DETERMINATION OF THE ENANTIOMER RATIO OF PBB 149 BY GC/NICI-TANDEM MASS SPECTROMETRY IN THE SELECTED REACTION MONITORING MODE

Roland von der Recke[1], Espen Mariussen[2], Urs Berger[3], Arntraut Götsch[1], Dorte Herzke[3], Walter Vetter[1]

[1]Institute of Food Chemistry, University of Hohenheim, Stuttgart, Germany
[2]Norwegian Institute for Air Research (NILU), Kjeller, Norway
[3]NILU, The Polar Environmental Centre, Tromsø, Norway

Introduction

Technical mixtures of polybrominated biphenyls (PBBs) have been extensively used as flame-retardants in textile and electronic industries and as additives in plastics [1]. Despite a continuous reduction of the worldwide annual production in the last decade, the presence of PBBs in the environment was recently confirmed in a wide range of samples [2]. PBBs exist in a theoretical variety of 209 congeners [1]. Many di-*ortho*, tri-*ortho*, and tetra-*ortho* PBBs form stable pairs of enantiomers, which was experimentally confirmed by enantioselective HPLC separation of chiral PBB in a technical mixture [3]. It is known from the literature, that chiral organohalogen compounds can be degraded enantioselectively [4,5]. In this work we used a chiral GC stationary phase and developed a method using GC/NICI-MSMS in the single reaction monitoring mode for the determination of the enantioratio of PBB 149 in extracts from Norwegian bird of prey eggs [6].

Material and Methods

Samples and Chemicals: The non-hatched white-tailed sea eagle egg was collected in Vikna, Nord-Trøndelag (Norway) after the hatching period in 1998 by the Norwegian Institute for Nature Research, Trondheim. Technical hexabromobiphenyl (Firemaster BP-6®, Michigan Chemicals) was used for identification of single PBB congeners as described elsewhere[3]. Solvents, reagents and gases were of best commercially available quality.

Sample clean-up and preparation: Sample clean-up including cold column extraction and clean-up of the extract by gel permeation chromatography and on a florisil column were performed as described elsewhere [7]. Organobromines were separated from polychlorinated biphenyls (PCBs) by group separation on 8 g of activated silica as described in the literature [8]. HPLC fractions was performed for separation of PBBs from PBDEs and further organochlorine compounds [6]. HPLC fractions containing PBB 149 were concentrated and subjected to enantioselectiv GC-MS analysis.

Gas chromatography in combination with mass spectrometry (GC/MS): Enantioselective GC/MS measurements were performed with a Varian CP-3800 GC coupled to a Varian 1200 triplequadrupole MS. Helium 5.0 was used as carrier gas. The injector and transfer line temperatures were set at 220 °C, respectively. The scan time was set at 1 s/cycle, and the filament emission was set at 150 µa.

Enantioseparations were performed with a 20 m x 0.25 mm i.d. capillary column coated with 0.15 µm of the chiral stationary phase (CSP). The CSP consisted of 35% randomly derivatised 6-*O*-*tert*.-butyldimethylsilyl-2,3-di-*O*-methyl-β-cyclodextrin diluted in PS086 (β-TBDM). The performance of the column was previously described in detail [6].

The GC oven temperature program for PBB 149 started at 80 °C (hold time 1 min), which then was raised at 20 °C/min to 190 °C (hold time 93.5 min), and finally at 5 °C/min to 210 °C/min (hold time 26 min). The total run time was 130 min. With this program PBB 149 eluted in the isothermal phase at 190 °C. Injections were performed in splitless mode (split opened after 4 min), using a pressure pulse at 15 psi for the initial 4 min. Thereafter, a constant flow rate of 1.0 mL/min was used throughout the measurements.

Non-chiral GC-analysis were performed with a Factor Four CP-Sil 8MS column (30 m x 0.25 mm i.d. x 0.25 µm d_f, Varian). The GC oven temperature program started at 70 °C (hold time 1.5 min), which then was raised at 30 °C/min to 140 °C, raised at 3 °C to 230 °C and finally at 4 °C/min to 270 °C/min (hold time 36.17 min). The total run time was 80 min. Injections were performed in splitless mode (split opened after 2 min), a constant flow rate of 1.0 mL/min was used throughout the measurements.

