High-Throughput Capillary Gas Chromatography for the Determination of Polychlorinated Biphenyls and Fatty Acid Methyl Esters in Food Samples

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Abstract

High-throughput capillary gas chromatography (CGC) methods, developed during the Belgian 1999 "dioxin" food crisis, for the determination of the contaminating polychlorinated biphenyls (PCBs) and the fatty acid composition of the lipids are described. For PCB analysis, the fat obtained by ultrasonic extraction is fractionated by matrix solid-phase dispersion, and the PCBs are analyzed by CGC–electron capture detection on a 10-mL × 100-µm-i.d. HP-5MS column. Analytical conditions for the high-speed column are deduced from analyses on conventional CGC columns using the method translation software. The concept of retention time locking is implemented to facilitate the elucidation of the PCB markers. The fatty acid methyl esters (FAMEs) are prepared by the sodium methylate procedure on part of the ultrasonic extract followed by analysis on 10-mL × 100-µm-i.d. HP-WAX or BPX-70 capillary columns. By optimizing both the sample preparation and CGC analysis, the throughput is more than fifty PCB and FAME samples per day with the same robustness as conventional methods.

Introduction

The year 1999 will be remembered in Europe as the year of the Belgian dioxin crisis. In January 1999, chicken farmers observed premature death (up to 25%) and nervous disorders among chicks combined with a high ratio of eggs failing to hatch. Two months after the start of the problems, a producer of animal feed took the initiative to analyze a sample of suspected animal feed and chicken fat for the presence of polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Both classes are referred to as "dioxins" but in fact consist of 75 dioxins and 135 furans. At the end of April, the Belgian authorities were informed that indeed high concentrations of dioxins were detected in the analyzed samples, and another set of samples was analyzed for confirmation. At the end of May, the public was informed about food contamination and measurements were taken, including the destruction of several lots of eggs, chicken meat, and related food products. At that stage, the source of the contamination was still unknown. A 1000-fold higher concentration of PCDDs and PCDFs in the animal feed fat and chicken fat (1–2 ng/g fat) versus normal background values (1–5 pg/g fat) (1) was detected.

We could not believe that dioxins as such could contaminate food products at a low-parts-per-billion level without the presence of other chlorine-containing contaminants at much higher levels, and one of us (Pat Sandra) advanced on national radio and television the hypothesis of a polychlorinated biphenyl (PCB) contamination. Within one day we received relevant samples and could prove that milligram-per-kilogram (parts-per-million) levels of PCBs were present in animal feed, chicken fat, and eggs. The link was made and it was obvious that the addition of used PCB transformer oil to the animal feed fat was the malefactor and source of the PCDDs and PCDFs. PCBs consist of a group of 209 congeners ranging from mono- up to decachlorobiphenyls. Although their acute toxicity is lower than that of dioxins, several studies have shown that PCBs are linked to several negative health effects including endocrine-disrupting activity, reproductive function disruption, developmental deficits in newborns, and decreased intelligence in school-aged children who had in uteri exposure. Also, the toxicity of dioxins and organochloropesticides (OCPs) increases in the presence of PCBs. PCBs accumulate in fat and build up in the food chain (2). Levels from 50 to 500 ppb (nanograms per gram fat) have also been detected in human fat samples (3) and mother's milk. It is also known that birds, fowls, and poultry are very sensitive to PCB poisoning.

Because PCBs are present at much higher levels than dioxins, PCB analysis is much faster and cheaper (by a factor of 10) compared with dioxin analysis. The analysis of PCBs instead of dioxins was accepted by the Belgian and European authorities, and starting on August 1 certificates were required for the export from Belgium of food products containing more than 2% fat. The norm was set at 200 ppb (200 ng/g fat) for the sum of seven PCB congeners named according to Ballschmiter (4) (PCBs 28, 52, 101, 118, 138, 153, and 180). Using the much faster PCB-monitoring

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analysis, some 50,000 analyses were performed in various laboratories, and all food products were able to be released at the end of December 1999.

