ION TRAP MS/MS AS ALTERNATIVE TO HRMS FOR THE ANALYSIS OF NON-ORTHO PCBS IN BIOTA SAMPLES

J. Malavia, F.J. Santos and M.T. Galceran

Dept. de Química Analítica, Universitat de Barcelona, Martí i Franqués 1-11, 08028 Barcelona. Spain

Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous toxic environmental pollutants of great concern. Among the 209 possible congeners, special attention has been focused on the study of non-ortho and mono-ortho PCBs because they show a similar toxicity as polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs)[1]. Non-ortho PCBs are usually present at low concentrations compared to the bulk of PCBs in the environmental matrices[2]. Therefore, the analysis of these compounds always involves extensive clean-up procedures and the use of gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS). In the last years, mass spectrometry based on ion-trap analysers (ITMS) have become an interesting alternative at low cost to high-resolution mass spectrometry (HRMS) for the analysis of these compounds in environmental and food samples[3,4]. The popularity of GC-ITMS is based on a favourable combination of excellent sensitivity in full-scan mode and high selectivity using tandem mass spectrometry (MS/MS). Nevertheless, further studies should be performed in order to determine the real applicability of this technique for the analysis of these compounds in environmental and food matrices.

The aim of this work is to demonstrate the suitability of the ion trap tandem mass spectrometry in the analysis of non-ortho PCBs in environmental samples. For this purpose, we have used an analytical method for the isolation of these compounds based on the use of multilayer silica columns and SPE cartidges pre-packed with Carbopack B. In addition, a GC-ITMS/MS method was optimised and validated participating in two European certification exercises for the determination of the CBs 71, 81, 126 and 129 in eel and chub samples, which are candidates to reference materials.

Methods and Materials

Standards

An individual standard solution of CB-77, 81, 126 and 169 was prepared in isooctane (200 $\mu g \cdot g^{1}$) from the individual solid congeners, purchased from AccuStandard Inc. (New Haven, USA). $^{13}C_{12}$ isotopically labelled CBs 77, 81, 126 and 169 were used as internal standards (99 % purity, Wellington Laboratories, Guelph, Canada) for the determination of the native CB compounds by GC-MS. The standard solution of $^{13}C_{12}$ isotopically labelled CBs 70, 111, 138 and 170 (WHO/EPA PCB-ISS), supplied by Wellington Laboratories (Guelph, Canada), was used as syringe standard for recovery determination.

Materials

All solvents (Merck, Darmstadt, Germany) were of high purity for pesticide residue. Silica gel 60 (0.063-0.2 mm) was obtained from Merck and was activated at 450ºC for 4 h before use. Superclean ENVI-Carb SPE tubes (3ml, 0.25 g) were provided by Supelco (Bellefonte, PA, USA).

GC-MS instrumentation

All analysis of non-ortho PCBs were carried out on a Trace GC 2000 gas chromatograph coupled with a GCQ/Polaris ion-trap mass spectrometer (ThermoFinnigan) . The chromatographic separation was

performed using a DB-5 (J&W Scientific, Folsom, USA) (5% phenyl, 95 % methylpolysiloxane) fused-silica capillary column (60 m ´ 0.25 mm I.D., 0.25 µm film thickness). Oven temperature program was 90 ºC (held for 3 min) to 200 ºC at 20 ºC/min (held for 1 min) and to 300 ºC at 2.5 ºC/min (held for 10 min). Helium was used as carrier gas at a flow rate of 33 cm/s at 90 ºC. Injector temperature was maintained at 275 ºC and splitless injection mode (1min) was used. Ion trap MS conditions were: ion source temperature 200ºC, transfer line temperature 290 ºC, trap-offset 10V and ionisation energy 70 eV. Xcalibur v. 1.2 was used for acquisition and treatment of the results. A HP-5890 Series II gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to an AutoSpec-Q (Micromass, Manchester, UK) mass spectrometer, operating in EI+ (32 eV) mode and at a resolving power of 10,000 was also used. Source and transfer line temperatures were set at 250 ºC and 280 ºC, respectively. The chromatographic conditions were the same as for GC-ITMS/MS. Monitorised masses in SIM mode were $[M]^{+\cdot}$ and $[M+2]^{+\cdot}$ for tetra-CBs and $[M+2]^{+\cdot}$ and $[M+4]^{+\cdot}$ for penta and hexa-CBs.

