SEMI-VOLATILE POPs NON-TARGETED SCREENING BY GC-APCI-HRMS TRAPPED ION MOBILITY SPECTROMETRY.

YANG F[1], AMINOT Y[2], MUNSCHY C[2], PREUD'HOMME H[1]

[1]Institut des Sciences Analytiques et de Physico-Chimie pour l'Environnement et les Matériaux (IPREM)-UMR5254, E2S UPPA, CNRS, Pau, France, 64000, fyang@univ-pau.fr;
[2]Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER)-DCN/RBE/BE/LBCO, Nantes, France, 44311.

Introduction

Persistent organic pollutants (POPs) and contaminants of emerging concern threaten the environment and human health due to their three main characteristics which are persistence, bioaccumulation and toxicity. Thus, these components (except PFASs) are lipophilic and easily accumulated in food chains, especially in high-fat food.[1] Although some of POPs have been identified and monitored in routine, these targeted compounds are only the tip of the iceberg when compared to what could be potentially harmful to the environment. This study was mainly focused on four classes of xenobiotic semi-volatile POPs including polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and their hydroxylated analogues (OH-PBDEs), which have been reported to have correlations to human health such as cancerogenicity, neurotoxicity, endocrine disruption and immunotoxicity [2]. Despite of the restriction or even ban the use of these chemicals for decades, parent molecules and their metabolites are still detectable at trace levels in the environment including in biota. Therefore, it is imperative to characterize the diverse family of POPs with ensured confidence identifications, then to assess a non-targeted screening (NTS) for other POPs and their metabolites. Ion mobility coupled with high resolution mass spectrometry (IM-HRMS), a novel platform that separates ions based on their size, shape, and charge state in the gas phase, offers an orthogonal separation dimension, in parallel with chromatographic separation and mass spectra. Moreover, atmospheric pressure chemical ionization (APCI), as a soft ionization mode, occurs predominantly molecular ion or/and protonated ions, which is advantage for NTS. Hence, GC-APCI-IMS-HRMS offers a new aspect of NTS analysis for semi-volatile POPs.

Materials and methods

PCBs, OCPs, PBDEs and OH-PBDEs standards were purchased from Wellington laboratories; PAHs and dioxin standards were obtained from Agilent. A Bruker GC SCION device with an atmospheric pressure chemical ionization (APCI-GC) was used for chromatographic separation. A Rxi-5SilMS 40m x 0.18mm x 0.18µm high-resolution column (Restek, France) was compared to a Rxi-5SilMS 30m x 0.25mm x 0.25µm high-resolution column (Restek, France). To estimate the ionization behavior of different classes of semi-volatile POPs, the APCI source was operated under the atmospheric (N2 transfer) condition and MeOH proton transfer condition. MS data were acquired on a trapped ion mobility spectrometry hybrid with a high-resolution time of flight mass spectrometry (timsTOF, Bruker Daltonics, Bremen Germany), which measures mobility value in 1/k0. The analytical method was optimized in APCI positive mode in full scan with a mass range from 200 to 1250 m/z. The total acquisition time was 25min. Raw data mining was treated by Data Analysis software (Bruker Daltonics) and Haloseeker 2.0 (ONIRIS, Nantes) [3]. Because limited databases are available for GC-APCI, and CCS databases, an in-house database of APCI-GC spectra with collision cross section (CCS in $Å^2$), converted from 1/k0 (at room temperature in N_2 buffer gas), was developed for semi-volatile POPs in the new TASQ® released 2021 software (Bruker Daltonics) for further non-targeted analysis.

Results and discussion

In this study, over 160 standards corresponding to PAHs, PCDD/Fs, PCBs, OCPs, PBDEs and OH-PBDEs were characterized by IMS-HRMS with retention time (RT), exact mass, isotope and CCS value.

To optimize the chromatographic separation of critical pairs, two columns with the same stationary phase, but different column dimensions were compared. In the same chromatographic condition, the 40m column always showed better peak resolution. To understand the capacity of IMS in isomer separation, the resolution was analyzed not only with column length but also by adding CCS value in the extracted-ion chromatogram (EIC), as shown in Table 1 and Figure 1.

