DETERMINATION OF PBDEs IN HUMAN BREAST ADIPOSE TISSUES BY GAS CHROMATOGRAPHY COUPLED TO TRIPLE QUADRUPOLE MASS SPECTROMETRY

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ABSTRACT

The potential of gas chromatography / tandem mass spectrometry with triple quadrupole analyzer for determination of 12 polybrominated diphenyl ethers in human breast tissues has been investigated. After extraction with hexane, two purification procedures -automated normal-phase HPLC and solid phase extraction – were assayed. Both electron impact ionization, in selected reaction monitoring mode, and negative chemical ionization, in selected ion recording mode, were tested for the optimum determination of analytes. Isotopically labelled standards were added before extraction as surrogates: [^{13}C] BDE47, [^{13}C] BDE99 and [^{13}C] BDE153 for EI, and *p,p'*-DDE-D_8 for NCI.

The method was validated in terms of accuracy, precision, limits of detection and limits of quantification, using human breast tissue spiked at three levels in the range 1-50 ng/g (5-250 ng/g for BDE 209). The analytical approach using SPE clean-up followed by GC-MS (NCI) led to lower detection limits (0.006-2 ng/g) and allowed the determination of the most problematic congener, BDE 209, whose poor sensitivity made difficult its determination at low residue levels. Special attention was given to the confirmation of the compounds detected in samples in order to avoid reporting false positives. Two MS/MS transitions or three m/z ions were selected for each analyte when using EI or NCI modes, respectively. In both cases, the transition/ion intensity ratio was used as confirmation parameter. The developed methodology was applied to the analysis of real human samples. Several BDEs (BDEs congeners 47, 100, 99, 154, 153 183 and 209) were detected in the range of 0.08-0.23 ng/g.

Keywords

Polybrominated diphenyl ethers, gas chromatography tandem mass spectrometry, human breast adipose tissue, triple quadrupole analyzer, electron impact, negative chemical ionization

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are structurally similar to polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs). They have a large number of congeners depending on the number and the position of the halogenated atoms on the two-phenyl rings. The total number of possible congeners of PBDEs is 209, going from mono to deca BDEs. These compounds have been widely used as reactive flame retardants in different consumer products and electronics **[1].** Toxicity studies show that environmental concentrations of PBDEs can produce thyroid hormone disruption, affect learning and memory functions in adults and induce developmental neurotoxic effects **[2-4].** Estrogenic effects of PBDEs have also been reported **[5].** So, these compounds are potentially negative for human health.

The resistance of PBDEs to degradation and their high lipid solubility are the cause of their persistence and bioaccumulation in the environment and along the food chain. PBDEs have been studied in different environmental samples like water, air particles, soil, sediments, and sewage sludge samples **[6].** The presence of BDEs in biotic samples has been reported by several authors. These compounds have been detected in animal tissues, including dolphin, seal and whale **[7]** and human biological samples as serum **[8, 9],** maternal milk **[10, 11]** and adipose tissues **[8, 12-14]**. In many cases, the concentrations of PBDEs were reported to be increasing over time.

Typically, tri- to hepta-BDEs have been detected in biological samples, including human adipose tissues **[15, 16].** Although BDE209 has been found in serum and in human adipose samples, the concentrations reported are normally at ng/g or sub-ng/g levels **[9, 11]**, its determination being difficult due to the decomposition in lower congeners and to its poor gastrointestinal adsorption **[7]**; thus, high sensitive methods are required to search BDE209 in human samples. Several reviews about the analysis of

PBDEs in different types of samples have been published in the last five years **[17-19].** Most recently, Covaci et. al. **[6]** have reviewed new developments in the analysis of PBDEs.

The determination of PBDEs in fatty samples usually requires a first sample pretreatment to dissolve the lipids in an appropriate solvent, followed by their extraction, which can be carried out by LLE or SLE with apolar solvents **[9],** Soxhet extraction **[20]**, column extraction with a mixture of apolar solvents **[21]** or sonication with appropriate solvent **[22]**. Alternative enhanced extraction techniques, such as pressurized liquid extraction or microwave assisted extraction have also been used **[23, 24].** The complexity of extracts requires further purification which can be made by gel permeation chromatography **[25, 26],** Florisil or acidified silica gel column chromatography **[27-29],** and automated normal-phase HPLC **[30].**

Nowadays, the most usual technique for the analysis of PBDEs is GC-MS. The selection of characteristics of the GC-system (stationary phase, column length, injection technique…) has a strong influence on the accuracy and precision of the analysis **[31]**. So, if chromatographic conditions are not correctly selected, low yields for nona- and deca-BDEs and poor precision for congeners with more than five bromine atoms can be obtained.

GC-MS methods using both EI **[12, 20, 32]** and NCI **[25, 26, 33]** have been proposed for the determination of PBDEs. However, several potential chromatographic interferences can hamper good quality data **[6]**. Thus, when working in EI-MS potential interferences originate from chlorinated compounds, like PCBs. When using NCI-MS, where only $[Br]^-$ ions are monitored, other brominated compounds might also interfere with the PBDEs determination. High resolution mass spectrometry (HRMS) is a good

option **[34]** to reach high sensitivity, selectivity and accuracy but at a considerable higher cost.

Tandem mass spectrometry (MS/MS), using ion trap detectors (ITD) or triple quadrupole (QqQ) analyzers, can be an interesting alternative to high resolution devices due to the specificity of MS/MS, which allows an improvement in selectivity and also in sensitivity **[8, 30, 35]**. Whereas ITD has been used for the trace analysis of PBDEs in differents types of samples **[8, 34]**, GC-MS/MS with QqQ analyzer has been rarely explored for the analysis of PBDEs in human samples, where high sensitive techniques are required. The use of QqQ in selected reaction monitoring (SRM) mode provides one of the most sensitive and selective techniques for the analysis of organic contaminants especially at low concentrations.

In the last years, GC-MS/MS with QqQ has been mainly applied to the determination of pesticides in fruits and vegetables **[36-38]** and in food samples **[39-41]** as well as several organic pollutants in environmental samples **[35, 42]**, providing excellent sensitivity, selectivity and gain on analysis time, and allowing the simultaneous determination, and confirmation of quite different target analytes. Recently, our own research group has also proved the efficiency of this technique for the reliable determination of organic pollutants in water **[35]** and xenoestrogen compounds in human breast tissues **[30]**.

The aim of this work is the development of analytical methodology for the sensitive determination and identification of PBDEs in human breast tissues based on the use of GC-MS/MS with QqQ analyzer. The PBDEs congeners most frequently detected ((BDEs congeners 28, 47, 66, 71, 85, 99, 100, 138, 153, 154, 183 and 209) have been included in the study. The application of two different clean-up procedures, based on normal phase HPLC and SPE, and the use of EI and NCI modes are investigated.

EXPERIMENTAL

Reagents and chemicals

Polibrominated Diphenyl Ether Analytical Standard Mixture "Lake Michigan Study", containing one triBDE (BDE28), two tetraBDEs (BDE 47 and 66), three pentaBDEs (BDE 85, 99 and 100) and three hexaBDEs (BDE 138, 153 and 154) (ca. 50 μg/mL in isooctane) was purchased from Chiron (Trondheim, Norway). Individual standards of BDE 71 (tetraBDE) and BDE 183 (heptaBDE) (50 μg/mL in isooctane each one) were supplied by Chiron, whereas BDE 209 (decaBDE) (50 μg/mL in isooctane:toluene (9:1)) was provided by Accustandard (New Haven, USA).

A standard mixture solution containing these 12 BDEs at a concentration level around 2.5 μg/mL, (except BDE209, at 5 μg/mL) was prepared in hexane and stored at 4° C. Working solutions were prepared by diluting this solution in hexane and stored at 4 ºC.