The analytical scheme of the official method, Beltest I 014, for the analysis of PCBs in food consists of several steps: sample drying, extraction, cleanup, and capillary gas chromatography (CGC) analysis (5). Sample drying can be performed by freeze-drying or chemically by sodium sulfate addition. In the second step, the lipophilic contaminants are extracted from the matrix using an apolar solvent. The extract contains the lipids, PCBs, PCDDs, PCDFs, and other apolar solutes such as OCPs, polycyclic aromatic hydrocarbons, and mineral oil. Different extraction techniques may be applied including Soxhlet extraction (or the automated versions Soxtec or Soxtherm), solvent extraction using ultrasonic agitation, accelerated solvent extraction (ASE), microwave-assisted solvent extraction (MASE), and supercritical fluid extraction. After optimization, all of these techniques perform equally well for the extraction of fat and PCBs, but it is obvious (especially in a crisis situation) that selection should be based on sample throughput and cost. Next, the PCBs are fractionated from the (coextracted) fat matrix. For this fractionation, column chromatography on acidic silica gel and aluminum oxide is advised, although other techniques such as gel permeation chromatography and solid-phase extraction (SPE) may be applied if validated. Both sample extraction and cleanup require a concentration step. Finally, the cleaned extract is analyzed using CGC with electron capture detection (ECD) or CGC with mass-selective detection (MS). CGC–ECD is extremely sensitive and in most cases sufficiently selective for the detection of PCBs extracted from fat. CGC–MS in the selected ion monitoring mode is somewhat less sensitive, but it is more specific because the presence of PCBs is confirmed by the detection of several ions per congener in a well-defined relative ratio. For samples positive in CGC–ECD, MS confirmation is mandatory. The limit of detection (LOD) for the seven congeners is 5 ppb (5 ng/g fat per congener).

At the beginning of the crisis, the time-consuming official method was followed, but step by step was replaced by a new method taking advantage of recent developments in sample preparation and CGC. The implementation, however, of a new methodology had to be accompanied with validation studies and the analysis of certified samples. This contribution describes the different steps of the optimization procedure that resulted in a high throughput and productivity for the determination of PCBs in food samples. At the same time, fast methods were developed to elucidate the fat in the food products through the analysis of the fatty acid methyl esters (FAMEs).

Table I. Comparison of PCB Concentrations in Three Contaminated Egg Samples Obtained by UE, MASE, and ASE

	Egg 1 (ppb)			Egg 2 (ppb)			Egg 3 (ppb)		
PCB	UE	MASE	ASE	UE	MASE	ASE	UE	MASE	ASE
118	170	68	147	556	410	550	945	1023	922
153	263	309	210	1142	1051	1184	1811	2032	2042
138	240	320	234	1019	1120	1105	2031	2323	2128
180	111	166	93	696	552	686	1015	1166	1259
Sum	**783**	**863**	**682**	**3412**	**3133**	**3525**	**5803**	**6544**	**6349**

Experimental

Sample preparation for PCB analysis

Samples were homogenized using a blender. From fat samples (chicken or pork fat) a 1-g sample was weighed in a 20-mL headspace vial. From eggs, 3 g egg yolk was taken. For animal feed samples or other meat products, a sample size corresponding with 200–500 mg fat was taken. To the sample, 2 g anhydrous sodium sulfate and 10 mL petroleum ether were added. The headspace vial was closed and placed in an ultrasonic bath at 30°C during 30 min. In this step, the fat and PCBs were transferred from the matrix in the petroleum ether phase. The sodium sulfate adsorbed the water present in the sample. After extraction, the sample was allowed to settle. A 5-mL aliquot was transferred to a test tube for the purpose of fractionating the PCBs by matrix solid-phase dispersion (MSPD) (6). Another aliquot (2 mL) was used to determine gravimetrically the fat content of the extract, and an aliquot corresponding with 10 mg fat was used for the FAME analysis. To the test tube containing the 5-mL sample, 2 g of acidic silica gel (44% sulfuric acid) was added, and the tube was vortexed for 10 s. After settlement of the adsorbent (which takes approximately 20 min), an aliquot of the clear solution was transferred to an autosampler vial.