It was observed that GC column resolution still played a major role in critical pair separation. CCS value provided as an additional but limited parameter to enhance chromatographic resolution. 2,4,4'-Trichlorobiphenyl (PCB-28) and 2, 4',5-Trichlorobiphenyl (PCB-31) are challenging critical pairs to separate. Both of these PCBs have 3 Cl atoms, extremely similar structure with only one different substitution pattern. Thus, PCB-28 and PCB-31 were co-eluted or

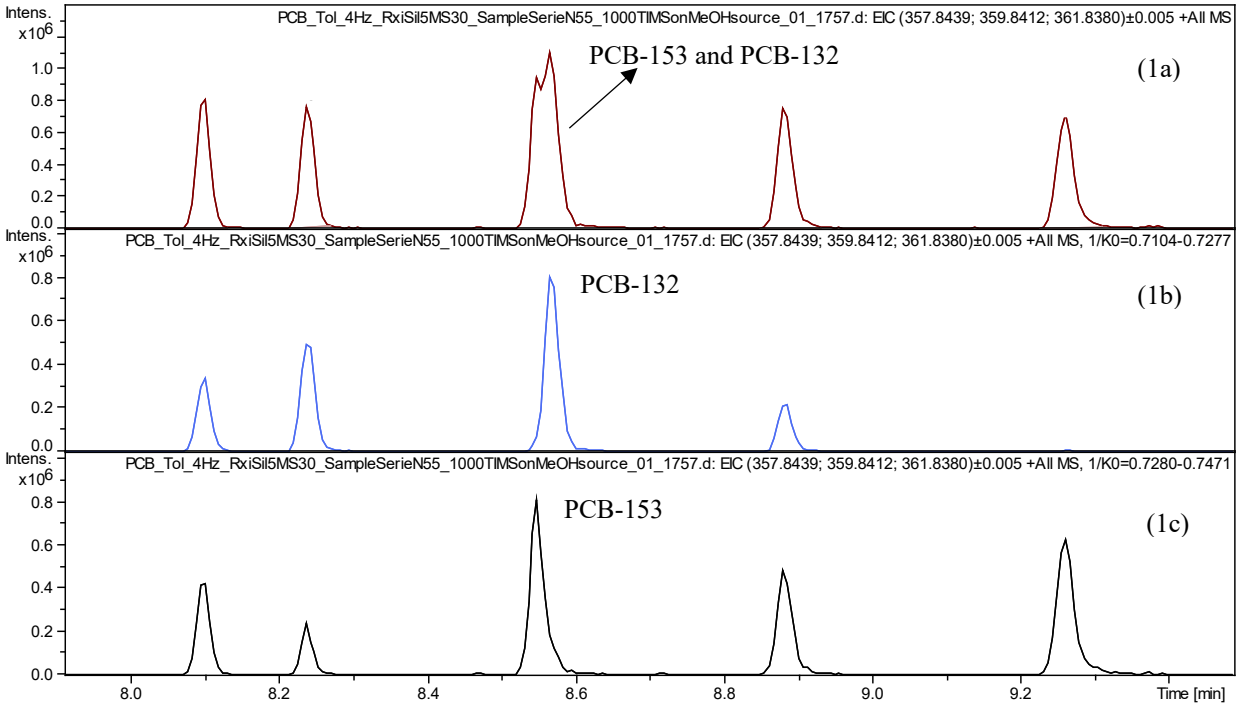
poorly separated with respectively a 30m x 0.25mm x 0.25µm (faster chromatography) and 40m x0.18mm x0.18µm (high resolution chromatography) column in chromatogram and mobilogram. A further, deeper and more specific study should be carried out to figure out the unique separation of this critical pair.

Rxi-5silMS 30 m and 40 m column provided adequate capacity to separate 2,2',5,5'-Tetrachlorobiphenyl (PCB-52) and 2,2',4,5'-Tetrachlorobiphenyl (PCB-49). While in mobilogram, their peak mutual overlap was over 73%. In this case, CCS value could not enhance peak separation. 2,2',4,4',5,5'-Hexachlorobiphenyl (PCB-153) and 2,2',3,3',4,6'-Hexachlorobiphenyl (PCB-132) were co-eluted in 30m GC column. When GC column length increased, the resolution and peak separation were significantly improved, while the peak mutual overlap on the mobilogram was less than 22%.

Furthermore, after adding 1/k0 mobility range in EIC, interferences were effectively eliminated in chromatogram and mass spectrum without losing peak intensity. Therefore, the additional, orthogonal CCS criteria can improve peak separation in case of a lack of GC column resolution (30m and faster chromatography) when ions provide sufficient different in IMS.

Table 1. Critical pair separation.

