Comparison of Atmospheric Pressure Gas Chromatography-Tandem Mass Spectrometry (APGC-MS/MS) and high resolution mass spectrometry for the Analysis of Polybrominated Diphenyl ethers (PBDEs)

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Introduction

Persistent organic pollutants (POPs) have been identified as a long term threat to the environment all over the world because of their bioaccumulation, wide distribution and potential toxicity. Aiming at the global restriction of the manufacture and use of POPs to protect human health and the environment, the Stockholm Convention was implemented in 2001. Tetra-BDE and penta-BDE have been be listed as an additional global POP under the Stockholm Convention in 2009 [1].Because toxic effects have been observed for some brominated flame retardants (BFRs), in particular polybrominated diphenylethers (PBDE) [2].

Currently the most sensitive and selective analytical methods for BDEs monitoring traditionally used are gas chromatography coupled to electron capture detector (GC/ECD) and more recently gas chromatography coupled to mass spectrometry (GC/MS) instrumentation. High resolution GC/high resolution MS (HRGC/HRMS) has been used for the analysis of POPs in samples with low levels or limited material available for analysis [3], and is considered as the reference method for several POPs. Using high resolution GC/MS provide reliable results at sub-parts-per-trillion (ppt) levels.

Atmospheric pressure chemical ionization (APCI) was initially developed in the seventies [4], and may, through recent developments be an alternative to both EI and CI ionization. APCI is a soft (low-energy) ionization technique in the gas phase using a corona needle for ionization. APCI often generates only molecular or quasi-molecular ions, commonly used in LC–MS/MS. It has been described as an alternative source for GC–MS and a way to couple GC to mass spectrometers initially developed for LC–MS [5]. Since 2009 several applications using GC-(APCI) TOF MS instrumentation have been published, including pharmaceutical development [6] and metabolic profiling [7]. More recently, developments in APCI or atmospheric pressure gas chromatography (APGC) has resulted in a very sensitive technique not only for the analysis of polycyclic aromatic hydrocarbon (PAHs), nitrogen-heterocyclic polyaromatic hydrocarbons (NPAHs) [8] and petroleum biomarkers [9], but also for brominated compounds [10] pesticides [11] and dioxins [12].

Materials and methods

Samples

Unhatched (dead) eggs from osprey (*Pandion haliaetus*) were collected in early July 2013 within a monitoring project that started in 1970. The content of each egg was homogenized and approximately 3 grams was subsampled for chemical analysis while the rest is stored frozen in the Swedish Environmental Specimen Bank, at Swedish Museum of Natural History, for future studies. Egg shell remains was saved as well for studies on egg shell thickness.

Chemicals

All organic solvents (methanol, ethanol, tetradecane, dichloromethane, n-hexane, and toluene) were of HPLC grade purity and obtained from Fluka (Steinheim, Germany). Anhydrous sodium sulfate (Na_2SO_4) and silica gel (60 Å, 70–230 mesh) were obtained from Fluka (Steinheim, Germany). Sulfuric acid (H_2SO_4) was purchased from Merck (Darmstadt, Germany). Potassium hydroxide (KOH) of reagent grade pellets (Ph, Eur) was obtained from Scharlau (Barcelona, Spain).

Nine PBDE were analytes of interest for the present study was analyzed. A PBDE solution containing BDE#28, #47, #66, #85, #99, #100, #138 #153, #154; and ^{13}C-labeled congeners BDE#77 and #139 used as internal

standard.; As recovery standard ^{13}C-labled PCB #178 was used at a concentration of 30 pg µL^{-1}. All compounds purchased from Wellington Laboratories (Guelph, Ontario, Canada). All standard solutions were prepared in toluene and kept in a fridge at 8 ℃.

Extraction and clean up

A laboratory blank and QC subsample of homogenized fish were analyzed in parallell with the eggs in each set of extraction and clean up. Eggs were thawed at room temperature and grounded with Na_2SO_4 in the ratio 1 to 10. Open column chromatography was applied for approximately 0.5 gram of egg. Briefly, the homogenate was spiked with ^{13}C-labelled internal standards prior to the lipid fraction extraction by a mixture of *n*-Hexane: dichloromethane (1: 1) on open column chromatography. The multilayer silica column was composed of KOH silica, neutral activated silica, 40% H_2SO_4 silica gel, 20% H_2SO_4 silica gel, neutral activated silica gel and Na_2SO_4. 50 mL of *n*-Hexane was used for rinsing and eluting the columns and samples later on. The final extracts were spiked with 25 µL of $^{13}C_{12}$-labelled injection standards and concentrated to ~25 µL.

