Journal of Chromatography, 595 (1992) 289–299
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 912

Automated clean-up procedure for the gas chromatographic–high-resolution mass spectrometric determination of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in milk

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(First received September 12th, 1991; revised manuscript received November 21st, 1991)

ABSTRACT

A highly automated extraction and clean-up method for polychlorinated dibenzodioxins and polychlorinated dibenzofurans is described. The method includes the use of gel permeation chromatography, alumina clean-up and porous graphitized carbon chromatography, followed by analysis by gas chromatography–high-resolution mass spectrometry. The procedure allows for the analysis of six milk samples per day in addition to two quality control samples and a blank. Detection limits on a fat basis for the individual congeners in milk samples are in the sub-ppt range. Long-term performance was investigated and data are given for reproducibility, precision and accuracy.

INTRODUCTION

Since the detection of polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in biological samples, analysis of these compounds has been subject of intense research [1–10]. The toxicological behaviour of these compounds has been reviewed by, *e.g.*, Van Zorge *et al.* [11]. Of the total of 75 possible PCDD congeners and 135 possible PCDF congeners, only the seventeen 2,3,7,8-chlorine-substituted compounds are of toxicological interest. Therefore, the development of methods of analysis has been focused on these most toxic congeners.

For convenience, the toxicity of all 2,3,7,8-chlorine-substituted congeners is expressed in toxic equivalents to the most toxic compound; 2,3,7,8-TCDD, by multipyling the amount of each congener with the toxic equivalence factors (TEF) published by Van Zorge *et al.* [11]. The total toxicity of all congeners can then be expressed as a single figure with the dimensions of toxic equivalent to 2,3,7,8-TCDD (TEQ).

The low concentration levels of these compounds in biological samples, typically in the low pg/g range, requires a highly sensitive and highly specific method of analysis. A combination of several different clean-up techniques must be used to obtain a sufficiently clean sample extract. Commonly used extraction and clean-up techniques include liquid–liquid partitioning [2–6,12,13], solid sorbent extraction [7], gel permeation chromatography [2–4,13], column chromatography using a variety of sorbents, *e.g.*, basic, acid or neutral alumina [2,4–6,8–10,12,13], silica [8,10], acid- or base-impregnated silica [5,8–10,13], Florisil [2–4], potassium silicate [8,10], caesium silicate [8,10] and carbon chromatography [2,5,6,8–10,13].

Carbon chromatography may be used for both the isolation and clean-up of the dioxins [5,6,12] or just for the separation of dioxins from interfering compounds [2,8–10,13]. Saponification for removal

0021-9673/92/$05.00

of the fat cannot be used as chemical modification of the dioxins may occur [14]. Even though extensive and selective clean-up is applied, some workers prefer to increase the selectivity even further by using tandem mass spectrometric methods [12].

Smith *et al.* [1] developed an analytical method employing several multilayer columns and carbon chromatography. This method has been adapted by several researchers [5,8,10] and, although the working procedure is laborious, it is probably one of the most often used methods of analysis. Recently, Liem *et al.* [6] developed an elegant method of analysis based on the use of a carbon sorbent for the isolation and clean-up of the dioxins and furans from milkfat.

In the Netherlands, the dioxin exhaust from municipal incinerators was recently found to be the main cause of unacceptably high dioxin levels in cows' milk in the locality. The large-scale inspection of cows' milk made the development of a method with a high capacity for clean-up and analysis necessary.

A common feature of all the methods described previously is the low sample throughput owing to laborious manual procedures. Therefore, these methods are not very suitable for routine analyses of large numbers of samples.

This paper describes a highly automated extraction and clean-up method based on gel permeation chromatography (GPC), alumina clean-up and carbon chromatography using a graphitized carbon high-performance liquid chromatographic (HPLC) column as described by Creaser and Al-Haddad [15]. Automated gas chromatographic–high-resolution mass spectrometric (GC–MS) measurement in combination with fully automated data processing is used for quantification.

The procedure allows for the clean-up and analysis of six milk samples per day in addition to two quality control samples and a procedural blank. The performance of the method with regard to repeatability and accuracy was tested by analysing samples of known content. The long-term performance was monitored using a set of two quality control samples which were analysed within each series of samples.

EXPERIMENTAL

Reagents and samples

All solvents were obtained from Merck (Darmstadt, Germany), except toluene, which was obtained from Promochem (Wesel, Germany). All solvents except toluene were distilled in glass prior to use. The toluene used was of nanograde quality and dit not introduce any interferences. BioBeads SX-3 were obtained from Bio-Rad Labs. (Veenendaal, Netherlands). Basic alumina, activity I, was obtained from Woelm (Eschwege, Germany). Prior to use, the alumina was deactivated with 7% demineralized water to achieve a quantitative recovery of the analytes.

