Measurement of Selected Halogenated Contaminants in Human Serum and Milk using Comprehensive Two-Dimensional Gas Chromatography and Isotope Dilution Time-of-Flight Mass Spectrometry

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INTRODUCTION

A new method using comprehensive two-dimensional gas chromatography and isotope dilution time-of-flight mass spectrometry (GCxGC-IDTOFMS) for the simultaneous measurement of selected polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and brominated flame retardants (BFRs) is presented. Conversely to reference methods based on classical GC-MS, a single injection of the extract containing all species of interest conducts to accurate identification and quantification. GCxGC ensures the chromatographic separation of most compounds and TOFMS allows mass spectral deconvolution of co-eluting compounds as well as the use of ^{13}C-labeled internal standards for quantification. Isotope ratio measurements of the most intense ions for both natives and labels ensure the required specificity. Potentially interfering matrix compounds are usually kept away from the compounds to be measured in the chromatographic space. The use of this new method with automated sample preparation procedures developed at the Centers for Disease Control and Prevention (CDC) for the analysis of human serum and milk [1,2] compared favorably to conventional isotope-dilution one-dimensional gas chromatography-high resolution mass spectrometry (GC-IDHRMS) for the different sample pools that were tested

EXPERIMENTAL

Standards, Chemicals and Supplies. All standard solutions were purchased from Cambridge Isotope Laboratories (Andover, MS, USA). The EC-5022 PCB 10-points calibration standard solution contained a mixture of 38 native PCBs: IUPAC no. 18, 28, 44, 49, 52, 66, 74, 87, 99, 101, 105, 110, 118, 128, 138, 146, 149, 151, 153, 156, 157, 158, 167, 170, 172, 177, 178, 180, 183, 187, 189, 194, 195, 196, 201, 203, 206, 209 spanning the concentration range 0.5 to 1000 pg/µL; as well as $^{13}C_{12}$-labeled PCBs at a concentration of 75 pg/µL in nonane. The EC-5087 $^{13}C_{12}$-labeled PCB internal standard spiking solution contained $^{13}C_{12}$-labeled PCBs at a concentration of 7.5 pg/µL in methanol. The EO-5159 BFR 10-points calibration standard solution contained a mixture of native, spanning the concentration range 0.2 to 2000 pg/µL, and $^{13}C_{12}$-labeled analytes, at a concentration of 75 pg/µL. The solution contained the following PBDEs: IUPAC no. 17, 28, 47, 66, 85, 99, 100, 153, 154, 183, 203, 209, as well as CB-153 and the polybrominated biphenyl BB-153. The EO-5158 $^{13}C_{12}$-labeled

BFR internal standard spiking solution $^{13}C_{12}$-labeled compounds at a concentration of 7.5 pg/µL in methanol. The ES-5019 persistent OCP 8-points calibration standard solution contained the following mixture of native and ^{13}C-labeled analytes: HCB, *β*-HCCH, *γ*-HCCH, heptachlor epoxide, oxychlordane, *trans*-nonachlor, dieldrin, *o,p'*-DDT, *p,p'*-DDT, mirex, *p,p'*-DDE. Natives were in the concentration range of 5 to 1000 pg/µL, and $^{13}C_n$-labeled at a concentration of 100 or 250 pg/µL. The ES-5177 $^{13}C_n$- labeled OCP internal standard spiking solution contained (10 pg/µL in methanol) was used. We prepared the multi-analyte calibration solution (59 native compounds) by combining equal volumes of the EC-5022, EO-5159, and ES-5019 solutions and reducing the final volume. All details concerning consumables, as well as the glassware pre-cleaning are available elsewere [1,2].

Samples. Human serum samples corresponded to a pool collected from 15 individuals in 2002 in 3 U.S. cities (Philadelphia, PA; Memphis, TN; Miami, FL) and obtained from the Menphis, TN Interstate blood bank. A mixture of water (3.5 mL) and calf serum (0.5 mL) (Bibco BRL; Grand Island, NY) was used as serum blank. Three human milk pools were analyzed. Pool A was obtained from the Mothers' milk bank (Denver, CO) and was a composite pool of 2 individuals collected in 2002, pool B and C both corresponded to 10 specimens collected in 2003 in California and in North-Carolina, respectively. A 10-fold water diluted bovine milk obtained in a local supermarket was used as method blank samples.