Analytical method

20 g of eel sample or 50 g of chub sample were respectively mixed with 40 g and 150 g of anhydrous sodium sulphate. The sample was transferred into a glass thimble and it was spiked with a standard mixture of $^{13}C_{12}$-labelled PCBs. The samples were Soxhlet extracted for 16 hours with 300 ml of n-hexane/dichloromethane (1:1). After evaporation, the extract was cleaned on a silica silica column (15 g of activated silica/22 % sulphuric acid and 30 g of activated silica/44% sulphuric acid), and the non-ortho PCBs were eluted with 100 ml of n-hexane. The extract was concentrated up to 2ml and the non-ortho PCBs were separated on a Superclean ENVI-Carb SPE cartidge[5]. Two fractions were obtained using the following eluents: (a) 15 ml of hexane for fraction 1, (b) 20 ml of hexane/toluene (99:1) for fraction 2 and (c) 20 ml of hexane/toluene (75:25) for fraction 3, which contained the non-ortho PCBs. Fraction 3 was evaporated and analysed by GC-ITMS/MS and GC-HRMS using $^{13}C_{12}$-PCBs 70, 138 and 170 as syringe standard.

Results and Discussion

GC-Ion-trap MS/MS optimisation

The different acquisition segments for each group of chlorination over the chromatographic run were established. The precursor ions of native and labelled non-ortho PCBs, and the $^{13}C_{12}$-PCBs of syringe standard were assigned to the corresponding segments along the chromatographic run (Table 1). For all studies, the mass isolation window of the precursor ion was set to 2 m/z in order to obtain a high selectivity and a good sensibility. CID parameters such as excitation time, excitation voltage and the stability q_z parameter were optimised. The effect of the resonant excitation voltage on the product ion yield was first investigated from 0.2 to 2.5 V in 0.1-V steps. For these experiments, the CID excitation time and the q_z were fixed to 15 ms and 0.45, respectively. At these conditions, the product ion spectra were dominated by fragment ions corresponding to the loss of one Cl atom from the precursor ion of each homologue group. The optimum excitation voltage for each PCB are shown in Table 1, and ranged from 1 to 1.3 V for all PCBs. Several studies were performed changing the excitation time from 10 to 30 ms and the q_z value from 0.225 to 0.45. The best fragmentation conditions were obtained at an excitation time of 15 ms and a q_z of 0.45. The product ions selected for each CB are given in Table 1.

Quality parameters of the GC-ITMS/MS method were established using a blank of eel sample. Repeatability (%RSD, n=5) was between 8.1 and 9.7 % and long-term precision (%RSD, 4 replicates 3 days) was from 8.4 % to 9.8 %. LODs were between 0.09 and 0.11 $pg \cdot g^{-1}$. Calibration curve was established between 0.1 and 500 $ng \cdot g^{-1}$, with correlation coefficients higher than 0.9999.

Table 1. GC-ITMS/MS conditions.

CB	Segment	MRM for quantification Precursor ion (m/z)	Product ion (m/z)	MRM for confirmation Precursor ion (m/z)	Product ion (m/z)	Excitation voltage (V)
CB-77, 81	1	2919	221.9	289.9	219.9	1.0
$^{13}C_{12}$-CB 77, 81		304.0	234.0	302.0	232.0	
$^{13}C_{12}$-CB 70		304.0	234.0	302.0	232.0	
CB-126	2	325.9	255.9	327.9	257.9	1.2
$^{13}C_{12}$-CB 126		337.9	267.9	339.9	269.9	
$^{13}C_{12}$-CB 138		371.9	301.9	373.9	303.9	
CB-169	3	359.8	289.8	361.8	291.8	1.3
$^{13}C_{12}$-CB 169	4	371.9	301.9	373.9	303.9	
$^{13}C_{12}$-CB 170		405.8	335.8	407.8	337.8	

Validation of the GC-ITMS/MS method

The method was validated participating in a European interlaboratory exercise based on the determination of non-ortho PCBs in an eel sample and in a certification exercise of non-ortho PCBs in a chub. Both exercises were organised under the aegis of the MAT (Measurement and Testing) Programme of the EU. The results obtained in the two exercises are summarised in Table 2, where the mean values obtained by our laboratory using the GC-ITMS/MS and the mean of all European laboratories which participated in the intercomparison exercises are given. As can be seen, the results obtained with the GC-ITMS/MS method agreed with the mean of all laboratories as well as with the GC-HRMS method for eel sample. For all PCBs, relative standard deviations lower than 10% were obtained. As an example, in Figure 1 are given the MRM traces for the non-ortho PCBs in the chub using the GC-ITMS/MS method. The GC-ITMS/MS method is being used in our laboratory for the analysis of polychlorinated dibenzo-p-dioxins and furans in biota samples.

