

# THE POLYBROMINATED FLAME RETARDANTS USED IN OUR CHILDRENS' SOFT DRINKS

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## Introduction

Brominated flame retardants (BFRs) have kept environmental chemists busy over the last two decades. While the most prominent first-phase BFRs, PBBs and PBDEs, have partly been banned, next generation BFRs are currently more and more in the focus of research. A huge structural diversity of BFRs has been produced or patented. In this study, we report on a group of polybrominated compounds used since decades mainly in best-seller soft drinks from the United States.<sup>1</sup> These soft drinks contain the so-called brominated vegetable oil (BVO, a.k.a. brominated soy oil) which has also been patented for use as flame retardants.<sup>2</sup> In soft drinks, BVO are used as solubility-transmitter disperser for flavoring citrus oils and other lipophilic compounds.<sup>3</sup> Since conventional fats have a lower density than water, in which they are insoluble, they would separate from the aqueous phase. By the addition of bromine to unsaturated fatty acids, the gravity increases and can be adjusted by blends with etheric oils to the desired density of the drink. In the soft drinks, they form the required matrix to dissolve oils and food colors such as carotinoids. In this investigation we studied the structures of BVO and suggested a method which involved extraction of the soft drink samples, conversion of the fatty acids into fatty acid methyl esters which were analyzed by GC/MS. The use of this food additive is common in the USA since decades, while its use was recently discontinued in Japan.<sup>4</sup> In Germany, their use is prohibited, and the German versions of the soft drinks contain natural hydrocolloids as a BVO substitute. However, imported US products containing BVO have been repeatedly seen in special shops in Germany. The effects of BVO were also studied in the past. It was found that brominated fatty acids were stored in the lipids, but toxic effects could not be observed.<sup>5</sup> High levels of lipid-bound bromine were found particularly in children up to the age of 15, the levels in adults being very much lower.<sup>5</sup> On the other hand, a person who daily consumed 2-4 L BVO-containing soft drink suffered from severe bromism and his serum bromide was at 3180 mg/L (39.8 mmol/L). The toxic symptoms were headache, fatigue, ataxia, and memory loss which progressed over 30 days.<sup>6</sup> Rats fed with particularly high amounts died within a few days.<sup>5</sup>

## Material and methods

**Samples.** Different brands of canned soft drinks (330-591 mL) were imported from the USA or US-imports were bought in special shops in Stuttgart and Berlin (Germany). Comparative samples from Germany (without BVO) were analyzed as well.

**Sample Cleanup.** Soft drink samples (100 mL) were spiked with the internal standard 10,11-dichloroundecanoic acid, NaCl was added for saturation, and lipophilic ingredients were extracted with 3 x 30 mL diethyl ether. The organic phase was washed with (i) 15 mL 2 M NaOH, (ii) 15 mL 2 M HCl, and (iii) 3x 15 mL H<sub>2</sub>O. Then the organic phase was concentrated in a rotary evaporator. The residue was transesterified with sulphuric acid (80 °C, 1 h) according to Vetter and Wegner<sup>7</sup>, except for the use of a lower transesterification temperature in order to prevent degradation of highly brominated fatty acids.

**Synthesis of the methyl esters of 9,10-dibromostearic acid (Br<sub>2</sub>-18:0) and 9,10-dibromopalmitic acid (Br<sub>2</sub>-16:0).** Syntheses followed the pathway reported in Matissek et al.<sup>8</sup> except that toluene was used as replacement for chloroform. About 30 mg of oleic acid methyl ester or palmitoleic acid methyl ester was dissolved in 10 mL toluene, and 2 mL 0.1 M aqueous bromine-solution was added and stored in the dark for 30 min. Excess of bromine was reduced with 1 M sodium thiosulphate solution. The organic phase was separated and evaporated (70 mbar, 40 °C). The reaction was quantitative. Higher amounts of Br<sub>2</sub>-18:0 were produced by bromination of 1g oleic acid methyl ester dissolved in 50 mL toluene; 50 mL bromine-solution was added and the liquid was kept in the dark for 3 h. 174 mg pure product and ~1.3 g oily brown raw product was obtained.