The performance of ultrasonic extraction (UE) was compared with two recently introduced sample-preparation techniques (i.e., ASE and MASE). The extraction efficiency of the three methods was evaluated with three egg samples contaminated at different levels (low, medium, and high). For ASE, a Dionex ASE 200 system (Dionex Corp., Sunnyvale, CA) was used. A 3-g sample was extracted at 100°C and 1500 psi using petroleum ether as the solvent. The extraction time was 5 min oven heat-up time, 5 min static extraction, and 3 cycles with 60% of the extraction cell volume (22 mL). The extract was then concentrated to 10 mL. For MASE, an ETHOS SEL system (Milestone, Analis, Gent, Belgium) was applied. A 3-g sample was extracted during 20 min at 95°C. The extraction solvent was *n*-hexane (10 mL in the extraction thimble and 10 mL outside the thimble) using a Weflon stir bar (Analis) to absorb the microwave energy in combination with the nonmicrowave absorbing solvent. After extraction, the extract was filtered and concentrated to 10 mL in order to obtain the same final concentration factor as the UE and ASE. Cleanup of the ASE and MASE extracts was performed in the same way as for the UE.

Preparation of the FAMEs

This procedure was based on the method developed by Schulte and Weber (7). The aliquot of the UE corresponding with 10 mg fat was diluted to 1 mL with petroleum ether. A 50-µL sample of a 10% sodium methylate solution in methanol was added, and the mixture was shaken for 30 s. After 10 min, 100 mg calcium chloride was added, and the mixture was centrifuged. The upper layer was transferred to an autosampler vial.

Analysis of PCBs

The extracts were analyzed by conventional or high-speed CGC–ECD and conventional

CGC–MS. For CGC–ECD, an HP 6890 GC (Agilent Technologies, Little Falls, DE) equipped with a split/splitless inlet and micro-ECD detection was used. Separations were performed on a 30-m × 0.25-mm-i.d., 0.25-µm film thickness HP-5MS column or a 10-m × 0.1-mm-i.d., 0.1-µm film thickness HP-5 column. An injection of 1 µL was performed in the splitless mode at 250°C. The carrier gas velocity and oven temperature program rate will be discussed in the Results and Discussion section. Nitrogen at 40 mL/min was used as the detector dilution gas, and the detector was set at 320°C. For CGC–MS analysis, the same conditions applied as for conventional CGC–ECD, except that helium was used as the carrier gas at 1 mL/min constant flow. Detection was done in the selected ion monitoring mode using two ions per congener group.

Analysis of FAMEs

The FAME mixtures were analyzed on an HP 6890 GC equipped with a 30-mL × 0.25-mm-i.d., 0.5-µm film thickness HP-WAX or a 10-mL × 0.1-mm-i.d., 0.1-µm film thickness HP-WAX (both from Agilent Technologies). Hydrogen was the carrier gas, and split injection at 250°C and FID detection at 250°C were applied. Other details are presented throughout the text. The separation of *cis*- and *trans*-FAME isomers was performed on a 10-mL × 0.1-mm-i.d., 0.2-µm film thickness BPX-70 capillary column from SGE (Melbourne, Australia). The column was operated isothermally at 180°C with hydrogen as the carrier gas at 50 or 95 cm/s. Injection was performed in the split mode at 1 to 500.

Table II. Translation of the Chromatographic Conditions from a Conventional to a Fast Capillary Column for PCB Analysis

Column	30-mL × 25-mm i.d., 0.25-µm film thickness HP-5MS	10-mL × 0.10-mm i.d., 0.10-µm film thickness HP-5MS
Injection	1-µL splitless, 250°C	1-µL splitless, 250°C
Detection	ECD, 320°C	ECD, 320°C
Carrier gas	Hydrogen	Hydrogen
Flow velocity	45.8 cm/s	67.2 cm/s
Oven temperature	70°C (2 min), 25°C/min to 150°C, 3°C/min to 200°C, 8°C/min to 300°C (2 min)	70°C (0.45 min), 110°C/min to 150°C, 13.2°C/min to 200°C, 35.2°C/min to 300°C (0.4 min)
Analysis time	36 min	8.2 min, speed gain = 4.40

Table III. Repeatability Tested on a Contaminated Egg and Pork Fat Sample

	Egg sample		Fat sample	
PCB	Concentration (ppb)	%RSD	Concentration (ppb)	%RSD
118	141	7	20	4
153	333	2	274	6
138	342	4	318	3
180	165	5	165	4
Sum	**982**	**2**	**776**	**4**

Results and Discussion

Most critical in the analytical scheme for PCB analysis is the new sample-preparation method. The performance of both UE and cleanup by MSPD has to be thoroughly evaluated and validated.

