Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1038 (2004) 189–199
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Separation of seventeen 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins and dibenzofurans and 12 dioxin-like polychlorinated biphenyls by comprehensive two-dimensional gas chromatography with electron-capture detection

P. Korytár[a,b,*], C. Danielsson[c], P.E.G. Leonards[a], P. Haglund[c], J. de Boer[a], U.A. Th. Brinkman[b]

[a] *Netherlands Institute for Fisheries Research, P.O. Box 68, 1970 AB IJmuiden, The Netherlands*
[b] *Department of Analytical Chemistry and Applied Spectroscopy, Free University, de Boelelaan 1083, 1081 HV Amsterdam, The Netherlands*
[c] *Department of Chemistry, Environmental Chemistry, Umeå University, SE-901 87 Umeå, Sweden*

Received 6 February 2004; received in revised form 10 March 2004; accepted 10 March 2004

Available online 17 April 2004

Abstract

Comprehensive two-dimensional gas chromatography (GC × GC) with electron-capture detection (ECD) has been optimized for the separation of seventeen 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins and dibenzofurans and 12 dioxin-like polychlorinated biphenyls, with emphasis on the selection of the first- and second-dimension, commercially available, columns. When eight second-dimension columns were subsequently combined with a 100% methylpolysiloxane stationary phase (DB-1) in the first dimension to create orthogonal conditions, a complete separation of all congeners with different TEF values was obtained with two column combinations, DB-1 × VF-23 and DB-1 × LC-50. When other types of first-dimension columns were used (and orthogonality was partly sacrificed), a DB-XLB column combined with 007-65HT, VF-23 and LC-50 was found to provide a complete separation of all 29 priority congeners. Next, the potential of these three column combinations for real-life analysis was preliminarily studied. With a spiked and fractionated milk extract, DB-XLB × LC-50 was found to be the most powerful column combination, because of the good separation of the 29 priority congeners from each other as well as from the matrix constituents. Quantitative performance (close to three-order linearity; LODs, 30–150 fg injected; R.S.D.s, 1.5–6.5% ($n = 10$)) was satisfactory.

Keywords: Gas chromatography, comprehensive two-dimensional; Polychlorinated dibenzo-*p*-dioxins; Polychlorinated dibenzofurans; Dioxin-like polychlorinated biphenyls

1. Introduction

Because of the high persistence and extreme toxicity of polychlorinated dibenzo-*p*-dioxins and dibenzofurans and so-called dioxin-like polychlorinated biphenyls (PCDDs, PCDFs and PCBs, respectively) [1], their trace-level determination in a wide variety of sample types is a topic of much interest [2–6]. For the present study, it is relevant to emphasize the highly lipophilic nature of these compounds which causes their bio-accumulation in the food chain; in other words, consumption of fatty food is an important route of exposure. As a consequence, the EU has recently set maximum residue levels for seventeen 2,3,7,8-substituted CDD/Fs in various types of food and feeding stuff, expressed in terms of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalent concentration, TEQ, with TEQ $= \sum_i \mathrm{TEF}_i \times c_i$, where TEF_i and c_i are the toxic equivalence factor and concentration, respectively, of compound i. These maximum levels vary from 0.75 to 6 pg TEQ/g fat [7]. Soon, maximum residue levels for dioxin-like CBs will be introduced. The rapidly growing number of samples offered for analysis makes further improving of the analytical procedure a rewarding task.

The separation and quantification of the set of 29 target compounds is challenging, because they typically occur at

* Corresponding author. Tel.: +31-255564607; fax: +31-255564644.
E-mail address: peter.korytar@wur.nl (P. Korytár).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.03.026

sub-ng/kg concentrations, have to be clearly separated from the many other, less toxic, congeners present in the samples, and also from a large number of (endogenous) sample constituents which may be present at much higher concentrations. Not surprisingly, therefore, current procedures for the quoted priority analytes include complicated and time-consuming multi-step sample pre-treatment. One way to improve the situation would be to considerably increase the separation efficiency of the gas chromatographic (GC) analysis by replacing conventional one-dimensional GC by so-called comprehensive two-dimensional gas chromatography (GC × GC). As is explained in, e.g., [8,9], in GC × GC two independent separations are applied to an entire sample which effects a considerably enhanced overall resolution and also, because of the analyte refocusing during modulation, an improved analyte detectability.