Two solutions of labeled compounds were used as surrogates. In EI experiments, a mixture containing one tetraBDE ([^{13}C] BDE47), one pentaBDE ([^{13}C] BDE99) and one hexaBDE ([^{13}C] BDE153), all purchased from Wellington Laboratories (Guelph, Ontario, Canada), was used. In NCI experiments, *p,p'*-DDE-D_8, purchased from Dr. Ehrenstofer (Augsburg, Germany), was used as surrogate. Working solutions of labeled standards (ca. 500 ng/mL for BDEs and 1 μg/mL for DDE) were prepared by dilution of the stock solutions with hexane and stored at 4 °C.

Ethyl acetate and *n*-hexane (ultra-trace quality) were purchased from Scharlab (Barcelona, Spain). Anhydrous sodium sulfate of pesticide residue quality (Scharlab) was dried for 18 hours at 300 ° C before use.

1 g Strata cartridges silica (Phenomenex, USA) were used for SPE.

Sample material

Human breast tissues were obtained from women with breast cancer with the exception of two samples that corresponded to healthy women**.** Samples were collected from the Oncology Institute of Cancer, at Valencia (FIVO). After collecting the samples, they were frozen at approximately -30° C until analysis. A pooled sample obtained by mixing several breast tissue samples was used as a "blank" to optimize the analytical procedure.

Equipment

LC Instrumentation. The LC system used for sample extracts clean-up was based on our previous work **[30]**. It consisted on a LC Pump Master 305 (Gilson), two six-way high-pressure valves VICI Valco (Europe Instruments, Schenkon, Switzerland), a sampler injector valve Rheodyne (Cotati, CA) with 1.0 mL loop, a silica column Novapack 150 x 3.9 mm i.d., 4µm (Waters, Mildford, MA), and a fraction collector Gilson FC 203B. Mobile phase used was hexane at a flow rate of 1 mL/min.

GC Instrumentation. A GC system (Agilent 6890N, Palo Alto, USA) equipped with an autosampler (Agilent 7683) was coupled to a triple quadrupole (QqQ) mass spectrometer, Quattro Micro GC (Micromass, Boston, USA), operating in EI and CI modes. The GC separation was performed using a DB-1HT capillary column with a length of 15 m x 0.25 mm i.d. and a film thickness of 0.1 µm (J&W Scientific, Folson, CA, USA). The oven temperature was programmed as follows: 140 ºC (1 min); 10 ºC/min to 220 ºC; 20 ºC/min to 300 ºC, 40 ºC/min to 340 ºC (5 min) and the injector temperature was 260 ºC. Splitless injections of 1 µL sample were carried out. Helium 99.999% (Carburos Metálicos, Valencia, Spain) was used as carrier gas at a flow of 1

mL/min. The interface temperature was set to 250 ºC and a solvent delay of 3 min was selected.

Working in EI, the source temperature was set at 250 ºC and the system operated in MS/MS (SRM) mode using argon 99.995% (Carburos Metálicos) as collision gas at a pressure of 0.28 Pa in the collision cell. Dwell times/channel between 0.05 to 0.3 s was chosen.

Working in NCI, the source temperature was set at 200ºC and the QqQ system operated in SIR mode. Methane 99.9995% (Carburos Metálicos) was used as reagent gas with an optimal flow of 60%.

The application manager Quanlynx was used to process the quantitative data obtained from calibration standards and from samples.

Analytical procedure. Sample preparation and extraction

Samples were thawed at room temperature. Approximately 1 g of tissue sample was spiked with 0.5 mL of surrogate labelled solution. The mixture was homogenized with 5-10 g of anhydrous sodium sulfate and extracted three times with 5 mL of *n*-hexane each time, shaking in a vortex. After filtration, the extract was preconcentrated under a gentle nitrogen stream at 40° C, and the final residue was adjusted to 10 mL with *n*-hexane.

Clean-up procedures

Two clean-up procedures were investigated. The first one was based on previous work carried out in our laboratory **[30].** The sample hexanic extract was purified by injecting 1 mL into the HPLC system. The mobile phase was *n*-hexane, at a flow rate of 1

mL/min. After 16 min, a pulse of 4 mL of modifier solvent (ethyl acetate) was introduced. The fraction eluting between minutes 2 and 8 was collected and then it was preconcentrated under a gentle nitrogen stream at 40 ° C to dryness, and redisolved in 0.5 mL of hexane.

The second procedure consisted into a SPE clean-up. 10 mL of the sample hexanic extract were passed through the silica SPE cartridge previously conditioned by passing 6 mL of hexane. The first 3 mL were discarded and the rest -approximately 7 mL- were collected together another additional fraction eluted by passing 3 mL of hexane. The cleaned-up extract was preconcentrated to dryness under a gentle nitrogen stream at 40°C and re-dissolved in 0.5 mL hexane.

GC Analysis

The final extracts obtained after clean-up procedure were injected into the Quattro Micro GC system working in (EI) MS/MS or in (NCI)MS mode under the experimental conditions shown in **Tables 1** and **2**, respectively. Quantification of samples was carried out by using calibration curves prepared with standards in solvent, using relative responses to the corresponding labeled internal standards (IS) added as surrogates to the samples. Three surrogates were used in EI experiments: [^{13}C] BDE47 for tri- and tetra-BDEs; [^{13}C] BDE99 for penta-BDEs and [^{13}C] BDE153 for hexa- and hepta-BDEs; while in NCI analysis *p,p'*-DDE-D_8 was used as surrogate for all congeners. The selection of each IS was made according to its retention/elution behavior in the clean-up procedure and to its gas chromatographic retention time.

Method validation

Validation of the method was performed evaluating the following parameters**:**

Linearity. The calibration curves were obtained by injecting reference standard solutions in duplicate. The concentration range tested was 0.1-40 µg/L for all congeners with the exception of BDE 209 (0.5-200 µg/L). Linearity was assumed when regression coefficient was >0.99 with residuals lower than 30 %.

Accuracy. It was evaluated by means of recovery experiments, analyzing blank breast tissue samples spiked at three levels: 1, 10 and 50 ng/g (5, 50 and 250 ng/g for BDE209), (n=5 each level). Previously, the blank sample was analyzed (n=5) to determine the analytes' concentration.

Precision. Precision, expressed as repeatability of the method, was determined in terms of relative standard deviation (R.S.D., in %) from recovery experiments at each fortification level (n=5, each).

Limit of Quantification (LOQ). The LOQ was firstly established as the lowest concentration that was validated following the overall analytical procedure with satisfactory recovery (70-110%) and precision (<20%). However, in NCI analysis, where the sensitivity was excellent, this value could be notably lowered leading to a more realistic LOQ. In this case, LOQ was statistically estimated for a signal-to-noise (S/N) = 10 from the chromatogram of samples spiked at the lowest fortification level tested, i.e. 1 ng/g.

Limit of Detection (LOD). The LOD value was estimated, from the quantification transition (EI) or ion (NCI), as the analyte concentration that produced a peak signal of three times the background noise from the chromatogram at the lowest fortification level tested. In the case of analytes showing higher sensitivity (congeners 28, 71, 47,

and 100), making the measurement of the noise manually unfeasible, the LOD was obtained using a software option for estimating the S/N ratio and referring/recounting this value to a S/N value of three.

Limit of confirmation (LOC). The LOC was estimated in the same way than LOD but considering the peak signal corresponding to the confirmation transition or ion.

Confirmation criteria. The Q/q ratio, defined as the ratio between the concentration obtained from the quantification transition (EI mode) or ion (NCI mode) (Q) and from the confirmation transition/ion (q), was used to confirm peak identity in samples. A safe confirmation was assumed when the Q/q concentration ratio was found to be between 0.8 and 1.2, i.e. a maximum tolerance of ± 20% was accepted to confirm a finding as an actual positive. Obviously, the agreement in the retention time in sample and reference standard was also required to confirm a positive.