For fat recovery, UE is compared with ASE and MASE, and the value of MSPD is evaluated through the analysis of some reference fat samples. In column chromatography and SPE, the analytes are eluted through a bed and the fat is retained. In MSPD, the fat matrix is allowed to bind on the adsorbent that is mixed with the sample while the solutes of interest stay in solution.

The extraction efficiency of the three methods (UE, ASE, and MASE) was evaluated with three egg samples received during the crisis and contaminated at different levels (Table I). The extracts were analyzed on a conventional capillary column applying retention time locking (RTL) (Table II). For the sample with the lowest concentration (egg 1), the relative standard deviation (RSD) on the PCB sums obtained by the three techniques was 12%; for the two other samples the RSDs were less than 6%. For the individual values, some small differences were noted, but in general these differences were within 10% of the average values. These results clearly demonstrate that there is no statistically significant difference between the three techniques and that equally good results are obtained. The ultrasonic method exhibits by far the highest throughput and is extremely cheap compared with ASE and MASE. The whole sample preparation takes approximately 1 h, and several samples can be prepared in parallel. One technician can easily handle more than 50 samples per day.

The repeatability of the method (n = 6) was evaluated by the analysis of a contaminated egg and fat sample (Table III). These samples were distributed in a round robin test for Belgian laboratories during the crisis. The RSDs were all below 10% for the congeners PCB 118, 153, 138, and 180 and below 5% for the sum of the congeners. The LOD (signal-to-noise > 3) of the micro-ECD and the MS were 0.2 and 0.5 pg, respectively. The linearity and

Table IV. Recovery, Linearity, and Sensitivity* for Pork Fat Spiked at Six Levels† with the Seven PCB Congeners

PCB	Mean %recovery	Linearity	Signal-to-noise at 5 ppb
28	110	0.9999	9
52	88	0.9903	8
101	91	0.9991	12
118	105	0.9996	12
153	101	0.9994	13
138	104	0.9991	11
180	105	0.9998	18

* Signal-to-noise ratio at 5 ppb.
† 5–200 ng/g fat.

method sensitivity were determined by spiking a blank pork fat sample at six levels with the individual PCB congeners. The spike levels were 5, 10, 25, 50, 100, and 200 ppb (nanograms per gram of fat) per congener. The recovery was determined versus an external standard, and the linearity was measured by plotting the absolute peak areas versus the spiked concentration. The signal-to-noise ratio was also measured at the lowest spiked concentration. The results are summarized in Table IV. All recoveries were within 80–110% for the individual congeners. The linearity was better than 0.995 except for PCB 52, for which an interfering peak was observed in the CGC–ECD trace. The signal-to-noise ratio was better than 6 for all of the congeners at the 5-ppb level, which is the value required in the official method as the limit of quantitation (LOQ).

Table V. Accuracy and Reproducibility Test for the Cod Liver Oil Sample*

PCB	Certified concentration (ppb)	Lab 1 ECD	Lab 1 MS	Lab 2 ECD	Lab 2 MS	Lab 3 ECD	Lab 3 MS	Lab 4 ECD	Lab 4 MS
28	68	61	65	73	64	*104*	68	*75*	70
52	149	126	*165*	141	164	144	*199*	*159*	148
101	370	333	394	385	404	296	*437*	356	373
118	454	390	467	421	448	479	*508*	397	458
153	938	975	989	886	1010	790	1030	810	1016
180	280	252	295	283	*312*	270	*326*	288	273
Sum	**2259**	**2137**	**2375**	**2189**	**2402**	**2083**	**2568**	**2085**	**2338**

* Values outside of the 80% to 110% range of the certified samples are noted in italic.

Table VI. Accuracy and Reproducibility Test for the Mackerel Oil Sample*

PCB	Certified concentration (ppb)	Lab 1 ECD	Lab 1 MS	Lab 2 ECD	Lab 2 MS	Lab 3 ECD	Lab 3 MS	Lab 4 ECD	Lab 4 MS
28	22.5	*25*	24	21	21	21	19	19	*16*
52	62	*75*	*72*	56	56	65	*71*	54	63
101	164	152	*181*	175	175	152	143	150	172
118	142	*163*	152	117	117	138	125	131	134
153	317	337	345	319	319	287	*350*	290	316
180	73	73	79	75	75	76	*83*	70	60
Sum	**778.5**	**825**	**853**	**763**	**739**	**739**	**791**	**714**	**761**

* Values outside of the 80% to 110% range of the certified samples are noted in italic.