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References

1. Ahlborg U.G., Becking G.C., Birnbaum L.S., Brouwer A., Derk H.J.G.M., Feeley M., Golor G., Hanberg A., Larsen J.C., Liem A.K.D., Safe S.H., Schlatter C., Waern F., Younes M. and Yrjänkeikki E. (1994) Chemosphere 28, 1049.
2. Cochran J.W. and Frame M. (2000) J. Chromatogr. A 843, 323.
3. Leonards P.E.G., Brinkman U.A.Th. and Cofino W.P. (1996) Chemosphere 32, 2381.
4. Hayward D.G., Holcomb J., Glidden R., Wilson P., Harris M. And Spencer V. (2001) Chemosphere 43, 407.
5. Molina X. L., Cabes M., Díaz-Ferrero J., Coll M., Martí R., Broto-Puig F., Comellas L. and Rodríguez-Larena M.C. (2000) Chemosphere 40, 921.

ANALYSIS II

Table 2. Results of the intercomparison exercises on non-ortho PCBs in eel and chub samples

	Intercomparison exercises on non-ortho PCBs in eel and chub samples				
	Eel sample (ng·g^{-1})			Chub sample (pg·g^{-1})	
CB	GC-HRMS [(a)]	GC-ITMS/MS [(a)]	Interlab. Results [(b)]	GC-ITMS/MS [(a)]	Interlab. Results [(b)]
	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.
CB-77	9.9 ± 0.7	11.6 ± 0.9	13.6 ± 4.5	192 ± 17	192 ± 19
CB-81	3.0 ± 0.5	2.0 ± 0.2	2.1 ± 0.9	12.3 ± 1.0	13.2 ± 1.7
CB-126	92.7 ± 7.4	93.0 ± 8.0	91.9 ± 9.0	17.2 ± 1.6	19.9 ± 2.0
CB-169	19.2 ± 1.2	19.7 ± 1.4	19.5 ± 3.7	1.42 ± 0.14	1.73 ± 0.29

(a) n=6; (b) n=12

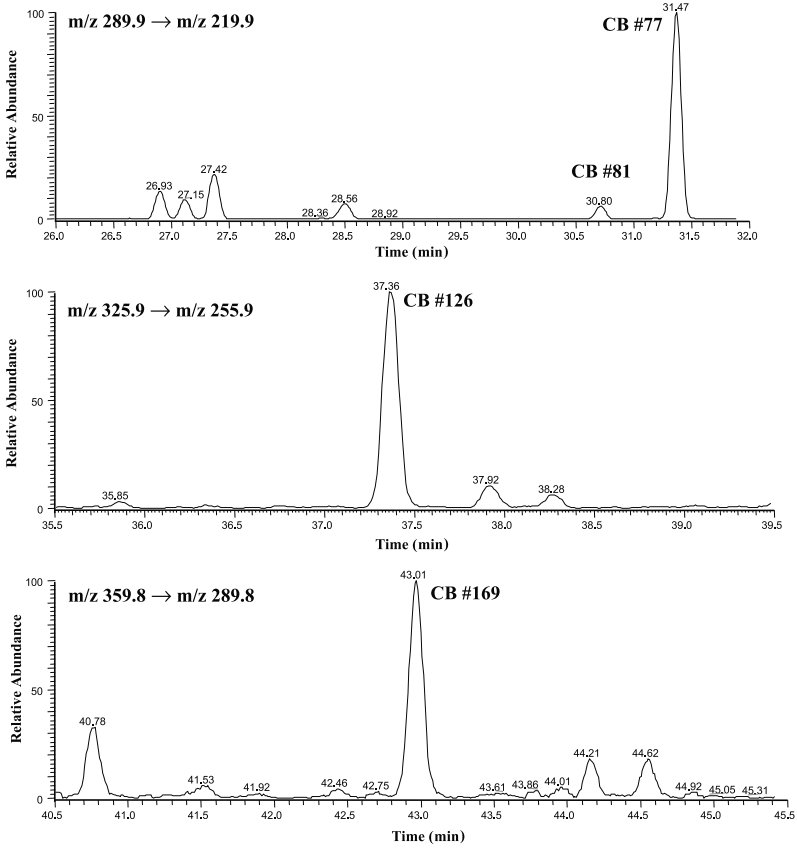

Figure1. MRM tracer for the non-ortho PCBs in the chub sample using the GC-ITMS/MS method.