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Ion-Trap Tandem Mass Spectrometry for the Analysis of Polychlorinated Dibenzo-*p*-dioxins, Dibenzofurans, and Dioxin-like Polychlorinated Biphenyls in Food

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This paper reports on the applicability of gas chromatography coupled to ion-trap tandem mass spectrometry (GC/ITMS/MS) for the analysis of polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (dl-PCBs) in food. MS/MS parameters were selected to achieve the high sensitivity and selectivity required for food analysis. Good precision (RSD = 5–18% for PCDD/Fs and 6–14% for dl-PCBs) and low limits of detection for PCDD/Fs (0.1–0.93 pg/g of fat) and dl-PCBs (0.1–0.89 pg/g of fat) were obtained. A comparative study of the congener-specific determination using both GC/ITMS/MS and GC−high resolution mass spectrometry (GC/HRMS) was performed by analyzing several matrices such as milk, fish oil, chicken, pork, fish, eggs, and a chicken compound feed, at low pg/g levels. The results using GC/ITMS/MS were in good agreement with those obtained by GC/HRMS. Consequently, GC/ITMS/MS is proposed for the analysis of PCDD/Fs and dl-PCBs in food and feed samples.

KEYWORDS: Dioxins; furans; dioxin-like PCBs; food; feed; gas chromatography−ion trap mass spectrometry; tandem mass spectrometry

INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (dl-PCBs), non-ortho- and mono-ortho-PCBs, constitute an important group of ubiquitous pollutants of great concern because of their high toxicity and persistence in the environment. PCDD/Fs have never been deliberately produced but have been released in the environment as byproducts from combustion processes and industrial synthesis of other chlorinated chemicals (*1*). Unlike dioxins, PCBs have been intentionally produced and widely used as commercial products in the chemical industry. Because these compounds have a highly hydrophobic and lipophilic character and are resistant to chemical and biological degradation (*2*), they tend to bioaccumulate through the food chain up to humans, resulting in a potential risk for human health. Seven 2,3,7,8-substituted PCDDs and 10 PCDFs are generally considered to be the most persistent and toxic PCDD/Fs congeners. In addition, four non-ortho and eight mono-ortho-substituted PCBs, known as dioxin-like PCBs, have toxicological properties similar to those of 2,3,7,8-substituted PCDD/Fs (*3*) and have thus been attributed toxic equivalency factors (TEFs) (*4*).

Because >90% of human background exposure to dioxins and dl-PCBs is estimated to occur through the diet (*5*, *6*), there is great interest in evaluating the presence of these compounds in foodstuffs. In this context it must be mentioned that nowadays a major proportion of the European population still exceeds the tolerable weekly intake (TWI) recommended by the Scientific Commission on Food (SCF), which is 14 pg of WHO-TEQ kg^{-1} of body weight (*7*). Therefore, continuous efforts should be made to reduce the contaminant releases and to control the safety of the food chain and food supply. Generally, foodstuffs of animal origin contribute to approximately 80% of the overall diet exposure, the main sources of contamination being meat, fish, and dairy products (*8*, *9*). Dioxin contamination incidents have occurred in various countries. For instance, in Belgium during the spring and the summer of 1999 (*10–12*), animal fats contaminated with PCBs were used to produce animal feed for pork and chicken, and dioxins were found in Spanish kaolinitic clay widely used as feed additive agents (*13*). More recently, dioxin contamination was found in choline chloride, a feed ingredient, due to the use of pentachlorophenol-treated wood shavings as a carrier (*14*). These incidents have pointed out the need for continuous monitoring of foodstuffs to allow rapid

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Table 1. Quality Parameters of GC/ITMS/MS Method for the Analysis of PCDD/Fs

	linearity				precision	
		RRF				
compound	correlation coeff (r^2)	mean[a]	RSD (%)	LOD (pg injected)	run-to-run[b] (RSD, %)	day-to-day[c] (RSD, %)
2,3,7,8-TCDF	0.9999	0.991	6.2	0.10	5.3	6.7
1,2,3,7,8-PeCDF	0.9994	1.010	6.1	0.10	5.8	5.2
2,3,4,7,8-PeCDF	0.9997	0.984	5.0	0.12	5.1	5.7
1,2,3,4,7,8-HxCDF	0.9999	1.015	3.9	0.14	5.4	6.2
1,2,3,6,7,8-HxCDF	0.9999	0.994	5.2	0.18	5.4	6.6
2,3,4,6,7,8-HxCDF	0.9999	0.960	7.6	0.17	4.9	7.9
1,2,3,7,8,9-HxCDF	0.9996	0.943	5.4	0.19	5.9	9.4
1,2,3,4,6,7,8-HpCDF	0.9999	1.092	3.5	0.16	5.7	6.2
1,2,3,4,7,8,9-HpCDF	0.9999	0.936	4.7	0.20	2.9	6.0
1,2,3,4,6,7,8,9-OCDF	0.9999	1.073	7.4	0.22	4.2	5.9
2,3,7,8-TCDD	0.9999	1.066	8.5	0.13	2.4	4.3
1,2,3,7,8-PeCDD	0.9999	1.031	7.8	0.20	5.3	5.5
1,2,3,4,7,8-HxCDD	0.9999	0.996	6.3	0.16	5.3	8.0
1,2,3,6,7,8-HxCDD	0.9999	0.966	8.2	0.16	2.0	7.1
1,2,3,7,8,9-HxCDD	0.9999	0.964	2.5	0.16	6.2	7.9
1,2,3,4,6,7,8-HpCDD	0.9999	1.045	3.6	0.19	3.5	7.1
1,2,3,4,6,7,8,9-OCDD	0.9999	1.073	1.9	0.28	2.4	6.6
PCB-77	0.9998	1.046	8.4	0.06	3.1	6.5
PCB-81	0.9996	1.018	2.4	0.07	2.5	4.9
PCB-126	0.9999	0.997	2.0	0.08	3.3	6.6
PCB-169	0.9996	0.980	7.7	0.08	3.8	6.4
PCB-123	0.9998	0.957	4.2	0.21	3.3	4.3
PCB-118	0.9997	0.987	3.9	0.19	2.9	4.7
PCB-114	0.9998	0.932	5.6	0.25	2.2	3.5
PCB-105	0.9999	0.977	7.9	0.10	2.7	4.0
PCB-167	0.9999	1.065	4.3	0.14	3.0	4.3
PCB-156	0.9996	0.996	2.8	0.14	2.7	5.0
PCB-157	0.9998	0.996	3.5	0.15	3.2	4.2
PCB-189	0.9999	0.946	8.7	0.14	3.2	6.7