Extraction and Cleanup. A semi-automated extraction and cleanup method has recently been developed for the measurement of the PCBs, OCPs, and BFRs in human serum and milk and is described in details elsewere [1-3]. Briefly, serum pre-treatment was carried out using the Gilson 215 liquid handler (Gilson, Middleton, WI). Solid phase extraction and cleanup were performed on a Zymark Rapidtrace Automated SPE workstation (Zymark Corp., Hopkinton, MA). Custom made Oasis HLB SPE cartridges (3 mL, 540mg) (Waters Corp., Milford, MA) were used for extraction of 4 mL serum samples. Removal of co-extracted biogenic materials was performed on a two layered custom made SPE cartridge (3 mL) packed with 100 mg silica and 1000 mg sulfuric acid silica. All extracts were evaporated using a Labconco Rapidvap evaporator (Labconco Corp., Kansas City, MO) and transferred to GC-vials prior GC-MS analyzes. Nonane was used as a keeper. QA/QC criteria, reproducibility and precision data, as well as details on the serum enzymatic lipid determinations are available in a previous report [1]. For human milk samples, the extraction step was performed on 1 g of milk by matrix solid phase dispersion using diatomaceous earth (3 mL cartridges, 900 mg) [2]. The amount of extracted lipids was determined gravimetrically on analytical balance. The same protocol as for serum was used for the remaining cleanup of human milk samples.

GC-IDHRMS analysis. They were performed on a MAT95XP instrument (ThermoFinnigan MAT, Bremen, Germany) interfaced with a 6890N gas chromatograph (Agilent Technologies, Atlanta, GA) fitted with a 15 m x 0.25 mm i.d. x 0.10 µm film thickness DB-5HT capillary column (J&W Scientific, Folsom, CA). Splitless injections were carried out with an injector temperature of 280^{o}C. The oven was programmed from 140^{o}C (1 min) to 320^{o}C with a ramp rate of 10^{o}C/min. The source temperature was 280^{o}C in the electron impact mode using a filament bias of 40 eV. Details of the GC-IDHRMS analyses are given elsewhere [1].

GCxGC-IDTOFMS analysis. The GCxGC-TOFMS instrument was the Pegasus 4D (Leco Corp., St Joseph, MI). This system was based on a non-moving quadruple jet dual stage modulator made of two cold nitrogen jets and two pulsed hot air jets responsible for trapping and refocusing of compounds eluting from the ^{1}D column.

This modulator was mounted in an Agilent 6890 GC oven and liquid nitrogen was used to create the cold jets. Details regarding the system have been reported elsewhere [4,5]. The automated 1.2 µL injections were performed using a MultiPurposeSampler MPS2 (Gerstel Inc., Baltimore, MD). The GC inlet temperature was 280°C for splitless injections. The purge time was 120 s at a flow of 20 mL/min. Carrier gas was helium and a constant flow of 0.8 mL/min was used. The GC column set used was made of the combination of a 15 m x 0.25 mm i.d. DB-1 100% dimethylpolysiloxane (J&W Scientific) with a film thickness of 0.25 µm as ^{1}D and a 1.2 m x 0.10 mm i.d. high temperature HT-8 (8% Phenyl)-polycarborane-siloxane (SGE, Austin, TX) with a film thickness of 0.10 µm as ^{2}D. Deactivated universal presstight connectors (Restek Corp., Bellefonte, PA) were used for connecting the capillary columns. Leak-free connections were ensured by careful inspection of the column cuts and cleanup of the column ends using acetone in order to remove any finger grease and soften the silica prior sealing. The connection was cured from 40°C to 320°C at a temperature ramp rate of 2°C/min and held at the final temperature for 2 hours. During chromatographic separation, the primary GC oven was programmed as follows: 90°C for 1 min, then to 150°C at 10°C/min, and finally to 300°C at 1°C/min. The ^{2}D column was coiled into the secondary oven that was 40°C higher than the primary oven and operated in the iso-ramping mode. The temperature of the modulator had an offset of 60°C compared to the temperature of the primary GC oven. Modulation was carried out on the very beginning of the ^{2}D column. The modulator period was 3 s (0.33 Hz modulation frequency) with a hot pulse duration set at 700 ms and a cool time between stages of 800 ms.