RESULTS AND DISCUSSION

GC-MS optimization

Optimization of GC-MS methods was performed by injecting hexanic standard solutions. First experiments were carried out using a fused-silica HP-5MS capillary column (30m x 0.35mm i.d. and 0.25 μm film thickness), but the results for BDE183 (hepta-brominated) and BDE209 (deca-brominated) were not satisfactory. This was probably due to partial or total degradation, as it has been pointed out by several authors **[33, 43]** who recommend the use of shorter columns with thinner film thickness. The best results where obtained with a fused-silica capillary column of 15m x 0.25 mm i.d. and 0.1 μm film thickness, and using a stationary phase of 100% methyl polisiloxane (DB-1HT), which can stand temperatures higher than 300 ºC required to elute higher brominated BDEs **[44].**

Using the selected column, the temperature program was optimized in order to achieve a satisfactory resolution and peak shape for the twelve PBDEs studied. It was necessary to set a high initial temperature (140 ºC) and final ramp (40 ºC/min) in order to avoid degradation of higher brominated congeners. A final temperature of 340ºC, with a holding time of 5 minutes, was required to elute BDE209.

GC-MS/MS method in EI ionization mode

Optimization of the MS/MS method was performed using triple quadrupole MS operating in EI ionization mode. Full scan spectra for all PBDE congeners showed the $[M]^+$ and/or $[M\text{-}Br_2]^+$ isotopic clusters as majoritary ions, so they were selected as precursor ions for every analyte. Different values of collision energy (between 10-60 eV) were tested to perform the subsequent fragmentation of selected precursor ions. The final purpose was to develop a SRM method with at least two MS/MS transitions,

normally the most sensitive ones, for each compound in order to have a reliable confirmation of the identity of the analyte.

The dwell time parameter was also optimized between 0.05 and 0.3 s in order to obtain a good chromatographic peak (with at least 10 points per peak) maintaining satisfactory sensitivity for each compound.

Table 1 shows the precursor and product ions corresponding to the quantitative (Q) and confirmative (q) transitions monitored in EI ionization mode. Optimum values of collision energy were found to be normally around 20 eV for the low brominated compounds, increasing for high brominated compounds until values as 50 eV.

Linearity of relative response of analytes was established by analyzing hexanic standard solutions, in duplicate, in the ranges 0.4-8 µg/L and 2-40 µg/L. Regression coefficients above 0.995 were obtained for all the compounds with residuals lower than 20 %.

GC-MS method in NCI mode

When working in NCI mode it was not feasible to developed a MS/MS method because only the clusters from the mass fragments $[Br]^-$ and $[HBr_2]^-$ were observed in the full scan spectra. The molecular cluster was not observed or constituted a minor peak; therefore, the unique transition feasible for the majority of compounds was the fragmentation of $[HBr_2]^-$ to give a bromine atom, with low sensitivity and poor selectivity. As a consequence, a SIR method was optimized monitoring the three most intense peaks of the mass spectra, which corresponded to *m/z* 79 ($[^{79}Br]^-$), *m/z* 81 ($[^{81}Br]$) and *m/z* 161 ($[H^{79}Br^{81}Br]$). The *m/z* 79 ion was used for quantification purposes and the other two ions were used for confirmation. BDE209 showed a different behavior, as its full scan spectra did not show the $[H^{79}Br^{81}Br]$ fragment. For this

congener the three most intense peaks were: *m/z* 79 ($[^{79}Br]^-$) -used for quantification- and *m/z* 487 $[C_6O^{79}Br_3{}^{81}Br_2]^-$ and *m/z* 81 ($[^{81}Br]^-$) -used for confirmation.

The NCI method was optimized injecting hexanic standard solutions. Different values of source temperature (100-150 ºC), electron energy (30-100 eV), emission current (100-500 μA) and methane flow (20-80%) were tested in order to improve the sensitivity, the optimum values being 200 ºC, 50 eV, 400 μA and 60%, respectively.

As the ions monitored in SIR method are the same for isotopically labeled (^{13}C) congeners, it was necessary to select another compound as surrogate. Based on the results obtained in our previous work **[30],** *p,p'*- DDE-D_8 was selected for analysis performed by NCI. The temperature program was slightly modified, decreasing the initial temperature to 120 ºC, in order to elute adequately the new surrogate.

As a summary, **Table 2** shows the quantitative (Q) and confirmative (q) *m/z* ions and the dwell time value selected for every compound.

Linearity of relative response of analytes was tested analyzing hexanic standard solutions, in duplicate, in the ranges 0.1-8 μg/L and 2-40 μg/L (2-40 μg/L and 10-200 μg/L for BDE209). Regression coefficients above 0.995 were obtained for all the compounds, except for BDE209 that was 0.993.

Clean-up optimization

First, we applied a clean-up procedure by automated normal-phase HPLC based on our previous work on human adipose tissues analysis **[30]**. 1 mL hexanic extract (0.1 g sample/mL) was injected into the LC-system, and every 1-mL fraction eluted with hexane was analyzed in order to determine the presence and recovery of analytes. Data obtained showed that all compounds and IS were collected in the fractions between minutes 2 and 8.

Once optimized the GC-MS measurement, the application of this clean-up procedure led to LODs around 5 ng/g. This value was considered too high for real samples; consequently, a second clean-up based on the use of SPE silica cartridges was optimized in order to improve sensitivity. 10 mL of a mixed hexanic standard solution of PBDEs, 100 ng/mL each, were loaded into the silica cartridge and every 1-mL fraction, eluted with hexane, was analyzed by GC-MS. Data obtained showed that, after discarding the first 3 mL, all analytes eluted in the next 10 mL. This procedure was subsequently applied to hexanic sample extracts in order to evaluate the fat content in the analytes' fraction, which was found to be approximately 30% of the total amount loaded into the cartridge. According to our experience, this amount of fat can be injected into the GC-MS without relevant damages neither in the chromatographic system or in the MS detector.

Validation results

Sample used in the validation process consisted of a pool of several human adipose tissue samples. This sample was previously analyzed (n=5), and any of the BDE congeners studied in this work were detected. So it was used as a blank in subsequent experiments.

Validation was carried out in terms of precision, accuracy, LOQs and LODs. Considering that two transitions or several ions were monitored for a reliable identification, both LOCs and Q/q ratios, were also evaluated. Labeled internal standards were added at the initial stage of the procedures as quality control (i.e. used as surrogates) in order to correct for possible losses along the overall procedure and/or instrumental deviations.

EI(MS/MS)procedure

Precision and accuracy were estimated by analyzing five replicate blank samples spiked at three concentration levels each: 1, 10 and 50 ng/g. Because of sensitivity differences, validation at the lowest level (1 ng/g) was only performed by applying the SPE clean-up procedure, while the LC clean-up procedure was applied to the other two levels (10 and 50 ng/g).

Recoveries were satisfactory, with average values between 70 and 120 % at the three levels tested, with the only exception of two congeners (BDE71 and BDE138) at the lowest level of fortification (recoveries around 60%). Precision was also satisfactory, with R.S.D. lower than 10% in the majority of the cases. **(Table 3)**

Application of the HPLC automated clean-up did not allow us to reach enough sensitivity to analyze real adipose tissue samples, where total PBDE levels found usually ranged between 0.3 and 70 ng/g **[16]**. However, the alternative method of

purification based on SPE led to a sensitivity about ten times higher, allowing the detection of analytes in real samples.

LODs were calculated from the quantification transition (Q) and were around 1 ng/g (SPE clean-up) or varied between 2-10 ng/g (HPLC clean-up). It can be pointed out that LOCs (obtained in a similar way to LOD, but considering the confirmation (q) transition) were quite similar to LODs, which means that confirmation of analytes was feasible at the same level than their detection. LOQs were established as the lowest level validated level (10 and 1 ng/g when using HPLC automated and SPE clean-ups, respectively), except for BDE28, whose greater response allows to obtain lower LOQ values (5 and 0.3 ng/g, respectively), which were estimated for an S/N=10. These values were considered too high for quantification of real samples. In the particular case of decabrominated BDE 209, the sensitivity was not sufficient for its determination at lows levels, requiring the application of the NCI (MS) mode.

In relation to Q/q concentration ratios, data obtained were excellent at the three levels of fortification, ranging between 0.84-1.14 (i.e. deviations bellow ±20%) and R.S.D.s were lower than 10%, except for some of the higher brominated congeners, possibly due to the lower sensitivity for these compounds.