**Bromination of  $\alpha$ -linolenic acid methyl ester.**  $\alpha$ -Linolenic acid methyl ester (13.7 mg) was dissolved in 10 mL toluene and 5 mL bromine solution was stirred overnight at 50 °C. Sample clean up was as shown above. The reaction led to a mixture of brominated fatty acids (see below).

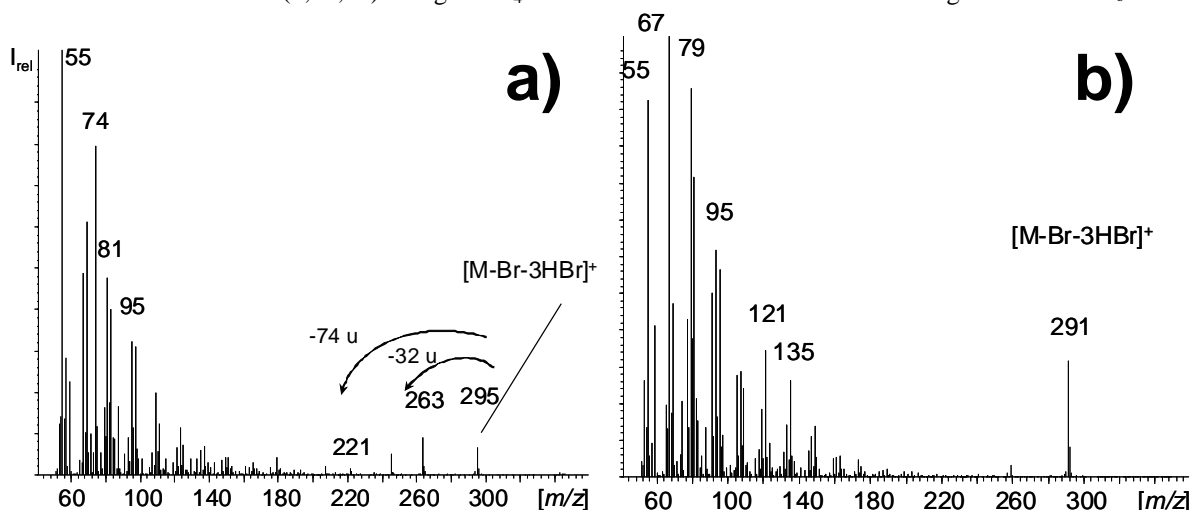
**Synthesis of the internal standard 10,11-diiodoundecanoic acid.** Iodination of 10,11-undecenoic acid was performed with 0.1 M ethanolic iodine solution. The cleanup was as shown above.

**GC/EI-MS.** An HP 5890 Series II GC/HP5972 mass selective detector was used in combination with an HP 7673 GC/SFC autosampler (Hewlett-Packard/Agilent, Waldbronn, Germany). A DB-5 column (30 m x 0.25 mm i.d. x 0.25  $\mu\text{m}$   $d_f$ ) was installed in the GC oven. The oven was programmed as follows: 60  $^{\circ}\text{C}$  (1 min), at 10  $^{\circ}\text{C}/\text{min}$  to 300  $^{\circ}\text{C}$  (10 min). Samples (1  $\mu\text{L}$ ) were splitless injected into an injector port heated at 250  $^{\circ}\text{C}$ . The transferline was set at 300  $^{\circ}\text{C}$ . The carrier gas helium (5.0) was transported in constant flow mode (1.0 mL/min). In the full scan mode,  $m/z$  50 – 350 was recorded. In the selected ion monitoring (SIM) mode, we recorded  $m/z$  287, 289, 291, 291, 293, 295, and 297 for methyl esters of brominated fatty acids and  $m/z$  199 for the IS 10,11-diiodoundecanoic acid.