The transfer line connecting the secondary column and the MS source was operated at a temperature of 250°C. The source temperature was 250°C with a filament bias voltage of -70 V. The unit *m/z* resolution mass spectrometer was capable of acquiring 5000 transients/s, which results in 500 summed complete mass spectra/s for the mass range from 10 to 1000 *m/z*. The data acquisition rate was set at 60 spectra/s for a collected mass range ranging from 100 to 750 *m/z*. Table 1 lists the masses selected for quantification.

The detector voltage was 1800 V. Data collection and processing were achieved using the 2.10 version of Leco ChromaTOF^{TM} software provided with the instrument. Peak apex finding was performed automatically and further manually corrected when required. The combination of slices corresponding to a compound was performed by automatically comparing the mass spectra under pre-established match criteria. Spectral searching was performed using the NIST library available with the software, as well as through the custom built ^{13}C-labeled compound library.

RESULTS AND DISCUSSION

The last compound (BDE-154) eluted after 45 min (analytical speed of 1.3 analytes /min). Most of the compounds were chromatographically resolved from each other. Heptachlor epoxide and Tetra-CB-74, as well as Hepta-CB-189 and Octa-CB-196 were two co-eluting couples that required the use of the deconvolution capability to be resolved. Figure 1 shows chromatograms of a multi-analyte standard solution and a real sample extract. The calibration curve covered 3 orders of magnitude with instrument LODs of 1 pg/µL injected (S/N>3 for the base peak). An example of quantification is shown in Figure 2 for Mirex and hepta-CB-170. It appeared to be important to carry out the quantification on the entire set of slices corresponding to one analyte. The ratio between corresponding native and ^{13}C-label slices varies over

the peak cluster. As illustrated in Table 2, the ratio between native and ^{13}C-label was calculated after the summation of the area of all combined slices.

Table 1. List of masses used to reconstruct deconvoluted ion currents (DICs) during the GCxGC-IDTOFMS quantification process

Compounds	^{12}C-native ions (*m/z*)	^{13}C-labeled ions (*m/z*)
PCBs		
Tri-CBs	186+188+256+258+260	198+200+268+270+272
Tera-CBs	220+222+255+257+290+292+294	232+234+267+268+302+304+306
Penta-CBs	254+256+291+324+326+328	266+268+303+336+338+340
Hexa-CBs	288+290+292+358+360+362	300+302+304+370+372+374
Hepta-CBs	322+324+326+394+396+398	334+336+338+406+408+410
Octa-CBs	356+358+360+362+426+428+430+432	368+370+372+374+438+440+442+444
Nona-CBs	390+392+394+396+398+460+462+464+466+468	402+404+406+408+410+472+474+476+478+480
Deca-CBs	424+426+428+430+432+494+496+498+500+502	436+438+440+442+444+506+508+510+512+514
OCPs		
HCB	247+249+251+282+284+286	253+255+257+288+290+292
β- and *γ*-HCCH	217+219+221+252+254+256+288+290+292	223+225+227+258+260+262+294+296+298
Heptachlor epoxide	351+353+355+357+388+390	361+363+365+367+398+400
Oxychlordane	385+387+389+391+422+424+426	395+397+399+401+432+434+436
trans-Nonachlor	407+409+411+413+442+444+446+448	417+419+421+423+452+454+456+458
Dieldrin	261+263+265+343+345+347+378+380+382	275+277+279+355+357+359+390+390+394
o,p'- and *p,p'*-DDT	235+237+239	247+249+251
Mirex	235+237+239+241+270+272+274+276	240+242+244+246+275+277+279+281
p,p'-DDE	246+248+250+316+318+320	258+260+262+328+330+332
PBDEs		
Tri-BDEs	246+248+404+406+408+410	258+260+416+418+420+422
Tetra-BDEs	324+326+328+482+484+486+488+490	336+338+340+494+496+498+500+502
Penta-BDEs	402+404+406+408+562+564+566+568	414+416+418+420+574+576+578+580
Hexa-BDEs	480+482+484+486+640+642+644+646+648	492+494+496+498+652+654+656+658+660
Hexa-BB	466+468+470+545+547+549+624+626+628+630	476+478+480+557+559+561+636+638+640+642

