FEASIBILITY OF TRIPLE-QUADRUPOLE GC-MS/MS AS AN ALTERNATIVE TECHNIQUE TO GC-HRMS FOR THE ANALYSIS OF PCDD/Fs AND DL-PCBs IN FOOD AND FEEDSTUFF SAMPLES

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Introduction

Surveillance programs to determine the levels of polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (DL-PCBs) in food and feedstuff samples have been intensified as a consequence of an increased safety concern about dietary exposure to these compounds as well as to other families of persistent organic pollutants (POPs). In general, despite some recent incidents causing an increase of the levels of PCDD/Fs and DL-PCBs for a certain period of time, usually low background concentrations are determined in food and feed matrices. Therefore, there is a need for analytical techniques providing enough precision, trueness and adequate limits of detection and quantification for routine analysis at these low levels.

Gas chromatography coupled to tandem mass spectrometry with a triple quadrupole analyser (GC-QqQ(MS/MS)) seems to potentially have sensibility, selectivity and robustness comparable to those of the present confirmation technique for PCDD/F and DL-PCB determination in food and feed matrices (*i.e.* gas chromatography coupled to high resolution mass spectrometry (GC-HRMS)[1,2]). Until now, other techniques such as gas chromatography coupled to ion trap tandem mass spectrometry (GC-IT(MS/MS))[3,4] and comprehensive two-dimensional gas chromatography (GC×GC) coupled to micro electron capture detection (μ-ECD)[5] or time-of-flight mass spectrometry (MS-ToF)[6,7] have been validated for acceptance as alternatives to GC-HRMS. In addition, bioanalytical methods have considerably improved in sensitivity and selectivity to the extent that they are used as screening methods to determine the total quantities of dioxin-like compounds[8]. However, none of the above mentioned approaches have been proved to be totally comparable to GC-HRMS for the analysis of PCDD/Fs and DL-PCBs in food and feedstuff samples. In this sense, GC-QqQ(MS/MS) has been considered as a promising technique for the analysis of these pollutants, being able for instance to provide a higher number of identification points compared to other mass spectrometry techniques[9].

In the present work, transitions from different precursors to product ions have already been evaluated at several collision energies in order to find the optimal conditions for the analysis of PCDD/Fs and DL-PCBs by GC-QqQ(MS/MS). Quality parameters (*e.g.* linearity, precision and limits of detection) will also be established before analysing different food and feedstuff extracts. Results will be compared to those obtained by GC-HRMS in order to asses the applicability of the technique for this kind of analysis.

Materials and methods

GC-QqQ(MS/MS): MS/MS analysis of PCDD/Fs and DL-PCBs was performed on TRACE GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) equipped with a triple quadrupole (TSQ Quantum XLS, Thermo Fisher Scientific, Bremen, Germany). Injections were performed in the PTV mode (1 μl; 80ºC, hold for 0.05 min, and then to 275ºC at 10ºC/s, hold for 1.2 min, and then to 300ºC at 10ºC/s, hold for 37 min; splitless time: 1.2 min) in a capillary HP-5ms column (30 m, 0.25 mm i.d., 0.25 μm film thickness) purchased from Agilent Technologies (Palo Alto, CA, USA). The column temperature was programmed from 90ºC (4 min) to 160ºC at a rate of 15ºC/min, then to 225ºC at 4ºC/min and then to 290ºC (4 min) at 7ºC/min. Helium was used as the carrier gas at a constant flow rate of 0.8 ml/min. The temperature of the transfer line and the MS source were set at 290ºC and 240ºC, respectively. Collision gas pressure was set to 1 mTorr for all the experiments.

GC-HRMS: Comparative analyses will be performed on a TRACE GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) coupled to a DFS high resolution mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) controlled by a Xcalibur data system. The chromatographic column used is a 60m x 0.25 mm i.d. x 0.25 µm film thickness DB-5ms fused silica column (J&W Scientific Inc., Folsom, CA, USA). DFS has an EB configuration combining a toroidal electrostatic analyser with a magnetic analyser. Positive electron ionisation (EI+) operating in the MID mode at 10000 resolving power is used.