In the negative ion chemical ionization mode (GC/NICI-MS), the electron energy was set at 150 eV. The ion source temperature was set at 150 °C. Methane 4.5 was used as the reagent gas at ~6.5 Torr. Initial GC/NICI-MS-SIM experiments were carried out with m/z 79 and 81, corresponding with the bromide ion, as well as the three most abundant isotope peaks of the $[M]^-$-ion of hexabromobiphenyls (625.5, 627.5, 629.5). The detector voltage was set at 1200 V for Firemaster BP-6® and at 1600 V for bird egg samples. The SIM peak width was set at 0.7 u.

GC/NICI-MSMS experiments were carried out using identical parameters except for the ion source temperature which was lowered to 150 °C or 100 °C in order to increase the abundance of the molecular ion in quadrupole 1.

In the single reaction monitoring (SRM) mode, fragmentation of the most abundant isotope peak of the molecular ion ($[M+4]^-$) was used. Prior to these experiments, the respective mass was analyzed in 0.1 u-steps (SIM mode). Highest abundance was found for m/z 627.5 $[C_{12}H_4{}^{79}Br_3{}^{81}Br_3]^-$. In the GC/NICI/MSMS mode, m/z 627.5 was fragmented in quadrupole 2 (collision cell). For this reason, Argon 4.5 was used as collision gas at a collision cell pressure of ~1 mTorr. The collision voltage was set at 1 V. The detector was set at 2000 V and the SIM peak width was set at 3 u. In quadrupole 3, both m/z 79 and m/z 81 were recorded.

Confirmatory GC/EI-MS experiments were performed with an electron energy of 70 eV and a filament emission current of 150 µa. The source temperature was set at 200 °C. In the GC/EI-MSMS mode the collision cell pressure was set at ~1 mTorr. Fragmentation of the most abundant $[M]^+$-isotope signal at m/z 627.6 ($[C_{12}H_4{}^{79}Br_3{}^{81}Br_3]^+$) to the most abundant $[M\text{-}Br]^+$ isotope signal at m/z 546.7 ($[C_{12}H_4{}^{79}Br_3{}^{81}Br_2]^+$) - corresponding with a loss of ^{81}Br - was performed at 20 V collision voltage, the detector voltage was set at 2000 V. Other SRM transfers were studied as well but provided lower sensitivity in the GC/EI-MS mode. m/z 305.9, 307.9, 309.9, 465.7, 467.7, 469.7, 625.5, 627.5, and 629.5 were measured with 0.7 u SIM peak width, the detector voltage was set at 1600 V.

Results and Discussion

As reported elsewhere, a close-to-baseline separation of the PBB 149 atropisomers was achieved on a 20-m-β-TBDM-column at an elution temperature of 190 °C [3]. Nevertheless, a long run time of ~80-90 min was required and the elution range of the PBB 149 enantiomers covered ~7 min (**Figure 1a**). Consequently, the good enantiomer resolution obtained during an isothermal phase had to be paid with loss of separation efficiency from other compounds. Another drawback was the significantly higher background level on the CSP as compared with the nonchiral, GC/MS-dedicated Factor Four column (see below).

Although GC/NICI-MS-SIM using m/z 79 and 81 provided a good sensitivity for PBBs, the coelution of another brominated compounds in the bird egg sample prevented a direct determination of the enantioratio (ER) of PBB 149 (**Figure 1b**). The lack of this interferent compound in Firemaster BP-6® indicated that this organobromine compound was not arising from another PBB congener. Plausible candidates may be both BDEs or halogenated natural products which have also been detected in Norwegian eggs of birds [9]. For this reason we attempted to use GC/NICI-MSMS in the SRM mode for solving the problem.

Hexabromobiphenyls are classic compounds for demonstrating the enormous affinity to the formation of the bromide ion in GC/NICI-MS. Over 90% of the TIC in the full scan mode (m/z 30-700), arose from the fragment ions m/z 79 and m/z 81 (**Figure 2**). Next to the bromide ion, only traces of the molecular ion were found in the GC/NICI-MS spectra of PBB 153 and PBB 149.