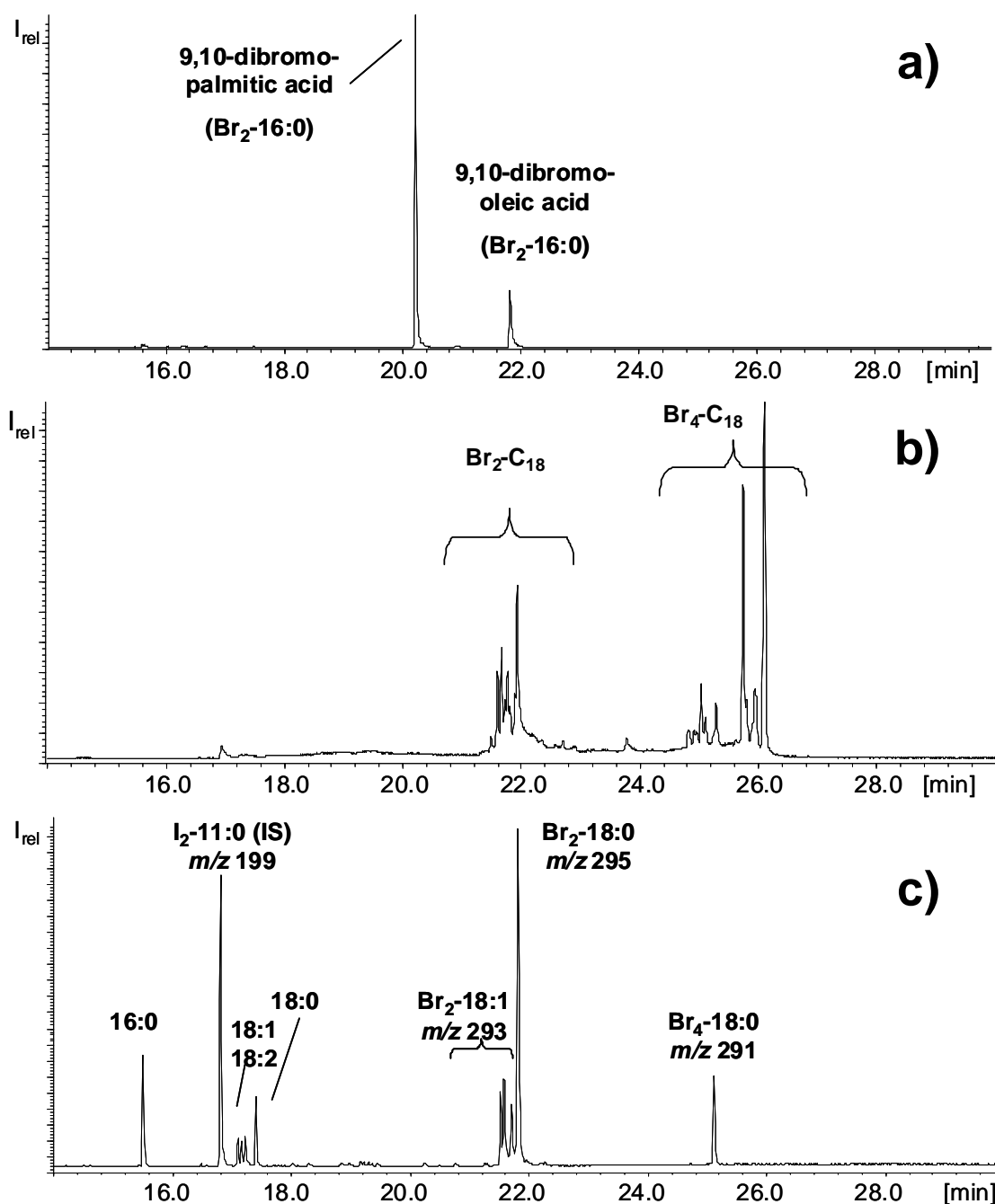
**GC/ECNI-MS.** Analyses were performed with an Agilent 7890A/5975C system equipped with a 7693A Automatic Liquid sampler and a PTV inlet. Injections (1  $\mu\text{L}$ ) were made in the pulsed splitless mode by using the following program: 80  $^{\circ}\text{C}$  for 0.01 min, then at 500  $^{\circ}\text{C}/\text{min}$  to 300  $^{\circ}\text{C}$  for 2 min, and at 10  $^{\circ}\text{C}/\text{min}$  to 260  $^{\circ}\text{C}$ . An injection pulse pressure of 25 psi was used in the first minute. Helium (5.0 quality) was used as carrier gas at a constant flow of 1.2 mL. A ZB-5MS 30 m x 0.25 mm i.d. x 0.25  $\mu\text{m}$   $d_f$  (Phenomenex) was installed in the GC oven which was heated with the following program: 50  $^{\circ}\text{C}$  for 1 min then 10  $^{\circ}\text{C}/\text{min}$  to 300  $^{\circ}\text{C}$  for 15 min (run time 41 min). The transfer line, ion source, and quadrupole temperatures were set at 300  $^{\circ}\text{C}$ , 150  $^{\circ}\text{C}$ , and 150  $^{\circ}\text{C}$ , respectively. Methane (5.5) was used as reagent gas with a flow of 2.0 mL/min. After a solvent delay of 8 min,  $m/z$  50-800 was recorded at 2 scans/min.

