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Comparison of Electron Impact and Electron Capture Negative Ionization for the Quantification of Polybrominated Diphenyl Ethers in Human Plasma

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Abstract

Polybrominated diphenyl ethers (PBDE) are persistent organic pollutants that are strongly associated with disrupted endocrine and immune functions. Due to the increasing health concerns, it is critical to quantify PBDEs in human specimens. Gas chromatography (GC) - mass spectrometry (MS) with electron impact (EI) and electron capture negative ionization (ECNI) sources were optimized to compare the quantification for ten PBDEs in 0.5 ml of human plasma. Source parameters, including electron energy, emission current, source temperature, focus lens and flow rate of the ECNI source reagent gas (methane), were optimized to achieve the best performances of EI-MS and ECNI-MS. The limits of quantification (LOQ) in human plasma ranged from 20 to 497 fg injected into GC/ECNI-MS compared to 519 to 2966 fg when using GC/EI-MS. Especially for highly brominated congeners, GC/ECNI-MS provided much lower LOQs than GC/EI-MS. Obtaining the necessary LOQs with GC/ECNI-MS, the method was validated according to the US Food and Drug Administration guidance for industry using standard materials purchased from the National Institute of Standards and Technology (NIST). The validated GC/ECNI-MS method was used to measure the concentrations of ten tetra-, penta- and hexa-PBDE congeners in maternal human plasma. Tetra-PBDEs, especially BDE-47, were identified as the predominant PBDE burdens in human plasma.

Keywords: Gas chromatography-mass spectrometry; Electron ionization; Electron capture negative ionization; Polybrominated diphenyl ethers (PBDEs); Quantification; Human plasma

Abbreviations: GC/ECNI-MS: gas chromatography coupled with electron capture negative ionization-mass spectrometry; GC/EI-MS: gas chromatography coupled with electron ionization – mass spectrometry; PBDE: polybrominated diphenyl ethers; NIST: National Institute of Standards and Technology; LOQ: limit of quantification

Introduction

Polybrominated diphenyl ethers (PBDEs) are flame retardants widely used in textiles, foams, building materials, electronic equipment and plastics since the 1960s [1]. PBDEs consist of 209 possible congeners (PBDE = $C_{12}H_{10-m}Br_mO$ (m = 1, 2, …, 10)) [2] and can be divided into 10 homolog groups (mono- to decabromodiphenyl ethers) based on the variations in both number and positions of bromination [3]. PBDEs are lipophilic and do not bind chemically to the material to which they are applied. Thus, they are easily transferred from the environment to the food chain and, subsequently, accumulated in humans [4].

The flame retardants have now become a major concern to public and have been documented in lay and scientific articles. Tetra- to hexa-PBDEs [5-7], among which BDE-47, -99, -100, -153 and -154 are reported to account for 90% of the total human body burden. The finding of PBDEs in human tissues is of concern because of their potential for endocrine system disruption, toxicity to the nervous and, reproductive systems [8], as well as their cancer promoting activity [9-10]. Recent epidemiological studies have associated neurodevelopmental effects with exposures to BDE-47, 99 and 100 [11]. In addition, PBDEs with bromine at the 5 and/or 5' positions (such as BDE-49) appear to be found in disproportionally high concentrations in human gestational tissues and blood [12,13]. These studies highlight the risk of PBDE exposure during pregnancy for their potential to affect mental development. Thus, a quick and reliable analytical approach is critical for the precise quantification of PBDE congeners in biological specimens to facilitate epidemiological and risk assessment studies.

Due to the structure characteristics of PBDEs, gas chromatography (GC) coupled with different detection systems is the typical analytical technique for the determination of PBDEs [6]. Methods using electron impact (EI) [2,7], electron capture negative ionization (ECNI) [4,14,15], inductively coupled plasma (ICP) [16-18], and metastable atom bombardment (MAB) [19] ion sources, after elution of PBDE congeners from capillary columns including ZB-5MS [2], DB-XLB [20], DB-5HT [4,21] and DB-5MS [22,23], have been described. Although GC/high resolution MS (GC/HRMS) [21] and GC/ion trap MS (GC/ITMS) [22] are successfully used for the determination of PBDEs [24], a unit-resolution mass spectrometer equipped with EI or ECNI [23] source in combination with selected ion monitoring (SIM) are commonly used for detection of PBDEs in many laboratories.