Figure 1 shows the GC-MS/MS chromatograms for a blank sample fortified at the lowest level validated, i. e. 0.1 ng/g (5 ng/g for BDE209), after application of the SPE clean-up. As can be seen, BDE209 congener could not be detected at this low level.

NCI (MS) procedure

The use of NCI in the determination of halogenated compounds like PBDEs leads to an increase in sensitivity respect to EI ionization, as several authors have reported **[14, 17]**. However, PBDEs MS spectra provides poor information with only two ions (Br^- and HBr_2^-), and determination of analytes in this mode may seem not sufficiently specific. It

must be pointed out that only a limited number of analytes containing one or more bromine atoms can be present in human adipose tissues analyzed by GC and ionized in NCI mode **[45]**, so the selectivity for these compounds is relatively high.

Validation of the overall procedure was carried out in a similar way than for EI, using in this case *p,p´*-DDE-D_8 as surrogate. Recoveries for BDE209 were quite good (98, 106 and 109%) without the necessity of the expensive use of [^{13}C]-labelled BDE 209 as reported in the literature **[6].**

Overall data for accuracy and precision were satisfactory at the three fortification levels tested, with recoveries between 70 and 110% and RSDs better than 15% for almost all compounds. **(Table 4)**

In relation to LODs, the NCI method led to values around 20 times lower than for EI mode, and ranged between 0.006-0.15 ng/g. These values are similar, and in some cases suppose an improvement, to data recently published **[6, 32, 46]**. In the particular case of BDE209, the LOD was found to be 0.5 ng/g. LOCs were quite similar to LODs, making feasible the confirmation of analytes at the detection level. In relation to LOQs, it seems quite evident that concentrations much lower than the lowest level validated (1 ng/g) could be quantified in the light of the high sensitivity of the method. In this case, LOQ values were estimated for S/N=10 from chromatograms of samples spiked at the lowest level tested. Values obtained ranged between 0.02 and 0.5 for tri- to hepta-BDEs, allowing the quantification of the concentrations typically found in human adipose samples **[15, 47]**.

Two set of values of Q/q concentration ratios were obtained: the first (Q/q_1) was calculated for the ions 79 [^{79}Br]$^-$ and 161 [H^{79}Br^{81}Br]$^-$ (except for BDE 209, ions 79 and 487[$C_6O^{79}Br_3{}^{81}Br_2$]$^-$), and the second (Q/q_2) for the ions 79 and 81 [^{81}Br]$^-$ for all compounds, BDE209 included. Experimental Q/q ratios were satisfactory and ranged

between 0.83-1.2, with the exception to BDE85 (1.3); so deviations were below ±20% and their RSD were mostly lower than 10%.

In conclusion, the NCI method led to a considerable improvement in sensitivity, making the detection of PBDEs at low levels feasible. As an illustrative example, **Figure 2** shows the SIR chromatograms corresponding to blank sample fortified at 1 ng/g level (5 ng/g for BD209) after applying the SPE clean-up followed by NCI (MS) analysis.

Application to real samples

The SPE clean-up followed by GC-(NCI) MS procedure was applied to the analysis of 15 human breast adipose tissue samples. The BDE congeners 47, 100, 99, 154, 153, 183 and 209 were identified in several samples, which is in accordance with data found in the literature **[12, 46-48]**. BDE 47, BDE99 and BDE153 were the most frequent congeners detected, (13, 10 and 14 out of 15 samples analyzed, respectively). BDE 100 was detected in 5 samples, and BDE 154, BDE 183 and BDE209 only were found in 2 samples. Concentrations of the mono- to hexa-BDEs ranged between 0.08-0.23 ng/g, these values being comparable to concentrations found in adipose tissues from Spanish population that ranged between 0.001-3 ng/g **[13]** and <0.07-6 ng/g **[28]**. Hepta- and deca-BDE could not be quantified because of their low concentrations, although they were detected in 2 out of 15 samples analyzed.

Illustrative chromatograms for real samples analyzed are shown in **Figure 3**. This figure illustrates the detection of several PBDEs at low levels. A reliable identification of analytes in samples was feasible by means of the Q/q experimental ratios, which varied between 0.84 and 1.2 in all findings

Moreover, in 12 of the samples analyzed one peak that did not correspond to any of the target compounds was observed (retention time 7.99 min). In addition, in four of these samples two additional peaks (at 8.94 and 14.59 min) were also present. We assumed that these peaks corresponded to BDE congeners not included in the study, although their identification was not feasible as reference standards were not available in our laboratory. Looking at the retention times, an estimation of the number of bromine atoms might be made. The peak at 7.99 min might correspond to a tri-congener, whereas the other two peaks might be tetra- and nona-BDEs, respectively.

CONCLUSIONS

Efficient and advanced analytical methodology has been developed for the determination of 12 BDE congeners, including BDE209, in human breast adipose tissues. The most difficult task of this work has been to determine BDE209, due to its partial degradation, which requires special chromatographic conditions.

Firstly a rapid method, based on GC-(EI)MS/MS with QqQ analyzer, has been developed for quantification and confirmation of all congeners studied, except BDE209, in one single determination step with chromatographic runs around 19 minutes. This method was satisfactorily validated in samples spiked at 50 ng/g and 10 ng/g, which were subjected to automated normal phase HPLC clean-up, previously to the GC-MS analysis. The fortification level could be lowered to 1 ng/g when applying a SPE clean-up procedure with silica cartridges. Later, a GC-(NCI)MS method was developed, which allowed a notable sensitivity improvement. It was validated at the same levels, obtaining satisfactory results for all congeners studied, including BDE209. The lowest LODs (0.006-0.5 ng/g) were obtained with this method, after performing the purification step by SPE. The overall method proposed in this work (extraction with hexane followed by SPE clean-up and analyses by GC-(NCI)/MS) was applied to the analysis of real human breast tissue samples, leading to the finding of several BDEs at low levels. The BDE209 congener, which has been rarely studied and detected in human samples, was also found in several of the samples analyzed.

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REFERENCES

1. Rahman F, Langford KH, Srimshaw MD, Lester JN (2001) Sci Total Environ 275:1-17

2. McDonald TA (2002) Chemosphere 46:745-755

3. Hallgren S, Darnerud PO (2002) Toxicology 177:227-243

4. Eriksson P, Jakobsson E, Fredriksson A (2001) Environ Health Perspect 109:903-908.

5. Meerts IATM, Letcher RJ, Hoving S, Marsh G, Bergman Å, Lemmen JG, van der Burg B, Brouwer A (2001) Environ Health Perspect 109:399-407

6. Covaci A, Voorspoels S, Ramos L, Neels H, Blust R (2007) J Chromatogr A 1153: 145-171

7. Hyötyläinen T, Hartonen K (2002) Trends Anal Chem 21:13-29

8. Gómara B, Herrero L, Bordajandi LR, González MJ (2006) Rapid Commun Mass Spectrom 20:69-74

9. Cariou R, Antignac J-P, Marchand P, Berrebi A, Zalko D, Andre F, Le Bizec B (2005) J Chromatogr A 1100:144-152

10. Kazda R, Hajšlová J, Poustka J, Ĉajka T (2004) Anal Chim Acta 520:237-243

11. Inoue K, Harada K, Takenaka K, Uehara S, Kono M, Shimizu T, Takasuga T, Senthilkumar K, Yamashita F, Koizumi A (2006) Environ Health Perspect 114:1179-1185

12. Li QQ, Loganath A, Ghong YS, Obbard JP (2005) J Chromatogr B 819:253-257

13. Fernandez MF, Araque P, Kiviranta H, Molina JMM, Rantakokko P, Laine O, Vartiainen T, Olea N (2007) Chemosphere 66:377-383