APGC-MS/MS

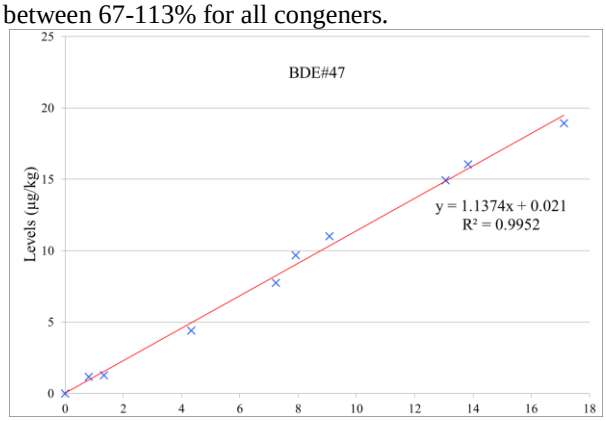
An Agilent 7890A gas chromatograph (GC) system (Palo Alto, USA) was coupled to a tandem quadrupole MS, Xevo TQ-S (Waters Corporation, UK), equipped with an APGC ion source. GC separation was achieved using a fused silica Rtx®-1614 capillary column, 15 m×0.25 mm i.d., film thickness 0.1 µm (Restek Corporation, PA, USA). The oven temperature was as follows: 80 ℃ (1 min); 20 ℃/ min to 250 ℃; 1.5 ℃/min to 260 ℃ (2 min); 25 ℃/min to 325 ℃ (2 min). Splitless injections of 1 µL of the final extract in tetradecane using a single Sky® 4.0 mm id cyclo double taper inlet liner from Restek, were carried out at 280 ℃. Helium was used as carrier gas at a constant flow rate of 1.0 mL/min. The GC interface temperature was set to 310 ℃ using N_2 as the make-up gas at 370 mL/min. The cone gas (N_2) was set at 170 L/hr, and the auxiliary gas (N_2) at 200 L/hr. The APCI corona needle was operated in the constant current mode at 1.5 µA. To reduce protonation which competes with charge transfer ionization, the source was kept dry at 150 ℃ without peak tubing. Two multiple reaction monitoring (MRM) transitions were measured for all compounds using argon as the reactant gas with collision energy of 20 to 30 eV depending on the compound.

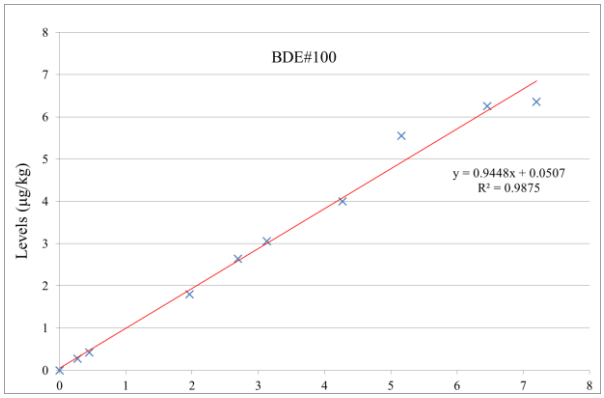
HRGC/HRMS

HRGC/HRMS analysis were performed on a Micromass Autospec Ultima (Waters, Milford, MA, USA) coupled with Agilent 6890N gas chromatography (Agilent Technologies, Atlanta, GA, USA) operating at >10000 resolving power using EI ionization at 35 eV. Measurements were performed in the selective ion recording (SIR) mode, monitoring the two most abundant ions of the molecular bromine or chlorine cluster. GC separation was achieved using a fused silica DB-5 capillary column, 30 m×0.25 mm i.d., film thickness 0.25 µm (SGE Analytical Science, Victoria, AUS). The oven temperature was as follows: 180 ℃ (2 min); 3.5 ℃/ min to 260 ℃; 6.5 ℃/min to 300 ℃ (4 min). Splitless injections of 1 µL were carried out at 275 ℃.

Results and discussion

With the exception of for BDE #85 and # 138 from the nine target BDEs BDE' concentration levels in the eggs of osprey were obtained and compared between APGC-MS/MS and HRGC/HRMS. The recovery varied between 67-113% for all congeners.





