Application Note: 10042

Confirmation and Quantitation of Polybrominated Biphenyls (PBBs) and Polybrominated Diphenyl Ethers (PBDEs) by MS/MS

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Key Words

- Polaris Q Ion Trap
- Flame Retardants
- GC-MS/MS
- Import Regulations
- PBDEs

Overview

Purpose

To investigate the sensitivity and selectivity of GC/MS/MS for the analysis of polybrominated pollutants in natural waters.

Method

Pond water extracts are analyzed by GC/MS/MS. Injection technique and trap parameters are adjusted to give maximum sensitivity and selectivity.

Introduction

In the 1970's, approximately nine million people were affected by exposure to polybrominated biphenyls (PBBs) in the Michigan area. The PBBs were accidentally added to cattle feed and subsequently ingested through the consumption of beef and dairy products. Currently, these people are 23 times more likely to have digestive cancers, and 49 times more likely to have lymphatic cancers. Polybrominated diphenyl ethers (PBDEs) have been shown to reduce thyroxin levels in those exposed, and mice exhibit abnormal behavior upon exposure to PBDEs. The European Union voted to ban pentabromodiphenyl ether at levels greater than 0.1 % in substances, primarily polyurethane. In addition, the levels of PBDEs in breast milk are doubling every five years. From this, it becomes apparent that the monitoring of these flame retardants in the environment is essential.

Environmental samples, as is their nature, are extremely complex. The extraction of soils and waste waters can be both time intensive and expensive. Cleanup can be reduced by removing unwanted matrix ions from the trap with the use of MS/MS.

Flame retardants, by their nature, are relatively non-volatile and so pose a special problem for the analytical chemist using gas chromatography. The use of an on-column injection technique allows for complete transfer of these non-volatile analytes to the column.

By using these two powerful analytical techniques, MS/MS and on-column injection, the recovery and sensitivity for polybrominated biphenyls and the polybrominated diphenyl ethers in matrix is greatly increased.

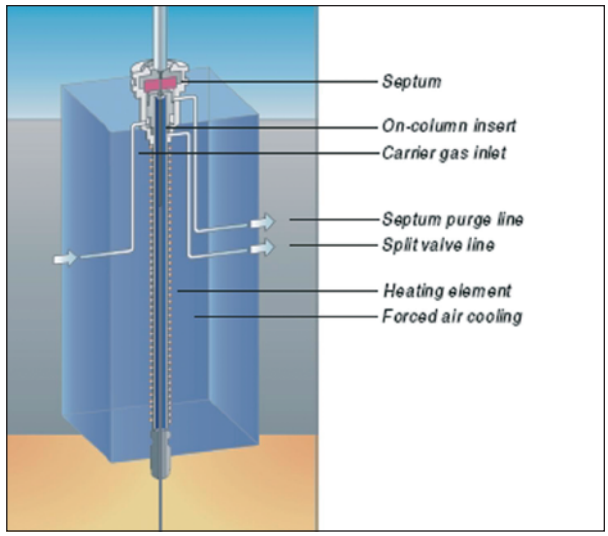

Figure 1: Injection on PTV showing the On-Column insert configuration

Sample Preparation

500 mL of city park pond water was extracted with three 80 mL portions of methylene chloride. The methylene chloride portions were dried with sodium sulfate then reduced to dryness using nitrogen gas. The residue was reconstituted to 5 mL with benzene. One 1 mL portion was spiked with 20 µl of 35 ng/µL Bromokal technical mixture and 10 µL of 60 ng/µL of Dow FR-250 technical mixture. Another 1 mL portion served as a matrix blank.

Standard curves were made with the same technical mixtures as noted above in concentration ranges from 1 pg/µL to 60 ng/µL using serial dilution.

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Instrument Parameters

Polaris*Q* Ion Trap

Source temperature: 275 °C
Ionization mode: Electron Ionization
AGC: 50
Injection waveforms: On at 0 scaling
Adjustable trap pressure flow: 3.00 mL/min
MS/MS parameters: See Table 1

TRACE GC Ultra

Column: Rtx™200, 0.25mm x 30mx 0.25 µm
Constant flow: 2 mL/min
Oven: 92 °C for 4 min
Ramp: 25 °C/min to 340 °C, hold for 4 min

PTV Injector

Mode: On-column
Initial temp: 92 °C for 4 min
Ramp: 0.5 °C/sec for 9 min, hold at 340 °C for 3.5 min