14. She J, Petreas M, Winkler J, Visita P, McKinney M, Kopec D (2002) Chemosphere 46 :697-707

15. Hites RA (2004) Environ Sci Technol 38:945-956

16. Richardson S (2006) Anal Chem 78:4021-4046

17. Covaci A, Voorspoels S, de Boer J (2003) Environnent International 29:735-756

18. Eljarrat E, Barceló D (2004) Trends Anal Chem 23:727-736

19. D´Silva K, Fernandes A, Rose M (2004) Crit Rev Environ Sci Technol 34:141-207

20. Johnson-Restrepo B, Kannan K, Rapaport DP, Rodan BD (2005) Environ Sci Technol 39:5177-5182

21. Lebeuf M, Gouteux B, Measures L, Trottier S (2004) Environ Sci Technol 38:2971-2977

22. Jacobs MN, Covaci A, Gheorghe A, Schepens P (2004) J Agric Food Chem 52:1780-1788

23. Eljarrat E, de la Cal A, Raldua D, Duran C, Barceló D (2004) Environ Sci Technol 38:2603-2608

24. Bayen S, Lee HK, Obbard JPh (2004) J Chromatogr A 1035:291-294

25. Akutsu K, Obana H, Okihashi M, Kitagawa M, Nakazawa H, Matsuki Y, Makino T, Oda H, Hori S (2001) Chemosphere 44:1325-1333

26. Saito K, Sjödin A, Sanadau CD, Davis MD, Nakazawa H, Matsuki Y, Patterson Jr DG (2004) Chemosphere 57:373-381

27. Smeds A, Saukko P (2003) Chemosphere 53:1123-1130

28. Meneses M, Wingfors H, Schuhmacher M, Domingo JL, Lindström G, Bavel BV (1999) Chemosphere 39:2271-2278

29. Covaci A, de Boer J, Ryan JJ, Voorspoels S, Schepens P (2002) Environ Res 88:210-218

30. Hernández F, Portolés T, Pitarch E, López FJ, Beltrán J, Vázquez C (2005) Anal Chem 77:7662-7672

31. Björklund J, Tollbäck P, Hiarne C, Dyremarck E, Östman C (2004) J Chromatogr A 1041:201-210

32. Covaci A, de Boer J, Ryan JJ, Voorspoels S, Schepens P (2004) Anal Chem 74:790-798

33. de Boer J, Wester PG, van der Horst A, Leonards PEG (2003) Environ Pollut 122:63-74

34. Larrazábal D, Martínez MªA, Eljarrat E, Barceló D, Fabrellas B (2004) J Mass Spectrom 39:1168-1175

35. Pitarch E, Medina C, Portolés T, López FJ, Hernández F (2007) Anal Chim Acta 583:246-258

36. Frenich AG, Rodríguez MJG, Arrebola FJ, Martínez Vidal JL (2005) Anal Chem 77:4640-4648

37. Lachenmeier DW, Nerlich U, Kuballa T (2006) J Chromatogr A 1108:116-120

38. Lachenmeier DW, Frank W, Kuballa T (2006) Rapid Commun Mass Spectrom 19: 108-112

39. Martínez Vidal JL, Liébanas FJA, Rodríguez MJG, Frenich AG, Moreno JLF (2006) Rapid Commun Mass Spectrom 20:365-375

40. Frenich AG, Vidal JLM, Sicilia ADC, Rodríguez MJG, Bolaños PP (2006) Analytica Chimica Acta 558:42-52

41. Leandro CC, Fussell RJ, Keely BJ (2005) J Chromatogr A 1085:207-212

42. Bucheli TD, Brändli RC (2006) J Chromatogr A 1110:156-164

43. Korytár P, Covaci A, de Boer J, Gelbin A, Brinkman UATh (2005) J Chromatogr A 1065:239-249

44. Korytár P, Covaci A, Leonards PEG, de Boer J, Brinkman UATh (2005) J Chromatogr A 1100:200-207

45. Čajka T, Hajšlová J, Kazda R, Poustka J (2005) J Sep Sci 28:601-611

46. Naert C, Piette M, Bruneel N, van Peterghem C (2006) Arch Environ Contam Toxicol 50:290-296

47. de Wit CA (2002) Chemosphere 46:583-624

48. Meironyté D, Bergman Å, Norén K (2001) Arch Environ Contam Toxicol 40:564-570

FIGURE LEGENDS

Figure 1. GC-MS/MS (EI) chromatograms of adipose breast tissue fortified at 1 ng/g with selected PBDEs (5 ng/g for BDE209) after application of the SPE clean-up procedure.

Figure 2. GC-MS (NCI) SIR chromatograms of adipose breast tissue fortified at 1 ng/g with selected PBDEs (5 ng/g for BDE209) after application of the SPE clean-up procedure.

Figure 3. GC-MS (NCI) SIR chromatograms corresponding to the analysis of four human breast adipose tissue samples. Chromatograms for the quantification ion (Q) and for the two confirmation ions (q_1 and q_2) are shown in each sample.

Table 1. Experimental conditions of the optimized GC-EI(MS/MS) method

t_R (min)	Window (min)	BDE	Precursor Ion (m/z)	Product Ion (m/z)	Q/q[b]	Collision Energy (eV)
6.4	3-7	*28*	248 [$C_{12}H_7O^{81}Br$] 406 [$C_{12}H_7O^{79}Br_2{}^{81}Br$]	139 [- $CO^{81}Br$] 246 [- $^{79}Br^{81}Br$]	Q q	15 15
8.1 8.3 8.6	7-9	*71* *47* *66*	486 [$C_{12}H_6O^{79}Br_2{}^{81}Br_2$] 326 [$C_{12}H_6O^{79}Br^{81}Br$]	326 [- $^{79}Br^{81}Br$] 219 [- $CO^{79}Br$]	Q q	15 20
8.3		*47* [a]	498 [$^{13}C_{12}$ $H_6O^{79}Br_2{}^{81}Br_2$]	338 [- $^{79}Br^{81}Br$]		15
9.7 10.1 10.5	9-10.5	*100* *99* *85*	566 [$C_{12}H_5O^{79}Br_2{}^{81}Br_3$] 406 [$C_{12}H_5O^{79}Br^{81}Br_2$]	406 [- $^{79}Br^{81}Br$] 297 [- $CO^{81}Br$]	Q q	25 15
10.1		*99* [a]	578 [$^{13}C_{12}$ $H_5O^{79}Br_2{}^{81}Br_3$]	416 [- $^{81}Br_2$]		20
10.9		*154*	644 [$C_{12}H_4O^{79}Br_3{}^{81}Br_3$] 484 [$C_{12}H_4O^{79}Br_2{}^{81}Br_2$]	484 [- $^{79}Br^{81}Br$] 217 [- $CO^{79}Br_2{}^{81}Br$]	Q q	10 50
11.2 11.6	10.2-11.9	*153* *138*	644 [$C_{12}H_4O^{79}Br_3{}^{81}Br_3$] 484 [$C_{12}H_4O^{79}Br_2{}^{81}Br_2$]	484 [- $^{79}Br^{81}Br$] 324 [- $^{79}Br^{81}Br$]	Q q	10 30
11.2		*153* [a]	656 [$^{13}C_{12}$ $H_4O^{79}Br_3{}^{81}Br_3$]	496 [- $^{79}Br^{81}Br$]		20
12.1	11.7-13	*183*	562 [$C_{12}H_3O^{79}Br_3{}^{81}Br_2$] 722 [$C_{12}H_3O^{79}Br_4{}^{81}Br_3$]	295 [- $CO^{79}Br_2{}^{81}Br$] 562 [- $^{79}Br^{81}Br$]	Q q	50 30
-	13-19	*209*	800 [$C_{12}O^{79}Br_4{}^{81}Br_4$] 960 [$C_{12}O^{79}Br_5{}^{81}Br_5$]	640 [- $^{79}Br^{81}Br$] 800 [- $^{79}Br^{81}Br$]	Q q	45 25

[a]: labeled congeners used as surrogates.

[b]: Q: quantification transition, q: confirmation transition.

