Separation of chlorinated and brominated persistent organic pollutants by pyrenyl-silica liquid chromatography

Belen Gomara[1], Carmen García-Ruiz[2], Maria Luisa Marina[2], Maria Jose Gonzalez[3]

[1]CSIC
[2]Alcala University
[3]Csic (iqog)

Introduction

Polybrominated diphenylethers (PBDEs) and polibrominated byphenils (PBBs) are two group of ubiquitous pollutants that are present in environmental samples due to their use as brominated flame retardants (BFR) to decrease the likelihood and intensity of fire in electronic components, plastics, clothing and a number of other commercial products[1]. Nowadays, there is a growing interest, mainly, in PBDEs analysis in environmental and food samples due to the marked increase in the levels of these compounds detected during the last decades[2]. In 2003 the European Community (EC) introduced a new regulation to control the presence of PBDEs in the environment[3], with the intention of introducing new regulations similar to those existing for PCDD/Fs and PCBs for foodstuffs[4]. These directives involve routine monitoring calling for adaptation of analytical methods in order to provide adequate sensitivity and selectivity to allow the unambiguous determination of PBDEs in complex matrices.

The analytical procedure is in most cases based on established methods for similar compounds such as polychlorinated byphenils (PCBs) and polichlorobibenzo-*p*-dioxins and furans (PCDD/Fs). The final clean up step of these methods is carried out to obtain three fractions (i) PCDD/Fs, (ii) planar (dioxin-like) PCBs and (iii) bulk of PCBs plus PBDEs and PBBs. While the first two fractions (PCDD/Fs and planar PCBs) are generally analysed by gas chromatography coupled to high resolution mass spectrometry (GC/HRMS)[5], the latter can be analysed either by gas chromatography with electron capture detection (GC/ECD)[6] or GC/MS[7]. The main limitation of both instrumental systems is GC coelutions among congeners of the three families (PCBs, PBDEs and PBBs). These coelutions may cause identification problems (in the case of ECD detection) and interference problems, increasing the background noise and detection limits (in the case of MS detection) which make very difficult to analyse the BFR, usually found a much lower concentration than PCBs in real samples. These problems can be solved if PCBs are separated from BFR compounds in an additional clean-up step. Different approaches have been proposed to solve coelution problems using open liquid chromatography columns with silica gel[8] and alumina[9], but any of them were completely satisfactory.

In this work, the separation of PCBs from BFR (PBDEs and PBBs) using HPLC, 2-(1-pyrenyl) ethyldimethylsilylated silica column (PYE), is presented. The different parameters investigated, affecting the HPLC separation, are showed. Finally, the feasibility of a complete methodology, including the HPLC (PYE) final fractionation step and GC/MS determination, for food samples analysis is evaluated.

Materials and Methods

HPLC grade solvents were employed for the preparation of HPLC mobile phases: n-hexane and n-heptane from Scharlau (Barcelona, Spain), toluene and isooctane from SDS (Peypin, France). Pestipur quality acetone and toluene from SDS (Peypin, France) and hexane from Merck (Darmstadt, Germany) were used for sample preparation[6,10].

Commercial mixture Firemaster BP-6 from Promochem (Barcelona, Spain) was used. Its major components are PBBs # 101, 118, 138, 149, 153, 167, and 180[11]. It was dissolved in hexane and a working solution containing 500 mg/L of the total PBBs congeners present in the mixture was prepared. Two working stock solutions from commercial mixtures were prepared in hexane containing 1 mg/L of each congener for the PCBs # 28, 52, 101, 138, 153, 170, 180 and 194 supplied by Dr. Ehrenstorfer (Augsburg, Germany) and for the PBDEs # 17, 28, 47, 66, 85, 99, 100, 153, 154 and 183 acquired from Wellington Laboratories (Ontario, Canada), because they are the most abundant in food samples[1,12]. All congeners were named according to IUPAC numbers[13].

A HP-1100 Series system from Hewlett-Packard (Waldbronn, Germany) equipped with an autosampler and variable wavelength UV-detector was used. Instrument control and data acquisition were performed with the HP-1100 Series ChemStation software. A Cosmosil 5-PYE column (2-(1-pyrenyl) ethyldimethylsilylated silica gel, 250 × 4.6 mm i.d., particle size 5 µm, from Nacalai Tesque, Promochem GmbH) and UV-absorption detection at 225 nm were employed.