Autosampler

Injection volume: 1 µL
Airgap: 0.2 µL
Solvent plug: 0.2 µL

Results and Discussion

The On-Column Programmable Temperature Vaporizing (OC-PTV) injector, shown in Figure 1, has a liner designed to allow the column to be inserted up to the top of the inlet. The needle can then be inserted inside the column allowing for a true on-column injection. Because of the rapid heating properties of the PTV inlet, it is able to track with the oven ramp temperature for the duration of the analysis. In effect, this recreates the principles of the traditional on-column injector. Figure 2 dramatically displays the increased analyte transfer with OC-PTV as compared to a traditional split/splitless inlet. While the OC-PTV was programmed with a temperature ramp to match that of the oven, so it was cool during the injection, the split/splitless injector was operated at a constant 275 °C. Figure 2 demonstrates that the OC-PTV transfers roughly 100 times more analyte versus the splitless injection, with 50 ng/µl of decabromodiphenyl ether injected in either case.

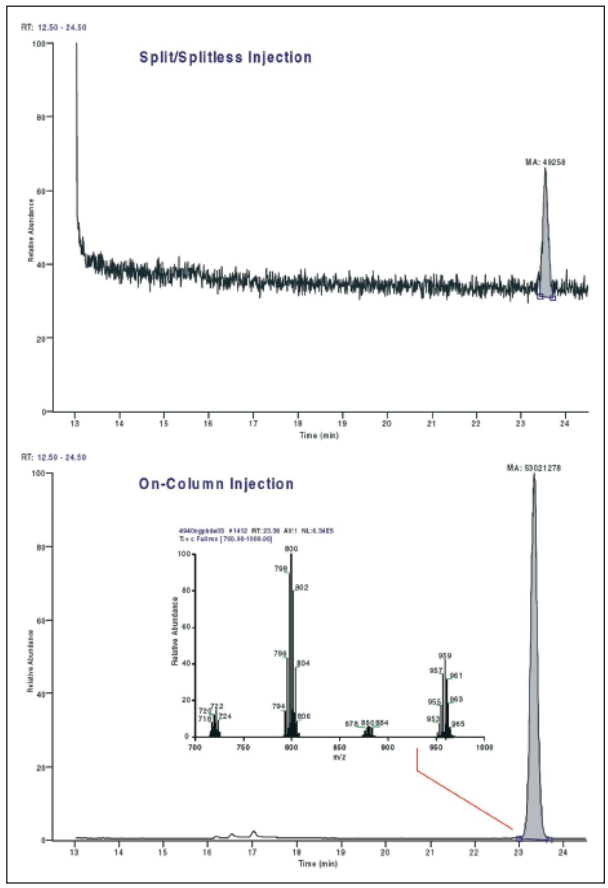

Figure 2: Comparison of transfer efficiency of splitless injection to on-column injection techniques with 50 ng decabromodiphenyl ether

DOW COMPOUND	RT	PRECURSOR ION	AMU WIDTH	EXCITATION VOLTAGE	Q MAX EXCITATION ENERGY	PRODUCT IONS	THEORETICAL RATIO M/(M+2)	CALCULATED RATIO M/(M+2)
Hexabromobiphenyl	13.09	627.5	15	5.2	0.3	542-552 (even nos.only)	1.02	0.86
Heptabromobiphenyl	13.5	706.4	17	5.8	0.3	620-632 (even nos. only)	1.36	1.32
Heptabromobiphenyl	13.6	706.4	17	5.8	0.3	620-632 (even nos. only)	1.36	1.31
Heptabromobiphenyl	13.97	706.4	17	6.8	0.3	620-632 (even nos. only)	1.36	1.43
Octabromobiphenyl	14.42	785.4	19	6.25	0.3	698-714 (even nos. only)	1.02	1.02
Decabromobiphenyl	14.9	943	19	6	0.3	857-871 (odd nos. only)	1.02	1.08

BROMOKAL COMPOUND	RT	PRECURSOR ION	AMU WIDTH	EXCITATION VOLTAGE	Q MAX EXCITATION ENERGY	PRODUCT IONS	THEORETICAL RATIO M/(M+2)	CALCULATED RATIO M/(M+2)
Tetrabromo Diphenyl ether	11.1	486	11	4.75	0.3	324-328 (even nos. only)	2.01	2.09
Pentabromo Diphenyl ether	11.6	564.6	13	4.6	0.3	402-408 (even nos. only)	1.01	1.01
Pentabromo Diphenyl ether	11.8	564.6	13	4.6	0.3	402-408 (even nos. only)	1.01	1.00
Hexabromo Diphenyl ether	12.2	643.5	17	4.7	0.3	480-488 (even nos. only)	1.52	1.47
Hexabromo Diphenyl ether	12.5	643.5	17	4.7	0.3	480-488 (even nos. only)	1.52	1.49

Table 1: MS/MS parameters

Using an automatic, trap pressure controller, Helium buffer gas rates from 0.3 mL/min to 5 mL/min were evaluated for maximum trapping efficiency. Within this range, 3.0 mL proved to be the optimum flow rate with no significant increase in trapping efficiency observed for higher flow rates. This gave an average of five times more signal for both the PBBs and the PBDEs from the default 0.3 mL/minute flow rate.