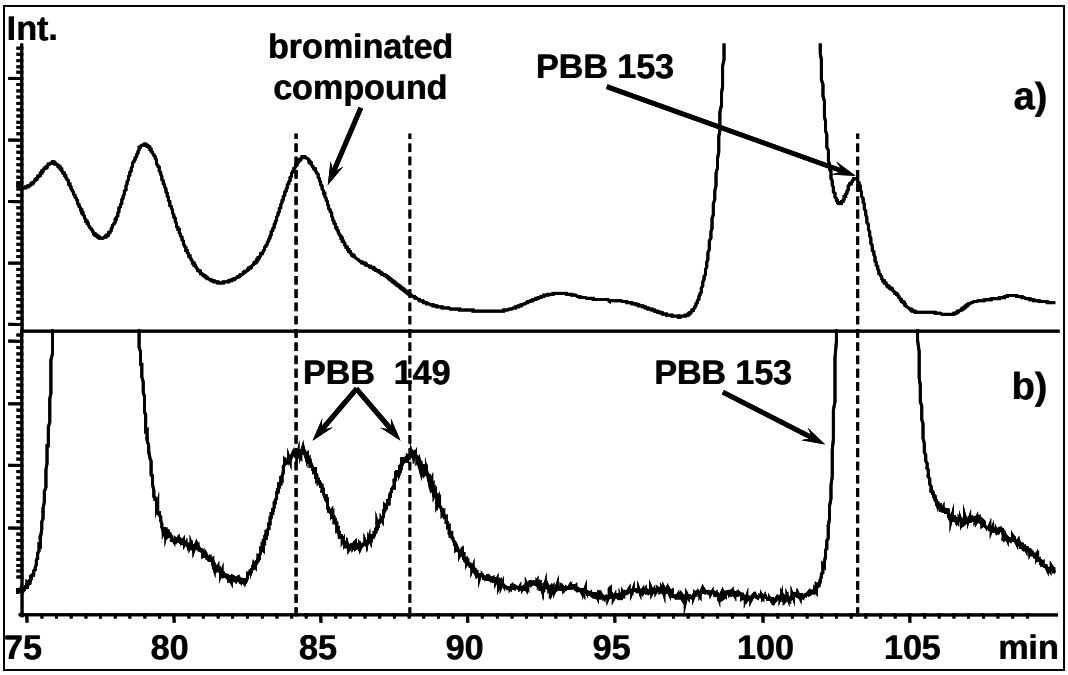

Figure 1: GC/NICI-MS-SIM chromatograms (m/z 79 and 81) of (a) the purified extract of a bird egg and (b) the technical product Firemaster BP-6®. Dotted lines refer to the retention times of PBB 149 enantiomers and PBB 153