The goal of this study was to compare the sensitivity of electron ionization (GC/EI-MS) to electron capture negative ionization (GC/ECNI-MS) of gas chromatography mass spectrometry (GC/MS) for the quantification of PBDE congeners in small volumes of human plasma. Our objectives were to: 1) optimize ion source parameters of EI-MS and ECNI-MS under SIM mode separately; 2) compare the limits of quantification (LOQ) of our targeted PBDE congeners determined by EI-MS and ECNI-MS; 3) validate the GC/MS method determined to provide more efficient ionization with the use of standard reference material (human serum) purchased from the National Institute of

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Standards and Technology (NIST); 4) apply the validated GC/MS method to determine PBDEs concentrations in human maternal plasma.

Material and Methods

Chemicals and consumables

All solvents used were of HPLC grade, including isooctane, methanol, and dichloromethane purchased from Fisher Scientific (Pittsburg, PA, USA). Waters Oasis HLB (poly (divinylbenzene-co-N-vinylpyrrolidone), 200 mg/3 ml) (Waters, Milford, MA, USA) were used as solid phase extraction (SPE) cartridges to extract plasma samples. Sep-Pak® Light Silica cartridges (55-105 µm, Waters, Milford, MA, USA) were serially combined with SPE cartridges for clean-up. Human serum (Sigma-Aldrich, St. Louis, MO, USA) was used as blank matrix for method development and validation. Ultrapure water (18 MΩ) was supplied by a Milli-Q system (Millipore, Billerica, MA, USA). All glassware used throughout the experiments was purchased from Fisher Scientific (Fisher Scientific, Pittsburg, PA, USA) and used only once to avoid any potential organic contamination.

Ten PBDE congeners in isooctane were obtained from AccuStandard (New Haven, CT, USA), and included the following: 2,4,4'-tribromodiphenyl ether (BDE-28), 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), 2,2',4,5'-tetrabromodiphenyl ether (BDE-49), 2,2',5,5'-tetrabromodiphenyl ether (BDE-52), 2,2',3,5',6-pentabromodiphenyl ether (BDE-95), 2,2',4,4',5-pentabromodiphenyl ether (BDE-99), 2,2'4,4',6-pentabromodiphenyl ether (BDE-100), 2,2',3,3',6,6'-hexabromodiphenyl ether (BDE-136), 2,2',4,4',5,5'-hexabromodipheyl ether (BDE-153), and 2,2',3,4,4',5',6-heptabromodiphenyl ether (BDE-183). A ^{13}C labeled reference standard, 2,3',4,4',5-pentabromodiphenyl ether (^{13}C-BDE-118) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA), and used as a surrogate internal standard going through the whole experimental procedure. Standard serum material fortified with PBDE congeners named SRM®1958, including known levels of congeners BDE-28, -47, -99, -100, -153, and -183, was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

Sample preparation

Plasma samples were stored at -80°C until preparation. Samples were thawed on ice in a fume hood overnight before preparation. An aliquot of 0.5 ml plasma was removed and placed into disposable glass tubes. Plasma samples were then spiked with 10 µL of 100 ng/ml ^{13}C-BDE-118 and 0.5 ml of pure formic acid before ultrasonication for 10 min. In the meantime, SPE cartridges were gravimetrically conditioned with two aliquots (2×3 ml) of pure methanol and two aliquots (2×3 ml) of water with formic acid and methanol (v/v/v, 95/0.5/4.5). After that, 1 ml of the plasma and formic acid matrix was applied to the cartridge. The cartridges were washed twice with 1 ml of water containing formic acid and methanol (v/v/v, 95/0.5/4.5). After drying the SPE cartridges under vacuum (-5 mm Hg) for 5 min, disposable Sep-Pak® cartridges were placed underneath the SPE cartridges and analytes were eluted with three aliquots (3×3 ml) of dichloromethane under a vacuum of -10 mm Hg. Extracts were collected and evaporated to dryness at 45°C under a gentle stream of nitrogen. The residue was reconstituted with 100 µL of isooctane, vibrated and centrifuged before being transferred into auto sampler vials for GC/MS analysis.

Standard serum material (NIST serum) was reconstituted according to the provided instructions. Briefly, the vial was brought to room temperature, lightly tapped at the bottom to dislodge anhydride. An aliquot of 10.7 ml of HPLC-grade water was added to the vial. Contents were mixed gently, and then stood for approximately 30 min. The vial was swirled again and stood for another 10 min. The vial was never shaken vigorously to avoid frothing. Total time for reconstitution was approximately 1 h. After reconstitution, NIST serum was extracted and analyzed as described above.

