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Gas chromatography–ion trap tandem mass spectrometry versus GC–high-resolution mass spectrometry for the determination of non-*ortho*-polychlorinated biphenyls in fish

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Abstract

Gas chromatography coupled with ion trap tandem mass spectrometry (GC–MS–MS) has been compared to gas chromatography–high-resolution mass spectrometry (GC–HRMS) for the analysis of non-*ortho*-chlorinated biphenyl (CB) congeners in fish samples. The MS–MS operating parameters related to the isolation and fragmentation of the precursor ions by resonant collision induced dissociation (CID) were optimised in order to achieve maximum sensitivity and selectivity. Analytical procedure consisting of Soxhlet extraction, clean-up using a multilayer silica column and the isolation of the target compounds with SPE commercial carbon cartridges packed with Carbopack B has been applied. Quality parameters have been established using standard solutions and fish samples. Good repeatability, long-term precision (lower than 10%), and limits of detection between 0.12 and 0.16 pg g^{-1} were obtained. The effect of potential interfering compounds such as polychlorinated naphthalenes in the quantification of non-*ortho*-CBs has been investigated. Using selective CID fragmentation conditions, the effect of these compounds was minimised. The GC–MS–MS method was validated by comparing the results with those obtained in two European intercomparison exercises.
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1. Introduction

Chlorinated biphenyls (CBs) constitute a class of ubiquitous persistent environmental pollutants of great concern because of their potential risks for human health. Owing to their physical, chemical and ecotoxicological properties, these compounds are extremely resistant to chemical and biological degradation, are easily bioaccumulated through the trophic pyramid and reach humans via the food chain [1]. Among the 209 possible CBs, some congeners present a chlorine substitution pattern that allows them to adopt a planar geometry. These particular compounds are called co-planar or non-*ortho*-CBs and show the same type of toxicity as 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) [2]. Due to their similar affinity to bind to the aryl hydrocarbon receptor (AhR) as PCDD/Fs, four non-*ortho*-CBs (IUPAC No. 77, 81, 126 and 169) as well as the mono-*ortho*-substituted congeners have been considered toxic compounds. In order to determine the human exposure to these compounds the World Health Organization (WHO) has assigned a toxic equivalent factor (TEF) based on their toxicity related to 2,3,7,8-TCDD, which is the most toxic congener [3–6]. Due to this dioxin-like toxicity, these compounds are known as dioxin-like CBs. Among them, the non-*ortho*-CBs 126 and 169 have the highest toxic equivalent factors (0.01 for CB 169 and 0.1 for CB 126) [3].

Individual non-*ortho*-CBs are often not detected in a general analysis of CBs due to their extremely low concentration compared with the bulk of CBs (two or three orders of magnitude) [7]. In addition, the presence of many other organic compounds at high concentration levels, which interfere in their instrumental determination, always involve extensive clean-up procedures. Therefore, the use of selective and sensitive analytical methods is required for the suitable determination of these compounds. Gas chromatography coupled

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with high-resolution mass spectrometry (GC–HRMS) as described in US EPA Method 1668 [8] is the method that is currently most often applied. Nowadays, this technique is considered a point of reference for the accurate and specific determination of these compounds in environmental and food samples, providing the required selectivity and detection limits (pg g^{-1} level). Nevertheless, the use of GC–HRMS instrumentation requires a considerable investment, since this technique is relatively expensive and qualified personnel is needed. In recent years, mass spectrometry based on ion trap analysers (ITMS) has become an interesting alternative to HRMS for the analysis of organic contaminants such as PCDD/Fs, polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs) and pesticides [9–14]. The recent establishment of new maximum residue values for PCDD/Fs in food and feed samples by the EU and the future inclusion of the dioxin-like CB levels in these values at the end of 2006 [15], has led to an important increase in the routine analysis of these compounds. Therefore, the development of more economical and reliable methods is required. Up to now few papers have been published [16–22] reporting the use of gas chromatography coupled with ion trap tandem mass spectrometry (GC–MS–MS) for the analysis of non-*ortho*-CBs.