Cows' milk samples were stored at −20°C until fat extraction. After fat extraction, the milk samples and the quality control samples were stored at room temperature.

Native and ^{13}C-labelled PCDD and PCDF standard mixtures were obtained from Cambridge Isotope Labs. (Woburn, MA, USA). All other reagents were of analytical-reagent grade.

Control samples

One quality control sample was a batch of milkfat originating from an industrial area in the Netherlands. The sample contained 2.62 pg of TEQ (toxicity equivalent to 2,3,7,8-TCDD) per gram of fat as revealed by repeated analysis. The other quality control sample was milkfat accurately spiked with PCDDs/PCDFs. Milkfat was first decontaminated using active carbon. Complete absence of PCDDs/PCDFs was confirmed by analysis. Thereafter, amounts of 2 pg per gram per compound of native PCDDs/PCDFs were added, the octachlorinated compounds were added at a level of 4 pg per gram of fat by addition of a dilute solution of the dioxin and furan congeners. This resulted in an artificially contaminated batch of milkfat containing 5.85 pg TEQ per gram.

Equipment

All glassware was successively washed with a detergent and flushed with hot water, acetone and demineralized water. The glassware was dried in an oven at 120°C and subsequently silanized using a 4% solution of dimethylchlorosilane (DMCS) in

toluene. Immediately before use, the glassware was rinsed with the organic solvent to be used.

The gel permeation chromatographic system consisted of a (Gilson Villiers le Bel, France) Model 305 HPLC pump, a Gilson Model 231-401 autosampler equipped to inject 12.5 ml of sample solution [16] and a Gilson Model 202 fraction collector adapted to collect fractions of 300 ml using 500-ml flasks as collection vessels. The GPC column, obtained from Spectrum (Los Angeles, CA, USA), was glass (60 cm × 2.5 cm I.D.), packed with BioBeads-SX_3 and equipped with adjustable plunger.

The basic alumina clean-up was performed with an instrument for automatic sample preparation with extraction columns (ASPEC, Gilson) for use with 3-ml disposable solid-phase extraction columns. Empty columns were purchased from Baker (Phillipsburg, NJ, USA). The columns were packed with 1.00 g of deactivated basic alumina shortly before use.

Carbon clean-up was performed with a HPLC system consisting of a Gilson Model 305 pump, a MUST column-switching device, including timer and solvent-select valve (Spark Holland, Emmen, Netherlands), a Gilson Model 231-401 autosampler, equipped with a 5-ml sample loop, and a Gilson Model 202 fraction collector adapted to take 100-ml flasks as collection vessels to collect fractions of 30 ml. A Hypercarb, porous graphitized carbon (PGC) column obtained from Shandon (Runcorn, UK) was used to separate the dioxins from non-planar co-extracted organic contaminants.

The GC–MS system consisted of an HP 5890 gas chromatograph (Hewlett Packard, Avondale, PA, USA), equipped with an HP 7673A autosampler, coupled to a VG Autospec-Q trisector (EBE) double-focusing mass spectrometer (VG Analytical, Manchester, UK) operated in the selected ion monitoring (SIM) mode at 10 000 mass resolution. Within each window, SIM was performed by adjusting the accelerating voltage. A dwell time of 50 ms per ion was used. Electron impact ionization was used with an electron energy of 35 V and a trap current of 500 μA.

Separation of the PCDDs and PCDFs was achieved on a 60 m × 0.25 mm I.D. DB5 column (film thickness 0.25 μm) obtained from J & W Scientific (Folsom, CA, USA).

Sample extraction and clean-up

Sample extraction. Milk samples were extracted using a quantitative liquid–liquid extraction method as described by Helrich [17]. To 150 ml of milk, sodium oxalate and ethanol were added. The fat was isolated by liquid–liquid extraction with diethyl ether and light petroleum (b.p. 40–60°C). The resulting fat-containing organic layer was dried over sodium sulphate and evaporated to dryness. The amount of fat remaining was determined by weight.

Gel permeation chromatography. An amount of 6 g of fat was dissolved in ethyl acetate–cyclohexane (1:1, v/v). This solution was fortified with 60 pg each of the ^{13}C-labelled PCDDs and PCDFs, resulting in a fortification level of 10 pg per compound per gram of fat. The solution was made up to 30 ml with ethyl acetate–cyclohexane (1:1, v/v) and mixed thoroughly. Two sample bottles were each filled with 15 ml of this solution. From the first bottle an aliquot of 12.5 ml, corresponding to 2.5 g of fat, was applied to the GPC column. The column was eluted at a flow-rate of 1 ml/min during sample transfer. After the sample had been applied, the flow-rate was increased to 5 ml/min. The dioxin-containing fraction was collected between 51 and 88 min. After 88.5 min, the flow-rate was reduced from 5 to 1 ml/min and from the second bottle again 12.5 ml were injected and the procedure was repeated. A second injection cycle is necessary to clean a total amount of 5 g of fat.