Instrumentation

GC/MS setup: Analysis was performed with an Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled with a Quattro micro™ mass spectrometer (Waters, Milford, MA, USA). A DB-XLB (30 m × 0.25 mm, i.d. × 0.25 µm film thickness, J &W, Agilent Technologies, Palo Alto, CA, USA) capillary column was used for seperation. Samples were injected using the solvent vent mode by the programmable temperature vaporization (PTV) injector. The initial temperature of the PTV was 120°C, and rose to 300°C at a rate of 120°C/min by the end of the injection. Solvent vent pressure was 70.0 psi at a flow rate of 100 ml/min for 0.3 min. The purge to clear out vaporized solvent was 50.0 ml/min and started at 2.1 min. The injection speed for PTV was optimized as 20 µL/second. After 3.5 min the gas saver was opened to allow a flow rate of 15.0 ml/min. The carrier gas was helium at a flow rate of 1 ml/min. The GC temperature program was as follows: initial temperature of 90°C was increased to 192°C at 30°C min^{-1}, that temperature was held for 1 min, then increased to 218°C at a rate of 1°C min^{-1}, held for 1 min, followed by another increase to 300°C at a rate of 3°C min^{-1}, which was held for 2 min. A post run procedure of 325°C lasted for 3 min, which contributed to a total run time of 65 min.

Optimization of ion source parameters: Two ionization methods with different monitoring parameters were compared and operated under selected ion monitoring (SIM). One ionization method was electron impact (EI) source with positive charges; the other was electron capture negative ionization (ECNI). Source temperature, electron energy, emission current and regent gas flowrate of ECNI were optimized. Neat standard solution of 4 ng/ml was used to optimize the source condition parameters; they were adjusted one at a time to identify the best settings. Chromatographic areas for the PBDE selected ions, either under EI or ECNI, were integrated and compared run-to-run. Specifically, the most and second most abundant ions generated either by EI or ECNI source were both recorded during optimization. To reduce the variability in absolute response between individual PBDE congeners, the response for each congener was recorded as the percent difference from the mean response for that congener across the experimental points of a given parameter. For example, the bromide ions' ($[Br]^-$) responses of BDE-47 at different ECNI electron energy (20-100 eV) were averaged, and it was determined that at an electron energy of 70 eV, BDE-47's bromide ions' abundance was 68% below the average.

Method Validation

Once optimum source parameters were determined, method validation was conducted according to the FDA guidance on bioanalytical method validation [25]. Considering all stages of the chemical measurement process in terms of the fit-for-purpose criterion, the method was also validated by NIST serum (SRM®1985).

Linearity: Calibration standards were prepared by 0.5 ml of blank human serum spiked with 0.025, 0.05. 0.2, 1, 5 and 10 ng of mixed

standard solution and 0.5 ng surrogate. Linear calibration curves were constructed by least-square regression of concentration versus peak area ratio (analyte/IS) of the calibration standards. The correlation coefficient was used as an indicator for linearity.

Recovery rates and precisions: Recovery rates and precisions were assessed by spiking 0.5 ml of NIST serum with 0.5 ng of surrogate followed by extracting the sample as described above. Recovery rates were calculated by comparing the measured concentrations of PBDEs in NIST serum to their theoretical concentrations in the NIST serum (SRM®1985). Six replicates of NIST serum were prepared to obtain the standard deviation (S.D.) and average concentration. Relative standard deviation (RSD) expressed in percentage was the ratio of S.D. divided by their average value, and was used to present the precision of determination.

Lower limits: The lower limit of quantification (LOQ) and limit of detection (LOD) were determined as the analyte concentration that induced a signal-to-noise ratio equal to 10 (S/N = 10) and 3 (S/N = 3), respectively. Initially, the LOQ and the LOD were determined by neat standards in isooctane (LOQ_i and LOD_i) for evaluating instrument performance, peak height and resolution. The LOQ and LOD in matrix (LOQ_m and LOD_m) were determined by analyzing human serum fortified with reference standards.

Data processing

Chromatographic peak areas were integrated for quantification. Least-square regression of concentration versus area ratios (analyte/I.S.) was performed to construct calibration curves. GC/MS data integration and analysis was performed on Masslynx 4.1 (Waters, Milford, MA, USA). During ion sources optimization, linear regression was performed of ion abundance versus the changes of individual ion source parameters. A p-value < 0.05 was considered significant. Statistical analysis was performed by SigmaPlot 11.0 (Systat Software Inc, San Jose, CA, USA).