In several recent studies from our, as well as other groups [10–14], it has been shown that proper selection of the stationary phases of the first- and second-dimension GC columns is extremely important when designing a GC × GC system, specifically when many structurally closely related compounds are the target analytes. In the present paper, this topic will be systematically studied and, while complete separation is of course one main goal, we shall also devote attention to separations in which analyte co-elution occurs only for target compounds having the same TEF values. Such separations will, after all, not adversely affect the final calculation of the TEQ concentration. While the principal aim is a study of the separation characteristics for the target compounds, as a next step the most promising GC × GC column combinations will be tested for their usefulness in analysing the target set in real-life samples. This should be of considerable help in selecting the column combinations to be used in the subsequent study on real-life screening and TEQ-based quantification.

Table 1
Composition of standard mixture and TEF values

Compound[a]	Code	Concentration (ng/ml)	TEF
Dibenzo-*p*-dioxins			
2,3,7,8-TCDD	4D1	100	1
1,2,3,7,8-PeCDD	5D1	250	1
1,2,3,4,7,8-HxCDD	6D1	250	0.1
1,2,3,6,7,8-HxCDD	6D2	250	0.1
1,2,3,7,8,9-HxCDD	6D3	250	0.1
1,2,3,4,6,7,8-HpCDD	7D1	250	0.01
OCDD	8D	500	0.0001
Dibenzofurans			
2,3,7,8-TCDF	4F1	100	0.1
1,2,3,7,8-PeCDF	5F1	250	0.05
2,3,4,7,8-PeCDF	5F2	250	0.5
1,2,3,4,7,8-HxCDF	6F1	250	0.1
1,2,3,6,7,8-HxCDF	6F2	250	0.1
1,2,3,7,8,9-HxCDF	6F4	250	0.1
2,3,4,6,7,8-HxCDF	6F3	250	0.1
1,2,3,4,6,7,8-HpCDF	7F1	250	0.01
1,2,3,4,7,8,9-HpCDF	7F2	250	0.01
OCDF	8F	500	0.0001
Non-ortho CBs			
3,3′,4,4′-TCB	77	1000	0.0001
3,4,4′,5-TCB	81	1000	0.0001
3,3′,4,4′,5-PeCB	126	1000	0.1
3,3′,4,4′,5,5′-HxCB	169	1000	0.01
Mono-ortho CBs			
2,3,3′,4,4′-PeCB	105	1000	0.0001
2,3,4,4′,5-PeCB	114	1000	0.0005
2,3′,4,4′,5-PeCB	118	1000	0.0001
2′,3,4,4′,5-PeCB	123	1000	0.0001
2,3,3′,4,4′,5-HxCB	156	1000	0.0005
2,3,3′,4,4′,5′-HxCB	157	1000	0.0005
2,3′,4,4′,5,5′-HxCB	167	1000	0.00001
2,3,3′,4,4′,5,5′-HpCB	189	1000	0.0001

[a] T: tetra; Pe: penta; Hx: hexa; Hp: hepta; O: octa.

2. Experimental

2.1. Samples and chemicals

A standard solution containing seventeen 2,3,7,8-substituted CDD/F congeners and 12 dioxin-like CBs was prepared by mixing two commercial standard mixtures, EPA 8290 STN and WP-STK, both from Wellington (Guelph, Ont., Canada) in *n*-nonane. The composition of the mixture is given in Table 1. A 10-fold-diluted mixture was used to test the various column combinations. Individual standards of CBs and CDD/Fs used for peak identification were purchased from Promochem (Wesel, Germany) and Cambridge Isotope Labs (Andover, MA, USA), respectively.