The dioxin-containing fractions were combined and 50 μl of dodecane were added. The solvent was evaporated at 40°C on a rotary evaporator to a volume of about 1 ml. The residue was quantitatively transferred into a sample tube using hexane as washing solvent and, using a gentle stream of nitrogen, evaporation was continued until only the dodecane was left. To the resulting residue 0.5 ml of hexane was added and the contents of the tube were mixed thoroughly using a vortex mixer.

Alumina clean-up. Using the ASPEC instrument, the alumina column was washed with 5 ml of hexane and the fraction of 0.5 ml resulting from the GPC clean-up was quantitatively transferred to the column. All solvent eluting from the column was collected starting from the moment of sample application. Then the dioxins were eluted with 3 ml of hexane and the eluate was collected. The resulting 3.5 ml of eluate were transferred to a sample bottle.

Graphitized carbon chromatography. The 3.5-ml hexane fraction resulting from the alumina clean-up was quantitatively transferred to the 5-ml sample loop of the autosampler. By switching the valve the sample was injected on to the PGC column. The column was successively eluted with cyclohexane–dichloromethane (1:1, v/v) and toluene, both at a flow-rate of 2 ml/min. When the column was eluted with cyclohexane–dichloromethane (1:1, v/v), the dioxins were retained whereas non-planar organic contaminants were eluted and discarded. After 30 min, the solvent was changed to toluene. After another 30 min the PGC column was eluted in the back-flush mode and the eluate was collected. The collection vessel was a 100-ml flat-bottomed flask which can be attached to the rotary evaporator. Collection took place during the first 15 min of the back-flush. Next the column was eluted in the back-flush mode with toluene for a further 15 min. The direction of the flow and the solvent was changed again and the column was regenerated with cyclohexane–dichloromethane (1:1, v/v) for 30 min. After this period the system was ready for injection of the next sample.

Preparation of the sample for GC–MS. The 30-ml toluene fraction resulting from the carbon chromatographic separation, was evaporated on a rotary evaporator to *ca.* 1 ml and quantitatively transferred to a tapered tube using hexane as washing solvent. The remaining solvent was evaporated under a stream of nitrogen. The walls of the tapered tube were flushed thoroughly with small volumes of hexane, typically decreasing from 200 to 50 μl. After complete evaporation of the solvent, the residue was dissolved in a 10-μl aliquot of the syringe standard solution containing 10 pg/μl of the syringe standard 1,2,3,4-[^{13}C]TCDD using a vortex mixer at low speed. The resulting sample extract was transferred to the insert of a GC autosampler vial using a syringe. A 50-μl insert was used (LC-Service, Emmen, Netherlands).

Gas chromatography–mass spectrometry

In order to perform GC–MS analyses continuously, the gas chromatograph was equipped with an autosampler. Aliquots of 2 μl of the samples were injected splitless on to a non-polar column and, using helium as the carrier gas, group separation of tetra-, penta-, hexa-, hepta- and octachlorinated congeners was achieved.

During sample injection the oven temperature of the gas chromatograph was set at 100°C and the injector temperature was kep at 280°C. After 3 min the temperature of the oven was increased to 200°C at 15°C/min and from 200 to 280°C at 4°C/min, the final temperature being maintained for 25 min. The interface between the GC and MS instruments was kept at 280°C. Injection by the autosampler, ramping of the GC oven temperature and acquisition of the MS data were synchronized by the use of the VG Sios interface.

The MS data acquisition is based on SIM. For each group of congeners with the same degree of chlorination, up to ten ions were monitored (see Table I). In general, the two largest peaks in the chlorine isotope cluster of the molecular ion were measured, except for [^{13}C]HxCDF and [^{13}C]HpCDF, because for these compounds MS interferences of the M + 4 peak from high levels of native HxCDD and HxCDF would be expected owing to a possible lack of GC resolution. Instead, for these two compounds the M^+ and $[M+2]^+$ ions were measured, these being the third largest and the largest peaks, respectively. An ion of perfluorkerosene (PFK) was used as lock-mass channel to correct for "magnet drift".

Relative response factors were determined from a calibration graph consisting of seven different levels of native congeners in the concentration range 100 fg/μl–10 pg/μl.