Results and discussion

Chromatographic separation

Baseline separation was achieved for monitored PBDE congeners (Figure 1A). The retention time, resolution factors and the monitored ions for chromatographic separation are listed in Table 1. Losses of 2Br and/or HBr from parent compounds were commonly observed for all congeners under EI source. Under ECNI, bromide ions were monitored for each congener. Because of the similarities in fragmentation of PBDE congeners with the same number of bromides, baseline separation of congeners is essential, denoted by a resolution factor that exceeded 1.5. The resolution factor is defined as twice the retention time difference divided by the sum of the half peak widths of two adjacent peaks. The smallest resolution factor was 1.6 (Table 1) and was between BDE-136 and BDE-153. A long running time (65 min) was used in this method to achieve feasible chromatographic separation for more PBDE congeners.

Mass Spectra

The characteristics of mass spectra obtained for the PBDE congeners were consistent with the mass spectra reported previously in the literature [26]. The most abundant ions, corresponding to the cluster for the loss of two bromide ions ($[M\text{-}2Br]^+$), were observed with EI detection, while the bromide ions ($[Br]^-$) were the dominant peaks under ECNI-CH_4 monitoring. Molecular ion clusters ($[M]^+$) and high-mass fragmentation ions ($[M\text{-}x\text{H}\text{-}y\text{Br}]^-$) were the second most abundant ions in full scan MS spectra of EI and ECNI mode, respectively. As an example, the mass spectra of BDE-47 under EI and ECNI mode and their isotopic patterns of abundant ion clusters are presented in Figures 1B & 1C. Bromide has two isotopes with atomic weights of 79 and 81, respectively. Their neural abundance ratio is nearly 1 to 1. BDE-47 is a tetra-BDE congener and, thus, has five spectra dominated by the parent ion $[M]^+$ under EI detection. Similarly, the isotopic cluster of its major ion $[M\text{-}2Br]^+$ was consistent of three spectra due to the remaining two bromide atoms in the fragment. In ECNI mode, the bromide isotopic patterns remained the same for both the high-mass fragmentation ions ($[M\text{-}x\text{H}\text{-}y\text{Br}]^-$) and bromide ions ($[Br]^-$).

Optimization of ion source parameters

EI Optimization: EI parameters including electron energy, source temperature, emission current and focus lens1 were optimized for ions $[M\text{-}2Br]^+$ and $[M]^+$ (Figure 2). In Figure 2, the average responses of all the selected PBDE congeners were marked with dots at each experimental point of every parameter while different lines were used for specific congeners in the figure. In the EI process, the sample of interest is vaporized into the mass spectrometer ion source, where it is impacted by a beam of electrons with sufficient energy to ionize the molecule. The optimized ranges of EI parameters were listed in Table 2. Adjustments of EI parameters had similar effects on the signal intensities of ions $[M\text{-}2Br]^+$ and $[M]^+$. Judging by the percentage of difference from the average abundance, either ion was the most sensitive to emission current, followed by electron energy, source temperature and focus lens1.

For all EI source parameters, there was minimal variability between congeners for either $[M\text{-}2Br]^+$ or $[M]^+$. The raise of EI parameters did not lead to a continuous increase of abundance of $[M\text{-}2Br]^+$ or $[M]^+$. Since ions $[M\text{-}2Br]^+$ and $[M]^+$ responded similarly to the changes of EI source parameters, the signal abundance of both ions was strongest at the same optimum experimental points. As a result, the best settings to achieve the strongest signal were as following: source temperature at 200°C, electron energy at 70 eV, emission ion current at 300 μA and focus lens1 at 100 V (Table 2).

Regression analysis between experimental points of each EI source

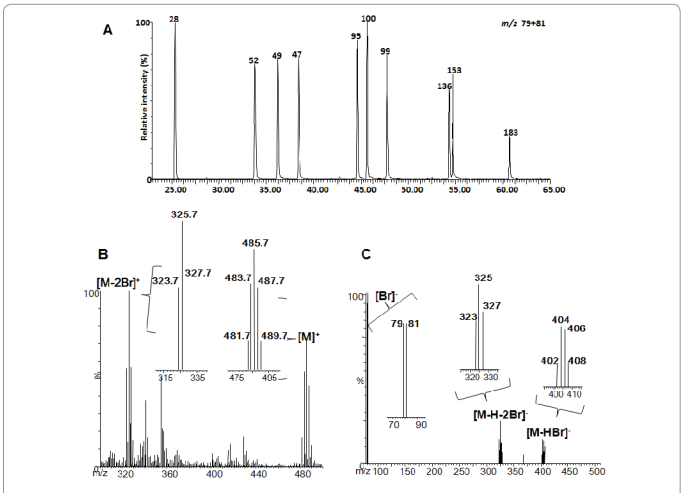

Figure 1: GC/MS characteristics of PBDEs (A) Chromatogram of bromide ions for selected PBDEs with the congener numbers listed. Mass spectra for BDE-47 under (B) EI and (C) ECNI (CH_4) ionization. The isotopic patterns for the dominant and second most abundant ions are shown next to their respective spectra.

parameter and the responses of ion $[M-2Br]^+$ and $[M]^+$ were conducted separately. There was no statistically significant relationships between adjustments of any EI source parameter and the signal intensity of either ion $[M-2Br]^+$ or $[M]^+$.