The aim of the present study was to evaluate the suitability of gas chromatography coupled with ion trap tandem mass spectrometry for the analysis of non-*ortho*-CBs in fish samples. For this purpose, MS–MS operating parameters such as precursor isolation time and mass isolation window, and collision induced dissociation (CID) parameters such as excitation time, excitation voltage and excitation energy (q_z), were studied and optimised in order to obtain the maximum sensitivity and selectivity. The GC–MS–MS method was evaluated by comparing the results with those obtained with GC–HRMS. Quality parameters such as repeatability, long-term precision and LODs for both MS methods were also determined. The GC–MS–MS method proposed was validated by participating in two certification exercises organized under the aegis of the European project CHRONO.

2. Experimental

2.1. Standard and materials

Individual analytical-reagent grade non-*ortho*-CB congeners: 77 (3,3′,4,4′-tetraCB), 81 (3,4,4′,5-tetraCB), 126 (3,3′,4,4′,5-pentaCB) and 169 (3,3′,4,4′,5,5′-hexaCB), were supplied with a purity higher than 99% by AccuStandard Inc. (New Haven, USA). Individual stock standard solutions of each compound at 200 μg g^{-1} were prepared by weight in isooctane. A standard solution of $^{13}C_{12}$-isotopically labelled CBs 77, 81, 126 and 169 was obtained from Wellington Laboratories (Guelph, Canada) at purity higher than 99%. These compounds were used as internal standards for quantification by isotopic dilution. A standard solution of $^{13}C_{12}$-isotopically labelled CBs 70, 111, 138 and 170 (WHO/EPA PCB-ISS), supplied by Wellington Laboratories, was used as syringe standard for recovery determination. Nine calibration standard solutions containing a mixture of the non-*ortho*-CB congeners at concentrations ranging from 0.1 to 500 ng g^{-1}, and the isotopically labelled internal standards for recovery and quantification at 50 ng g^{-1}, were prepared by dilution of the corresponding individual stock standard solution in isooctane.

Toluene, *n*-hexane, isooctane, acetone and dichloromethane of residue analysis grade were purchased from Merck (Darmstadt, Germany). Silica gel (0.063–0.2 mm) for column chromatography and anhydrous sodium sulphate and sea sand for analysis were also supplied by Merck. Before use, silica gel was activated for at least 4 h at 450 °C. Supelclean ENVI-Carb SPE cartridges packed with Carbopack-B (3 ml, 0.25 g) were provided by Supelco (Bellefonte, PA, USA). All glass materials were cleaned with AP-13 Extran alkaline soap (Merck) for 24 h, rinsed consecutively with Milli-Q water and acetone, and dried overnight. The eel (*anguilla anguilla*) and chub (*leuciscus cephalus*) samples, candidate reference materials, were provided by the Netherlands Institute for Fisheries Research (RIVO).

2.2. Sample preparation

Soxhlet extractor was used for the extraction of CBs from fish samples. Approximately 20 g of eel sample or 50 g of chub sample were mixed with 40 and 150 g of anhydrous sodium sulphate and 30 and 50 g of pre-cleaned sea sand, respectively. The sample was transferred into a glass thimble and it was spiked with 125 μl of the $^{13}C_{12}$-isotopically labelled CBs standard mixture (10 pg μl^{-1}). An incubation time of 16 hours in darkness at 4 °C was used before extraction. The sample was Soxhlet extracted for 18 h with 300 ml of *n*-hexane/dichloromethane (1:1). The extract was then concentrated to ca. 5 ml using a rotary evaporator, and it was cleaned-up in a multilayer-silica column (from bottom to top: glass wool, 5 g of silica, 30 g of silica/44% H_2SO_4 conc., 5 g of silica, 15 g activated silica/22% H_2SO_4 conc. and 5 g of anhydridous sodium sulphate) using 100 ml of *n*-hexane as solvent. After evaporation up to ca. 1 ml, the extracts were applied to Supelclean ENVI-Carb SPE cartridge [2,23] and two fractions were obtained using the following eluents: 15 ml of *n*-hexane and 20 ml of *n*-hexane/toluene (99:1) for Fraction 1, which contained the *ortho*-CBs; and for Fraction 2, 20 ml of *n*-hexane/toluene (75:25, v/v), where the non-*ortho*-CBs were eluted. Fraction 2 was evaporated under reduced pressure up to 1 ml, and afterwards it was carefully concentrated under a gentle nitrogen stream to ca. 25 μl, using nonane as keeper. After addition of 25 μl of $^{13}C_{12}$-isotopically labelled CBs 70, 138 and 170 (50 pg μl^{-1}) as syringe standard, the extract was analysed with GC–MS–MS and GC–HRMS. This sample