In Table I, for each level of chlorination of dioxins and furans, the monitored ions and the limits of their isotope ratios are given. The complete process from sequential injections up to and including quantification is fully automated.

RESULTS AND DISCUSSION

For positive identification of the congeners, the following criteria are adopted: the relative retention time of the unknown and the labelled compound must match, *i.e.*, the ratio of the retention time of the analyte (B) to that of the internal standard (A) should be equal to the relative retention time of the analyte in a calibration mixture within a margin of ± 5 s/A; and the isotope ratios, measured within the cluster of ions monitored, should be equal to the theoretical value within a margin of 15% (see Table I). If these criteria are satisfied, the recorded data

TABLE I

SOME ACQUISITION AND IDENTIFICATION PARAMETERS FOR SEVENTEEN 2,3,7,8-CHLORINE-SUBSTITUTED DIOXINS AND DIBENZOFURANS INCLUDING THEIR ^{13}C-LABELLED ANALOGUES

T = Tetra; Pe = penta; Hx = hexa; Hp = hepta; O = octa.

Compound	Selected ion ratio A/B[a]	*m/z*		Theoretical abundance, A/B
		A	B	
TCDF	M/M+2	303.90	305.90	0.77
TCDD	M/M+2	319.90	321.89	0.77
[^{13}C]TCDF	M/M+2	315.94	317.94	0.77
[^{13}C]TCDD	M/M+2	331.94	333.93	0.77
PeCDF	M+2/M+4	339.86	341.86	1.55
PeCDD	M+2/M+4	355.85	357.85	1.55
[^{13}C]PeCDF	M+2/M+4	351.90	353.90	1.55
[^{13}C]PeCDD	M+2/M+4	367.89	369.89	1.55
HxCDF	M+2/M+4	373.82	375.82	1.24
HxCDD	M+2/M+4	389.82	391.82	1.24
[^{13}C]HxCDF	M/M+2	383.86	385.86	0.51
[^{13}C]HxCDD	M+2/M+4	401.86	403.85	1.24
HpCDF	M+2/M+4	407.78	409.78	1.03
HpCDD	M+2/M+4	423.78	425.77	1.03
[^{13}C]HpCDF	M/M+2	417.83	419.82	0.44
[^{13}C]HpCDD	M+2/M+4	435.82	437.81	1.03
OCDF	M+2/M+4	441.74	443.74	0.89
OCDD	M+2/M+4	457.74	459.73	0.89
[^{13}C]OCDD	M+2/M+4	469.78	471.78	0.89

[a] M = Molecular ion, containing ^{35}Cl exclusively.

are used for quantification. The quantification was performed using the isotope dilution method [13], thus correcting for possible losses of compounds during the extraction and clean-up procedure.

The described method was initially tested for accuracy and repeatability by analysing six samples of an artificially contaminated milkfat containing 2 pg of each compound per gram of fat, except for the octachlorinated congeners, which were present at 4 pg/g. Expressed in 2,3,7,8-TCDD equivalents (TEQ), the sample contains 5.85 pg TEQ per gram of fat.

The mean concentration of the individual congeners (pg/g fat), as determined for these six samples, are given in Table II. From these data, it follows that both the accuracy and repeatability are good: the mean accuracy expressed in pg TEQ per gram of milkfat as compared with the content known from the addition of dioxins was 106%; the accuracy ranged from 97% to 131%, except for OCDD, which showed an accuracy of 158%, indicating too high results. In general, the results of analysis for a few compounds are systematically high. Analysis of the contaminated fat does not show any background contribution for these compounds. Until now the reason for these high results is unknown. Especially for OCDD several other groups seem to have had the same problem concerning high results.

The determination of the TEQ value of the sample, *i.e.*, the sum of TEQ values of the individual congeners, shows a relative standard deviation (R.S.D.) of 6.8%. The mean R.S.D. is 11.8% for the determination of the individual congeners. Obviously, to some extent, random fluctuations in the amounts of the individual congeners are ruled out by calculation of the TEQ value for the seventeen congeners.