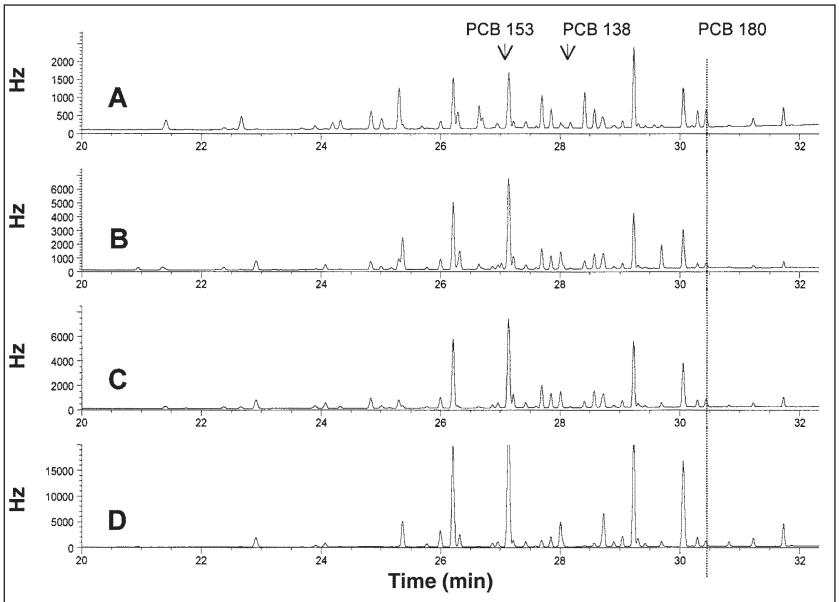

Figure 1. RTL-CGC–ECD analyses of (A) Aroclor 1260, (B) egg extract, (C) pork fat extract, and (D) mink fat extract. Conditions can be found in the text and Table II.

The performance of MSPD and the reproducibility and accuracy of the method was evaluated through the determination of the PCB content in two certified reference materials of the European Community, namely the cod liver oil sample CRM 349 and the mackerel oil sample CRM 350 (IRMM, Geel, Belgium). The analyses of these reference materials were performed by four laboratories, namely RIC and three laboratories to which the method was transferred having the same CGC–ECD and CGC–MS instrumentation. The results are summarized in Table V for cod liver oil and Table VI for mackerel oil. Most values were within 80% and 110% of the certified samples. The values outside these ranges are noted in italic. For all of the laboratories and for both techniques, the sum values were always between these limits. The tables also illustrate that the MSPD fractionation of fat from PCBs works very efficiently.

Implementation of the RTL concept provided very reproducible retention data for PCB elucidation via CGC–ECD analysis (8). The temperature program of 70°C (2 min) to 150°C at 25°C/min, ramped to 200°C at 3°C/min, and then to 300°C (2 min) at 8°C/min was selected, and an initial head pressure of 71 kPa hydrogen was applied. The pressure was then adjusted via the RTL software to obtain a retention time of 26.999 min for p,p'-dichlorodiphenyltrichloroethane. The total analysis time under these conditions was 36 min. Figure 1 shows the RTL-CGC–ECD profiles of Aroclor 1260, egg, pork fat, and mink fat recorded with a time interval of one month. As an example, the retention time for PCB 180 varied from 29.230 to 29.234 min (mean = 29.232, s = 0.002 min, %RSD < 0.01%). The chromatogram of the mink fat extract was interesting. The minks were fed with contaminated eggs, and although the same Aroclor 1260 profile was present, some differences were noted in the relative abundances of the PCB congeners. This can be explained by the different metabolisms between the animal species. In the mink sample, the PCB concentration (measured as the sum of the seven congeners) was as high as 25 ppm (25 mg/kg fat). This concentration was fatal for most minks.