preparation method can be applied to the analyses of fish samples with a fat content up to 40%. For samples with a higher content, a reduction of sample intake should be performed to prevent the saturation of the multilayer-silica column.

2.3. GC–MS instrumentation

The GC–ion trap MS–MS experiments were performed using a Trace GC 2000 gas chromatograph coupled to a GCQ/Polaris ion trap mass spectrometer (ThermoFinnigan, Austin, TX, USA) equipped with an AS2000 autosampler. A DB-5 (5% phenyl, 95% methylpolysiloxane), 60 m × 0.25 mm i.d., fused-silica capillary column (J&W Scientific, Folsom, USA) of 0.25 μm film thickness was used for chromatographic separation. The oven temperature program was: 90 °C (held for 3 min) to 200 °C at 20 °C/min (held for 1 min) and to 300 °C at 2.5 °C/min (held for 10 min). Helium was used as carrier gas at a constant flow-rate of 1 ml min^{-1} held by electronic pressure control. Injector temperature was maintained at 280 °C and splitless injection mode (1 min) was used. The MS operating conditions were the following: ion source and transfer line temperatures 200 and 290 °C, respectively. The instrument was tuned in EI positive mode using perfluorotributylamine (FC-43) according to manufacturer's recommendations in order to achieve the best sensitivity. Parameters such as automatic gain control (AGC) and multiplier (1350 V, 10^5gain) were set by automatic tuning. The electron energy was 70 eV and the emission current 250 μA. In MS–MS mode, for native and labelled tetra-, penta- and hexa-CBs the $[M + 2]^{\bullet+}$ ion of the cluster molecular ions, was selected as precursor ion. The $[M - 2^{35}Cl]^+$ and $[M - {}^{35}Cl^{37}Cl]^+$ product ions were monitorised for quantitative purposes. The resonance excitation voltage applied for all CBs congeners was 1.4 V. The MS–MS acquisition method was time programmed in three segments for tetra-, penta- and hexa-CBs, respectively. Xcalibur version 1.2 software was used for data acquisition and processing of the results.

GC–HRMS analyses were performed on an HP-5890 Series II gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to an AutoSpec-Q hybrid (E_1BE_2qQ) high-resolution mass spectrometer (Micromass, Manchester, UK), operating in EI+ mode at an electron energy of 32 eV and at a resolving power of 10,000 (10% valley definition). The ion source and transfer line temperatures were set at 250 and 280 °C, respectively. The chromatographic conditions were the same as described above for GC–MS–MS. Selected ion monitoring mode at an accelerating voltage of 8000 V, dwell time 80 ms and delay time 20 ms was used. The total scan cycle was 1 s. Verification of the resolution in the working mass range was obtained by measuring the PFK reference peaks on a mass-calibrated oscilloscope. The two major molecular ions of each CB homologue group were used for mass monitoring.

3. Results and discussion

3.1. Optimisation of the GC–MS–MS method

Preliminary experiments were conducted to select a characteristic precursor ion for each non-*ortho*-CB congener. Generally, the EI-MS fragmentation pattern of non-*ortho*-CBs as well as mono-*ortho*-CBs is characterised by successive losses of chlorine atoms from the molecular cluster ions with a dominant fragment ion corresponding to the loss of two chlorine atoms. In contrast, for di-, tri- and tetra-*ortho*-substituted congeners, successive losses of a single chlorine atom occurred [19,22]. In order to achieve maximum sensitivity, the most intense ion of the molecular cluster ions of each homologue group was selected as precursor ion. In addition, the loss of two chlorine atoms from each precursor ion was chosen as selective transition for MS–MS studies. In this way, the two most intense $[M - 2Cl]^+$ ions corresponding to the loss of two ^{35}Cl ($-m/z$ 70) or $^{35}Cl^{37}Cl$ ($-m/z$ 72) from the precursor ion $[M + 2]^{\bullet+}$ were selected as product ions.