Figure 6A on page 4 shows tetrabromodiphenyl ether from the Bromokal standard in full-scan. The base peak cluster at 482-488 Daltons (m/z) is also the molecular ion cluster. The ion trap, with external ionization, is able to collect a mass range containing the isotopic mass cluster, and selectively remove all other ions outside this designated mass range from the trap. This step alone significantly cleans up the detected spectra, as a quick comparison of Figure 6B, showing the molecular ion cluster, to Figure 6C showing the full scan spectrum, illustrates. After the molecular ion cluster, now also defined as the precursor ion, is isolated, an excitation voltage is applied for an optimum period of time. The precursor ions absorb this excitation voltage as rotational and vibrational energy. Once the rotational and vibrational energy becomes great enough, collision with another molecule in the trap, helium for instance, will then cause the bond(s) to break, giving a consistent, resultant, product ion spectrum.

By identifying and quantifying using this product ion spectrum for a particular analyte, the chance of generating false positives by a co-eluting component having the same precursor ion spectrum, as in a SIM analysis, is eliminated. The non-analyte precursor ion, although equivalent in mass to charge ratio, will have a different bond composition, and will fragment to a different product ion pattern. The tetrabromodiphenyl ether resultant product ion (Figure 6B) can be detected and quantitated in coeluting matrix, as shown in Figure 6D. In the full scan mode (Figure 6C), the matrix from the pond water obscures the ability to see the molecular ion pattern. However, by applying the MS/MS technique, the interfering matrix is removed, which allows the product ion, and its characteristic isotopic pattern, to be clearly seen and quantitated in the same pond water sample (Figure 6D).

An approximately half liter sample of the local park pond water was collected, extracted, and a portion was spiked to give a 700 pg/µL concentration of the Bromokal and a 600 pg/µL concentration of the Dow FR-250 technical mixtures to the extract. The resultant percent recoveries for the spiked sample of city pond water were all between 80 and 120 % with the exception of decabromobiphenyl from the Dow technical mixture.

Calibration curves generated for both the technical mixtures and the individual polybrominated biphenyls are illustrated in Figures 3 and 4. For the individual polybrominated biphenyls, 350 fg on column was the lowest concentration injected and detected, which resulted in signal-to-noise ratios ranging from 10 to 24 for the polybrominated biphenyls studied. The calibration was linear for the range of concentrations tested from 350 fg to 35 ng on column. Relative standard deviations were between 6-8 % for the three polybrominated biphenyls, based on 17 replicate injections.

Finally, since the individual standards for all of the components determined in this study were not available, technical mixtures were used for the polybrominated diphenyl ethers, and some assumptions were made about their actual concentrations. Technical mixtures present an interesting problem with regards to quantitation. Since the exact concentration of each component in a technical mixture is not known, it is difficult to quantitate the individual components to any degree of accuracy. In addition, technical mixtures are not equimolar in component concentration, so one can only estimate the percent concentration relative to another component within in the same mixture. Given these two facts, it was decided to simply define each component peak at a particular concentration, as the concentration of the total technical mixture. As a result, the concentrations assigned to each of the components in the calibration curve of Figure 3 is approximate. For all components contained in the two technical mixtures, good correlation coefficients were generated, and each component had a dynamic range spanning across 4 or more orders of magnitude.