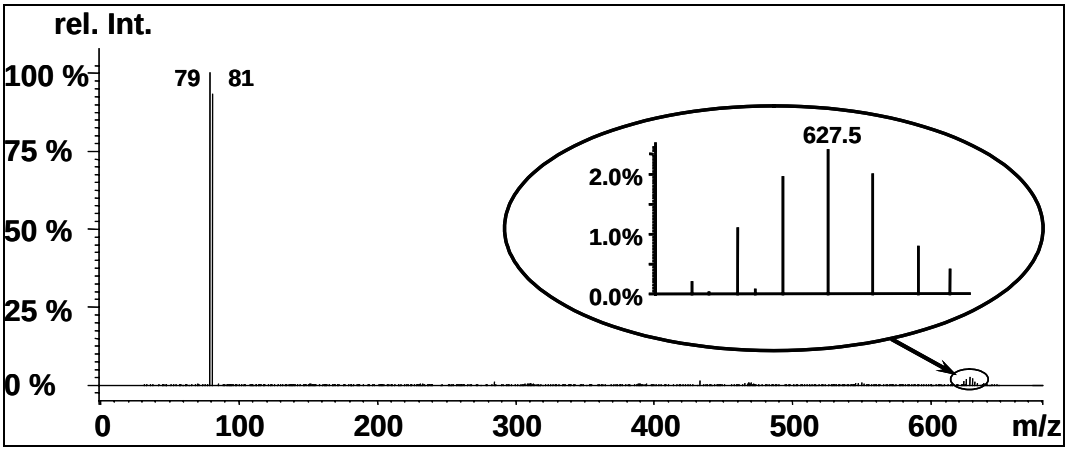

Figure 2: GC/NICI-MS full scan mass spectrum of PBB 153

With the information that the interfering compound is no hexabromobiphenyl (see above), GC/NICI-MSMS experiments should allow for a mass spectrometric separation of the interference from the enantiomers of PBB 149. Initial GC/NICI/MSMS experiments were performed in the product ions scan mode using the most abundant isotope peak of the molecular ion of hexabromobiphenyl (m/z 625.5) as precursor ion, and by monitoring of m/z 70 - 600 in quadrupole 3. Different collision voltages were successively tested, but the bromine ion (m/z 79 and 81) was the only product detected in any experiment. This is in agreement with the typical finding in GC/NICI-MSMS of halogenated compounds. Based on these experiments in the product ions scan mode, subsequent GC/NICI-MSMS investigations in SRM mode involved transitions m/z 625.5 → m/z 79 and m/z 625.5 → m/z 81. The best S/N-ratio was achieved with a collision voltage of 1 V. The ion source temperature also had a great impact on the intensity of the $[M]^-$-ions. When the ion source temperature was lowered from 200 °C to 150 °C and 100 °C, the quotient of the intensity of the $[M]^-$ to $[Br]^-$ ions was increased by factor ~4 and ~10 respectively. Therefore the samples of bird eggs were analysed using an ion source temperature of 100 °C (**Figure 3**).
Using GC/NICI-MSMS the interference found in by conventional GC/NICI-MS (**Figure 1a**) was no more visible (**Figure 3**). Furthermore, the abundant peak prior to PBB 153 detected by conventional GC/NICI-MS in the bird egg sample (**Figure 1a**) was not found in the GC/NICI-MSMS-SRM chromatogram (**Figure 3**). In contrast, the two peaks prior and shortly after the PBB 149 enantiomers are most likely hexabromobiphenyls as well. **Figure 3** also shows that the first eluting PBB 149 enantiomer was slightly depleted, corresponding with an ER of 0.63 ± 0.04 (n=3). Although the reproducibility of the ER in the sample was good, comparative measurements with a Firemaster BP-6® standard showed remarkable deviations from the excepted racemic ratio (ER = 1.05 ± 0.31; n=12). No explanations could be found for these variations from run to run. However, it should be noted that the concentration of PBB 149 in the Firemaster BP-6® standard was about ten fold higher than in the egg sample.

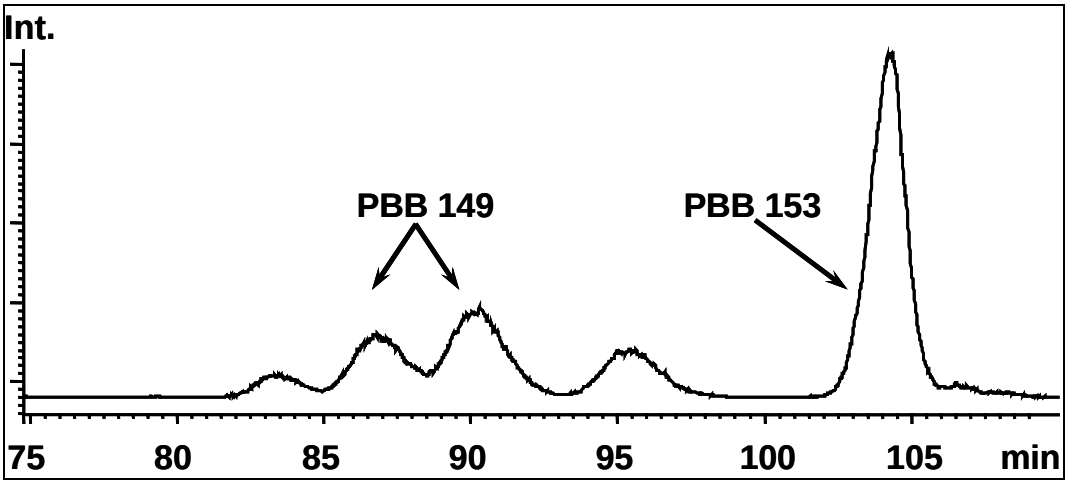

Figure 3: GC/NICI-MSMS-single reaction chromatogram (sum of m/z 625.5→m/z 79 and m/z 625.5→m/z 81) of a purified extract of a bird egg