The milk sample was prepared by the Institut Quimic de Sarria (Barcelona, Spain) according to a method validated for GC–HRMS. A brief summary is as follows [15]. Two-hundred grams of milk was mixed with ethanol containing 15% potassium oxalate and thereafter liquid–liquid extracted three times: first, with diethyl ether–*n*-hexane (7:10, v/v), second and third time, with *n*-hexane. The extract was purified on a multilayer silica column (containing from top to bottom layers of anhydrous sodium sulphate, sulphuric acid–silica, activated silica, sodium hydroxide–silica, activated silica, silver nitrate–silica, and glass wool). The purified extract was transferred to SPE carbon tubes (Supelco, Bellefonte, PA, USA) with *n*-hexane for fractionation. The first fraction, containing CBs, was eluted with *n*-hexane and *n*-hexane–toluene (72:25, v/v) and the second fraction, containing CDD/Fs, with toluene in reverse mode. The CB-containing fraction was further fractionated on an HPLC pyrenyl column using *n*-hexane as eluent in order to separate the dioxin-like CBs (second fraction) from the bulk of the CBs (first fraction). Both fractions of interest—those containing the dioxin-like CBs and the CDD/Fs—were finally purified on a silica column (containing layers of anhydrous sodium sulphate, activated silica, sulphuric acid–silica and glass wool). The sample was spiked at the end of the clean-up process with 10 pg of TCDD/F, 50 pg of Pe-, Hx-

and HpCDD/Fs and 100 pg of OCDD/F and concentrated to 25 μl.

2.2. GC × GC system

The GC × GC system was built from an HP6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph equipped with a loop-type carbon dioxide jet modulator (KT2002 CO_2 system; Zoex, Lincoln, NE, USA). Principles, working characteristics and optimisation of parameters are extensively described in [16]. The hot air pulse duration was 200 ms, and the modulation period varied between 6 and 8 s, depending on the experiment. At the start of each run, the CO_2 flow was adjusted by using a needle valve to keep the cold-jet temperature at 0–10 °C, at an initial oven temperature of 90 °C. Helium gas (Hoek Loos, Schiedam, The Netherlands) with a purity of 99.999% was used as carrier gas. A micro-ECD (Agilent) was operated at 300 °C, with 99.999% pure nitrogen (Hoek Loos) as make-up gas at a flow-rate of 150 ml/min. The data acquisition rate was 50 Hz. One microlitre samples were injected manually into a split/splitless inlet port operated in the splitless mode at 280 °C with split opening after 2 min.

The GC columns used in the first and second-dimensions are listed in Table 2. The columns were coupled via a 1.5 m × 0.1 mm i.d. uncoated fused-silica deactivated column (BGB Analytik, Aldiswil, Switzerland), which serves as the modulator loop. For the comparison of the first-dimension separations (see Table 4 further), all columns were coupled to a 2.5 m × 0.1 mm i.d. retention gap to create the same pressure profile in the column as in a GC × GC run, but without influencing the separation by the stationary phase in the second column. No modulation was applied in this case, because it is virtually impossible to exactly measure the first-dimension peak width if it is modulated into a number of second-dimension peaks. Mini press-fits (Techrom, Purmerend, The Netherlands) were used for the connections. HP Chemstation software (Agilent) was used to control the GC instruments and to acquire data. ChromCard software (ThermoFinnigan, Milan, Italy) was used for GC × GC data processing, evaluation and visualisation. Transform software (Fortner Research, Sterling, VA, USA) was used for producing two-dimensional chromatograms.