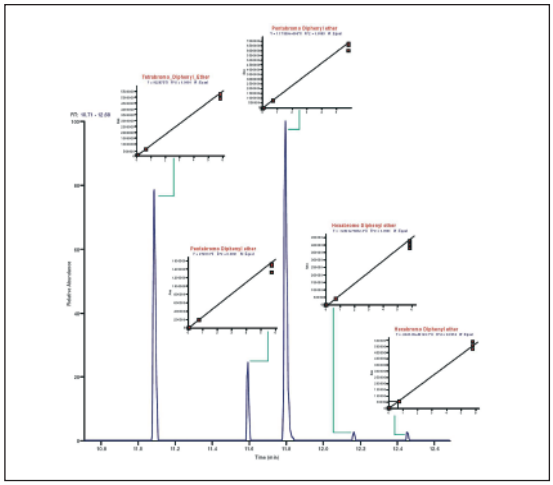

Figure 3: Linearity of Bromokal technical mixture components

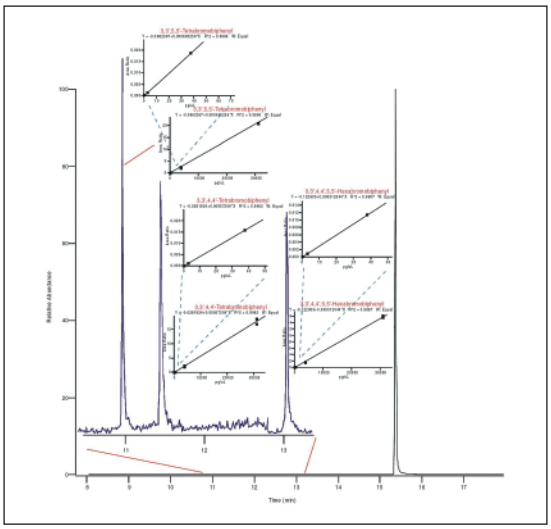

Figure 4: Linearity of Dow FR-250 technical mixture components

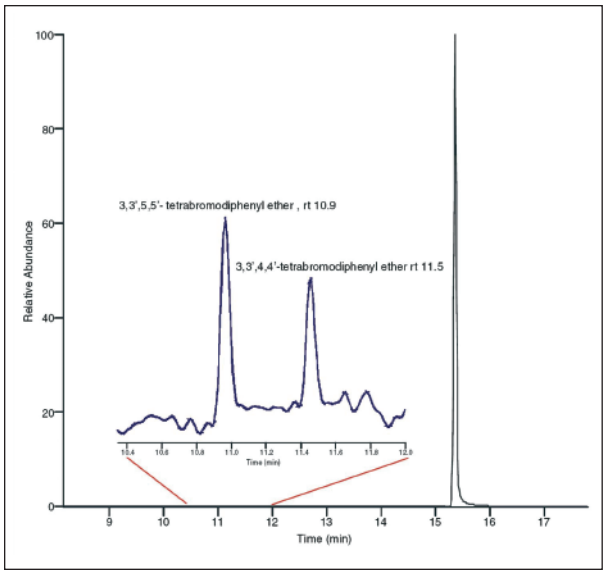

Figure 5: Chromatography of 1 µL injection of Polybrominated Biphenyls at a concentration of 350 fg/µL

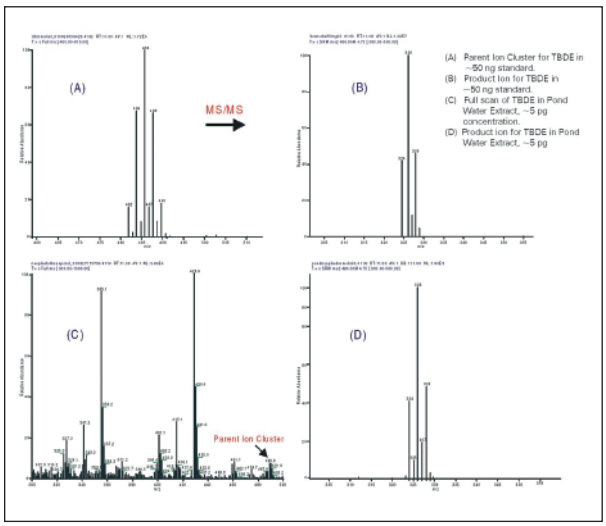

Figure 6: Comparison of selectivity of full-scan vs. MS/MS

Conclusion

The GC/MS/MS technique is ideal for samples with matrix, giving both selectivity, and linearity with a large dynamic range. MS/MS was proven to minimize the effect of matrix found in environmental samples. Finally, use of the on-column PTV injector greatly increased analyte transfer allowing for lower detection limits.

Greatly enhanced sensitivity for compounds that are marginally volatile with the use of the PTV inlet in the On-Column mode.

- Decabromodiphenyl ether increased by two orders of magnitude in sensitivity with the use of OC-PTV
- Elimination of matrix interference through the use of MS/MS
- Tetrabromodiphenyl ether found at picogram levels in extracted pond water
- Actual isotope ratio close to theoretical ratio for all ions studied

Method is linear across 5 orders of magnitude

- All polybrominated biphenyls studied were detectable at 350 femtograms delivered, corresponding to 70 ppt, and linear to 35 ng delivered.
- Bromokal technical mixture linear from approximately 10 pg to 50 ng injected on column. Estimated lower detection limit for the individual components of the technical mixtures at the same level as the polybrominated biphenyls.

Acknowledgement

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