Compound	Resolution (30m)	Resolution (30m+CCS)	Resolution (40m)	Resolution (40m+CCS)
PCB-28/PCB-31	coeluted	coeluted	0.14	0.14
PCB-52/PCB-49	0.8	0.8	>1.5	>1.5
PCB-153/PCB-132	0.44	0.59	0.73	0.73



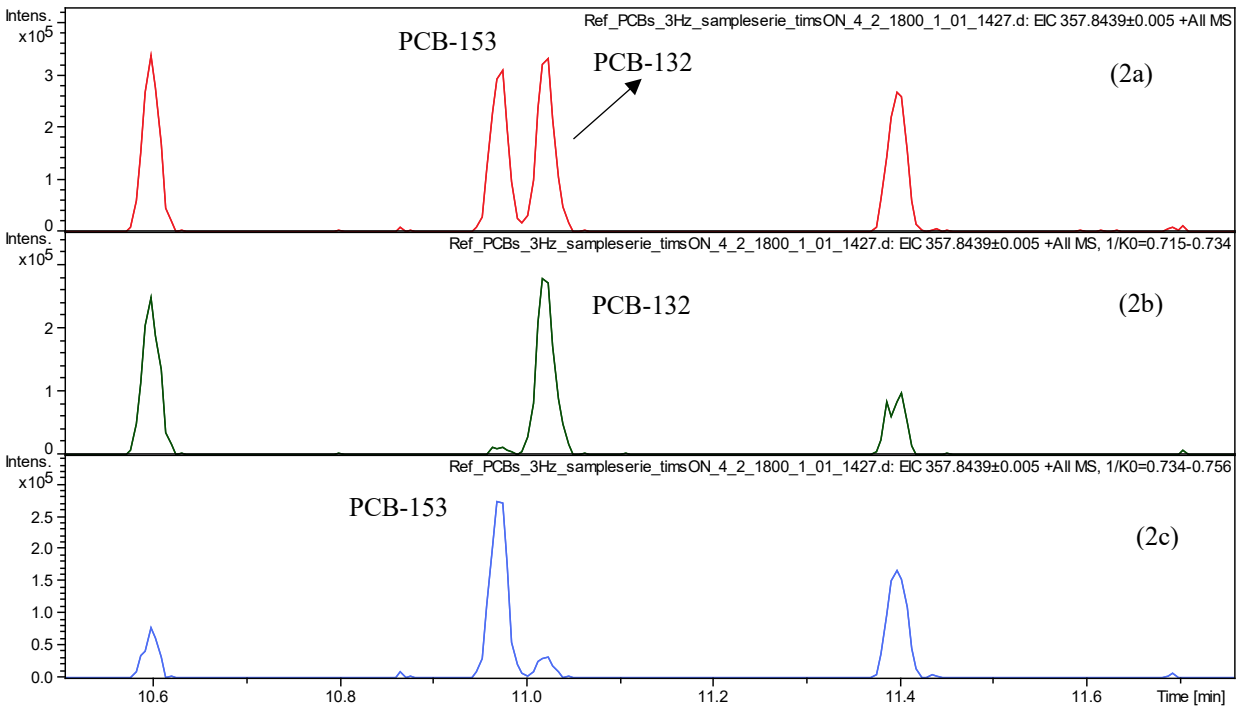


Figure 1. PCB-153 and PCB-132 were co-eluted in 30m GC column, shown in (1a), while adding 1/k0 mobility range in EIC (1b and 1c), the chromatographic peak resolution was enhanced from 0.44 to 0.59. Using a 40m GC column, the separation was increased significantly(2a) to 0.73, whereas IM capacity was limited by the structural similarity of PCB-153 and PCB-132, which results in a 22% peak mutual overlap and resolution of IMS.

The ionization behavior was also investigated according to POPs classes and their precursor ion. Dioxins, PAHs, and PCBs produce preferentially the molecular ion $[M]^{+\cdot}$ in the APCI source. However, with the increase of bromine substitution level, PBDEs become difficult to be ionized under the atmospheric APCI source. Adding MeOH as a modifier in the APCI source promotes ionization and protonation of PBDEs to $[M]^{+\cdot}$ and $[M+H]^{+}$.

To investigate the relationship between CCS value and chemical structure, an IMS (CCS) – MS (m/z) trend line was plotted by chemical's family, as shown in Figure 2.
PAHs which are not halogenated and not substituted, have in general for the same number of carbons a small CCS. It is interesting and logic to note also that an increase of aromatic ring for PAHs induce a smaller CCS gap than an increase of 1 halogen substitution. Inversely, considering a similar exact or nominal mass, the PAHs family demonstrate always a higher CCS than for PCBs or PBDEs, as the mass density is higher for halogenated substituted planar or semi planar compounds.

