# PULSED LARGE VOLUME INJECTION GC-MS IN ELECRON CAPTURE NEGATIVE IONIZATION MODE UTILIZING ISOTOPIC DILUTION FOR SIMULTANEOUS DETERMINATION OF PBDEs, PBBs, PCBs AND OCPs

Zhao Yuli[1)], Yang Limin[1)] and Wang Qiuquan* [1), 2)]

[1)] Department of Chemistry & the MOE Key Laboratory of Modern Analytical Sciences, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China

[2)] State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China

## Introduction

Over the past few decades, analytical method for measuring halogenated persistent organic pollutants (H-POPs), such as polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), polychlorinated biphenyls (PCBs) and organochlorinated pesticides (OCPs), have been rapidly developed primarily based on gas chromatography (GC) coupled with mass spectrometry (MS). Major problems in the determination of the H-POPs in environmental and biological samples are intra- and/or interferences caused by the numerous congeners of similar physicochemical properties in each and/or different classes of H-POPs, as well as the interferences caused by complex matrices. Moreover, the H-POPs often occur in the environment at extremely low level. Thus, analytical methods with both high selectivity and high sensitivity are demanded. This study aimed to develop a selective and sensitive method of pulsed large volume injection GC coupled with electron-capture negative ionization quadrupole MS (*p*LVI-GC/ECNI-*q*MS) for simultaneous determination of 28 typical H-POPs, including 5 OCPs, 10 PCBs, 1 PBB and 12 PBDEs, using isotopic dilution of $^{13}$C-labelled and perdeuterated analogues[1].

## Material and methods

The target H-POPs are 5 OCPs including α, β, γ, δ-hexachlorocyclohexane isomers (HCHs) and hexachlorobenzene (HCB), 10 PCBs (PCB-28, 52, 101, 105, 118, 138, 153, 163, 180 and 209), 1 PBB (PBB-153) and 12 PBDEs (BDE-28, 47, 49, 66, 85, 99, 100, 138, 153, 154, 183 and 209). The internal standards for quantification include $^{13}C_6$-HCB, $D_6$-α-HCH, $^{13}C_{12}$-PCB-28, 52, 101, 138, 153, 180 and $^{13}C_{12}$-BDE-77, 126, 209. All native and $^{13}$C-labeled standards of PBDEs, PBB, PCBs and HCB in nonane or isooctane were purchased from Cambridge Isotope Laboratories (CIL; Andover, MA); neat HCH standards were purchased from AccuStandard (New Haven, CT); while the $D_6$-α-HCH standard in cyclohexane was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All GC/MS analyses were performed on a GCMS-QP2010 (Shimadzu, Japan). A DB-17MS capillary column of 30 m length × 0.25 mm I.D. × 0.25 μm film thickness (J&W Scientific, Folsom, CA) was used to analyze all the H-POPs except for BDE-209, which was separately analyzed on a shorter DB-XLB column of 15 m length × 0.25 mm I.D. × 0.25 μm film thickness (J&W Scientific, Folsom, CA) in order to reduce possible thermal degradation.

A standard reference material (SRM 1946) of Lake Superior fish tissue was purchased from NIST (Gaithersburg, MD) for method validation. The fish tissue was analyzed in two batches at an interval of four

months, each comprises 5 replicate samples, a method blank and a spiked method blank sample (method blank sample spiked with the corresponding standards at a concentration of about 5-10 times the signal to noise ratio). Each aliquot of 0.5 g (wet weight) fish tissue was mixed with 15 g $Na_2SO_4$ and wrapped into a clean filter paper. After spiking with the internal standards, the samples were subjected to Soxhlet extraction with a dichloromethane/hexane (V/V=1/1) mixture for 24 h, throughout which the container was wrapped with aluminum foil to minimize possible photodegradation of the analytes, especially in BDE-209. The resultant extract was reduced to 15 mL using a rotary evaporator, and further to approximately 1 mL using $N_2$ stream, followed by solvent exchange into 5 mL hexane. Approximately 5 mL concentrated $H_2SO_4$ was then added into the extract and was shaken for 5 min to destroy most of the lipids. After centrifugation, the upper hexane phase was decanted and then concentrated to 1 mL using $N_2$ stream. The extract was finally loaded onto an alumina column (1.0 cm I.D. × 30 cm length) packed with 10 g deactivated neutral alumina for further cleanup. The first eluate of 10 mL hexane was discarded, while the second fraction of 30 mL dichloromethane/hexane (V/V=1/1) solution was collected, and then concentrated under a $N_2$ stream, finally solvent exchanged into 0.5 mL hexane followed by GC-MS analysis.

## Results and discussion

### *ECNI-qMS with monitoring characteristic ions for determining the H-POPs*

Based on the thorough comparison of three ionization modes of electron impact, positive chemical ionization and electron-capture negative ionization (ECNI) in their selectivity and sensitivity for the H-POPs, the ECNI-qMS with monitoring the characteristic ions was proposed. When analyzing a real sample, selectivity predominates over sensitivity, in terms of many possible chlorinated and/or brominated chromatographic interferences have been reported including the naturally-occurring compounds that vary from matrix to matrix and sometimes are more abundant than the analytes[2, 3], as well as from non-targeted coexisting anthropogenic compounds or between the analytes[4]. GC/ECNI-*q*MS with monitoring the characteristic ions provided more structural information and thus higher selectivity regardless of the 1-200 times less sensitive for the majority of the H-POPs studied compared with the GC/ECNI-*q*MS with monitoring the base peak ions. Besides, monitoring the characteristic ions of $[M\text{-}HBr]^-$ for the PBDEs, $[M]^-$ for the PBB and $[M\text{-}Cl_x]^-$ for the low-chlorinated PCBs but not their base peak ions of $^{78.9/80.9}Br^-$ and $^{35.0/37.0}Cl^-$ allowed using $^{13}C$-labelled analogues as their internal standards for quantification, which ensure more reliability of the quantification results. However, the lost sensitivity could be compensated by introducing a larger volume of samples into the analyzing system.