[a] $n = 5$ for PCDD/Fs, $n = 7$ for dl-PCBs. [b] $n = 5$. [c] $n = 5$ replicates $\times$ 3 days.

identification of potential contamination before it becomes out of control (*9*). In response to such incidents, the European Union (EU) implemented comprehensive regulation on foodstuffs and feedingstuffs and launched large monitoring programs to test food and feed. In 2001, the EU established regulations about the maximum residue levels (MRLs) of PCDD/Fs in food products and feedingstuffs to reduce human exposure to these compounds (*15, 16*). In 2006, the MRLs for food and feed products were revised according to the new data available about the background levels and the contribution of dl-PCBs in the total WHO-TEQ values (*17, 18*). To check compliance with these regulatory limits cost-effective analytical methods are required to carry out large-scale monitoring programs.

The analysis of PCDD/Fs and dl-PCBs is usually performed using high-resolution gas chromatography coupled to high-resolution mass spectrometry (GC/HRMS) with isotope dilution for quantification (*19–21*). Sector instruments that routinely operate above 10000 of mass resolution are currently used for HRMS. This technique offers the required specificity and sensitivity down to the femtogram level. Nevertheless, this technique requires a great investment and highly skilled scientists, so alternative methods are under investigation in several laboratories (*19*). Among these methods, gas chromatography coupled to ion trap mass spectrometry (GC/ITMS) working in tandem mode (MS/MS) appears to be one of the most promising techniques, because it makes it possible to counterbalance the potential decrease in selectivity due to the low mass resolution by operating the instrument in tandem mode. In addition, the significant increase of the signal/chemical background noise ratio provided by this approach makes it possible to achieve acceptable detection limits. Until now, GC/ITMS/MS has been applied to the analysis of PCDD/Fs and dl-PCBs in environmental (*22–25*) and food (*26–29*) samples. However, the number of papers demonstrating the real applicability of this technique to the analysis of these compounds, especially for PCDD/Fs, in food and feed is still limited (*27, 29*), probably due to the very high sensitivity and selectivity required for the analysis of these compounds in this kind of sample.

Table 2. Analysis of PCDD/Fs in the Fish CARP-1 (NCR Reference Material)

	concentration (pg/g of product)		
compound	mean $\pm$ SD	RSD (%)	certified value $\pm$ uncertainty
2,3,7,8-TCDF	12.7 ± 1.0	8	11.9 ± 2.7
1,2,3,7,8-PCDF	6.9 ± 0.5	7	5.0 ± 2.0
2,3,7,8-TCDD	7.1 ± 0.6	8	6.6 ± 0.6
1,2,3,7,8-PCDD	5.1 ± 0.4	8	4.4 ± 1.1
1,2,3,4,7,8-HxCDD	1.2 ± 0.1	6	1.9 ± 0.7
1,2,3,6,7,8-HxCDD	5.9 ± 0.5	9	5.6 ± 1.3
1,2,3,7,8,9-HxCDD	0.9 ± 0.1	11	0.7 ± 0.4
1,2,3,4,6,7,8-HpCDD	8.2 ± 0.8	10	6.5 ± 1.8
OCDD	8.0 ± 0.8	10	6.3 ± 1.9

In a recent paper, we demonstrated that GC/ITMS/MS is a useful technique for the analysis of PCDD/Fs and dl-PCBs in vegetables oils. In the present work we evaluate if this technique is also applicable for the analysis of these compounds in food samples at concentrations close to the MRLs established by the EU. Quality parameters such as limit of detection, repeatability, and long-term precision were determined. In addition, a critical comparison about the results obtained using both GC/ITMS/MS and GC/HRMS methods was performed. The applicability of the GC/ITMS/MS method proposed was studied in several European interlaboratory exercises (*30, 31*).

MATERIALS AND METHODS

Chemicals. Acetone, dichloromethane, toluene, *n*-hexane, cyclohexane, and ethyl acetate of residue analysis grade were purchased from Merck (Darmstadt, Germany), whereas nonane of organic trace analysis grade was supplied by Fluka (Buchs, Switzerland). Water from a Milli-Q purification system (Millipore Corp., Bedford, MA) and sulfuric acid 95–97% from Merck were used. 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.

PCCD/Fs and dl-PCBs Standards. A set of five calibration standard solutions (CS1−CS5) of the 17 toxic 2,3,7,8-chloro-substituted PCDD/Fs containing the corresponding $^{13}C_{12}$-labeled compounds in nonane, EPA-1613 CVS, were obtained from Wellington Laboratories Inc. (Guelpth, ON, Canada). The concentrations of native compounds ranged from 0.5 to 2000 ng/mL, and the labeled compound concentrations were 100 ng/mL except for [$^{13}C_{12}$]OCDD, which was 200 ng/mL. The ^{13}C-labeled internal and recovery standard solutions, EPA-1613 ISS and EPA-1613 LCS, were supplied by Wellington Laboratories Inc. in nonane. For dl-PCBs (PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189), a set of seven calibration standards solutions, WP-CVS (CS1−CS7, Wellington Laboratories Inc.) containing native and ^{13}C-labeled compounds at concentrations ranging from 0.1 to 800 ng/mL and 50 ng/mL, respectively, were used. The ^{13}C-labeled internal standard solution WP-ISS (^{13}C-PCB 70, 111, 138, and 170) at a concentration of 1000 ng/mL and the recovery standard solution WP-LCS (1000 ng/mL) were also purchased from Wellington laboratories Inc. All solutions were of purity >99%.