3. Results and discussion

3.1. Tuning of second-dimension separation

In order to fully profit from the advantages provided by GC × GC, it is generally recommended to use a truly orthogonal system, because then, next to a high peak capacity and enhanced sensitivity, also chemically meaningful structured chromatograms can be obtained [8]. Therefore, eight second-dimension columns (Table 2) which cover the whole range of stationary phases available in columns with dimensions as are required for the second separation—typically 1 m long with an i.d. of 0.1 mm and a film thickness of 0.1 μm—were subsequently combined with a 100% methylpolysiloxane stationary phase (DB-1,

Table 2
Survey of first- and second-dimension GC columns used

Commercial code	Stationary phase	Temperature limit[a] (°C)	Dimensions	Producer[b]
First-dimension columns				
DB-1	100% methylpolysiloxane	325/350	30 m × 0.25 mm × 0.25 μm	J&W Scientific
VF-1 ms	100% methylpolysiloxane	325/350	30 m × 0.25 mm × 0.25 μm	VARIAN
VF-1 ms	100% methylpolysiloxane	325/350	50 m × 0.25 mm × 0.25 μm	VARIAN
HT-5	5% phenyl-methylpolysiloxane (carborane)	400/410	30 m × 0.25 mm × 0.25 μm	SGE International
DB-XLB	Proprietary	340/360	30 m × 0.25 mm × 0.25 μm	J&W Scientific
DB-DIOXIN	Proprietary (44% methyl, 28% phenyl, 20% cyanopropyl, 8% polyoxyethylene-polysiloxane)	250/270	30 m × 0.25 mm × 0.25 μm	J&W Scientific
LC-50	50% liquid crystalline-methylpolysiloxane	−/270	10 m × 0.18 mm × 0.10 μm	J&K Environmental
Second-dimension columns				
BPX-50	50% phenyl-methylpolysiloxane (silphenylene)	360/370	1 m × 0.10 mm × 0.1 μm	SGE International
007-65HT	65% phenyl-methylpolysiloxane	−/360	1 m × 0.10 mm × 0.1 μm	Quadrex
HT-8	8% phenyl-methylpolysiloxane (carborane)	360/370	1 m × 0.10 mm × 0.1 μm	SGE International
OV 1701	14% cyanopropylphenyl-methylpolysiloxane	−/280	1 m × 0.10 mm × 0.1 μm	Quadrex
VF-23 ms	Proprietary (high cyano containing polymer; with absolute cyano content 70–90%)	−/260	1 m × 0.10 mm × 0.1 μm	VARIAN
SupelcoWax-10	Polyethylene glycol	−/280	0.5 m × 0.10 mm × 0.1 μm	Supelco
007–210	50% trifluoropropyl-methylpolysiloxane	−/280	1.0 m × 0.10 mm × 0.1 μm	Quadrex
LC-50[c]	50% liquid crystalline-methylpolysiloxane	−/270	0.35 (0.9) m × 0.18 mm × 0.1 μm	J&K Environmental

[a] Maximum isothermal temperature/maximum programmed temperature.
[b] J&W Scientific, Folsom, CA, USA; VARIAN, Middelburg, The Netherlands; SGE International, Ringwood, Australia; J&K Environmental, Sydney, Nova Scotia, CA; Quadrex, New Haven, CT, USA; Supelco, Bellefonte, PA, USA.
[c] 0.9 m long column used only for milk sample analysis.