TABLE II

RESULTS OF THE ANALYSIS OF ARTIFICIALLY CONTAMINATED MILKFAT (QCS 2), USED TO ESTABLISH THE INITIAL PERFORMANCE OF THE DESCRIBED METHOD OF ANALYSIS ($n=6$)

Compound	TEF	Mean (pg/g)	S.D. (pg/g)	R.S.D. (%)	Accuracy (%)
2,3,7,8-TCDF	0.1	2.05	0.28	13.6	97.4
2,3,7,8-TCDD	1	2.22	0.26	11.7	99.3
1,2,3,7,8-PeCDF	0.05	2.03	0.16	7.8	104.5
2,3,4,7,8-PeCDF	0.5	2.04	0.16	7.8	106.2
1,2,3,7,8-PeCDD	0.5	1.96	0.21	10.5	99.2
1,2,3,4,7,8-HxCDF	0.1	2.20	0.18	8.3	114.4
1,2,3,6,7,8-HxCDF	0.1	2.25	0.18	8.1	108.9
2,3,4,6,7,8-HxCDF	0.1	2.51	0.29	11.4	120.7
1,2,3,7,8,9-HxCDF	0.1	2.22	0.19	8.5	125.3
1,2,3,4,7,8-HxCDD	0.1	2.34	0.43	18.4	109.8
1,2,3,6,7,8-HxCDD	0.1	2.46	0.25	10.1	109.0
1,2,3,7,8,9-HxCDD	0.1	2.13	0.31	14.7	98.7
1,2,3,4,6,7,8-HpCDF	0.01	2.55	0.38	15.0	120.9
1,2,3,4,7,8,9-HpCDF	0.01	2.27	0.30	13.3	115.7
1,2,3,4,6,7,8-HpCDD	0.01	2.83	0.56	19.9	130.9
OCDF	0.001	4.42	0.47	10.7	106.7
OCDD	0.001	5.77	0.69	11.9	157.6
Total TEQ (pg/g)		6.23	0.43	6.8	98.6

The analyses of the other quality control sample originating from an industrial area in the Netherlands showed similar results on repeatability; the mean R.S.D. is 9.6% ($n=4$) for the determination of individual congeners; the R.S.D. of the TEQ value of this sample is 6.3%. The accuracy cannot be estimated for this sample as its true value is unknown.

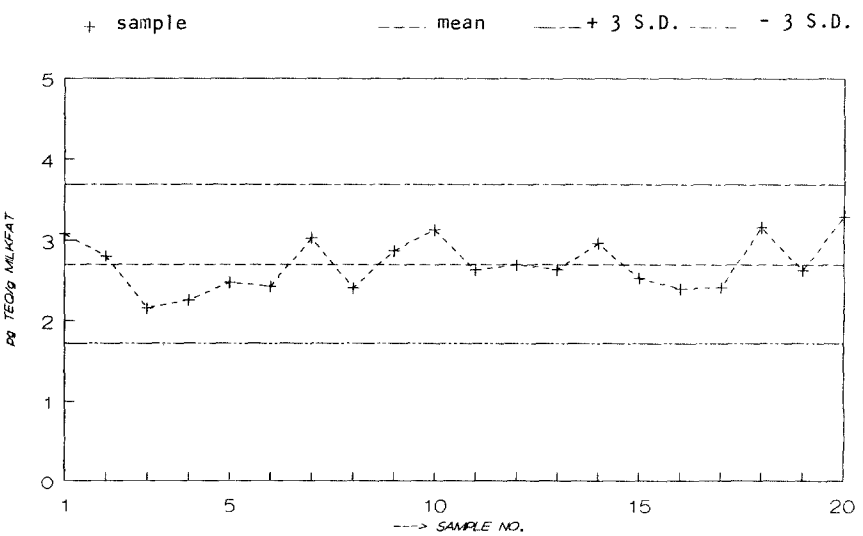

Fig. 1. Quality control chart based on data resulting from the analysis of quality control sample 1 (QCS 1) (see text) during a period of 3 months.

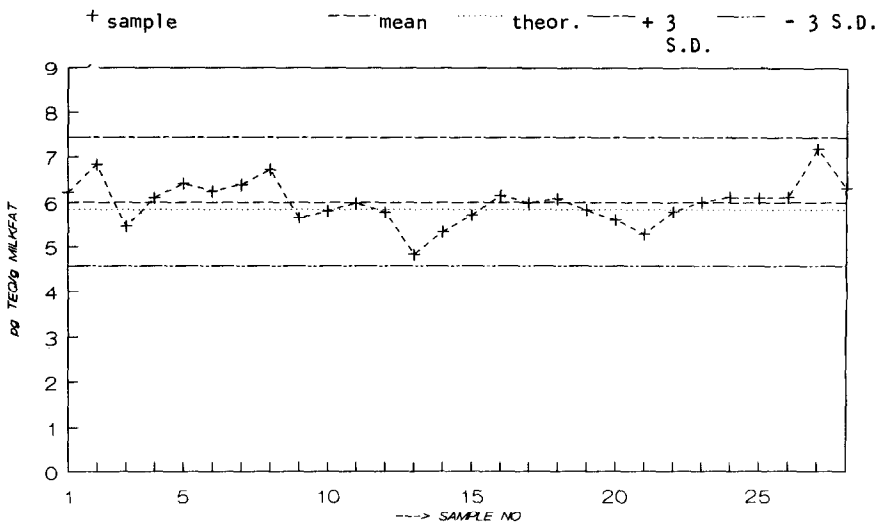

Fig. 2. Quality control chart based on data resulting from the analysis of quality control sample 2 (QCS 2) (see text) during a period of 3 months.

