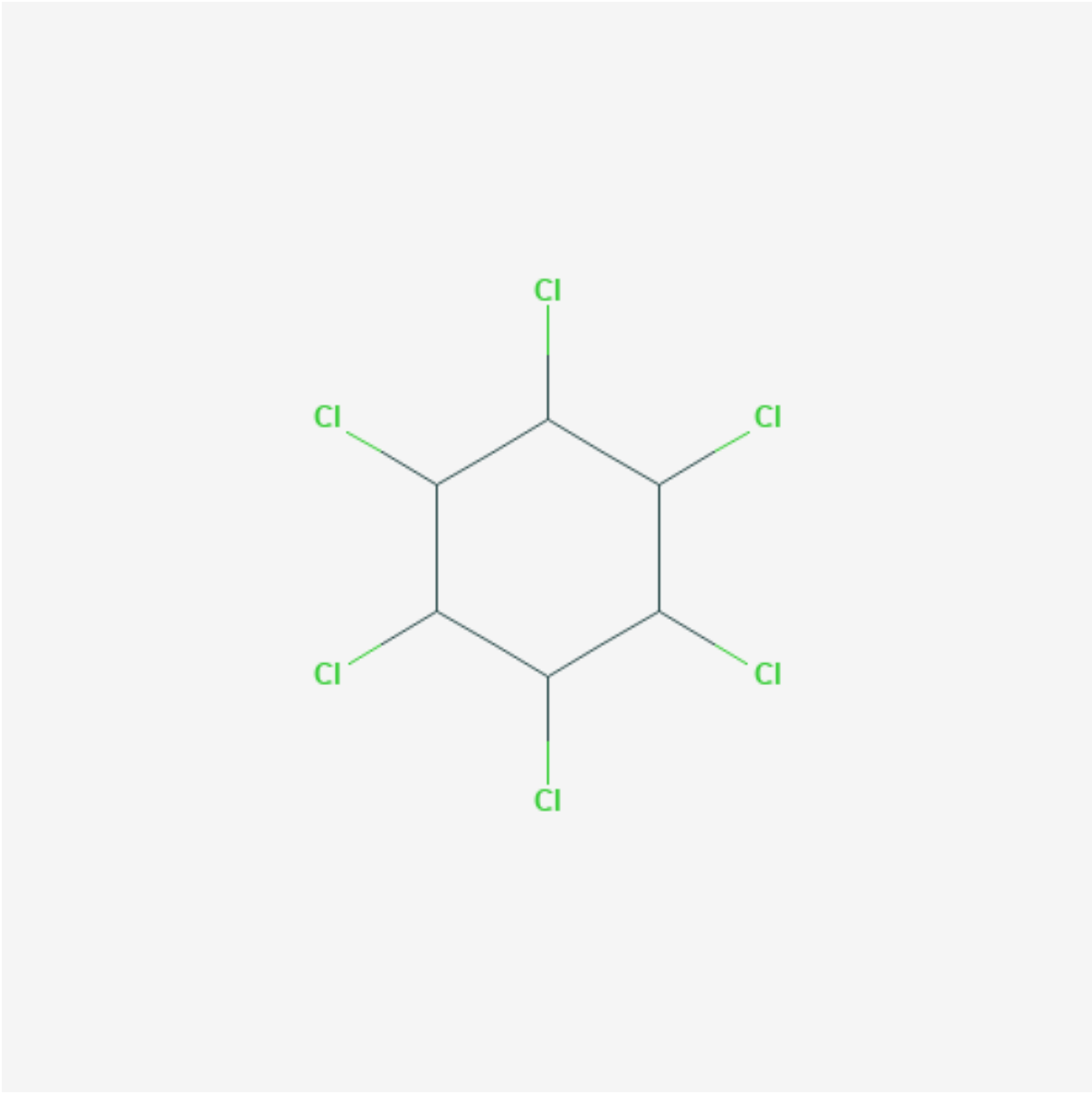

Figure 26: Molecular structure of ‘2-HCH

4 References

References

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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.