The abundance of ion $[M-2Br]^+$ and ion $[M]^+$ were compared under their optimum EI source parameters, respectively. Ion $[M-2Br]^+$ was the base ion for all evaluated PBDE congeners. The relative abundance ratios of these two ion clusters ranged from 14% ~94% (Table 1). Given the same settings of source parameters, PBDEs with a high degree of bromination had a low relative abundance ratio of ion $[M]^+$ to base ion $[M-2Br]^+$.

ECNI Optimization: The optimization of ECNI parameters for bromide ions and high-mass fragment ions of PBDE standards were evaluated based on source temperature, electron energy, emission current, focus lens 1 and the flow rate of reagent gas methane (CH_4) (Figure 3). Methane proved to be more consistent in providing higher sensitivity than isobutene [26], and resulted in less variation than ammonia [27]. Thus, we chose methane as the reagent gas with helium as the carrier gas. The optimized ranges of ECNI parameters were listed in Table 2.

The response of high-mass fragmentation ions and bromide ions differed when adjusting the same ECNI source parameters, such as source temperature and focus lens1. According to the percentage of difference from the average abundance, the response of bromide ions was most sensitive to changes of electron energy, followed by source temperature, focus lens1, reagent gas flow and emission current. For high-mass fragment ions of PBDEs, adjustments of reagent gas flow and electron energy resulted in comparable intensity followed by emission current, source temperature and focus lens1.

Positive associations were observed between the adjustments of ECNI source parameters and the responses of $[Br]^-$, which were consistent across selected PBDE congeners. In contrast, there was congener-to-congener variability in $[M–xH–yBr]^-$ response to source temperature, emission current and CI reagent gas flow rate. For example, when the source temperature was over 150°C, the $[M–xH–yBr]^-$ response of penta- (BDE-95, -99, -100) and hexa-BDEs (BDE-136, 153) decreased, while that of tetra-BDEs (BDE-47, -49 and -52) kept increasing until 250°C. The congener-to-congener differences of $[M-xH-yBr]^-$ response to changes in source temperature was a unique finding. This is in disagreement with a previous study [26] which described the opposite response of $[Br]^-$ and $[M-xH-yBr]^-$ to changes of source temperature. We also observed congener-to-congener variations of $[M-xH-yBr]^-$ from tetra-, penta-, and hexa-BDEs with changes in emission current and CI reagent gas flow rate.

Regression analysis was used to examine the associations between adjustments of each ECNI parameters and signal abundances of bromide ions as well as high-mass fragment ions, respectively. Changes of electron energy ($p<0.001$), emission current ($p<0.05$), focus lens1 ($p<0.01$) and CI reagent gas flow ($p<0.01$) all presented statistically significant linear correlations with bromide ion abundance, while source temperature did not ($p>0.05$). Similarly, statistically significant linearity was found between responses of high-mass fragment ions and all adjusted source parameters, including source temperature ($p<0.05$), electron energy ($p<0.01$), emission current ($p<0.001$), focus lens ($p<0.05$) and CI reagent gas flow ($p<0.001$).

We compared the abundance of bromide ions and high-mass fragment ions under their optimum settings of source parameters. The signal intensities of bromide ions were much greater than those of high-mass fragment ions. The relative abundance ratios ranged from 2.2% to 16.4% (Table 1), which was derived from the quotient of the integrated chromatographic peak areas of high-mass fragment ions and that of bromide ions. High-mass fragmentation of BDE-136 had the highest relative abundance of 16.4% compared to its bromide ion.

Comparison of EI-MS and ECNI-MS: The base ions of EI-MS and ECNI-MS responded differently to the same adjustment of a certain source parameter. As for EI source, changes in the emission ion current had the biggest impact by producing a relative response ranging from -34 to 42% (Figure 2). For ECNI source, changes of electron energy had the strongest impact with a relative response ranging from -99 to 154% (Figure 3). In contrast, changing the electron energy for EI only resulted in a relative response ranging from -40 to 31%. Adjustments of source temperature changed the relative response from -19% to 47% for ECNI source, and -15 to 23% for EI source.