Table 2. Variation in the native over ^{13}C-label ratio for the different slices of a peak cluster

			^{13}C-Label		Native		
Compound	$^{1}t_R$ (S)	$^{2}t_R$ (S)	area	S/N	area	S/N	Ratio[a]
BDE-99-1	2493	1.72	4755	22.00	786	4.00	0.17
BDE-99-2[b]	2496	1.68	20523	102.00	4351	20.00	0.21
BDE-99-3[c]	2499	1.71	18341	78.00	6335	26.00	0.35
BDE-99-4	2502	1.65	3940	21.00	2210	10.00	0.56
Sum	-		47559	-	13682	-	0.29

[a]Ratio of native over ^{13}C-label. [b]Base peak for ^{13}C-label. [c]Base peak for natives.

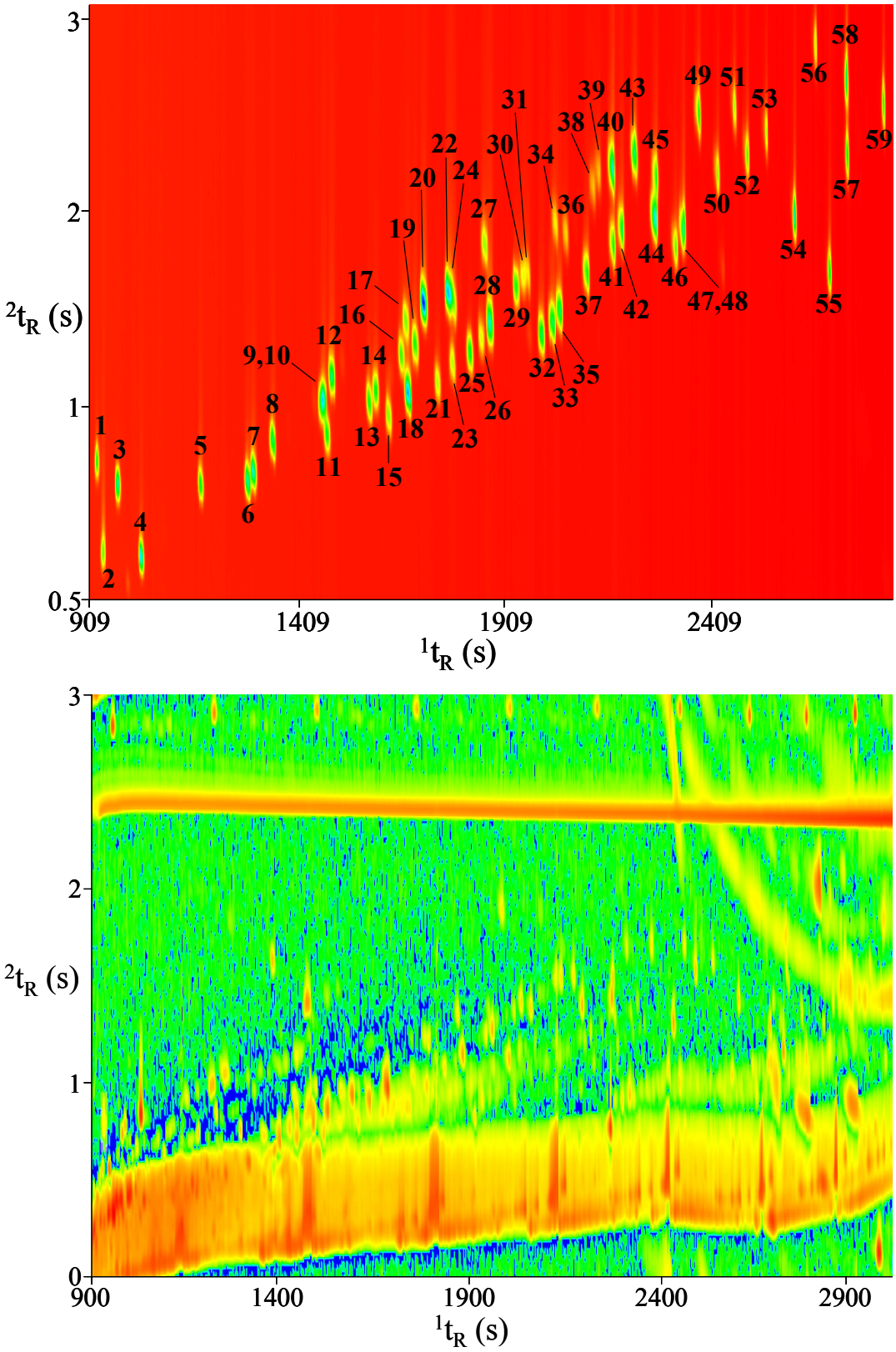

Figure 1. Comprehensive two-dimensional gas chromatograms for a contour plot of TIC chromatogram of a 100 pg/µL native compound multi-analytes calibration solution (top) and a real human serum sample (bottom).