The method has been applied over a period of 3 months to the analyses of 180 samples of cows' milk in an excretion experiment with lactating cows [18]. In each series of twelve unknown samples, a set of at least three quality control samples (QCS) is analyzed, *i.e.*, one aliquot of the milk fat from a contaminated area (QCS 1), one aliquot of the artificially contaminated milkfat (QCS 2) and a blank (QCS 3) that is taken through the entire procedure. The blank is processed after quality control samples 1 and 2. From these data, the long-term reproducibility of the results is found to be good. The mean result for QCS 1 from twenty measurements (over a 3-month period) was 2.62 pg TEQ/g milkfat with R.S.D. = 11.5% (Fig. 1). The mean result for QCS 2 from 28 measurements (within the same period) was 6.04 pg TEQ/g milkfat with R.S.D. = 9.0% (Fig. 2).

The blanks did not contain any dioxins above the limit of determination. These results show that the long-term reproducibility, even with the low TEQ values occurring in biological samples, can be very good. The mean recovery, as calculated from all 228 samples that were analysed during the 3-month period (*i.e.*, including both unknown samples and quality control samples), ranged from 54.7 ± 26.5% for 2,3,7,8-TCDD to 76.9 ± 54.2% for OCDD. It should be noted that with isotope dilution, the percentage is of limited importance as the calculation of the amounts should not be a function of the recovery. A low recovery, however, results in a high limit of determination. For this reason, we require minimum recovery of about 25% for the three most important congeners, *viz.*, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF, which together contribute about 75% to the total TEQ value of Dutch cows' milk.

Fig. 3 and 4 show parts of chromatograms of a calibration standard and a milk sample, respectively. Only the tetra- and pentachlorinated congeners are shown as these are the most important compounds with respect to their TEF values. The chromatograms reveal that milk samples (Fig. 4) are effectively cleaned by the described method; no major interferences are present. The total TEQ value for the milk sample shown is 2.65 pg TEQ per gram of fat. Therefore, it is obvious that the method is sufficiently sensitive to analyse even low-contaminated biological samples.

The limit of determination (LOD) is estimated to be about 0.5 pg TEQ per gram of milkfat. Environmental contamination may have its own specific pattern of congeners depending on the local sources of pollution. As the LOD is dependent on the relative amounts of the seventeen congeners, it is therefore also dependent on the type of sample and on