Given the same optimizing ranges of each source parameter, EI-MS and ECNI-MS had different optimum settings to achieve the most abundant signals. ECNI-MS required higher source temperature, electron energy, emission current and focus lens1 to generate its base ion $[Br]^-$ (Table 2). This suggests that ECNI is a softer ionization method than EI. In addition, the base ions of both EI-MS and ECNI-MS had a smaller mass compared to the second most abundant ions. Further, the response of monitored ions in ECNI-MS mode responded in a linear fashion to the changes of ECNI source parameters. Such linear relationship was not observed during the optimization of EI-MS. The optimum settings of the most and second most dominant ion under EI-

Table 1: Selected PBDE congeners, retention times, selected ions, and fragment ions ratios.

PBDE congener	t_R (min)	Resolution Factor*	Base EI ion $[M-2Br]^+$ (m/z)	Molecular EI Ion $[M]^+$ (*m/z*)	$[M]^+/[M-2Br]^-$ (%)$^{\$}$	Base ECNI ion $[Br]^-$ (m/z)	ECNI ion $[M-xH-yBr]^-$	*m/z*	$[M-xH-yBr]^-/[Br]^-$ (%)$^{\#}$
BDE-28	25.40		245.8	405.8	93.87	78.8, 80.8	$[M-Br]^-$	326.9	3.29
BDE-52	33.79	33.56	325.7	485.7, 487.7	26.74	78.8, 80.8	$[M-H-2Br]^-$	324.8	2.58
BDE-49	36.21	9.68	325.7	485.7, 487.7	44.73	78.8, 80.8	$[M-H-2Br]^-$	403.8, 401.8	2.81
BDE-47	38.42	8.84	325.7	485.7, 487.7	79.69	78.8, 80.8	$[M-H-2Br]^-$	324.8	2.24
BDE-95	44.63	24.84	403.6, 405.6	563.6	27.5	78.8, 80.8	$[M-H-2Br]^-$	402.8, 404.8	6.46
BDE-100	45.66	4.12	403.6, 405.6	563.6	79.26	78.8, 80.8	$[M-H-2Br]^-$	402.8, 404.8	9.29
BDE-99	47.73	8.28	403.6, 405.6	563.6	51.9	78.8, 80.8	$[M-H-2Br]^-$	402.8, 404.8	2.93
BDE-136	54.30	26.28	483.5	643.5	14.13	78.8, 80.8	$[M-H-Br]^-$	563.7, 561.7	16.45
BDE-153	54.70	1.60	483.5	643.5	33.13	78.8, 80.8	$[M-Br]^-$	564.7, 562.7	4.74
BDE-183	60.69	24.00	563.6	723.6	23.24	78.8, 80.8	$[M-2Br]^-$	561.6, 559.6	10.45

*Resolution factor indicates the degree of separation of analytes on column. The resolution factor (R) of two species, A and B, where B is eluted later than A, $R=2X[(t_R)_B - (t_R)_A]/W_A+W_B$, where W_A and W_B are the width of each peak at baseline. Baseline resolution is achieved when R=1.5.

$: Ratio of abundance of $[M]^+$ and $[M-2Br]^+$ at their optimum EI source parameters.

#: Ratio of abundance of $[M-xH-yBr]^-$ and $[Br]^-$ at their optimum ECNI source parameters.

Table 2: MS source parameters optimized for EI and ECNI detection.

Parameter	EI Optimized	Optimum EI $[M-2Br]^+/[M]^+$	ECNI Optimized	Optimum ECNI $[Br]^-/[M-xH-yBr]^-$
Source Temperature (°C)	100-300	200/200	100-300	250/150
Electron Energy (eV)	40-80	70/70	20-100	100/80
Emission Current (μA)	100-600	300/300	100-600	600/600
Regent Gas Flow (%)	-	-	20-100	100/100
Focus Lens1 (V)	60-160	100/100	60-160	160/120

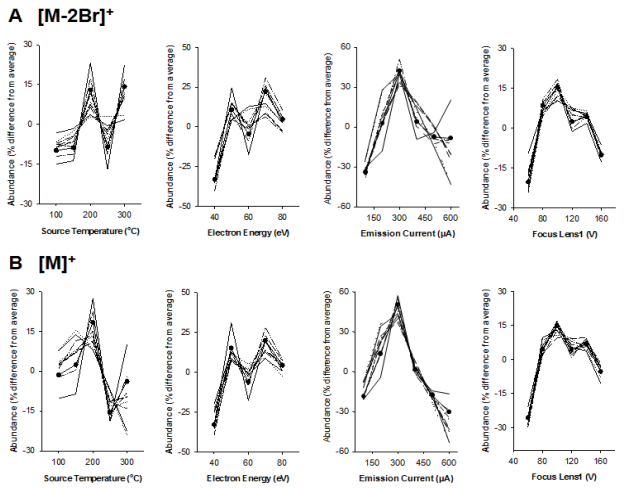

Figure 2: Profile of EI source parameters optimized for PBDE congeners (-) and their average response (-●-) by (A) $[M - 2Br]^+$ and (B) $[M - Br]^+$. Optimized parameters include source temperature, electron energy, emission current, and focus lens1.