Table 2. Experimental conditions of the optimized GC-NCI(MS) method

t_R (min)	Window (min)	BDE	Quantification Ion (m/z)	Confirmation Ion (m/z)		Dwell(sec)
8.1 7.6	3-9	*28* *p,p'-DDE-D$_8$* [a]	79 [^{79}Br]$^-$ 289 [$C_{14}D_8{}^{35}Cl_3$]$^-$	161 [H^{79}Br^{81}Br]$^-$ 81 [^{81}Br]$^-$	q_1 q_2	0.1
9.8 10.0 10.3	9-10.8	*71* *47* *66*	79 [^{79}Br]$^-$	161 [H^{79}Br^{81}Br]$^-$ 81 [^{81}Br]$^-$	q_1 q_2	0.1
11.5 11.8 12.3	10.8-12.6	*100* *99* *85*	79 [^{79}Br]$^-$	161 [H^{79}Br^{81}Br]$^-$ 81 [^{81}Br]$^-$	q_1 q_2	0.1
12.7 13.0 13.4	12.5-14	*154* *153* *138*	79 [^{79}Br]$^-$	161 [H^{79}Br^{81}Br]$^-$ 81 [^{81}Br]$^-$	q_1 q_2	0.1
14.0	13.5-15	*183*	79 [^{79}Br]$^-$	161 [H^{79}Br^{81}Br]$^-$ 81 [^{81}Br]$^-$	q_1 q_2	0.1
16.5	15-19	*209*	79 [^{79}Br]$^-$	487[$C_6O^{79}Br_3{}^{81}Br_2$]$^-$ 81 [^{81}Br]$^-$	q_1 q_2	0.05

[a]: used as surrogate.

Table 3. Validation of the GC-EI-MS/MS method for analysis of BDEs in human breast tissue samples (n=5, at each fortification level).

	LC clean up							SPE clean up				
	Fortification level							Fortification level				
	50 ng/g		10 ng/g					1 ng/g				
Compound	**Recovery (R.S.D.)**	**Q/q[a]**	**Recovery (R.S.D.)**	**Q/q[a]**	**LOQ (ng/g)**	**LOC (ng/g)**	**LOD (ng/g)**	**Recovery (R.S.D.)**	**Q/q[a]**	**LOQ (ng/g)**	**LOC (ng/g)**	**LOD (ng/g)**
BDE28	86(8)	1.01(3)	89(3)	1.07(3)	5	3	2	72(5)	1(6)	0.3	0.3	0.1
BDE71	95(4)	1.02(0)	96(9)	1(7)	10	5	3	64(6)	1.01(10)	1	0.6	0.3
BDE47	99(4)	0.99(2)	93(7)	0.99(5)	10	5	3	71(2)	1.14(4)	1	0.5	0.2
BDE66	100(5)	1.02(2)	91(6)	1(5)	10	10	6	77(15)	1.05(10)	1	1	0.6
BDE100	94(8)	1.01(2)	100(5)	1.06(6)	10	4	3	72(6)	1.12(7)	1	0.3	0.3
BDE99	91(11)	0.97(3)	92(4)	1.03(7)	10	6	3	75(12)	1.03(8)	1	0.4	0.4
BDE85	110(6)	0.91(1)	117(9)	0.88(9)	10	7	5	82(9)	1.04(10)	1	1	0.8
BDE154	113(18)	0.98(11)	94(17)	0.86(14)	10	10	5	72(10)	1.02(17)	1	1	0.7
BDE153	92(11)	0.97(11)	101(14)	1.15(8)	10	10	8	92(12)	0.95(10)	1	1	1[b]
BDE138	102(16)	0.98(4)	111(5)	1.02(13)	10	10	10[b]	61(7)	0.84(7)	1	1	1[b]
BDE183	100(7)	0.96(9)	95(14)	1.08(8)	10	10	10[b]	77(15)	0.97(13)	1	1	1[b]
BDE209	-	-	-	-	-	-	-	-	-	-	-	-

[a]: Average value calculated from Q/q concentration of five replicates at each level of fortification.
[b]:LOD estimated for a S/N = 3 was coincident with the lowest level that was fully validated in spiked samples with satisfactory recovery and precision

Table 4. Validation of the GC-NCI-MS method for determination of BDEs in human breast tissue samples (n=5, at each fortification level).

	LC clean up									SPE clean up					
	Fortification level									Fortification level					
	50 ng/g[a]			10 ng/g[b]						1 ng/g[c]					
Compound	**Recovery (R.S.D.)**	**Q/q^d_1**	**Q/q^d_2**	**Recovery (R.S.D.)**	**Q/q^d_1**	**Q/q^d_2**	**LOQ (ng/g)**	**LOC (ng/g)**	**LOD (ng/g)**	**Recovery (R.S.D.)**	**Q/q^d_1**	**Q/q^d_2**	**LOQ (ng/g)**	**LOC (ng/g)**	**LOD (ng/g)**
BDE28	95(6)	1.15(5)	1(2)	104(5)	1(5)	0.99(1)	0.2	0.07	0.05	72(6)	0.93(9)	1.03(2)	0.02	0.02	0.006
BDE71	94(6)	1.14(7)	1(0)	99(9)	1.09(3)	0.99(1)	0.5	0.09	0.045	69(4)	0.97(3)	1(2)	0.07	0.03	0.02
BDE47	101(7)	1.06(7)	0.99(0)	100(8)	1.09(1)	1(1)	1	0.06	0.3	94(12)	1.11(4)	1(0)	0.03	0.01	0.01
BDE66	101(8)	1.09(10)	1(1)	96(8)	1.06(3)	1.01(1)	0.3	0.2	0.1	87(7)	0.98(4)	1.02(3)	0.2	0.05	0.05
BDE100	98(11)	1.14(8)	1(0)	95(7)	1.05(3)	1.01(1)	0.3	0.1	0.1	88(8)	1.07(1)	1.03(4)	0.02	0.008	0.005
BDE99	95(12)	1.11(10)	1(1)	96(7)	0.97(8)	1.01(1)	0.3	0.1	0.1	91(11)	0.95(4)	1.01(2)	0.03	0.01	0.01
BDE85	99(15)	1.13(6)	1.3(18)	91(6)	1.03(9)	1.01(2)	0.5	0.2	0.15	78(5)	1.01(7)	1.01(2)	0.1	0.03	0.03
BDE154	107(12)	1.07(6)	0.99(4)	89(4)	1.02(2)	1.01(2)	0.2	0.07	0.05	89(7)	1.09(6)	1.01(3)	0.02	0.01	0.005
BDE153	107(12)	1.2(5)	1(3)	92(6)	1.04(1)	1.01(2)	0.3	0.1	0.1	91(11)	1.08(2)	1(3)	0.03	0.01	0.01
BDE138	101(15)	1.11(6)	0.97(2)	88(5)	1.01(5)	1(4)	0.7	0.2	0.2	77(12)	0.96(2)	1.04(5)	0.1	0.03	0.03
BDE183	101(20)	1.16(8)	0.98(1)	80(4)	0.98(7)	0.98(6)	0.5	0.3	0.15	85(8)	0.99(2)	0.96(4)	0.5	0.2	0.15
BDE209	106(4)	0.83(15)	1.2(13)	109(19)	0.9(9)	0.99(6)	40	10.7	12.5	98(16)	0.95(7)	1.02(9)	2	1	0.5

[a] 250 ng/g for BDE209, [b] 50 ng/g for BDE209, [c] 5 ng/g for BDE209.

[d] Average value calculated from Q/q concentration of the five replicates for each level of fortification.

Q/q_1 =79/161, Q/q_2 =79/81 and Q/q_1 =79/487, Q/q_2 =79/81 for BDE 209.