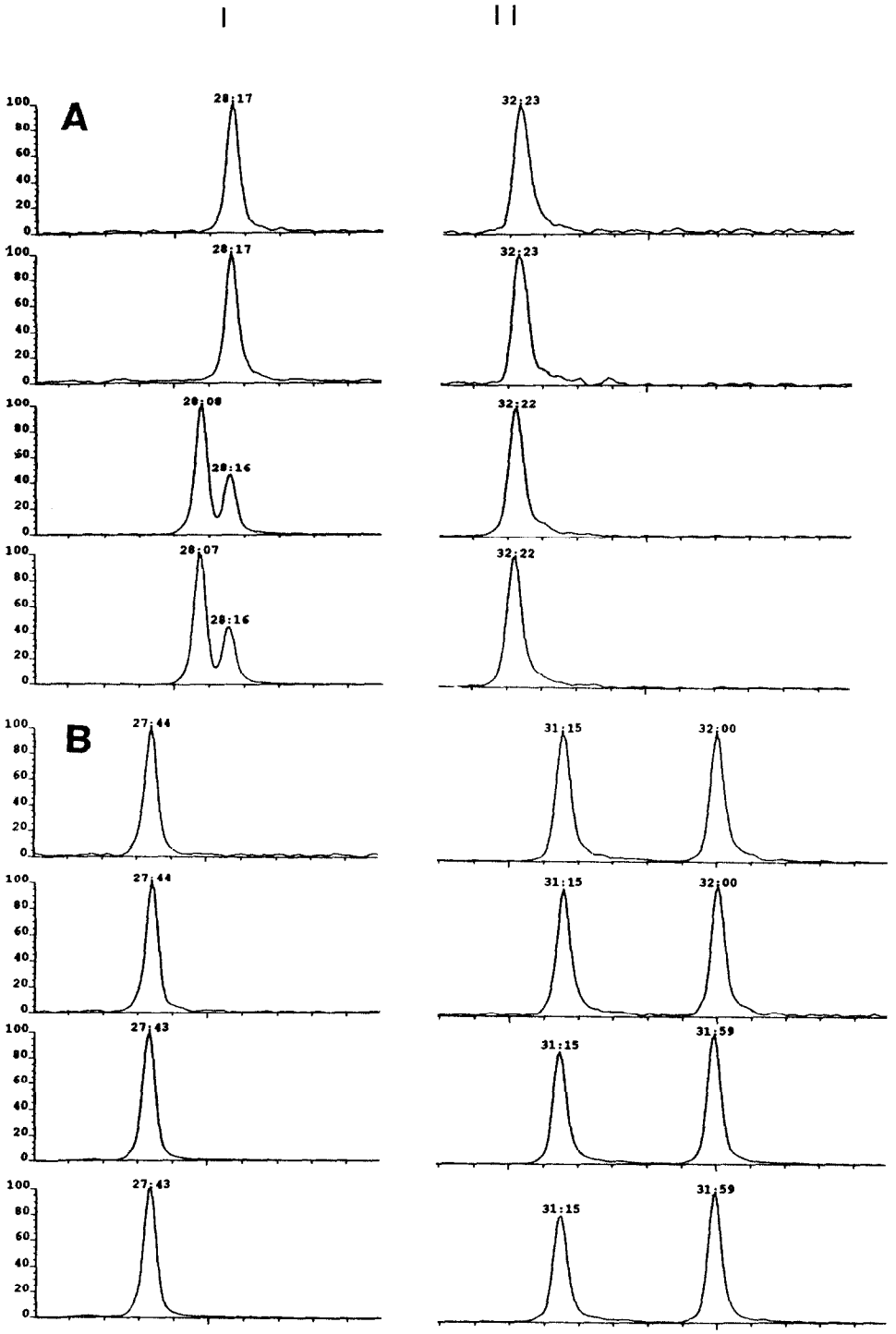

Fig. 3. Parts of a chromatogram of a calibration standard at a level of 1 pg/μl showing 2,3,7,8-TCDD and 1,2,3,4-[^{13}C]TCDD (internal standard) (A, I), 1,2,3,7,8-PeCDD (A, II), 2,3,7,8-TCDF (B, I) and 1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF (B, II). The two upper traces show the ions of the native compounds and the lower traces show the ^{13}C-labelled analogues. Ions monitored according to Table I. From top to bottom, ions are of increasing *m/z* value. Peaks are labelled with retention time (min)