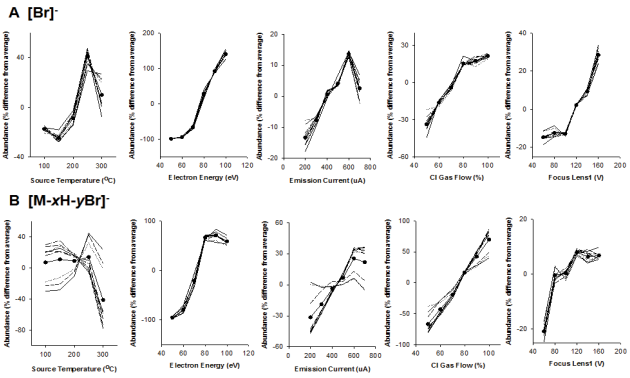

Figure 3: Profile of ECNI source parameters optimized for PBDE congeners (-) and their average response (-●-) by (A) bromide ion ($[Br]^-$) and (B) high-mass fragment ions ($[M-xH-yBr]^-$). Optimized parameters include source temperature, electron energy, emission current, CI reagent gas flow and focus lens 1.

MS were the same, while they were different under ECNI source. This might suggest that ECNI-MS is not as robotic as EI-MS. The optimum source parameters and the most abundant ions for ECNI-MS detection are instrumentally dependent.

In summary, both EI and ECNI have their advantages and disadvantages in quantifying PBDE congeners in human plasma. One critical advantage of ECNI-MS is the achievement of a much lower LOQ than EI-MS. The signal intensity of the base ion ($[Br]^-$) in ECNI-MS mode is almost ten times higher than that of the base ion ($[M-2Br]^+$) in EI-MS mode, which makes it a superior method for PBDE congener quantification. However, ECNI-MS is based on the detection of compounds containing any amounts of bromide atoms and is therefore not as selective as EI-MS. Since our goal was to establish a method that provides extremely high sensitivity (to reach sub-ng/ml) for quantification of PBDE congeners in small volumes (0.5 ml) of human plasma, GC/ECNI-MS met the criteria set forth in this study and was subsequently validated.

Validation of the optimized GC/ECNI-MS Method

Linearity: The calibration range for each PBDE congener was based on body burden data [7,28-33] and was constructed from 0.02 ~ 20 ng/ml (concentrations based on plasma volume). Excellent linearity was obtained with coefficients of determination (R^2) of >0.998 and RSDs of <18%.

Recovery rates and precisions: The recovery and precision of this method was assessed by NIST serum (SRM®1958). The results in Table 3 illustrate that the GC/ECNI-MS method was efficient at extracting and quantifying the selected congeners. Recovery rates of spiked PBDE congeners in NIST serum ranged from 76.3% to 115.3% with RSDs of <23%.

Lower limits of quantification: The limit of quantification of PBDE congeners with and without matrix (human plasma) is listed in Table 4. LOQ_m, presented by the injected amount into the mass spectrometer, varied from 20 fg to 497 fg for ECNI-MS and 519 fg to 2966 fg for EI-MS, generally ten times better than EI. The limits of quantification achieved in the current study were much lower than previous results published by Ackerman et al, which provided the lowest LOQ to our knowledge [26]. In addition, even the high-mass fragment ions of ECNI-MS in our study provided much better sensitivity than what was previously reported of the high-mass fragment ions [26]. It is possible that the performance of the ECNI source varies by instrument, even when analyzing the same compound.

Analysis of maternal plasma

After validation, the optimized GC/ECNI-MS method was used to determine 10 selected PBDE congeners in seven maternal plasma samples selected from the longitudinal study of autism risk MARBLES (Markers of Autism Risk in Babies-Learning Early Signs) currently underway UC Davis. Each sample was extracted and analyzed once. Error bars presented individual deviations. As shown in Figure 4, BDE-47 (0.088 – 0.459 ng/ml, average level: 0.248 ng/ml) was detected in all samples, contributing 52% total burdens of PBDEs. This is in agreement with other studies such as NHANES [34] and CHAMACOS [33], where BDE-47 was identified as the predominant PBDE congener in humans. Interestingly, the sum of tetra-BDE (BDE-47, -49, -52) concentrations (mean of seven individuals: 0.307 ng/ml) was 3.2 and 5-fold ($p < 0.01$) higher than the sum of penta- (mean of seven individuals: 0.096 ng/ml) or hexa-BDEs (mean of seven individuals: 0.062 ng/ml) concentrations, respectively. This is in contrast to their concentrations reported in the environment, where tetra-BDEs are not considered the major congeners [35].