MS–MS operating conditions and CID parameters such as precursor ion isolation window, excitation voltage, excitation time (CID time), and excitation energy, were optimised in order to maximize the sensitivity and selectivity in the detection of non-*ortho*-CBs [24,25]. The effect of each parameter upon the MS–MS process was studied by varying only one of the parameters while keeping the others constant. For these experiments, a standard mixture of native and $^{13}C_{12}$-isotopically labelled non-*ortho*-CB congeners (100 ng g^{-1}) was used. The effect of the mass isolation window on the selective isolation of the precursor ion was first investigated from 1 to 3 *m/z*. For these experiments, an isolation time of 10 ms and an excitation time of 15 ms, were applied. As a compromise between the sensitivity and the selectivity, a window of 1 *m/z* was chosen for all non-*ortho*-CBs in order to avoid potential interferences. The effect of the CID resonant excitation voltage on the product ion yield was also studied from 0.4 to 2.0 V in 0.2 V steps, using an isolation window of 1 *m/z*. A similar optimum excitation voltage of 1.4 V was obtained for all compounds, and it was used for further studies. In order to achieve maximum abundances for the product ions, the excitation time was then studied from 10 to 30 ms in 5 ms steps. An increase of the excitation time produces an enhancement of the fragmentation yield, but the MS–MS scan time also increases and therefore a decrease in the response occurs. For these compounds, the best results were obtained at an excitation time of 15 ms. Using the optimum CID conditions, additional experiments were performed in order to optimise the excitation energy, which is related to the stability of the product ions and can be controlled by the q_z parameter. The influence of this parameter in the fragmentation yield and the stabilisation of the product ions was studied at three q_z levels, 0.225, 0.300 and 0.450, maintaining constant the CID parameters previously optimised. Maximum abundances of the product

Table 1
Quality parameters for GC–MS–MS and GC–HRMS methods

CB	GC–HRMS			GC–MS–MS		
	LOD (pg)	Repeatability (R.S.D. %)[a]	Long-term precision (R.S.D. %)[b]	LOD (pg)	Repeatability (R.S.D. %)[a]	Long-term precision (R.S.D. %)[b]
Standard solution						
77	0.1	1.5	2.6	0.1	1.8	3.6
81	0.1	1.6	2.5	0.1	1.9	3.2
126	0.1	1.2	2.6	0.1	1.1	3.0
169	0.1	1.7	2.0	0.1	2.7	4.1
Eel sample						
77	0.2	7	7	0.2	9	9
81	0.2	9	9	0.2	8	8
126	0.1	8	9	0.1	9	9
169	0.1	7	9	0.1	10	10

[a] $n = 5$.
[b] $n = 4$ analyses × 3 days.

ions were observed at the highest q_z value (0.450), while at medium or low excitation energy no fragmentation was obtained. In summary, the optimal CID conditions for the analysis of non-*ortho*-CBs were: isolation windows of the precursor ions ±1 *m*/*z*, isolation time 10 ms, excitation time 15 ms, resonant CID voltage 1.4 V and excitation energy 0.450. Quantification was performed by isotopic dilution and the sum of the responses of the two most intense product ions was used in order to achieve high sensitivity.

3.2. Quality parameters

Repeatability, long-term precision and limits of detection of the GC–MS–MS method were established using both standard solutions and eel samples. For repeatability studies, five analyses of a standard mixture of non-*ortho*-CBs (20 ng g^{-1}) were consecutively analysed using both the proposed GC–MS–MS method and GC–HRMS on one day. In the same way, five replicates of ca. 20 g of a blank eel sample spiked at 20 pg g^{-1} were performed using both detection systems. For long-term precision four replicates were analysed on three different days along two consecutive weeks. Relative standard deviations (R.S.D.) for repeatability and long-term precision were similar for the proposed GC–MS–MS method and the reference method and ranged from 3 to 4% for the standard solutions and between 8 and 10% for the eel sample (Table 1).