Food Samples. Seven food samples (milk, fish oil, pork tissue, herring tissue, chicken tissue, egg yolk, and egg white) and a chicken compound feed sample supplied by The Netherlands Institute for Fisheries Research (RIVO) were analyzed. The crude fish oil sample

Table 3. PCDD/F and dl-PCB Mean Concentrations of Food Samples ($n = 6$) and Their RSDs for GC/ITMS/MS and GC/HRMS Methods

	milk (pg/g of fat)				fish oil (pg/g of product)				pork (pg/g of fat)				chicken compound feed (pg/g of product)			
	GC-ITMS/MS		GC-HRMS		GC-ITMS/MS		GC-HRMS		GC-ITMS/MS		GC-HRMS		GC-ITMS/MS		GC-HRMS	
	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)
2,3,7,8-TCDF	0.54	12	0.46	13	4.33	11	4.37	12	0.06	13	0.05	28	0.10	13	0.12	4
1,2,3,7,8-PeCDF	1.07	15	0.87	16	0.91	16	1.09	11	0.08	11	0.07	14	0.35	9	0.30	5
2,3,4,7,8-PeCDF	1.65	12	2.00	15	3.71	11	4.70	11	0.29	11	0.28	8	0.39	10	0.31	5
1,2,3,4,7,8-HxCDF	1.20	13	1.06	11	0.50	15	0.42	15	0.36	11	0.32	5	0.36	8	0.32	4
1,2,3,6,7,8-HxCDF	1.27	18	1.02	16	0.51	18	0.41	15	0.26	13	0.27	7	0.29	9	0.30	2
1,2,3,7,8,9-HxCDF	0.93	17	0.77	15	0.36	17	0.31	24	0.16	13	0.18	6	0.29	9	0.30	4
2,3,4,6,7,8-HxCDF	0.87	11	0.91	16	0.38	17	0.45	13	<0.14[a]		0.09	20	0.30	10	0.29	4
1,2,3,4,6,7,8-HpCDF	1.69	10	1.37	19	0.32	18	0.26	13	0.36	9	0.30	9	0.33	10	0.35	6
1,2,3,4,7,8,9-HpCDF	1.04	8	0.85	14	0.21	15	0.17	33	0.16	13	0.16	13	0.29	11	0.28	9
OCDF	2.95	15	3.33	34	0.36	13	0.28	25	<0.33[a]		0.15	19	0.34	10	0.46	16
2,3,7,8-TCDD	0.65	13	0.53	6	0.36	7	0.34	11	0.17	10	0.17	5	0.12	12	0.13	6
1,2,3,7,8-PeCDD	1.24	16	1.08	10	1.20	10	1.26	10	0.31	12	0.36	8	0.33	10	0.32	3
1,2,3,4,7,8-HxCDD	<0.80[a]		0.90	9	<0.40[a]		0.29	24	0.40	11	0.39	9	0.33	9	0.31	5
1,2,3,6,7,8-HxCDD	<0.93[a]		1.15	12	<0.63[a]		0.70	15	0.37	11	0.34	8	0.25	11	0.27	6
1,2,3,7,8,9-HxCDD	<0.80[a]		0.84	12	<0.50[a]		0.29	25	0.20	9	0.18	12	0.31	10	0.30	5
1,2,3,4,6,7,8-HpCDD	3.77	18	3.74	10	0.47	18	0.41	11	0.69	10	0.60	21	0.37	10	0.32	4
OCDD	34.9	18	30.3	10	0.96	12	0.76	35	2.15	8	2.03	10	0.96	10	0.91	8
PCB-77	47.7	12	49.6	12	70.2	8	63.6	10	26.1	7	26.9	7	10.8	8	11.1	5
PCB-81	7.32	12	7.32	13	1.92	14	2.17	12	1.75	8	2.03	7	4.49	7	4.62	6
PCB-126	27.4	5	24.9	13	33.4	8	28.5	12	1.42	8	1.60	4	5.05	5	5.12	7
PCB-169	4.49	11	4.19	15	12.0	14	10.4	10	2.48	8	2.39	4	4.40	5	4.35	8
PCB-123	78.9	7	74.6	12	96.7	14	94.3	45	6.66	7	5.51	6	8.99	7	9.43	8
PCB-118	36703	8	31490	19	5669	11	5589	21	984	7	979	3	1350	7	1431	3
PCB-114	70.6	9	86.8	16	112	8	110	14	22.6	5	23.4	5	8.86	6	9.21	4
PCB-105	831	5	796	12	2042	8	2063	8	136	5	143	6	838	4	884	3
PCB-167	590	5	553	8	366	7	334	17	142	3	147	3	10.9	4	10.5	5
PCB-156	677	6	634	7	635	8	576	15	236	5	245	3	124	6	142	3
PCB-157	85.6	9	78.15	7	184	7	177	15	27.2	6	27.3	3	15.0	7	15.1	5
PCB-189	67.6	7	61.18	7	69.8	8	60.6	16	21.1	8	20.0	2	8.81	7	8.88	2

[a] Lower than the limit of detection.

was obtained from herrings caught west of the Shetland Islands (60.50° N/0.300 W) (*32*) and the herring tissue material from herrings from the North Sea (52.30° N, 02.00 E). Pork tissue, chicken tissue, eggs, and chicken compound feed were obtained from a feeding experiment conducted at the Institute for Animal Science and Health (ID-Lelystad, Lelystad, The Netherlands). The milk sample consisted of a spiked sterilized whole milk with dioxin and dl-PCB congeners at the levels currently found in Dutch raw milk. Before analysis, all of these samples were stored in the dark at 4 °C except egg yolk and white and chicken compound feed, which were frozen at −20 °C.