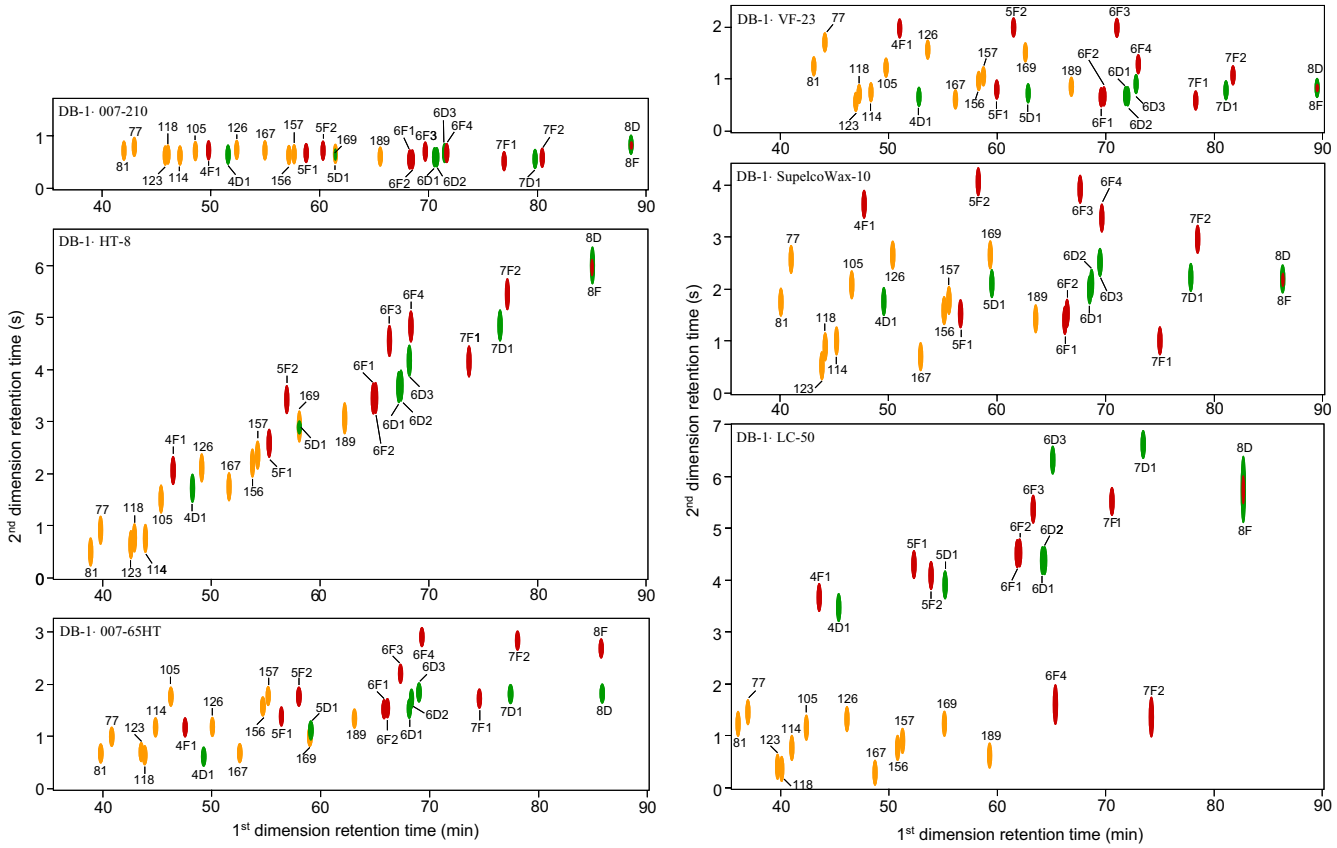

Fig. 1. GC × GC–μECD chromatograms of the mixture of 10 CDFs (red), 7 CDDs (green) and 12 dioxin-like CBs (orange) for a DB-1 column combined with second-dimension columns as indicated in the frames. Temperature programme for both columns: 90 °C (2 min), at 30 °C/min to 170 °C (5 min), then at 1.5 °C/min to 290 °C (10 min). Final temperature for LC-50 and 007–210 was 270 and 280 °C, respectively, because of temperature limits. Inlet pressure for DB-XLB × LC-50, 39 p.s.i., for other combinations, 45 p.s.i. Modulation period, 7 s.

30 m × 0.25 mm × 0.25 μm) in the first dimension to be tested for separation of the 17 CDD/Fs and 12 dioxin-like CBs. For six out of the eight combinations, the separations are shown in Fig. 1. The separations achieved with OV1701 and BPX-50 as second column are not shown, because the two-dimensional elution patterns were very similar to those achieved on VF-23 and 007-65HT, respectively, but with slightly less resolution in the second-dimension. There were six pairs of congeners which were not resolved on the first column. One of these pairs, viz. 169/5D1, is of particular interest, because it is the only case where the members have different TEF values. The second-dimension separation of these congeners was expressed in terms of their resolution, ${}^2R_S$. The calculated values for all tested columns are given in Table 3.