Limits of detection (LODs), based on a signal-to-noise ratio (S/N) of 3:1, were determined experimentally using an eel sample without detectable quantities of non-*ortho*-CBs, spiked at low concentration levels. In these conditions, the LODs of the proposed method ranged from 0.12 to 0.16 pg g^{-1}. Similar LODs were obtained using GC–HRMS, demonstrating the capacity of the GC–MS–MS to detect these compounds at low concentration levels. Using standard solutions, the LODs obtained with both techniques were also similar and ranged from 0.08 to 0.12 pg injected. Linearity in the calibration range (0.1–500 ng g^{-1}) was studied and correlation coefficients higher than 0.9999 were obtained.

3.3. Analysis of fish samples

In order to examine the feasibility of the GC–MS–MS method for the analysis of non-*ortho*-CBs in fish samples, two samples were analysed using the method developed and participating in two intercomparison exercises organised in the framework of the European project CHRONO. In the first exercise, an eel sample with a low content of non-*ortho*-CBs

Table 2
Analysis of non-*ortho*-CBs in the eel and chub samples using GC–HRMS and GC–MS–MS

CB	Eel sample (pg g^{-1})			Chub sample (pg g^{-1})	
	GC–HRMS[a] (mean ± S.D.)	GC–MS–MS[a] (mean ± S.D.)	Interlaboratory results[b] (mean ± S.D.)	GC–MS–MS[c] (mean ± S.D.)	Interlaboratory results[b] (mean ± S.D.)
Intercomparison exercises of non-*ortho*-PCBs in eel and chub samples					
PCB-77	9.9 ± 0.7	11.6 ± 0.7	13.6 ± 4.5	192 ± 17	192 ± 19
PCB-81	3.0 ± 0.5	2.0 ± 0.2	2.1 ± 0.9	12.3 ± 1.0	13.2 ± 1.7
PCB-126	92.7 ± 7.4	93.0 ± 8.0	91.9 ± 9.0	17.2 ± 1.6	19.9 ± 2.0
PCB-169	19.2 ± 1.2	19.7 ± 1.4	19.5 ± 3.7	1.42 ± 0.14	1.73 ± 0.29

[a] $n = 4$ replicates.
[b] $n = 12$ laboratories.
[c] $n = 6$ replicates.

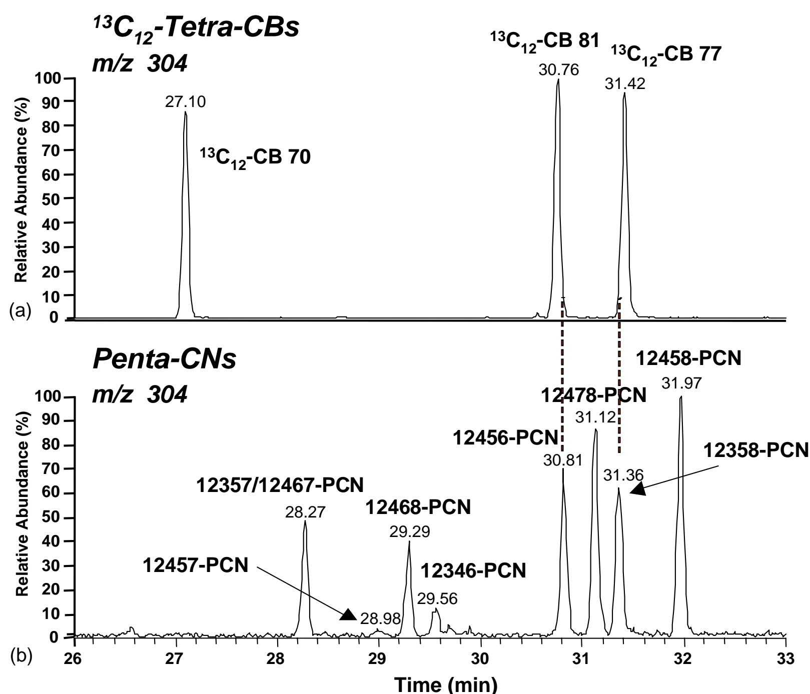

Fig. 1. GC–MS chromatograms of the *m/z* 304 for (a) non-*ortho*-CBs 77, 81 and (b) the interfering penta-CNs.