A certified reference material, CARP-1 (common carp, *Cyphinus carpio*), obtained from the National Research Canada Council (Ottawa, ON, Canada) was used to validate the GC/ITMS/MS method for PCDD/Fs analysis.

Analytical Procedure. Appropriate amounts of fresh sample for egg yolk and white (125 g), herring tissue (60 g), chicken tissue (120 g), and pork tissue (60 g) were freeze-dried prior to the extraction. These samples and the chicken compound feed (45 g) were spiked with known amounts of [$^{13}C_{12}$]PCDD/Fs (EPA-1613 LCS) and [$^{13}C_{12}$]-dl-PCBs (WP-LCS) mixtures and were Soxhlet extracted for 24 h with 300 mL of toluene/cyclohexane (1:1, v/v). For the whole milk sample (130 g), the lipid fraction was extracted by liquid–liquid extraction using diethyl ether and petroleum ether (1:1, v/v). The fish oil sample (5 g) was directly diluted with *n*-hexane. The milk extract and the diluted fish oil were spiked with known amounts of [$^{13}C_{12}$]PCDD/Fs and [$^{13}C_{12}$]-dl-PCBs congeners. All extracts obtained by Soxhlet and liquid–liquid extraction were rotary concentrated at 40 °C up to 100 mL, changing the solvent to *n*-hexane. Fat and organic matter were removed from the extracts by a sulfuric acid treatment, using 50–100 mL of 95–97% sulfuric acid. Finally, the extracts were rotary concentrated and filtered (particulate size did not exceed 1 μm) before the cleanup process. Purification of the different extracts was accomplished using the automated Power-Prep System (Fluid Management System Inc., Waltham, MA) based on the use of multilayer silica, basic alumina, and PX-21 carbon sorbents, prepackaged in columns made of Teflon and hermetically sealed. Two main fractions containing (i) PCDD/Fs and non-ortho PCBs and (ii) mono-ortho PCBs were obtained. The final volume of these extracts were adjusted to ca. 15 μL after addition of the corresponding $^{13}C_{12}$-isotopically labeled PCDD/FS and dl-PCB congeners as internal standards, and the final extracts were analyzed by GC-ion trap MS/MS. An independent analysis of this set of samples was carried out for the determination of PCDD/Fs and dl-PCBs by GC-HRMS.

GC/Ion Trap MS/MS and GC/HRMS. The GC/ion trap MS/MS analysis was performed using a Trace GC 2000 series gas chromatograph coupled to a GCQ/Polaris ion trap mass spectrometer (ThermoFinnigan, Austin, TX) equipped with an AS2000 autosampler. The chromatographic separations of PCDD/Fs and dl-PCBs were conducted on a DB-5MS (5% phenyl, 95% methylpolysiloxane) fused-silica capillary column (J&W Scientific, Folsom, CA), 60 m × 0.25 mm i.d. × 0.25 μm film thickness, with helium as the carrier gas at 1 mL/min. For PCDD/Fs, the oven temperature was programmed from 140 °C (held for 1 min) to 200 °C with a ramp rate of 20 °C/min (held for 1 min) and then to 300 °C at 2.5 °C/min (held for 20 min). For dl-PCBs, the oven was programmed from 140 °C (held for 2 min) to 180 °C at 20 °C/min (held for 1 min) and then to 300 °C with a ramp rate of 2.5 °C/min (held for 5 min). Helium was used as carrier gas at a constant flow rate of 1 mL/min held by electronic pressure control at 90 °C. Injector temperature was kept at 280 °C, and samples and standards were injected (2 μL for PCDD/Fs and 1 μL for dl-PCBs) in the splitless injection mode (1 min). MS operating conditions were the following: positive electron ionization (EI+) mode using automatic gain control (AGC) with electron energy of 70 eV and an emission current of 250 μA. The transfer line and ion source temperatures were kept at 280 and 200 °C, respectively. The instrument was tuned using perfluorotributylamine (FC-43) according to the manufacturer's recommendations to achieve the best sensitivity. The electron multiplier voltage was set to 1350 V (10^5 gain) by automatic tuning. In MS/MS mode, for native and labeled PCDD/Fs and dl-PCBs the $[M + 2]^{\bullet +}$ ion for the cluster molecular ions was selected as precursor ion, except for OCDF, OCDD, and [$^{13}C_{12}$]OCDD, which were the corresponding $[M + 4]^{\bullet +}$. The $[M - CO^{35}Cl]^+$ and $[M - CO^{37}Cl]^+$ product ions for PCDD/Fs and [M