Table 3 and Fig. 1 demonstrate that the introduction of a trifluoropropyl group into the siloxane stationary phase (column 007–210) does not add to the separation of the target compounds at all: ${}^2R_S$ is invariably equal to zero and all compounds show up in a horizontal line in the

Table 3
Second-dimension resolution, ${}^2R_S$, on eight stationary phases for analyte pairs co-eluting on first-dimension DB-1 column

Co-eluting pairs on DB-1	Resolution in second-dimension on[a]							
	007–210	HT-8	BPX-50	007-65HT	OV-1701	VF-23	SupelcoWax-10	LC-50
123/118	–	–	–	–	–	0.8	0.9	–
169/5D1	–	–	–	0.4	1.2	3.3	1.1	5.7
6F1/6F2	–	–	–	–	–	–	0.4	–
6D1/6D2	–	–	–	0.4	–	–	–	–
6D3/6F4	–	0.9	2.1	2.2	0.7	1.8	1.5	2.5
8D/8F	–	–	1.7	1.7	–	–	Decomposition	–

[a] (–) Peaks not resolved (${}^2R_S < 0.4$).

GC × GC plane. Moreover, tailing peaks were observed with this stationary phase. The HT-8 column has strong boiling-point-based selectivity, as is indicated by the ordering of the peaks in a diagonal line. Although carborane containing several per cent of phenyl groups is known to provide some extra selectivity, which causes congener separation based on the number of chlorine substituents [13,14], not much added value was observed in the present case. Only the separation of the 6D3/6F4 pair was significantly improved. A higher proportion of phenyl groups in the stationary phase, as in the BPX-50 and 007-65HT columns, yielded stronger retention further improves the separation of this pair; in addition, separation of 8D/8F is achieved. However, the 169/5D1 pair, the separation of which is essential for TEQ calculation, still is not satisfactorily resolved. The cyanopropyl-containing stationary phases, OV1701 and VF-23, specifically the latter, which has a higher cyano content, provide an excellent separation of 169/5D1 and, consequently, allow TEQ determination. The resolution of the 6D3/6F4 pair is similar to those achieved on the phenyl phases, but the separation of 8D/8F is, unfortunately, completely lost.

A main characteristic of the polyethylene glycol phase, SupelcoWax-10, is the very high retention of the target analytes. In order to avoid the need to install a second oven to speed up the second-dimension separation, a 0.5 m instead of a 1 m long second-dimension column was used. Even so, peaks were broader than with the other stationary phases. Despite the rather promising results—which are similar to those for VF-23—the decomposition of 8F and, slightly less, also of 7F2 disqualifies this phase. The decomposition is probably due to surface-catalysed dehalogenation of the chlorines in positions one and nine due to steric congestion [17]. A liquid crystalline LC-50 phase, which provides planarity selectivity, was also included in the test set, although at the time of analysis it was available only as a 0.18 mm i.d. column. Fig. 1 shows that this column is extremely effective in separating the CDDs and CDFs, which show up in the top part of the chromatogram, from the CBs, which have much shorter second-dimension retention times. The selectivity for planar compounds is so high, that although only a 0.35 m instead of 1 m long column was used, compounds such as 6F4, 7F2, 8D and 8F showed wrap-around. As regards selectivity, the LC-50 column provides the best result for the critical pair 169/5D1. The 6D3/6F4 pair is also clearly separated, but the results for the other pairs leave much to be desired.

In conclusion, one aim of the present study—the base-line separation of all congeners with different TEF values—has been achieved. There are two column combinations which meet this demand. One of these, moreover, the DB-1 × LC-50 set, provides an interesting separation of the priority CBs from the CDD/CDFs. The second aim, the separation of all six co-eluting pairs, cannot be achieved with any of the column sets tested. Especially because no other types of second-dimension column are commercially available today, it is obvious that improved performance should preferably come from optimisation of the first-dimension separation.