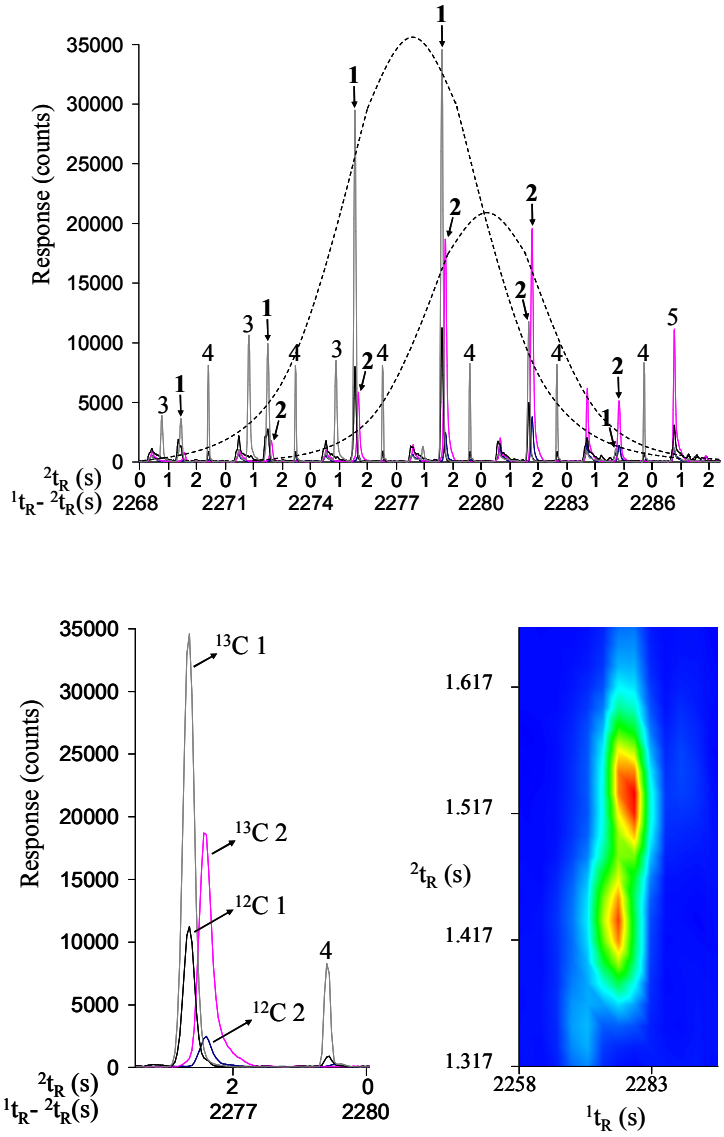

Figure 2. Region of the chromatogram of a real human serum sample (top) where Mirex and CB-170 elute. Extracted ion chromatograms are based on ions listed in Table 1 for those 2 compounds (native and labeled). Peaks are: (1) Mirex, (2) CB-170, (3) phthlalate, (4) siloxane bleed, (5) tetracosane. The dashed Gaussian shapes are artificial and are only shown to help to locate the elution windows of the 2 compounds. Expanded section (bottom) showing one of the slices illustrating chromatographic and mass spectral resolution of the 2 compounds and their corresponding ^{13}C labels (left) as well as the corresponding contour plots (right).