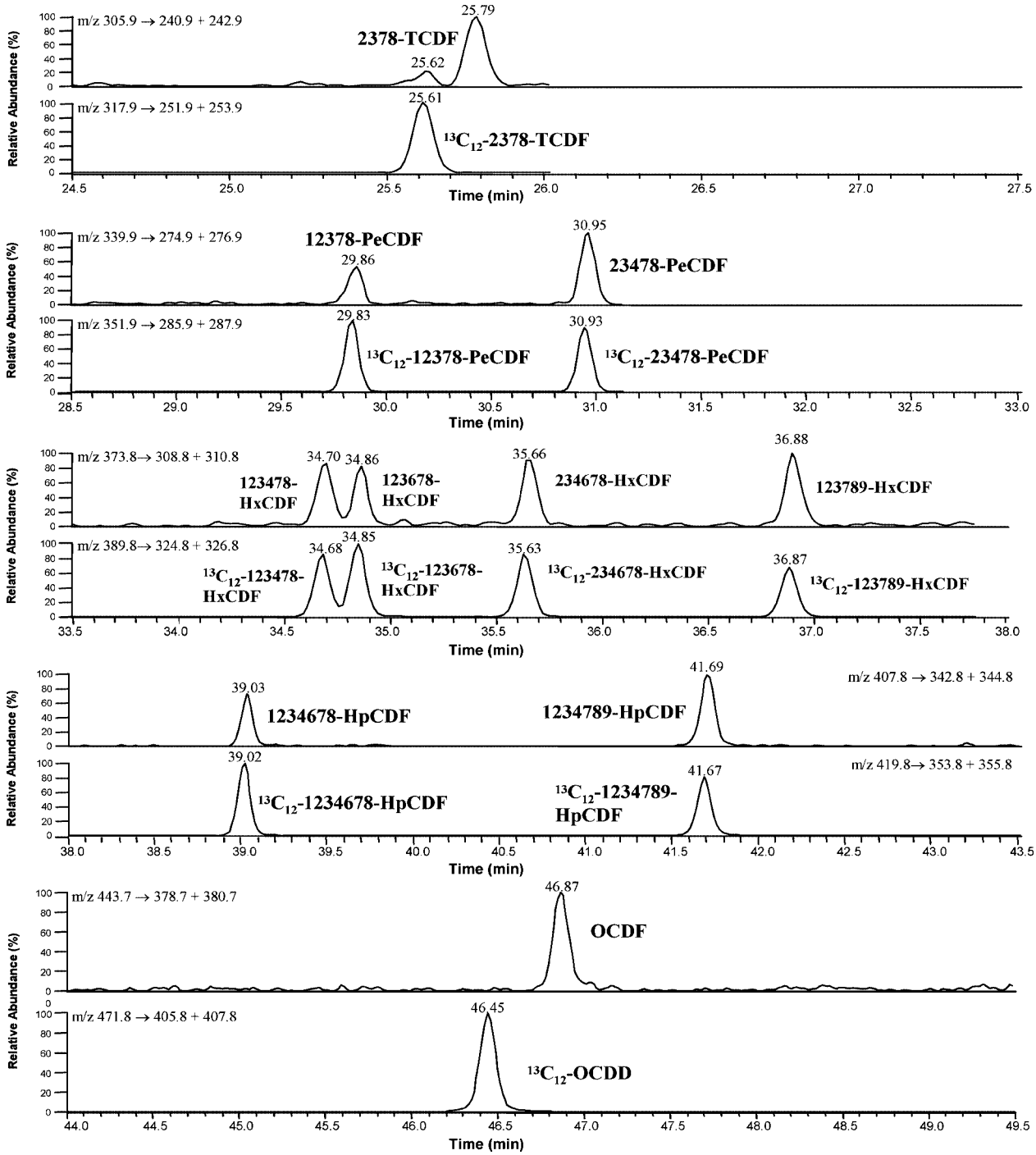

Figure 1. GC/ITMS/MS chromatograms of the PCDFs for a chicken compound feed sample.

$- 2^{35}Cl]^+$ and $[M - {}^{37}Cl^{35}Cl]^+$ product ions for dl-PCBs were monitored for quantitative purposes. After optimization, the resonance excitation voltage applied for the compounds was between 1.4 and 1.9 V. The MS/MS acquisition method was time programmed in eight segments for the different homologue groups. For non-ortho PCBs, three segments were selected in time for determining the tetra-, penta-, and hexa-CBs, whereas for mono-ortho PCBs the segments were chosen for the monitoring of penta-, hexa-, and hepta-CBs. Xcalibur version 2.0 software was used for data acquisition and processing of the results.

The GC/HRMS measurements were performed on an AutoSpec Ultima (Micromass, Manchester, U.K.) high-resolution mass spectrometer coupled to a GC 8000 series gas chromatograph (Carlo Erba Instruments, Milan, Italy) equipped with a CTC A 200S autosampler. Positive electron ionization (EI+) mode was used with an electron energy of 32 eV, a current trap of 500 μA, and an acceleration voltage of 8000 V, operating in the selected ion monitoring (SIM) mode at a resolving power of 10000 (10% valley definition). Verification of the resolution in the working mass range was obtained by measuring perfluorokerosene (PFK) reference peaks. The ion source and transfer line were set at 250 and 280 °C, respectively. The two most abundant ions of the molecular ion cluster of each homologue group for PCDD/Fs and dioxin-like PCBs were monitored at a 50 ms dwell time and a delay time of 20 ms. Chromatographic separations were performed using the same GC column and the same working conditions as those previously described for GC/ITMS/MS measurements.

The target compounds were quantified by isotopic dilution according to the EPA procedure. The relative response factor (RRF) of each individual 2,3,7,8-chloro-substituted PCDD/F and dl-PCB congeners was obtained from the calibration standard solutions. The results are expressed in picograms of WHO-TEQ per gram (*4, 33*), and the concentrations for nondetectable compounds (upperbound values) were calculated using the limit of detection (LOD) (*34*).