## Results and Discussion

**GC/EI-MS spectra of BVO.** Brominated fatty acids did not show the molecular ion. Instead,  $[\text{M}-159]^+$  ( $m/z$  295) was obtained for dibromostearic acid methyl ester ( $\text{Br}_2$ -18:0) (**Figure 1a**). This corresponds with the molecular ion of methyl oleate shifted by 1 u to lower mass. Aside from this, the mass spectrum was virtually identical with that of methyl oleate. For instance it featured the typical fragmentation of monoenoic fatty acid methyl esters, i.e.  $[\text{M}-\text{Br}-\text{HBr}-32]^+$ ,  $[\text{M}-\text{Br}-\text{HBr}-74]^+$ , and in the low mass range the characteristic McLafferty ion  $m/z$  74, accompanied with further abundant fragment ions.<sup>9,10</sup> The fragment ions characteristic for  $\text{Br}_2$ -18:0 were  $m/z$  295,  $m/z$  263, and  $m/z$  221, and  $m/z$  74. Likewise the corresponding fragment ions of dibromopalmitic acid methyl ester ( $\text{Br}_2$ -16:0) were  $m/z$  267,  $m/z$  235,  $m/z$  193, and  $m/z$  74. GC/EI mass spectra of saturated tetrabrominated fatty acids were characterized by ions corresponding with  $[\text{M}-\text{Br}-3\text{HBr}]^+$  while  $\text{M}^+$  was not detected. The corresponding high mass ion of  $\text{Br}_4$ -18:0 is found at  $m/z$  291 (**Figure 1b**). The MS formally agrees with the methyl esters of 18:3 isomers (no abundant McLafferty ion,  $m/z$  79 >  $m/z$  81)<sup>9</sup>. Likewise, the tetrabromination of 18:3(9,12,15) will give  $\text{Br}_4$ -18:1 isomers with the characteristic fragment ion at  $m/z$  289.