The relatively long analysis times under RTL

conditions prompted us to evaluate fast high-resolution CGC for PCB analysis (9). Presently with state-of-the-art CGC instrumentation, capillary columns with internal diameters of 0.1 mm and lengths of 10 m can be used, thus drastically decreasing the analysis time while maintaining the resolving power compared with a conventional capillary column. This is a prerequisite for PCB analysis and fast CGC techniques such as the use of multicapillary columns; flash GC or vacuum outlet CGC–MS could not be applied because the resolution is too low to separate the specific congeners. With the help of the method translation software, chromatographic conditions of the conventional RTL-CGC–ECD analysis discussed previously can be translated to a high-speed column keeping the resolution intact (9). The translated conditions are given in Table II. The program predicted that the speed gain factor will be 4.4, which means an analysis time of 8.2 min. The analyses of an Aroclor 1260 reference sample and an animal feed sample are shown in Figure 2. Compared with Figure 1, PCB 180 eluted then at 6.462 min instead of 29.230 min. The experimental speed gain factor was thus 4.5, corresponding very well with the predicted speed gain factor of 4.4. From the chromatograms, it is clear that the resolution has not been compromised compared with the analysis on the conventional columns. Important to note is that splitless injection can be applied in fast high-resolution CGC. This resulted in an increased sensitivity (LOD = 0.03 pg), and the RSDs for the contaminated egg samples (Table III) were of the same order as for the conventional column (all below 10%). The RTL concept was also implemented for the high-speed column, and the variation was less than 0.02% over a two-month period.

Some 4000 samples (including animal feed, eggs, chicken fat, pork fat, pork meat, and meat products such as ham and sausages) were analyzed using this methodology. From a practical point of view, the splitless liner was replaced after each 100 analyses and the column after 1000 injections. On the column selected and the chromatographic conditions applied, the PCB congeners 28 and 31 were not separated, but this was not critical because both congeners were not relevant for the Aroclor-1260-type pollution.

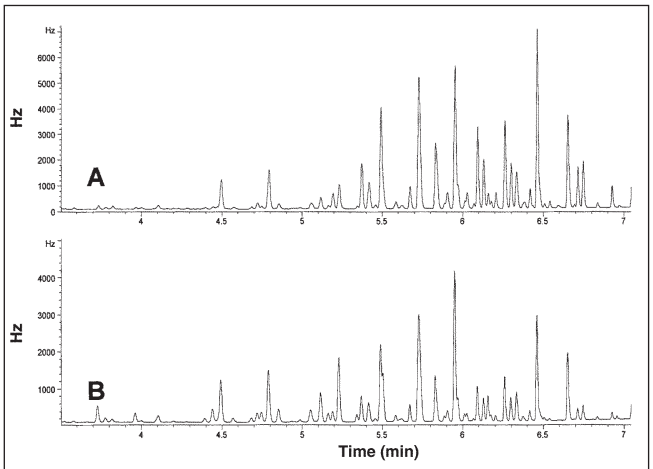

Figure 2. Fast high-resolution CGC–ECD analyses of (A) Aroclor 1260 and (B) a contaminated egg extract. Conditions can be found in the text and Table II.

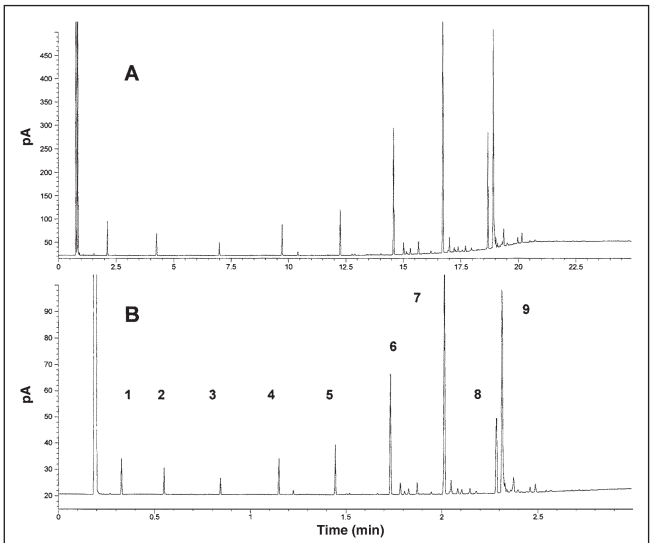

Figure 3. FAME chromatograms of a milk fat sample by (A) conventional and (B) high-speed CGC on HP WAX columns. The peaks are: *n*-butanoic acid (C4:0), 1; *n*-hexanoic acid (C6:0), 2; *n*-octanoic acid (C8:0), 3; *n*-decanoic acid (C10:0), 4; *n*-dodecanoic acid (C12:0), 5; tetradecanoic acid (C14:0), 6; *n*-hexadecanoic acid (C16:0), 7; *n*-octadecanoic acid (C18:0), 8; and *n*-*cis*-9-octadecenoic acid (C18:1 *cis*), 9. Conditions can be found in the text and Table VII.