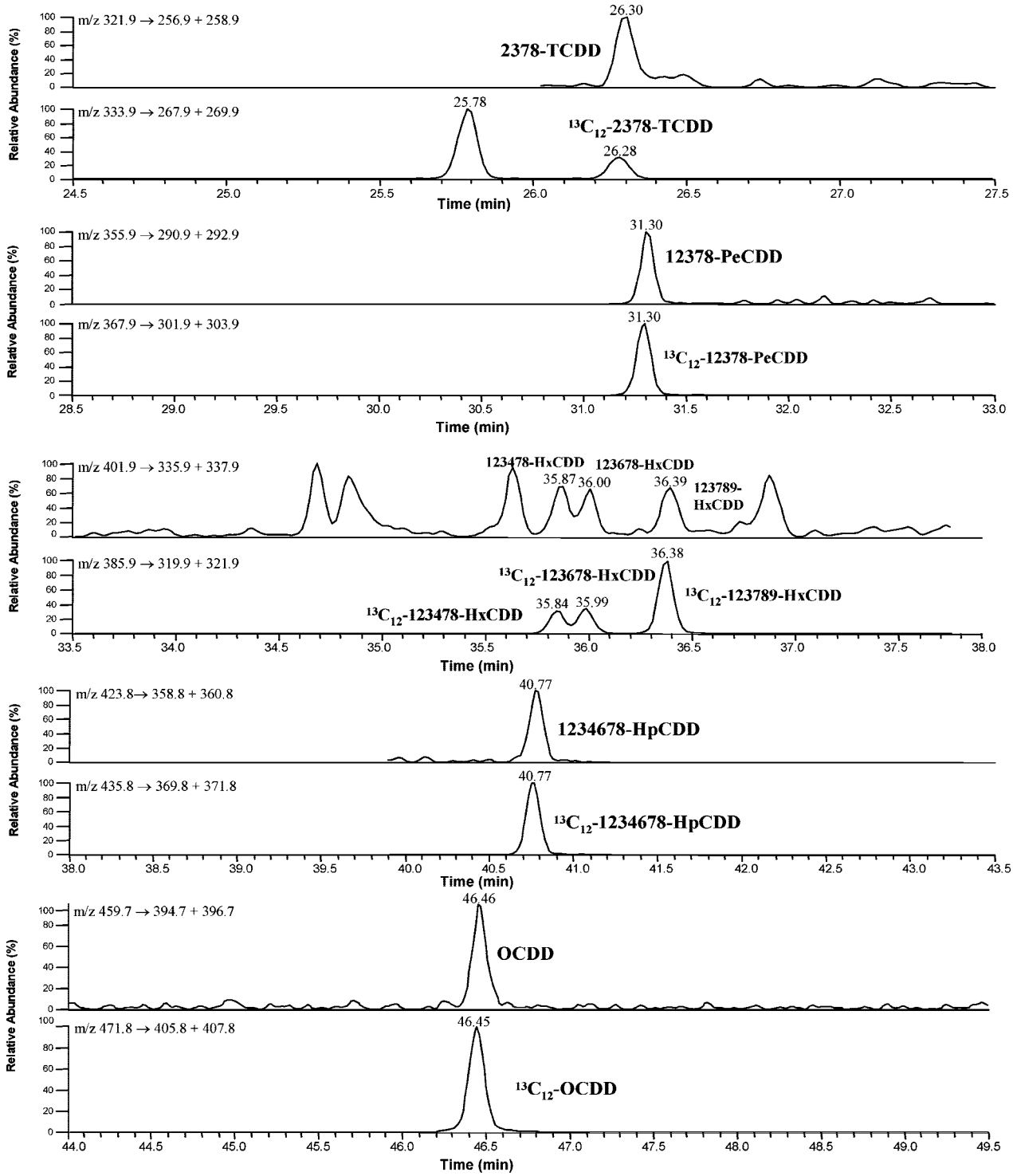

Figure 2. GC/ITMS/MS chromatograms of the PCDDs for a chicken compound feed sample.

Quality Control Criteria. A daily isomer-specific GC separation test, sensitivity test, and calibration test were carried out. To prevent any loss of sensitivity and to detect changes in LODs during analysis, standard solutions of PCDD/Fs and dl-PCBs at low concentration levels (0.5 ng/mL for PCDD/Fs and 0.1 ng/mL for dl-PCBs) were analyzed in the same GC run sequence as the samples (one standard every two extracts). Other performance checks were 10000 resolution power for HRMS, S/N ratio higher than 3 for ITMS/MS, procedural blanks of both instruments and methods, and recovery rates of the labeled compounds (40–120%). Additionally, a certified reference material (CARP-1) was analyzed to ensure that the analytical method was maintained under control.

To confirm the identification of PCDD/Fs and dl-PCBs the following restrictive criteria were applied: (a) the signal-to-noise ratio should be greater than 3 for each congener, (b) the isotope ratios between the two monitored product ions should be within ±20% of the theoretical value, and (c) the retention time should be within the margin of ±2 s of those observed for standards. Quantification of PCDD/Fs and dl-PCBs was carried out by isotope dilution using relative response factors (RRFs) (*35*).

RESULTS AND DISCUSSION

GC-ITMS/MS Method. The optimal MS/MS operating parameters for the determination of PCDD/Fs and dl-PCBs were those previously established (*28*, *29*). Because for each analyte it is necessary to monitor at least two different product ions to check the isotope ratio for confirmation purposes, precursor ions containing at least one ^{37}Cl atom were used to ensure the