3.2. Tuning of first-dimension separation

3.2.1. Orthogonal approach

Somewhat surprisingly, the first-dimension separation could be improved by using another type of 100% dimethylpolysiloxane stationary phase (VF-1 ms) than used in the previous experiments (DB-1). This is shown in Table 4, which compares GC separations on DB-1 and VF-1 ms under the same chromatographic conditions. A major improvement was observed for the 6D3/6F4 pair, which is clearly separated on VF-1 ms ($^1R_S = 1.4$), while there is no separation at all on DB-1. However, the improvement has little importance for the final GC × GC separation, because this pair is easily separated in the second-dimension on, e.g., VF-23 and LC-50. Small gains of selectivity were also observed for two other co-eluting pairs, 6F1/6F2 and 6D1/6D2, but no improvement was observed for the 123/118, 169/5D1 and 8D/8F pairs, which remain totally unresolved. The very limited usefulness of selecting a longer (50 m versus 30 m) first-dimension column is also illustrated in Table 4, *viz.* for VF-1 ms.

A better option to improve the first-dimension separation is to use a more selective stationary phase. Of course, by doing so, the orthogonality and possibly also the ordered structure will be lost. However, while ordered structure is extremely useful when analysing complex samples containing many unknowns, because it allows a fast provisional identification, in target analysis, i.e. in this study, the most important criterion is the separation of the priority analytes, and 'structure' is not really required.

3.2.2. Non-orthogonal approach

Next to the two methylpolysiloxane phases, four more stationary phases were tested in the first dimension (Table 2). Achieving the required separation is one criterion to be considered. However, the stability of the stationary phase is also important, because column bleed will also be modulated and may cause system peak(s) in the second-dimension chromatograms [18]. Co-elution of the target compounds with such system peaks will adversely affect their detectability and additional tuning will be required to improve separation, if at all possible. Therefore, the noise levels of all tested columns were studied. In order to minimize the contribution of injector bleeding (contaminants, septum, O-ring), the injector temperature was held at 60 °C in all analyses and no injection was performed. The baseline profiles observed at an elution temperature of 240 °C are shown in Fig. 2. The difference between the 'special phases', DB-DIOXIN and LC-50, and the non-polar phases, DB-1, VF-1 ms, HT-5 and DB-XLB, is dramatic. The absolute noise level for the former two phases is *ca.* 100 Hz, as against around 10 Hz for the latter four. Moreover, the non-polar stationary phases show only one negative system peak at the start of each

Table 4
Elution order, retention times (^1t_R) and resolution of partially resolved congeners (^1R_S)[a] for the 29 test compounds on four non-polar first-dimension columns[b]

Compound	DB-1 (30 m)		VF-1 ms (30 m)		VF-1 ms (50 m)		HT-5 (30 m)			DB-XLB (30 m)		
	^1t_R [min]	^1R_S	^1t_R [min]	^1R_S	^1t_R [min]	^1R_S	Compound	^1t_R [min]	^1R_S	Compound	^1t_R [min]	^1R_S
81	42.7		44.0		55.3		81	41.1		81	58.0	
77	43.9		45.3		56.7		77	43.1		77	59.8	
123	47.7		49.1		60.6		123	45.7		123	62.2	
118	48.1	1.3	49.5	1.3	61.0		118	46.4		118	63.2	
114	49.5		50.9		62.7		114	47.4		114	64.8	
105	51.4		52.9		64.8		105	50.6		105	67.5	
4F1	52.9		54.6		66.7		4F1	53.0		4F1	72.5	
4D1	55.4		57.0		69.2		4D1	54.5		4D1	74.3	
126	56.5		58.1		70.3		126	56.8		126	74.3	–
167	60.1		61.6		73.8		167	59.6		167	76.6	
156	63.2		64.8		77.2		156	63.2		156	80.5	
157	63.8		65.5		77.9		157	64.2		157	81.1	
5F1	65.2		67.1		79.9		5F1	65.2		5F1	85.2	
5F2	67.5		69.4		82.2		5F2	69.2		169	88.3	
169	69.3		70.9		83.5		5D1	70.0		5F2	89.1	
5D1	69.3	–	70.9	–	83.9	1.4	169	70.7		5D1	89.8	
189	75.3		77.0		89.6		189	76.7		189	93.4	
6F1	78.9		81.0		94.1		6F1	79.9		6F1	100.1	
6F2	79.3	0.9	81.4	1.1	94.5	1.4	6F2	80.2	1.0	6F2	100.5	1.4
6F3	81.1		83.1		96.2		6D1	84.1	0.7	6F3	103.2	
6D1	82.4		84.3		97.3		6D2	84.3		6D1	103.5	1.1
6D2	82.6	0.7	84.6	0.9	97.6	1.0	6F3	84.5	0.5	6D2	104.1	
6D3	83.8		85.7		98.8		6D3	86.3		6D3	105.1	
6F4	83.8	–	86.2	1.4	99.4		6F4	87.1		6F4	106.6	
7F1	91.7		93.8		107.1		7F1	93.8		7F1	112.8	
7D1	96.0		98.0		111.2		7D1	99.2		7D1	117.6	
7F2	96.8		99.2		112.5		7F2	100.5		7F2	120.2	
8F	108.3		110.7		124.0		8D	112.7		8D	130.2	
8D	108.5	–	110.7	–	124.1	–	8F	113.0	0.7	8F	131.2	