Results and discussion

For the optimisation of the multiple reaction monitoring (MRM) method, different transitions were studied in order to select the most intense and, if possible, to achieve the highest number of identification points[9]. For PCDD/Fs, the loss of $COCl^{\bullet}$ group was the most abundant when collision induce dissociation (CID) voltages around 25 and 30 V were applied. Table 1 summarised the most intense transitions selected for each native PCDD/Fs. As can be observed, collision energies were always higher in the case of PCDFs. In addition, some other transitions were observed and studied when CID was set to higher values (40 – 50 V). The loss of 135 uma from the molecular cluster was common for PCDFs and the relative abundance of this transition varied with the chlorination degree, being more intensive for lower chlorinated congeners (*i.e.* TCDF and PeCDFs) than for HxCDFs to OCDF. On other hand, PCDDs used to present transitions from the molecular cluster to $[M\text{-}126]^{+}$ if the voltage applied in the collision cell is higher than 40 V. Some other transitions were registered (*e.g.* losses of $Cl^{\bullet}$ for HxCDD/Fs and of $COCl_2^{\bullet}$ for OCDD/F) although the relative abundance, in comparison with those summarised in Table 1, was very low in all cases.

For DL-PCBs, similar behaviour was observed, being the most intense transitions those from the molecular cluster to the loss of two atoms of chlorine for the congeners investigated, as is reported in Table 2.

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Table 1. Chromatographic segments, retention times and MRM transitions and CID voltages selected for native PCDD/Fs.

Segment No.	Time (min)	Congener	Precursor ion	Product ion	CID (V)
1	**24.0 – 27.0**	**2,3,7,8-TCDF**	304	241	28
			306	243	27
		2,3,7,8-TCDD	320	257	21
			322	259	20
2	**27.0 – 30.0**	**1,2,3,7,8-PeCDF**	340	277	27
			342	279	25
		2,3,4,7,8-PeCDF	340	277	27
			342	279	25
		1,2,3,7,8-PeCDD	356	293	19
			358	295	20
3	**30.0 – 32.4**	**1,2,3,4,7,8-HxCDF**	374	311	28
			376	313	28
		1,2,3,6,7,8-HxCDF	374	311	29
			376	313	28
		2,3,4,6,7,8-HxCDF	374	311	28
			376	313	29
		1,2,3,4,7,8-HxCDD	390	327	17
			392	329	20
		1,2,3,6,7,8-HxCDD	390	327	19
			392	329	19
		1,2,3,7,8,9-HxCDD	390	327	20
			392	329	17
		1,2,3,7,8,9-HxCDF	374	311	28
			376	313	29
4	**32.4 – 35.2**	**1,2,3,4,6,7,8-HpCDF**	408	345	29
			410	347	29
		1,2,3,4,6,7,8-HpCDD	424	361	20
			426	363	20
		1,2,3,4,7,8,9-HpCDF	408	345	27
			410	347	28
5	**35.2 – 37.0**	**OCDD**	458	395	19
			470	397	18
		OCDF	442	379	27
			444	381	27

Table 2. Chromatographic segments, retention times and MRM transitions and CID voltages selected for native DL-PCBs.

Segment No.	Time (min)	Congener	Precursor ion	Product ion	CID (V)
1	**21.0 – 22.8**	**CB77**	290	220	23
			292	222	24
		CB81	290	220	23
			292	222	24
2	**22.8 – 26.2**	**CB123**	326	254	25
			328	256	26
		CB118	326	254	25
			328	256	25
		CB114	326	254	23
			328	256	25
		CB105	326	254	25
			328	256	26
		CB126	326	254	24
			328	256	26
3	**26.2 – 29.4**	**CB167**	360	290	26
			362	290	27
		CB156	360	290	26
			362	290	26
		CB157	360	290	26
			362	290	26
		CB169	360	290	25
			362	290	25
4	**29.4 – 32.0**	**CB189**	394	324	27
			396	326	26