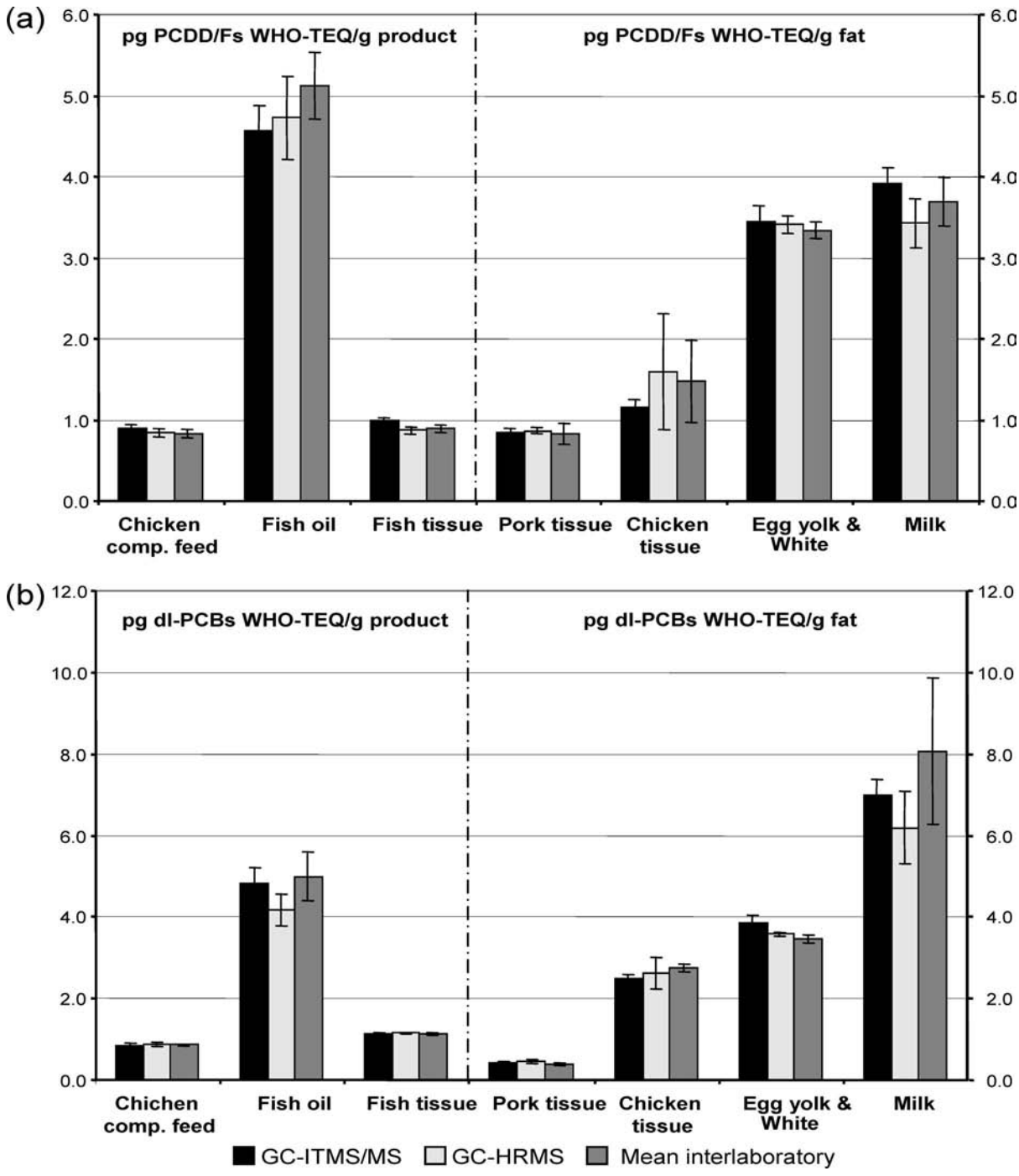

Figure 3. Total PCDD/F (**a**) and dioxin-like PCB (**b**) WHO-TEQ values obtained for the selected food samples using GC/ITMS/MS and GC/HRMS along with the means of an interlaboratory exercise.

production of both $[M - CO^{35}Cl]^+$ and $[M - CO^{37}Cl]^+$ ions for PCDD/Fs and $[M - {}^{35}Cl_2]^+$ and $[M - {}^{35}Cl^{37}Cl]^+$ ions for dl-PCBs. Therefore, for tetra-CDD/Fs to hepta-CDD/Fs and for dl-PCBs the $[M + 2]^{\bullet+}$ ions were selected as precursor ions, whereas for OCDD and OCDF the $[M + 4]^{\bullet+}$ ions were used (*28*, *29*). Other MS/MS parameters that affect the sensitivity and selectivity of the detection of the target compounds were set according to those previously reported for vegetable oils (*28*, *29*), and some experiments were conducted to confirm the suitability of these values for the analysis of the selected food samples.

Quality parameters of the GC/ITMS/MS method such as linearity, limits of detection, repeatability, and long-term precision were established using standard solutions. For this purpose, five standard solutions in the range of 0.5−200 ng/mL for 2,3,7,8-TCDD/F, 2.5−1000 ng/mL for penta- through hepta-CDD/Fs, and 5.0−2000 ng/mL for OCDD/F containing the corresponding labeled compounds were used to determine the linearity. For dl-PCBs, calibration curves using seven calibration solutions at concentrations between 0.1 and 800 ng/mL were established. Good linearity was obtained for all of the compounds, with correlation coefficients >0.9994 (**Table 1**). As expected, the mean values of the RRF for each congener obtained from the calibration solutions were close to 1.0 with relative standard deviations of <9%. In addition, the RRF values were found to be reproducible through 24 months, with a relative standard deviation (RSD) of <15%. Limits of detection (LODs), defined as the concentration that produces a signal-to-noise ratio

Table 4. Synthesis of ANOVA Results Using Fisher's Test for the PCDD/F and dl-PCB Congeners

	calculated *F* values			*P* values ($\alpha = 0.05$)		
compound	method	matrix	interactions	method	matrix	interactions
			Milk, Fish Oil, Pork Tissue and Chicken Compound Feed			
PCDD/Fs WHO-TEQ	0.1873	604.80	2.137	0.6675	2.42E−33	0.1108
dl-PCBs WHO-TEQ	0.2961	189.57	1.260	0.5893	1.12E−23	0.3024
Total WHO-TEQ	0.4778	463.85	2.618	0.4934	4.27E−31	0.0641
			Chicken Tissue, Eggs Yolk and White, and Herring Tissue			
PCDD/Fs WHO-TEQ	0.3368	398.46	2.259	0.5776	4.79E−09	0.1587
dl-PCBs WHO-TEQ	1.7598	6023.89	2.937	0.2212	9.43E−14	0.0991
Total WHO-TEQ	0.1042	2724.07	0.6045	0.7552	2.25E−12	0.6303

(S/N) greater than 3, were determined experimentally using standard solutions at low concentration levels. Instrumental LODs (**Table 1**) were 5−10-fold higher than those found in HRMS and ranged from 0.1 to 0.28 pg injected for PCDD/Fs and between 0.06 and 0.21 pg injected for dl-PCBs. These values are in agreement with those reported in the literature for PCDD/Fs (*19*, *22*, *25*, *27*, *36*) and dl-PCBs (*35*–*37*). The precision of the GC/MS/MS method was assessed by consecutively analyzing five replicates of a standard mixture of PCDD/Fs at low concentration levels (2 ng/mL) and dl-PCBs (0.5 ng/mL) on one day for run-to-run and on three different days for day-to-day. Good precision was achieved with RSDs between 2.1 and 6.2% and from 3 to 9.4% for run-to-run and day-to-day precision, respectively. To evaluate the accuracy of the developed method, a carp tissue certified reference material, CARP-1 (National Research Canada Council), was analyzed. Triplicate analysis was carried out following sample treatment proposed for the analysis of fish tissue (see Materials and Methods) and the established GC/ITMS/MS method. As can be seen in **Table 2**, the results obtained using GC/ITMS/MS agreed with the certified concentrations of the material. RSDs from 7 to 11% were obtained, and recoveries ranging from 85 to 95% were achieved. These results show that the GC/ITMS/MS method can be considered as an attractive alternative to HRMS for the analysis of PCDD/Fs in food samples.