This led to a more critical interpretation of the ER found in the bird eggs sample. However, additive GC/EI-MSMS measurements confirmed the non-racemic proportions of PBB 149 in the sample[6]. Additional investigations of the signal-to-noise ratio of PBB 149 and PBB 153 using GC/NICI-MSMS were performed with a non-chiral Factor Four column using Firemaster BP-6® as a reference standard. On the non-chiral Factor Four column, the S/N ratio was ~20 fold better than on the CSP column. In the technical product the S/N of PBB 149 was approximately 60 times lower than of PBB 153.
GC/NICI-MS-SIM provided a two fold higher S/N ratio than GC/NICI-MSMS-SRM. In addition, significant alterations in the peak intensities were observed run-by-run in GC/NICI-MSMS-SRM measurements. Since ER determinations are relative measurements, this would not impact the measured value. However, we cannot exclude that these variations did not occur within the same run (see variations in the ER of PBB 149 in Firemaster BP-6®).

Conclusions

The present investigation demonstrated that, under certain experimental conditions such as interference of the PBB enantiomers by another brominated compound, GC/NICI-MSMS-SRM may be a suitable tool for the elimination of the interference. Once stable conditions are obtained, a proper determination of enantioratios may be obtained. This method currently scarcely used in analytical chemistry[10].

References

1 de Boer J., de Boer K. and Boon J.P. (2000) Polybrominated biphenyls and diphenylethers; in: The Handbook of Environmental Chemistry, Vol 3, Part K, Paasivirta J. Ed., Springer Verlag.

2 Anonymous (2001), BFR 2001: The Second International Workshop on Brominated Flame Retardants, Stockholm, Sweden, May 14–16, 2001, Proceedings, The Swedish Environmental Protection Agency, 404.

3 Berger U., Vetter W., Götsch A. and Kallenborn R. (2002) Chromatographic enrichment and enantiomer separation of axially chiral polybrominated biphenyls in a technical mixture, J. Chromatogr. A 973, 123-133 .

4 Kallenborn R. and Hühnerfuss H. (2001) Chiral Environmental Pollutants, Springer Verlag.

5 Vetter W. (2001) Enantioselective fate of chiral chlorinated hydrocarbons and their metabolites in environmental samples. *Review*, Food Rev. Int. 71, 113-182.

6 Götsch A., Mariussen E., von der Recke R., Herzke D., Vetter W. and Berger U. (2004) Enantioselective separation of atropisomeric BB 132 and BB 149 in extracts from a Norwegian white-tailed sea eagle egg. BFR 2004: The Third International Workshop on Brominated Flame Retardants, Toronto, Ontario, Canada, June 6-9, 2004, Proceedings.

7 Herzke D., Berger U., Kallenborn R., Nygård T. and Vetter W.; Polybrominated diphenylethers and biphenyls and other organobromines in eggs from Norwegian birds of prey – Species specific considerations. Manuscript in preparation.

8 Krock B., Vetter W. and Luckas B. (1997) PCB/toxaphene group separation on silica prior to congener specific determination of toxaphene residues in fish and other samples by GC/ECD. Chemosphere 35, 1519-1530.

9 Herzke D., Berger U., Nygård T. and Vetter W. (2003) Organochlorines, organobromines and their metabolites in eggs of Norwegian birds of prey. Organohalogen Compds. 61, 466-469.

10 Watzer B., Seyberth H.W., Schweer H. (2002) Determination of misoprostol free acid in human breast milk and serum by gas chromatography/negative ion chemical ionization tandem mass spectrometry. Journal of Mass Spectrometry 37, 927-933.