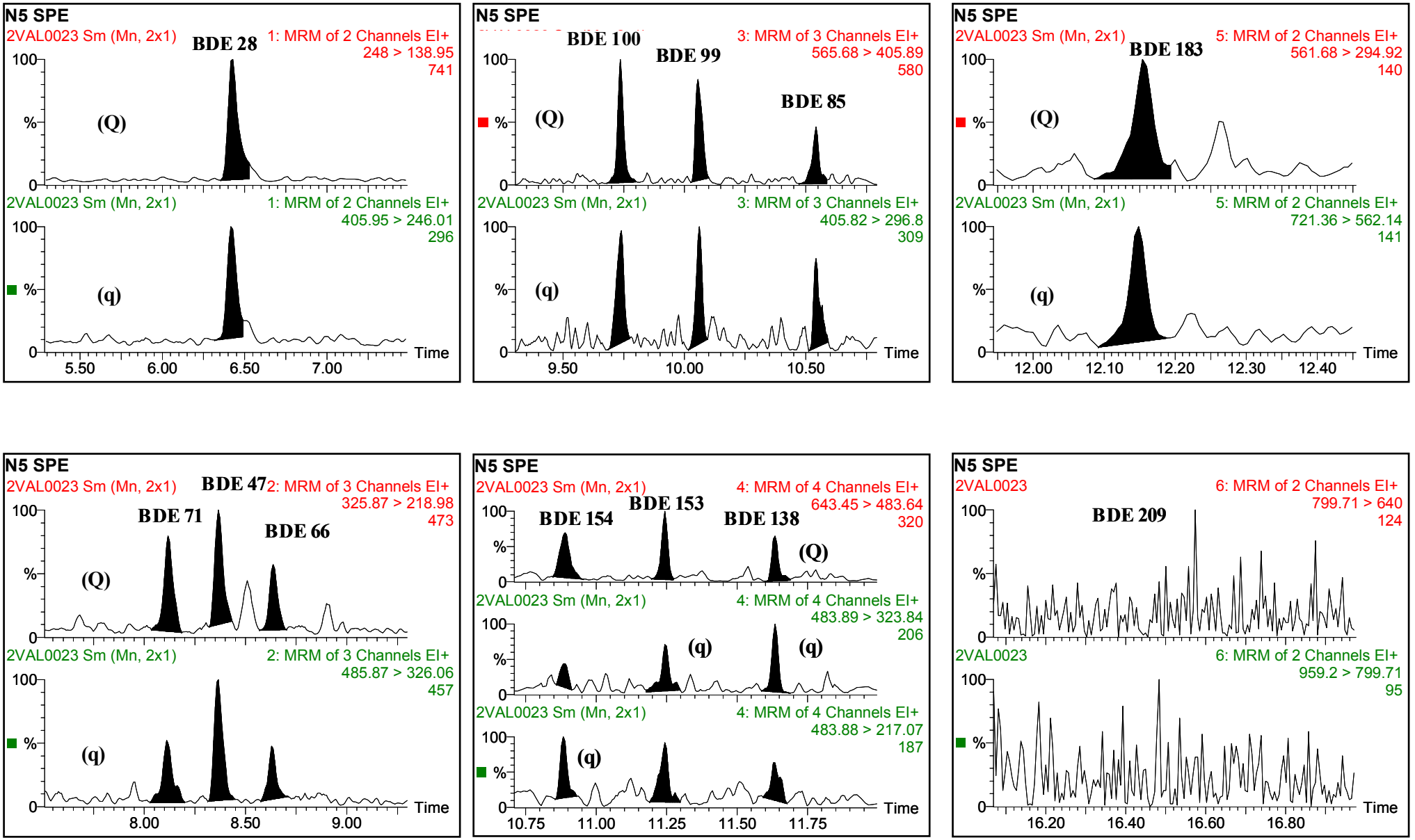
N5 SPE
2VAL0023 Sm (Mn, 2x1)
BDE 28
1: MRM of 2 Channels EI+
248 > 138.95
741
(Q)
2VAL0023 Sm (Mn, 2x1)
1: MRM of 2 Channels EI+
405.95 > 246.01
296
(q)
Time
N5 SPE
BDE 100
BDE 99
BDE 85
3: MRM of 3 Channels EI+
565.68 > 405.89
580
(Q)
2VAL0023 Sm (Mn, 2x1)
3: MRM of 3 Channels EI+
405.82 > 296.8
309
(q)
Time
N5 SPE
2VAL0023 Sm (Mn, 2x1)
BDE 183
5: MRM of 2 Channels EI+
561.68 > 294.92
140
(Q)
2VAL0023 Sm (Mn, 2x1)
5: MRM of 2 Channels EI+
721.36 > 562.14
141
(q)
Time
N5 SPE
2VAL0023 Sm (Mn, 2x1)
BDE 47
BDE 71
BDE 66
2: MRM of 3 Channels EI+
325.87 > 218.98
473
(Q)
2VAL0023 Sm (Mn, 2x1)
2: MRM of 3 Channels EI+
485.87 > 326.06
457
(q)
Time
N5 SPE
2VAL0023 Sm (Mn, 2x1)
BDE 154
BDE 153
BDE 138
4: MRM of 4 Channels EI+
643.45 > 483.64
320
(Q)
2VAL0023 Sm (Mn, 2x1)
4: MRM of 4 Channels EI+
483.89 > 323.84
206
(q) (q)
2VAL0023 Sm (Mn, 2x1)
4: MRM of 4 Channels EI+
483.88 > 217.07
187
(q)
Time
N5 SPE
2VAL0023
BDE 209
6: MRM of 2 Channels EI+
799.71 > 640
124
2VAL0023
6: MRM of 2 Channels EI+
959.2 > 799.71
95
Time

Figure 1

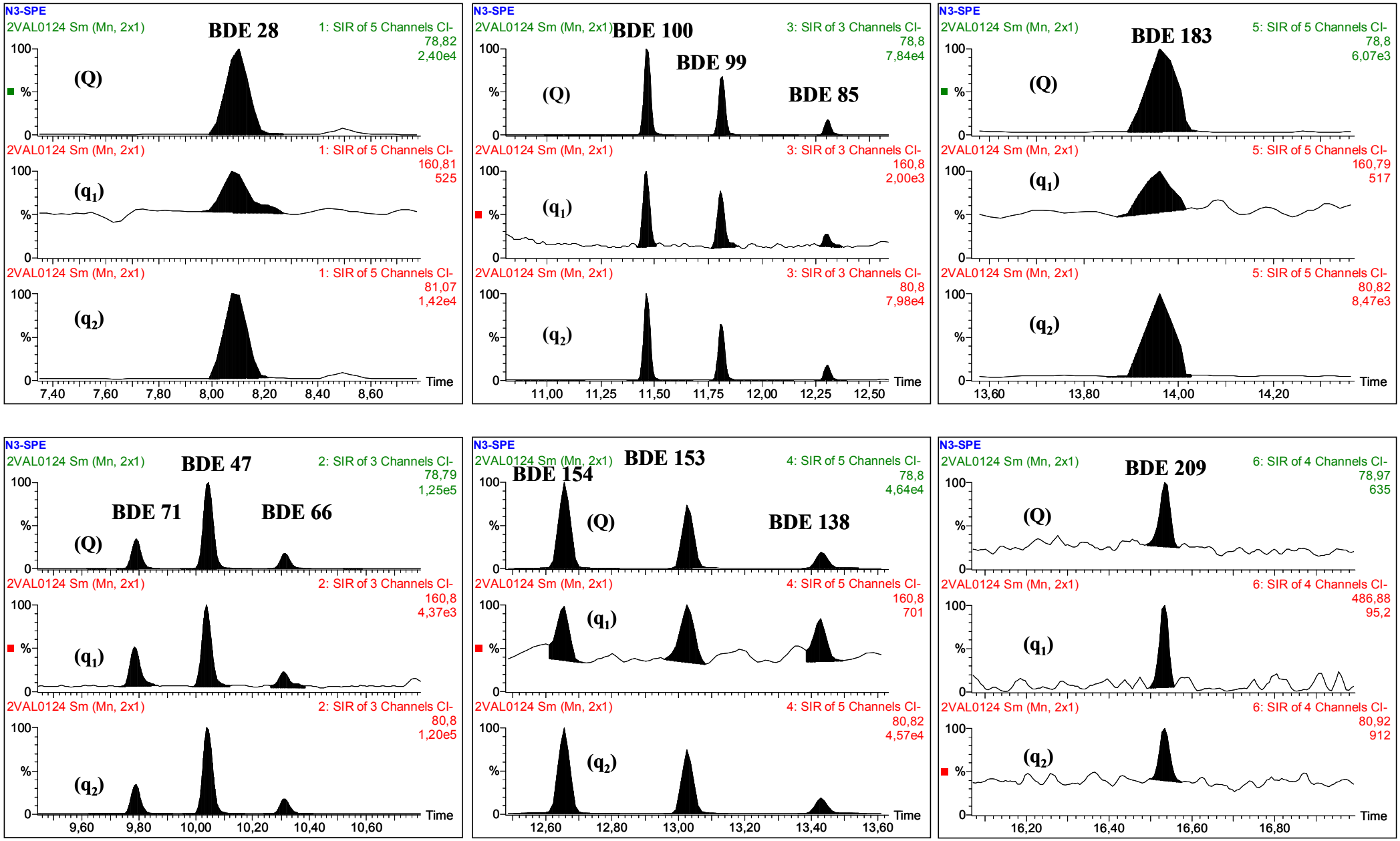
N3-SPE
2VAL0124 Sm (Mn, 2x1)
BDE 28
1: SIR of 5 Channels CI-
78,82
2,40e4
(Q)
160,81
525
(q1)
81,07
1,42e4
(q2)
Time
BDE 100
BDE 99
BDE 85
3: SIR of 3 Channels CI-
78,8
7,84e4
160,8
2,00e3
80,8
7,98e4
BDE 183
5: SIR of 5 Channels CI-
78,8
6,07e3
160,79
517
80,82
8,47e3
BDE 47
BDE 71
BDE 66
2: SIR of 3 Channels CI-
78,79
1,25e5
160,8
4,37e3
80,8
1,20e5
BDE 153
BDE 154
BDE 138
4: SIR of 5 Channels CI-
78,8
4,64e4
160,8
701
80,82
4,57e4
BDE 209
6: SIR of 4 Channels CI-
78,97
635
486,88
95,2
80,92
912