PCBs and PBDEs show a positive linear correlation between CCS and chlorine or bromine substitution degree. In the similar mass level PCBs have higher CCS than PBDEs, since the carbon-carbon bond that connects the two aromatic rings is freely rotation, resulting in a larger structure. Furthermore, PBDEs and PCBs isomers with structural difference are annotated by CCS values. Within PCBs and PBDEs, ortho-substitutions have smaller CCS than meta-substitution, then para-substitution. For example, 2,6-dichlorobiphenyl (PCB-10) had a measured CCS value at 127 A^2, against 3,5-dichlorobiphenyl (PCB-14) at 129 A^2. Further study is undergoing for measured CCS value within isomers and their substitution patterns.

In this study, PAHs and PCBs parent molecules were in molecular ion form, PBDEs produced both molecular ion and protonated ion. The IMS(CCS) – MS (m/z) trendline was based on molecular ion CCS value. Other interesting study which was investigated different ionization sources to produce other types of parent ion such as $[PBDE\text{-}Br\text{-}H]^-$ and $[PCB\text{-}Cl+O]^-$ a similar result was illustrated [4]. Additionally, mass/CCS trend lines for the specific PFAS groups were also concluded CCS increased linearly with the chain lengths of CF_2 within each PFAS group.[5].

The CCS-m/z trend line can be applied to other component categories and their metabolites in NTS to estimate the function group or halogenated substitution level of a compound without any available standard. By comparing the CCS within isomers could be also interesting to predict substitution patterns, therefore it can elucidate the structure of unknown in NTS.

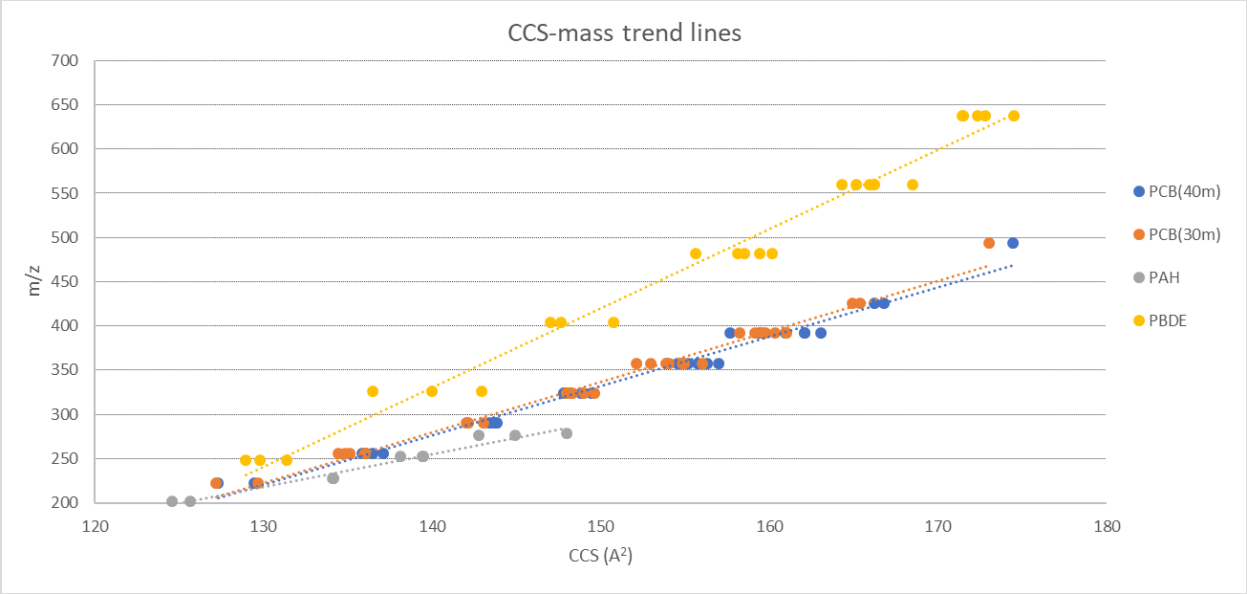


Figure 2. IMS (CCS)- MS(m/z) trend lines for semi-volatile POPs

GC-APCI-timsTOF coupling with IMS provides a novel approach to characterize diverse chemicals with exact mass, isotopic pattern, retention time and CCS value. An in-house database is under construction in TASQ® 2021 software (Bruker Daltonics) to identify and quantify semi-volatile POPs in various matrices.

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