Results and Discussion

In a previous paper[14], the 5-PYE column was used for the simultaneous separation of coplanar and chiral PCBs using hexane as mobile phase at 0.5 ml/min and 25 ºC for column temperature. Since these conditions were not appropriate for the separation of PCBs from PBDEs and PBBs, different chromatographic conditions were studied. Firstly, toluene was added to hexane in order to increase the elution power of the mobile phase and to decrease the retention time of the most retained congeners of each family. Figure 1 shows the chromatograms corresponding to PCBs, PBDEs, and PBBs congeners for a mobile phase of hexane without toluene (a) and with a 2% (v/v) toluene (b). It can be observed that the addition of toluene decreased the retention times of the studied compounds, especially for the latter eluting congeners of each family. Although higher percentages of toluene would be necessary to obtain a higher retention of the latter eluting congeners, it was not possible to increase that percentage because there was an important decrease of sensitivity (signal decreased approximately six times due to the UV-absorption of toluene in the UV region). Secondly, 2 % (v/v) toluene in heptane and isooctane was tested. When isooctane:toluene (98:2, v/v) was used as mobile phase (Figure 2a), retention times for the PBDEs and PBBs congeners were longer than for the PCBs congeners favouring the separation between the latter eluting PCBs congeners and the first eluting PBDEs and PBBs congeners (comparison of Figure 2a and Figure 1b show this results). The latter chromatograms were recorded at a flow rate of 1 mL/min instead of 0.5 mL/min to decrease the analysis time. Under these chromatographic conditions, different column temperatures were investigated. Figure 2 shows the chromatograms corresponding to PCBs, PBDEs, and PBBs congeners at two different column temperatures: 25 ºC (a) and 45ºC (b). A better separation between the latter eluting congeners of PCBs and the first eluting congeners of PBDEs and PBBs was

achieving selecting 45 ºC as separation temperature. Temperatures higher than 45 ºC were not tested because the maximum operating temperature for 5-PYE column is 50 ºC.

The selected chromatographic conditions were used for the separation of selected PCBs congeners from PBDEs and PBBs congeners in a mixture of standard solutions at different concentrations. Finally, the HPLC (PYE) fractionation step was included in a classical methodology for PCBs and BFR analysis in different food samples to test its performance in real samples.

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Figure 1. Chromatograms corresponding to hexane standard solutions of PCBs congeners (~1 mg/L each), PBDEs congeners (~1 mg/L each), and PBBs congeners (~500 mg/L total) eluted with (a) hexane and (b) hexane:toluene (98:2, v/v). Chromatographic conditions: temperature 25 ºC; flow-rate, 0.5 mL/min and UV-detection at 225 nm.

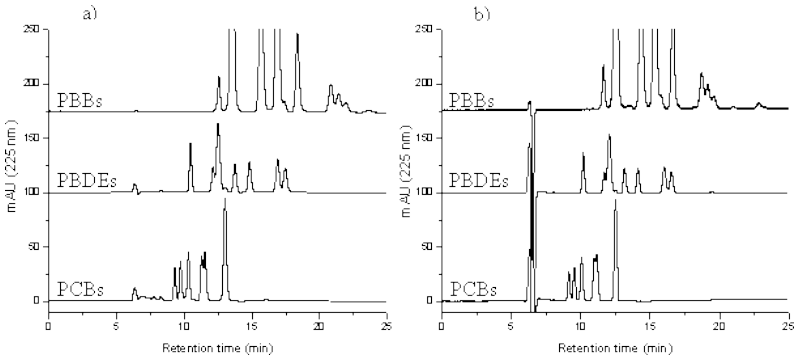

Figure 2. Chromatograms corresponding to hexane standard solutions of PCBs congeners (~1 mg/L each), PBDEs congeners (~1 mg/L each), and PBBs congeners (~500 mg/L total) eluted at (a) 25ºC and (b) 45ºC. Chromatographi

conditions: mobile phase, isooctane:toluene (98:2, v/v); flow-rate, 1.0 mL/min and UV-detection at 225 nm.