Figure 2

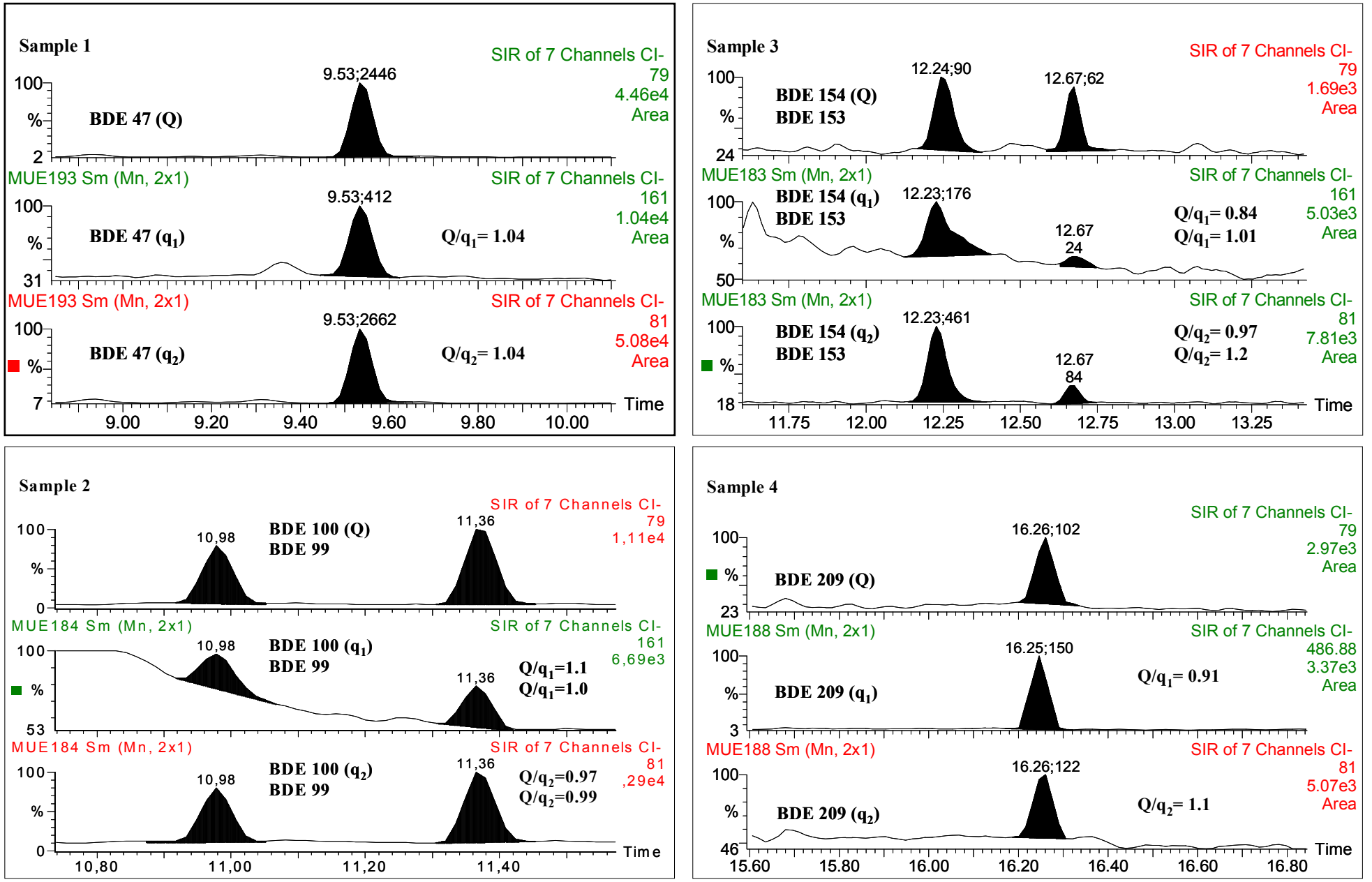
Sample 1
SIR of 7 Channels Cl-
79
4.46e4
Area
9.53;2446
BDE 47 (Q)
MUE193 Sm (Mn, 2x1)
SIR of 7 Channels Cl-
161
1.04e4
Area
9.53;412
BDE 47 (q1)
Q/q1= 1.04
MUE193 Sm (Mn, 2x1)
SIR of 7 Channels Cl-
81
5.08e4
Area
9.53;2662
BDE 47 (q2)
Q/q2= 1.04
Time
Sample 3
SIR of 7 Channels Cl-
79
1.69e3
Area
12.24;90
12.67;62
BDE 154 (Q)
BDE 153
MUE183 Sm (Mn, 2x1)
SIR of 7 Channels Cl-
161
5.03e3
Area
12.23;176
BDE 154 (q1)
BDE 153
Q/q1= 0.84
Q/q1= 1.01
12.67
MUE183 Sm (Mn, 2x1)
SIR of 7 Channels Cl-
81
7.81e3
Area
12.23;461
BDE 154 (q2)
BDE 153
Q/q2= 0.97
Q/q2= 1.2
12.67
Time
Sample 2
SIR of 7 Channels Cl-
79
1,11e4
10,98
BDE 100 (Q)
BDE 99
11,36
MUE184 Sm (Mn, 2x1)
SIR of 7 Channels Cl-
161
6,69e3
10,98
BDE 100 (q1)
BDE 99
Q/q1=1.1
Q/q1=1.0
11,36
MUE184 Sm (Mn, 2x1)
SIR of 7 Channels Cl-
81
,29e4
10,98
BDE 100 (q2)
BDE 99
Q/q2=0.97
Q/q2=0.99
11,36
Time
Sample 4
SIR of 7 Channels Cl-
79
2.97e3
Area
16.26;102
BDE 209 (Q)
MUE188 Sm (Mn, 2x1)
SIR of 7 Channels Cl-
486.88
3.37e3
Area
16.25;150
BDE 209 (q1)
Q/q1= 0.91
MUE188 Sm (Mn, 2x1)
SIR of 7 Channels Cl-
81
5.07e3
Area
16.26;122
BDE 209 (q2)
Q/q2= 1.1
Time

Figure 3