Table 3: Analysis of NIST serum (SRM®1958) by established GC/ECNI-MS method.

Congener	Theoretical concentration (ng/ml)	Theoretical standard deviation (ng/ml)	Determined concentration (ng/ml)	Determined standard deviation (ng/ml)	Recovery rate (%)	RSD (%)
BDE-28	0.376	0.026	0.287	0.066	76.3	23.0
BDE-47	0.529	0.025	0.439	0.018	83.0	4.1
BDE-99	0.399	0.009	0.351	0.009	87.8	2.6
BDE-100	0.386	0.019	0.381	0.045	98.7	11.8
BDE-153	0.368	0.043	0.381	0.068	103.4	17.8
BDE-183	0.369	0.026	0.425	0.097	115.3	22.8

Table 4. Limits of detection and quantification for PBDE congeners using optimized GC/MS method.

PBDE Congener	EI $[M - 2Br]^+$ (fg)					ECNI $[Br]^-$ (fg)				
	LOD_i	LOD_m	LOQ_i	LOQ_m	LOQ of ref Ackerman	LOD_i	LOD_m	LOQ_i	LOQ_m	LOQ of ref Ackerman
BDE-28	264	306	871	1010	2880	3	14	11	47	NA
BDE-47	71	157	235	519	1590	6	9	20	30	118
BDE-49	147	313	486	1032	2210	6	8	19	28	238
BDE-52	357	899	1179	2966	NA	4	6	15	20	NA
BDE-95	170	269	566	898	NA	13	29	43	97	NA
BDE-99	154	237	515	791	1590	24	55	81	183	146
BDE-100	224	227	748	756	1710	14	31	45	102	62.6
BDE-136	332	640	1107	2135	NA	62	72	208	241	NA
BDE-153	425	637	1417	2123	732	56	64	186	212	97.2
BDE-183	648	702	2160	2341	1540	140	149	465	497	10.4

NA: Not applicable
LOD_i: Limit of detection of instrument; LOD_m: Limit of detection in matrix (human plasma)
LOQ_i: Limit of quantification of instrument; LOQ_m: Limit of quantification in matrix (human plasma)

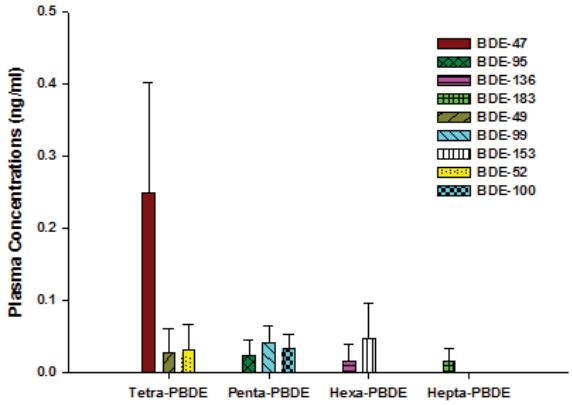

Figure 4: Concentrations of selected PBDEs in seven maternal plasma samples determined by validated GC/ECNI-MS method. Data shown is mean with standard deviation (n=7).

Conclusions

The source parameters of GC/EI-MS and GC/ECNI-MS were optimized and evaluated to provide high sensitivity and precision for the analysis of PBDE congeners in human plasma. While GC/EI-MS and GC/ECNI-MS are both suitable for PBDEs analysis, GC/ECNI-MS is approximately ten times more sensitive than GC/EI-MS. To meet the requirements for the detection of biologically meaningful concentrations in small volumes of human plasma, GC/ECNI-MS was superior to GC/EI-MS and was further validated according to method validation guidelines of the FDA by NIST serum. Special attention was given to chromatographic separation to achieve baseline separation for selected PBDE congeners. The GC/ECNI-MS method has been successfully applied to analyze small volume (0.5 ml) maternal plasma samples. Determined results showed that despite the commercial profile of PBDEs, tetra-PBDEs, especially BDE-47, were present in significantly higher concentrations in maternal plasma when compared to penta- and hexa-PBDEs.

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