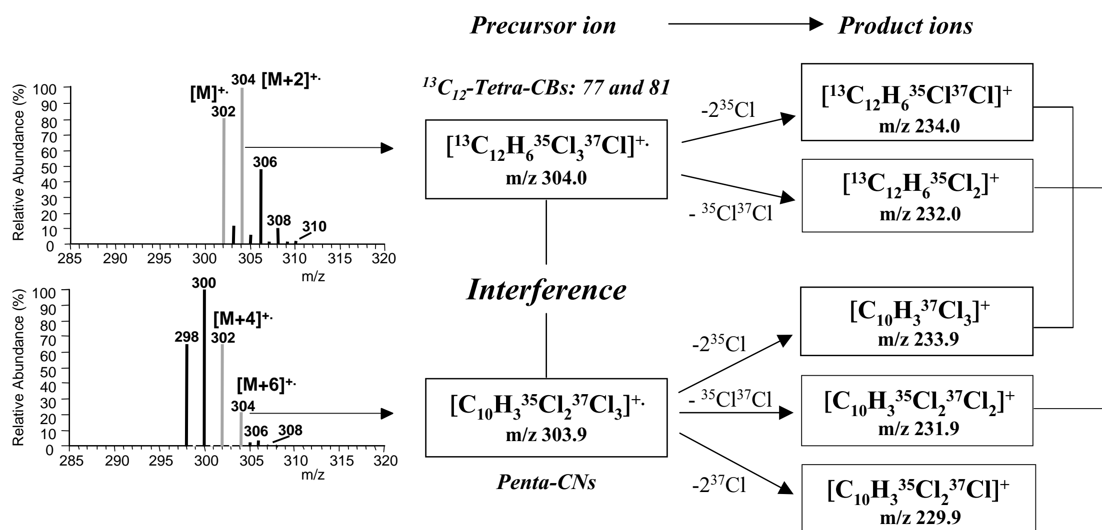

Fig. 2. Interfering mass of penta-CNs on the molecular ions of $^{13}C_{12}$-labelled tetra-CBs.

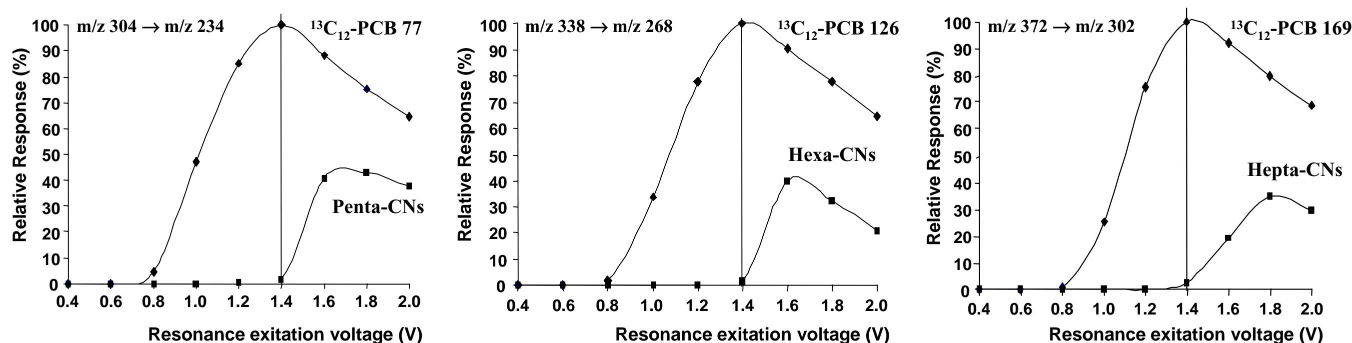

Fig. 3. Relative abundance of product ion vs. resonance excitation voltage (V) for the $^{13}C_{12}$-labelled non-*ortho*-CB congeners and the interfering PCNs.