Human serum QC samples were used to build up QC charts for both GC-IDHRMS and GCxGC-IDTOFMS methods for all investigated analytes. Figure 3 illustrates the case of DBE-47, DBE-100 and CB-153. The SD values are higher with GCxGC-IDTOFMS partly because several slices of different intensities had to be integrated and also due to the low mass resolution of the TOFMS. Finally, OCP (Figure 4) and BDE (Table 3) data are shown for human serum and milk samples, respectively.

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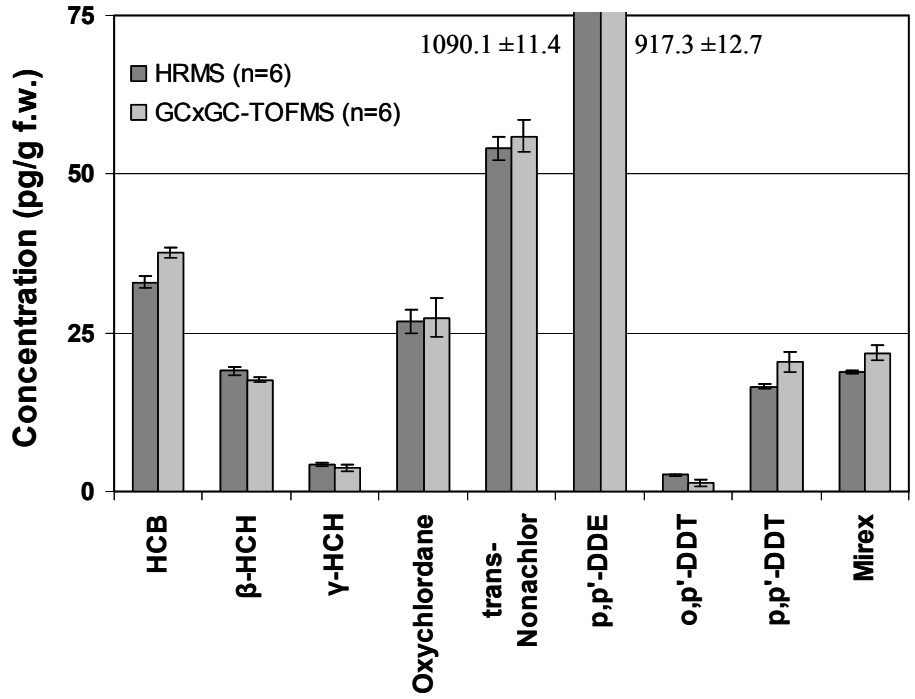

Figure 4. Comparison between GCxGC-IDTOFMS and the reference GC-IDHRMS for the measurement of OCPs in human serum samples.