### *Pulsed large volume injection GC*

Pulsed large volume injection (*p*LVI) was carried out by pulsed injections (12 s per injection) with the autosampler and a programme temperature vaporizer (PTV) operated in solvent vent mode. The maximum allowable sample introduction volume (employing hexane as solvent) for *p*LVI was initially determined by injecting the same amounts of the H-POPs in different volumes, followed by optimizing a number of PTV parameters, such as sample volume per injection, split ratio, initial and final PTV temperature as well as column inlet pressure during the sample transfer. The optimum PTV parameters were determined based on the

requirement that, for each analyte, the mean injection efficiency and repeatability of 11 successive injections must be comparable to those of 1-μL splitless injection, and that the decomposition of thermally labile components was insignificant. As illustrated in Figure 1, 120 μL was finally introduced onto the column by 6 pulsed injections of 20 μL each at an initial temperature of 75 °C with the split valve open; between adjacent injections, the hexane was evaporated selectively and eliminated via the split vent at the split ratio of 200:1, while the analytes were retained on the packing material (8 mg silanized quartz wool) in the liner (straight tube design, 1.2 mm I.D. × 9.5 cm length, deactivated using dimethyldichlorosilane) by cold trapping. After sample injection and solvent evaporation, the split valve was closed (split ratio = 0:1) and the PTV rapidly heated up to the final temperature of 280 °C for transferring the analytes under a pulsed high pressure of 200 kPa. Once the analyte transfer was finished, the split valve was opened at the split ratio of 10:1 to vent the non-volatile matrix components.

***Performance of the pLVI-GC/ECNI-qMS***

Baseline separation was achieved by using a DB-17MS column within 60 min divided into 20 retention time windows for all the H-POPs except for the partial overlap between BDE-154 and PBB-153, although many chromatographically co-eluted interferences have been previously reported when using a usual column of 5%-diphenyl-95%-dimethylsiloxane, typically including the interferences between BDE-28 and PCB-118, BDE-47and PCB-180, BDE-154 and PBB-153, PCB-163 and PCB-138 [5]. Chromatographic interference between PBB-153 and BDE-154 was solved by monitoring their different characteristic ions at m/z = 627.5 for PBB-153 and m/z = 561.6 for BDE-154. By using *p*LVI, the sample amount finally introduced onto the column was increased by more than two orders of magnitude compared with the normal 1-μL splitless injection.

The *p*LVI(120-μL)-GC/ECNI-*q*MS with monitoring characteristic ions provided lower instrument detection limits (IDLs, 3σ) for the H-POPs, ranging from 10 fg $mL^{-1}$ of HCB to 87.5 pg $mL^{-1}$ of PCB-52, and the sensitivity generally increased along with the increase in the number of Cl or Br substitutions in the molecules. The IDLs were significantly improved by factors of 81.0-208 compared with those using the splitless injection(1-μL)-GC/ECNI-*q*MS with monitoring characteristic ions, while improved by factors of 1.63-121 (except for the 0.011-0.87 of BDE-28, 47, 49, 66, 99, 138 and 153) compared with those using the splitless injection(1-μL)-GC/ECNI-*q*MS with monitoring base peak ions. The method also exhibited good linearity with correlation coefficients of 0.9904-0.9999, satisfied repeatability of 0.1-2.2 % and reproducibility of 2.1-8.4 % for the H-POPs. On the other hand, the method allowed the use of $^{13}C$-labeled and/or perdeuterated analogues as internal standards for reliable quantification of all the H-POPs studied, which is not possible for the HCHs, PCB-28, PCB-52, PBDEs and PBB-153 when monitoring their corresponding base peak ions.

***Validation of pLVI-GC/ECNI-qMS and comparison with GC/HR-MS***

The H-POP concentrations in SRM 1946 were determined using the developed *p*LVI-GC/ECNI-*q*MS with monitoring the characteristic ions. The determined concentrations are well in accordance with the certified and/or reported values[6, 7], demonstrating good accuracy of the method. Moreover, concentrations of the β-, δ-HCH, and BDE-49, 138, 209 in SRM 1946 were determined for the first time as 0.98 ± 0.07, 0.13 ± 0.01, 1.81 ± 0.08, 0.35 ± 0.06 and 0.93 ± 0.12 ng $g^{-1}$ wet weight, respectively. Most of the H-POPs were not detected in the

method blank samples except for γ-HCH (8.6 pg $g^{-1}$), PCB-138 (2.6 pg $g^{-1}$), PCB-153 (2.2 pg $g^{-1}$), BDE-99 (2.3 pg $g^{-1}$), BDE-100 (1.2 pg $g^{-1}$), BDE-183 (0.4 pg $g^{-1}$) and BDE-209 (1.21 ng $g^{-1}$). All the method blanks, with the exception of BDE-209, were below 1% of the concentrations determined in SRM 1946, and the results reported were blank-corrected only for BDE-209. Recoveries of the internal standards from SRM 1946 were 74.2-97.9 %, and the precision of the 10 replicate measurements was less than 18.4%. Method detection limits (MDL, determined from spiked method blank samples as MDL = Method Blank + 3σ) of the *p*LVI-GC/ECNI-*q*MS were 0.01-63 pg $g^{-1}$ for the H-POPs; most of which were lower than those listed by US EPA in Method 1668 and 1614 using the GC/high resolution-MS[8, 9].

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**Figure 1: Parameters and procedures of *p*LVI-GC/ECNI-*q*MS for simultaneous determination of the 28 H-POPs**