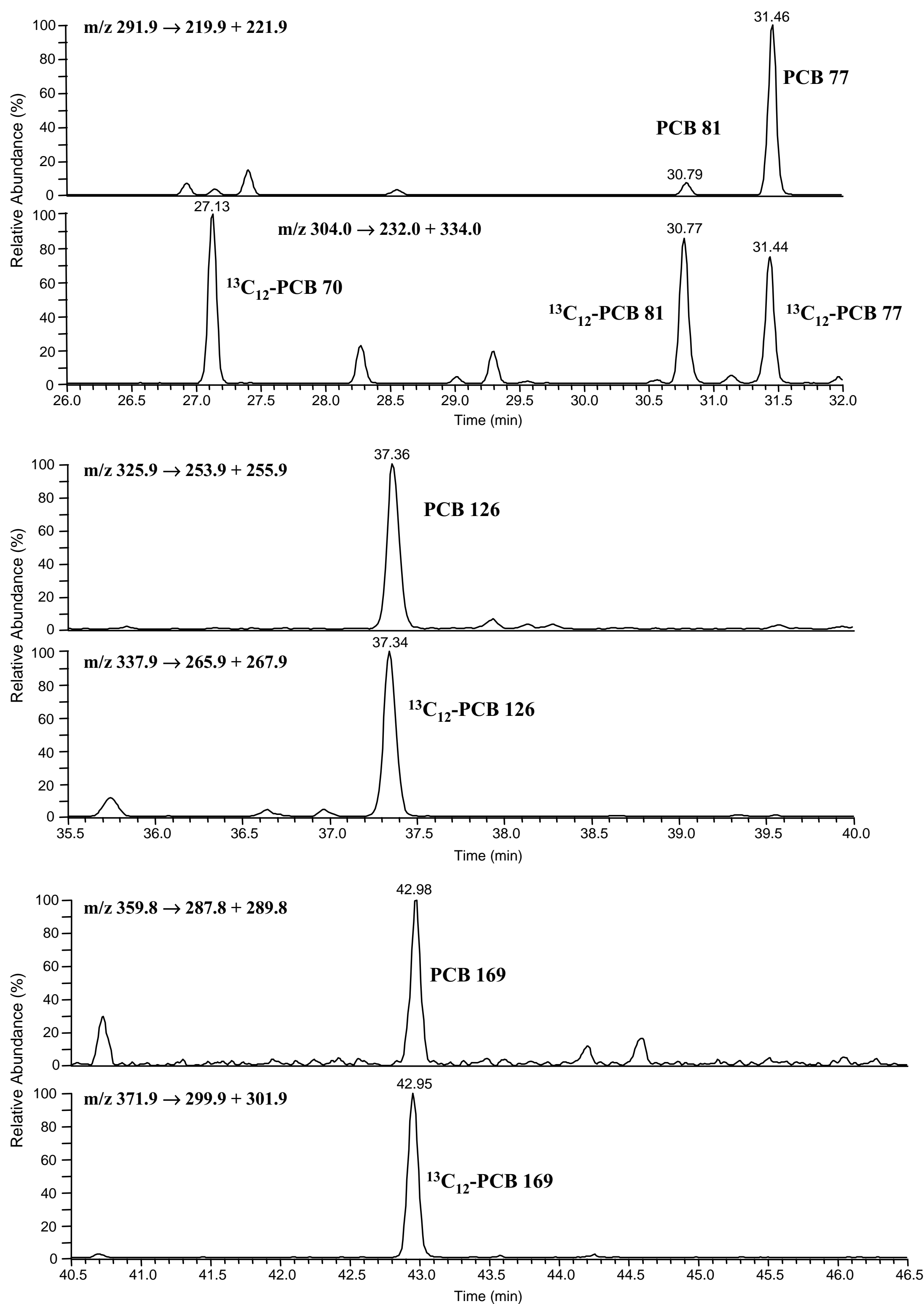

Fig. 4. GC–MS–MS chromatograms of the non-*ortho*-CBs for a chub sample.

was analysed using both the developed GC–MS–MS method and GC–HRMS. The results obtained by our laboratory are given in Table 2, where the mean of all the European laboratories are also given. As can be seen, the results obtained using GC–MS–MS agreed with the mean values obtained by our laboratory using GC–HRMS, and comparable standard deviations were obtained (R.S.D.% lower than 10%) showing that the GC–MS–MS method can be considered as

a good alternative to the GC–HRMS. In addition, the results were in agreement with those reported in the intercomparison exercise.