**Figure 1: GC/EI-MS of the methyl esters of (a) dibromostearic acid and (b) tetrabromostearic acid**

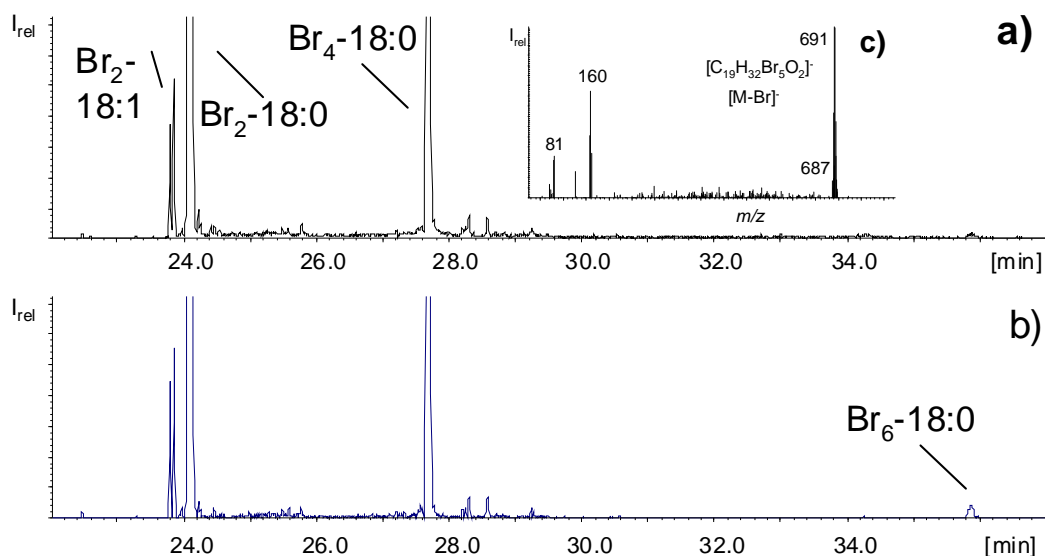


**Figure 2: GC/EI-MS full scan chromatograms of synthesized (a) 9,10-dibromopalmitic acid methyl ester and 9,10-dibromostearic acid methyl ester (mixture) and (b) a sample of the bromination of  $\alpha$ -linolenic acid methyl ester, and (c) a GC/EI-MS-SIM-chromatogram of a transesterified sample extracted from a soft drink**

$Br_2-18:1$  isomers originating from the incomplete bromination of linoleic acid will show  $m/z$  293 whereas  $Br_6-18:0$  from 18:3(9,12,15) methyl ester will produce  $m/z$  287. Since vegetable oils contain almost exclusively unsaturated  $C_{18}$  homologues screening of the values mentioned above in SIM-mode is recommended for the determination of BVO.

The synthesized brominated fatty acid methyl esters were used to study the retention range of polybrominated fatty acids. Bromination of  $\alpha$ -linolenic acid led to the formation of different dibromo- and tetrabromo homologues (Figure 2b). The range detected agreed with that observed in soft drink samples after transmethylation. Noteworthy, the soft drink samples still contained unsaturated fatty acid methyl esters (18:1, 18:2, and 18:3) (see minor peaks in Figure 2c). The internal standard I<sub>2</sub>-11:0 methyl ester (Figure 2c) was added before the extraction.

**GC/ECNI-MS analysis of BVO.** After these assessments, the soft drink samples were analyzed in the negative ion mode which was about two orders of magnitude more sensitive for BVO than GC/EI-MS. GC/ECNI-MS spectra of brominated fatty acid methyl esters were characterized by intense Br<sup>-</sup> ( $m/z$  79, 81) and Br<sub>2</sub><sup>-</sup> ( $m/z$  158, 160, 162) fragment ions in the low mass range. The molecular ion was not detected but the [M-Br]<sup>-</sup> fragment ion was abundant in higher brominated fatty acid methyl esters, i.e.  $m/z$  531 (Br<sub>4</sub>-18:0) and  $m/z$  687 (Br<sub>6</sub>-18:0). The intensity of the [M-Br]<sup>-</sup> fragment ion increased with increasing number of bromine. The sample contained traces of the latter (Figure 3a-c) which were detected only by GC/ECNI-MS. Semi-quantitative data allow determining that the concentration of BVO in the soft drinks was ~2 mg/100 mL.



**Figure 3: GC/ECNI-MS ion chromatograms isolated from the full scan run of an extract of the brominated fatty acid methyl esters extracted from a soft drink sample. (a)  $m/z$  79, (b)  $m/z$  160 and (c) full scan GC/ECNI-MS spectrum of hexabromostearic acid methyl ester (Br<sub>6</sub>-18:0)**

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