Table VII. Translation of the Chromatographic Conditions from a Conventional to a Fast Capillary Column for FAME Analysis

Column	30-mL × 0.25-mm i.d., 0.50-µm HP-WAX	10-mL × 0.10-mm i.d., 0.10-µm HP-WAX
Injection	1-µL split 1/150, 250°C	0.6-µL split 1/1000, 250°C
Detection	FID 100 Hz, 250°C	FID 100 Hz, 250°C
Carrier gas	Hydrogen	Hydrogen
Flow velocity	78.5 cm/s	103.8 cm/s
Oven temperature	50°C to 250°C at 10°C/min, 5 min isothermal	50°C to 250°C at 79.3°C/min, 0.63 min isothermal
Analysis time	25 min	3 min, speed gain = 7.93

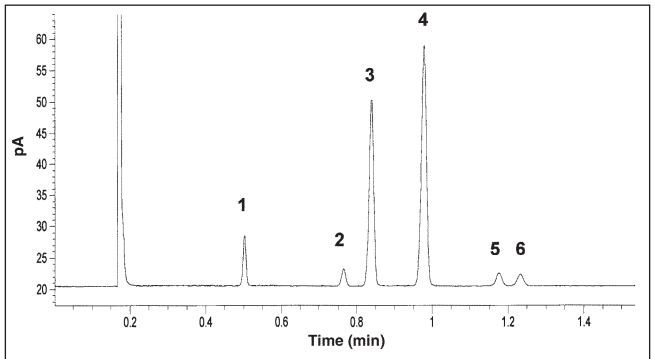

Figure 4. FAME chromatogram of a standard mixture on a BPX-70 column: *n*-hexadecanoic acid (C16:0), 1; *n*-octadecanoic acid (C18:0), 2; *n*-*cis*-9-octadecenoic acid (C18:1 cis), 3; *n*-*cis*,*cis*-9,12-octadecadienoic acid (C18:2 *cis*,*cis*), 4; *n*-*cis*,*cis*,*cis*-9,12,15-octadecatrienoic acid (C18:3 *cis*,*cis*,*cis*), 5; and eicosanoic acid (C20:0), 6. Conditions can be found in the text; 413.7 kPa hydrogen was used at 180°C.

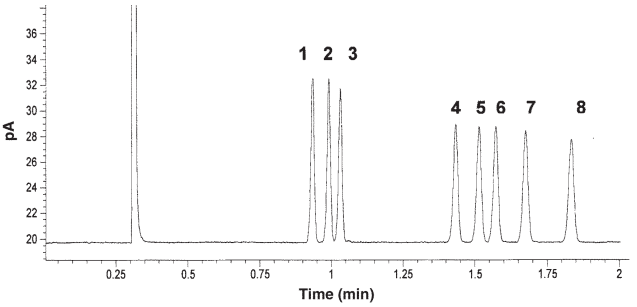

Figure 5. Fast FAME *cis*/*trans* separation on a BPX-70 column: *n*-hexadecanoic acid (C16:0), 1; *n*-trans-9-hexadecanoic acid (C16:1 *trans*), 2; *n-cis*-9-hexadecanoic acid (C16:1 *cis*), 3; *n*-octadecanoic acid (C18:0), 4; *n-trans*-9-octadecenoic acid (C18:1 *trans*), 5; *n-cis*-9-octadecenoic acid (C18:1 *cis*), 6; *n-trans,trans*-9,12-octadecadienoic acid (C18:2 *trans,trans*), 7; and *n-cis,cis*-9,12-octadecadienoic acid (C18:2 *cis,cis*), 8. Conditions can be found in the text; 206.8 kPa hydrogen was used at 180°C.