The second intercomparison exercise was organised with the objective to certify the non-*ortho*-CB content in a chub sample. However, the presence of polychlorinated naphthalenes (PCNs) in the sample was detected and the effect of these potential interfering compounds on the optimised ITMS–MS method was investigated. PCNs have a planar geometry similar to non-*ortho*-CBs or PCDD/Fs [26] and, therefore, they can be eluted in the same fraction from which the non-*ortho*-CBs were obtained. Experiments conducted to determine the order of elution of these compounds in the ENVI-Carb SPE cartridges were performed and the results obtained confirmed their presence in the non-*ortho*-PCB elution fraction. Moreover, these compounds co-eluted with the non-*ortho*-CBs in the chromatographic separation (see Fig. 1) and the molecular ions of each homologue group interfere with the precursor ions selected for the $^{13}C_{12}$-isotopically labelled non-*ortho*-CBs. Since the fragmentation pattern of the two families of compounds is similar, the product ions of the labelled non-*ortho*-CBs would be interfered with by the corresponding product ions of the PCNs. For instance, in Fig. 2 the interference of the $[M + 6]^{\bullet+}$ (*m/z* 303.9) molecular ion of pentachloronaphthalenes with the $[M + 2]^{\bullet+}$ (*m/z* 304.0) molecular ion of the labelled PCB 77 and 81 is shown. In addition, the corresponding product ions produced in the CID process (*m/z* 234.0 and *m/z* 232.0) could also interfere. In the same way, the hexa- and heptachloronaphthalenes interfere with the signal of the labelled PCB 126 and 169, respectively. In order to avoid these interferences in the quantification of the non-*ortho*-CBs using the GC–MS–MS method, several experiments studying the effect of the CID excitation voltage on the fragmentation of the PCNs were carried out to prove that the MS–MS measurements provided enough selectivity. Fig. 3 shows the relative abundance of the $[M - 2Cl]^{+}$ product ions for the penta-, hexa- and hepta-CNs and the corresponding abundance profiles for the labelled non-*ortho*-CBs. As can be seen, the optimum CID excitation voltages for PCNs were higher than those selected for the labelled compounds (1.4 V).

The results obtained with the proposed GC–MS–MS method in the certification exercise are given in Table 2, and as an example, Fig. 4 shows the GC–MS–MS chromatograms of a chub sample. The mean values obtained by our laboratory (Table 2) agreed with the mean of all the European laboratories, which participated in the intercomparison exercise using the GC–HRMS technique. Relative standard deviations from 7 to 9.3% were obtained for the chub sample and recoveries ranging from 80 to 95% were achieved. All these results show that the appropriate selection of the excitation CID voltage in ITMS–MS provides enough selectivity to prevent PCNs interferences. As a conclusion, this method is proposed for the analysis of non-*ortho*-CBs in fish samples.

4. Conclusions

The suitability of GC–MS–MS for the determination of non-*ortho*-CBs in fish samples has been demonstrated. The accurate optimisation of the CID parameters made it possible to achieve maximum sensitivity and selectivity in the determination of the non-*ortho*-CBs avoiding interferences of related compounds such as polychlorinated naphthalenes. LODs ranging from 0.12 to 0.16 pg g^{-1} for fish samples and repeatability and long-term precision with relative standard deviation lower than 10% were obtained The comparison of the results obtained by GC–MS–MS and GC–HRMS showed that GC–MS–MS is an interesting low-cost alternative to GC–HRMS for the analysis of these compounds. In view of the good results obtained in two European intercomparison exercises, the GC–MS–MS method developed is proposed. Further studies are being carried out in order to demonstrate the general applicability of this technique for the analysis of these compounds in biota samples.

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