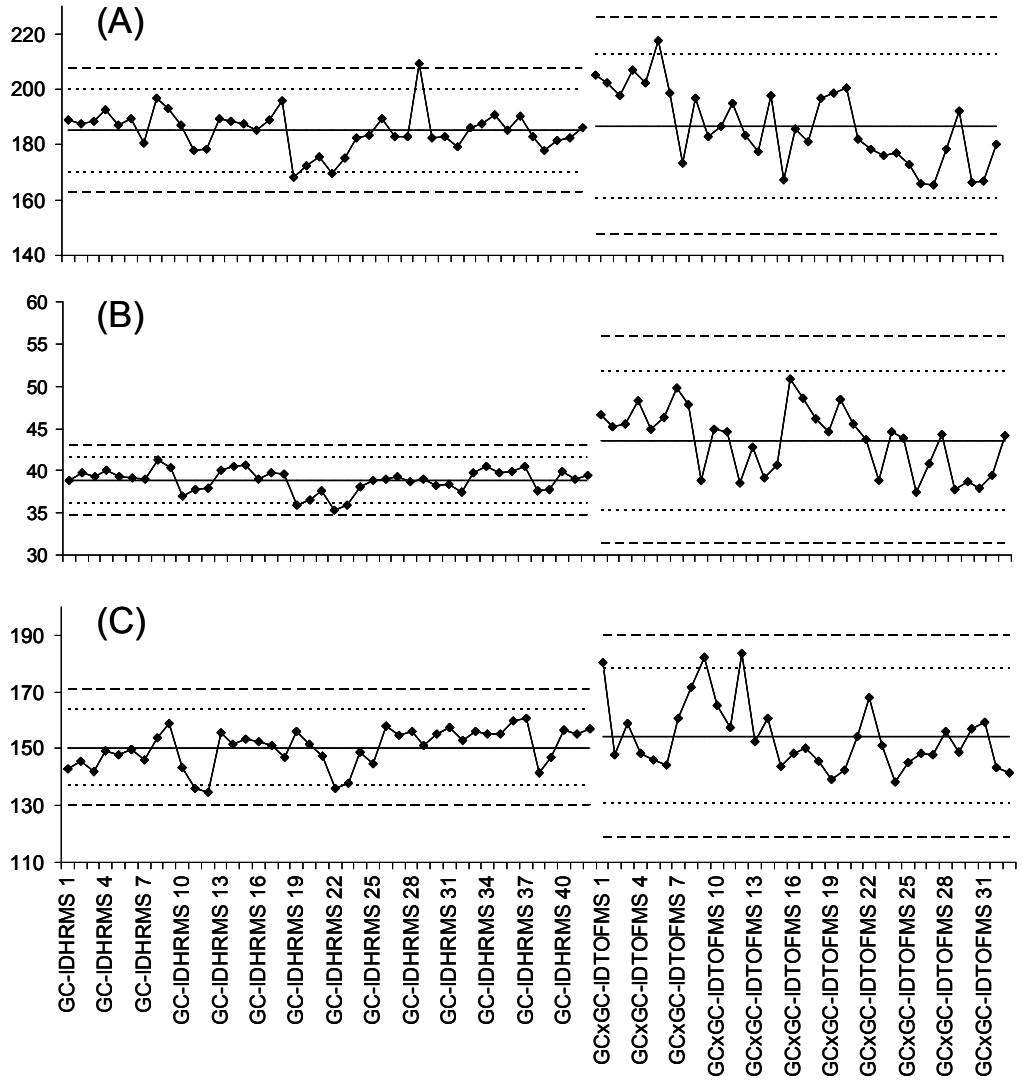

Figure 3. QC charts for BDE-47 (A), BDE-100 (B), and CB-153 (C).

Table 3. Comparison between the new GCxGC-IDTOFMS and the reference GC-IDHRMS method for the measurement (ng/g lipids) of selected BDEs in natural human milk pools

	Pool A					Pool B					Pool C				
	GC-HRMS n=3		GCxGC-TOFMS n=4		Dev.[b] (%)	GC-HRMS n=3		GCxGC-TOFMS n=4		Dev. (%)	GC-HRMS n=120		GCxGC-TOFMS n=4		Dev. (%)
Analyte	Mean	SEM[a]	Mean	SEM		Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM	
BDE-28	6.6	0.6	7.7	0.2	15.3	12.7	0.9	11.7	1.0	8.3	2.9	0.1	4.0	0.5	41.0
BDE-47	230.4	8.2	227.4	17.4	1.3	284.5	14.0	308.3	24.4	8.4	64.0	2.8	65.7	0.4	2.6
BDE-100	46.1	1.7	49.4	0.9	7.3	45.6	2.2	53.9	3.7	18.3	11.4	0.6	11.4	0.7	0.3
BDE-99	71.6	2.9	76.7	1.6	7.1	74.0	2.6	81.7	6.4	10.4	19.3	0.7	19.7	0.6	1.9
BB-153	0.7	0.1	1.0	0.3	52.7	1.2	0.0	1.6	0.5	27.7	6.7	0.4	9.0	0.7	33.6
BDE-154	5.9	0.2	4.9	0.4	17.3	3.7	0.2	4.7	1.1	27.3	1.1	0.0	0.9	0.2	18.3
BDE-85	7.9	1.0	6.1	0.3	22.9	6.9	0.2	7.4	0.8	7.1	1.6	0.0	-	-	
BDE-153	18.5	1.4	18.6	0.3	0.7	21.4	0.9	25.8	5.4	20.4	9.2	0.4	9.8	0.9	6.1
ΣBDEs	386.9	36.5	390.8	16.2	1.0	448.8	20.9	493.5	39.0	9.9	109.5	4.7	111.5	2.1	1.8

[a] SEM, standard error of the mean. [b] Deviation between the 2 methods.