For the preparation of FAMEs by the transesterification of the triglycerides, several procedures can be applied. The sodium methylate procedure is by far the simplest and fastest approach, giving similar results as other methods. Several samples can be handled at the same time, and the pre-CGC time (time before the first CGC analysis is started) is less then half an hour. From then on samples can be prepared in time with the CGC analysis. The offline sample procedure is, however, so fast that conventional CGC analysis cannot follow the high sample-preparation throughput. This is illustrated in Figure 3, in which the analysis of the FAMEs of butter fat on the 30-mL × 0.25-mm-i.d., 0.5-µm film thickness HP-WAX column is shown. On polyethylene glycol the fatty acids were separated according to the carbon number and the number of double bonds. The fatty acids ranging from isobutyric acid (peak 1) to oleic acid (peak 9) were nicely separated in a 25-min run (Table VII). The translation of the conditions to a 10-mL × 0.1-mm-i.d., 0.1-µm film thickness HP-WAX is given in Table VII, and the obtained chromatogram is shown in Figure 3. The total analysis time was 3 min with the same resolution as obtained on the conventional capillary column. The standard deviation on the retention times was better than 0.002 min and on the absolute peak areas better than 2% for large peaks and 5% for small peaks. The importance of the FID hertz rate on the RSDs has been discussed previously (9). The nature of the stationary phase has a strong influence on the analysis time (10), and the more selective a phase is, the shorter is the analysis time. This is illustrated in Figure 4, in which the isothermal analysis of a FAME standard mixture (Alltech 625024, Lokeren, Belgium) on the 10-mL × 0.1-mm-i.d., 0.2-µm film thickness BPX-70 capillary column is shown. The main fatty acids of a vegetable oil were remarkably baseline separated in 1.3 min. The column was operated at 95 cm/s hydrogen and 180°C isothermally. By reducing the velocity to 50 cm/s, an excellent separation was obtained between the *cis*/*trans* isomers of C16:1, C18:1, and C18:2 (Figure 5). The *cis*/*trans* distribution in margarines for example is important because of health aspects associated with *trans* fatty acids such as high cholesterol levels and heart diseases (11).

Conclusion

The contamination of Belgium food products has initially been referred to as a "dioxin crisis". The main contamination source, however, was PCBs. PCBs can be analyzed much faster and cheaper than PCDDs and PCDFs; therefore, the analysis of PCBs was "the" solution to ban all contaminated food. A new sample preparation method using UE followed by MSPD cleanup and CGC–ECD or CGC–MS analysis has been developed and validated. RTL and fast high-resolution CGC completed the introduction of new methodologies in PCB screening. Elucidation of the fatty acids in fats and oils can be done at high throughput by analyzing the FAMEs obtained by sodium methylate hydrolysis followed by fast CGC on polar capillary columns.

References

1. A. Bernard, C. Hermans, F. Broeckaert, G. De Poorter, A. De Cock, and G. Houins. *Nature* **401(6570):** 231 (1999).
2. http://www.fda.gov.
3. A. Pauwels, F. David, P. Schepens, and P. Sandra. *Intern. J. Environ. Anal. Chem.* **73(3):** 171 (1999).
4. K. Ballschmiter and M. Zell. *Frenesius J. Anal. Chem.* **302:** 20 (1980).
5. "Bepaling van Polychloorbifenylen in Dierenvoer, Dierlijke Vetten, El en Eiproducten, Zuivelproducten en Andere Voedingsmiddelen". http://beltest.fgov.be/docs_pdf/I14_NL.pdf, July 2000.
6. S.A. Barker. Supplement. *LC-GC* **May:** S37–S40 (1998).
7. E. Schulte and K. Weber. *Fat. Sci. Technol.* **91:** 181 (1989).
8. V. Giarrocco, B.D. Quimby, and M.S. Klee. Hewlett-Packard Application Note. Hewlett-Packard, Little Falls, DE, December 1997, pp. 228–392.
9. F. David, D.R. Gere, F. Scanlan, and P. Sandra. *J. Chromatogr. A* **842:** 309 (1999).
10. P. Sandra. *LC-GC* **5(3):** 236 (1987).
11. J.M. Hodgson, M.L. Wahlqvist, J.A. Boxall, and N.D. Balazs. *Atherosclerosis* **120:** 147 (1996) 147.

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