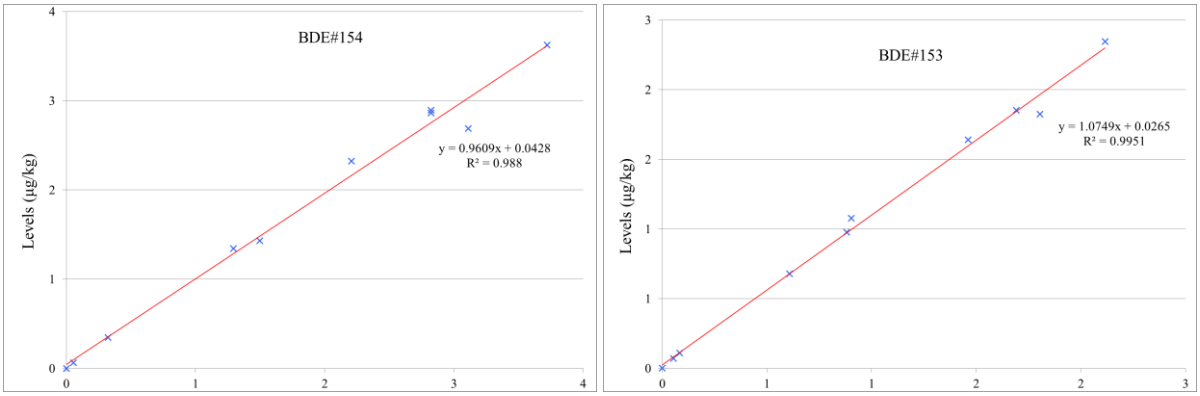


Figure 1. Comparison on PBDEs concentrations analyzed by APGC-MS/MS and HRGC/HRMS

Figure 1 displays the linear fits of the four detected compounds between APGC-MS/MS and HRGC/HRMS. As can be seen in Figure 1, the results showed very good agreement. Figure 2 illustrates typical overlay chromatograms of the native compounds in a standard.

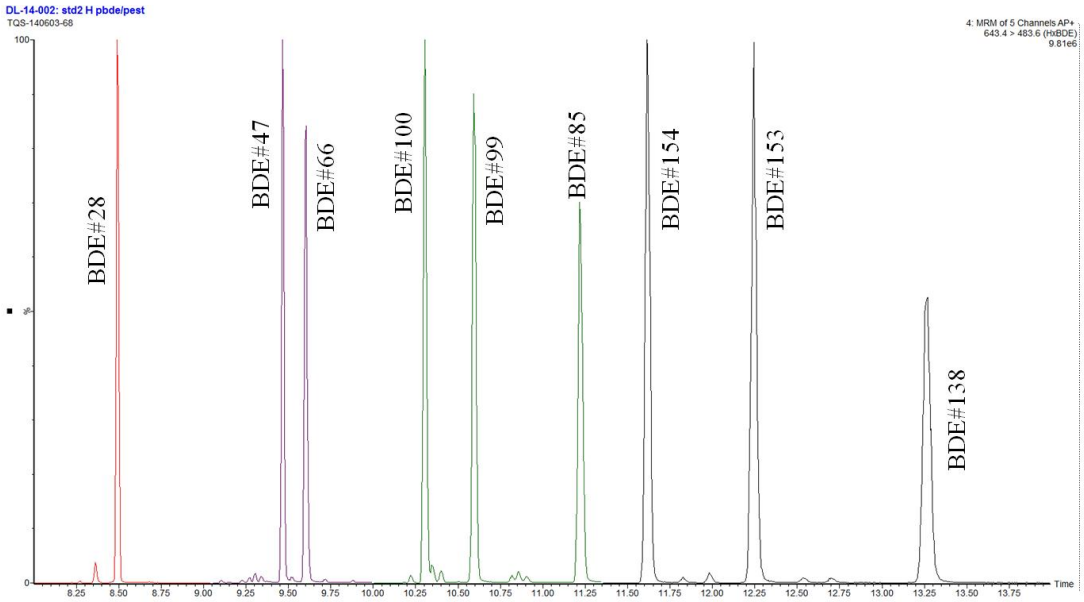


Figure 2. Combined chromatograms of the native compounds in the standard on APGC-MS/MS

As can be seen from Figure 1 and 2 the analysis of BDEs with APGC and high resolution GC/MS were comparable. Also the limit of detection was very similar, although for example S/N value of MS/MS data and high resolution MS data are difficult to compare.

Especially for compounds showing good response in NCI, the APGC showed to be a very good alternative to high resolution GC/MS.

Conclusion

The application of APGC-MS/MS method provided comparable results as HRGC/HRMS. It is a promising technique for the targeted analysis of different classes of groups of compounds, best used for compounds usually requiring the usage of chemical ionization.

Acknowledgements
This work is supported in part by the scholarship from China Scholarship Council (CSC) under the Grant CSC No. 201206400003; by the project „Employment of Best Young Scientists for International Cooperation Empowerment"(CZ.1.07/2.3.00/30.0037) co-financed from European Social Fund and the state budget of the Czech Republic.

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