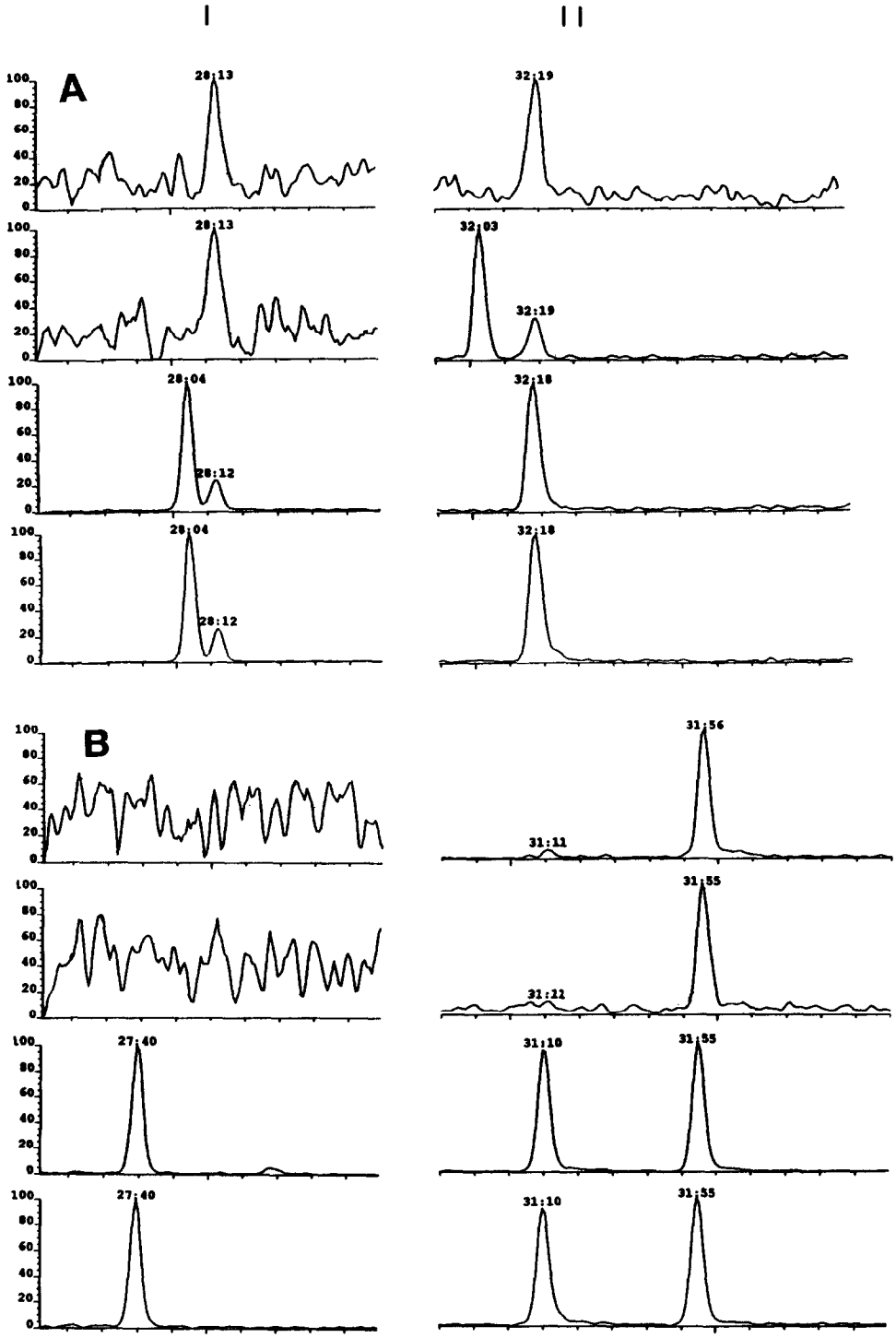

Fig. 4. Parts of a chromatogram of an extract of milkfat showing the acquisition windows of tetra- and pentachlorinated congeners as in Fig. 3. The two upper traces show the ions of the native compounds and the lower traces show the ^{13}C-labelled analogues. Ions monitored according to Table I. From top to bottom, ions are of increasing m/z value. Peaks are labelled with retention time (min). It can be seen that 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF are absent in milkfat.

the area from which it originates. Further, the LOD is dependent on the presence of interferences which may be matrix dependent. As mentioned before, there are no significant interferences caused by extracts of milkfat, which may be partly due to the fact that milkfat is relatively clean in comparison with other matrices, but it is also a result of the highly selective method used.

One problem involving interfering compounds was the occurrence of large amounts of phthalates in the part of the chromatogram where the pentachlorinated congeners elute. These amounts were large enough to disturb the ionization conditions in the ion source of the mass spectrometer. The phthalates are suspected to be present in solvents and packing material. We reduced this problem to such an extent by introducing the alumina clean-up that the analysis was not hindered by the presence of the phthalates.

The graphitized carbon column shows some tailing on the elution profile of the dioxins. When samples with widely differing contents are analysed, some cross-contamination may occur. This cross-contamination is of the order of 1–4% of the preceding sample, but may be decreased by eluting with toluene in the back-flush mode for a longer period, at the expense of a lower sample throughput.

CONCLUSIONS

The proposed method gave reproducible and accurate results for milkfat samples. When the results are expressed in pg TEQ per gram of fat, the mean accuracy was 103% ($n=28$) for a sample that was artificially contaminated at the Dutch tolerance level. The repeatability was good, with R.S.D. of 8.6% for the determination of TEQ values and 11.8% for the determination of individual congeners. The long-term performance was evaluated using two different quality control samples and was excellent. QCS 1 gave a mean result of analysis of 2.65 pg TEQ/g with R.S.D. = 11.5% ($n=20$). The mean result of analysis of QCS 2 was 6.04 pg TEQ/g with R.S.D. = 9.0% ($n=28$).

The chromatograms show efficient clean-up of the sample extracts, as no interferences are present. By applying a high degree of automation and organization, high sample throughputs can be achieved which cannot be matched by other methods at the same level of contamination. The described clean-up method is highly automated using Gilson x–y–z robots for injection, fraction collection and solid-phase extraction. This results in a high sample throughput as compared with other methods [10]. With three skilled technicians, the clean-up and MS analysis of six unknown samples per day are possible. In each series of twelve unknown samples, a set of three quality control samples can be handled at the same time by the same technicians. To achieve this, strict organization of the procedure is necessary.

ACKNOWLEDGEMENTS

The authors thank Mr. P. W. J. Kolkman and R. J. van Mazijk for their assistance.

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