Analysis of Food Samples. To examine in depth the feasibility of the proposed method for the determination of PCDD/Fs and dl-PCBs in food samples, a validation study comparing both GC/ITMS/MS and GC/HRMS methods was performed. For this purpose, four matrices, milk, fish oil, pork tissue, and chicken compound feed, were analyzed. Six independent analyses (three analyses of each sample on two different days) were carried out using the same sample intake and sample preparation procedures for both MS techniques. PCDD/F and dl-PCB concentration mean values with their corresponding RSDs are given in **Table 3**. Results were expressed for each individual congener in picograms per gram of fat or product, depending on the matrix. As can be seen, good agreement between GC/ITMS/MS and GC/HRMS techniques was obtained for all individual congeners with differences in the PCDD/Fs mean values of <20%, except for milk and fish oil, for which the differences were slightly higher, between 15 and 30%, probably due to matrix interferences because positive deviations were observed for nearly all PCDD/Fs in GC/ITMS/MS. For dl-PCBs, good agreement between both MS methods was achieved for all congeners with differences in the mean values of <20%. Although the sensitivity obtained using GC/ITMS/MS was enough to determine the target compounds, some PCDD/Fs such as HxCDDs in milk and fish oil and 2,3,4,6,7,8-HxCDF and OCDF in pork tissue were found to be at lower concentrations than the LODs of the GC/ITMS/MS method. Regarding the precision achieved by the two MS methods, comparable RSDs ranging from 7 to 18% for PCDD/Fs and between 6 and 14% for dl-PCBs were obtained. As an example, the GC/ITMS/MS chromatograms corresponding to the PCDFs and PCDDs obtained for a chicken compound feed sample are given in **Figures 1** and **2**, respectively. As can be seen, high selectivity and good S/N ratio were obtained using the GC/ITMS/MS method. LODs of the method were calculated for all PCDD/Fs and dl-PCBs in milk and pork tissue and ranged from 0.10 to 0.93 pg/g of fat for PCDD/Fs and between 0.10 and 0.89 pg/g of fat for dl-PCBs. For chicken compound feed and fish oil, PCDD/F LOD values were from 0.06 to 0.35 pg/g of product, whereas for dl-PCBs LODs ranged between 0.1 and 0.89 pg/g of product. These values were for PCDD/Fs 10-fold higher than those obtained by GC/HRMS. In contrast, for dl-PCBs LODs found with both MS techniques were generally similar. To maintain these low LOD values a thorough control of the GC/ITMS/MS system is mandatory. Frequent tests using standard solutions at low concentration levels must be performed to ensure the final quality of the results. Moreover, analysis of certified reference materials is recommended to control the performance of the whole analytical method.

To complete the evaluation of the capability of GC/ITMS/MS to produce reliable results in the analysis of PCDD/Fs and dl-PCBs, three additional food samples (chicken tissue, herring tissue, and egg yolk and white) were analyzed ($n = 2$) following the sample treatment previously described under Materials and Methods. The TEQ values for PCDD/Fs and dl-PCBs, expressed as picograms of WHO-TEQ per gram fat or product (upper bound), obtained using both GC/ITMS/MS and GC/HRMS methods for the analysis of all the selected food samples, were calculated and are shown in **Figure 3**. In addition, TEQ values obtained in an interlaboratory exercise when most of the participants used GC/HRMS are also included. TEQ values for PCDD/Fs ranging from 0.78 pg of WHO-TEQ/g of product for fish tissue to 4.27 pg of WHO-TEQ/g of product for fish oil, and for dl-PCBs from 0.43 pg of WHO-TEQ/g of fat for pork tissue to 6.98 pg of WHO-TEQ/g of fat for milk, with standard deviations between 1 and 9.3% were obtained. As can be seen, the results obtained with the proposed GC/ITMS/MS and the GC-HRMS methods were in good agreement. To compare these results and to be able to draw global conclusions, a statistical treatment of the data was performed using analysis of the variance (ANOVA). The assumption of homogeneity of variances was tested with Barlett's test prior to the ANOVA. Two-way ANOVA was applied at a significant level of 0.05, and the ANOVA results considering WHO-TEQ PCDD/Fs, WHO-TEQ dl-PCBs, and total WHO-TEQ values are given in **Table 4**. For each WHO-TEQ value, the calculated *F* values and the *P* values are reported. The method *P* values obtained are always higher than the significance level ($P > 0.05$), showing that no significant differences between the two MS techniques occurred. With regard to the effect of the food matrices, this factor

obviously affects the variance of the data because the concentrations of PCDD/F and dl-PCB in the selected samples are clearly different. Nevertheless, *P* values indicating that the influence of the matrix on the results obtained by a determined MS method (factor of interaction between matrices and MS methods) was not significant. These results show that the GC/ITMS/MS method can be proposed for the determination of PCDD/Fs and dl-PCBs in a wide range of food samples and is able to provide results similar to those obtained by GC/HRMS.

In conclusion, the suitability of GC/ITMS/MS for the analysis of PCDDs, PCDFs, and dl-PCBs in food samples has been demonstrated. MS/MS provided enough sensitivity and selectivity for the determination of PCDD/Fs and dl-PCBs at pg/g levels (picograms per gram) in food samples, and it can be proposed as an alternative to GC/HRMS.

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