[a] (–) Not possible to identify peak maxima ($^1R_S < 0.5$).
[b] Conditions: column outlets coupled to a 2.5 m × 0.1 mm i.d. retention gap; temperature programme, 90 °C (2 min), at 30 °C/min to 170 °C, then at 1.0 °C/min to 300 °C; inlet pressure, 45 and 53 p.s.i. for 30- and 50 m long columns, respectively.

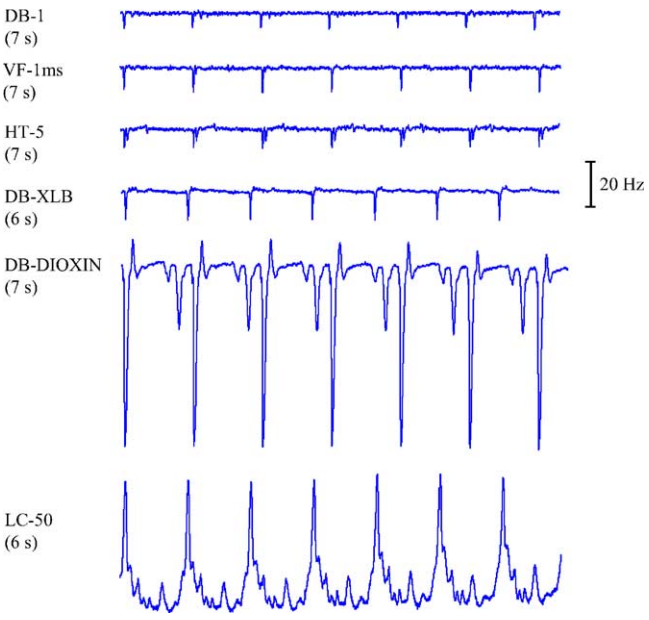

Fig. 2. Noise/bleed comparison at 240 °C for the six stationary phases used in the first-dimension column; modulation period added in brackets.

second-dimension chromatogram. Consequently, the system can easily be tuned to create separation of all analytes from the system peak, which will improve detectability. The noise levels then are 2 Hz for DB-1, VF-1 ms and DB-XLB, and 3 Hz for HT-5; that is, the limits of detection (LODs) will be 30–50-fold lower than for the special phases. Because of this huge difference, DB-DIOXIN and LC-50 cannot really be used as first-dimension columns for the present application, and they were not further considered.

The DB-XLB and HT-5 stationary phases were further evaluated for the separation of the 29 test compounds. First, elution order, retention times (^1t_R) and resolution (^1R_S) of partially resolved congeners were determined under the same conditions as for the 100%-methylpolysiloxane phases. All columns had the same dimensions; they were coupled to a 2.5 m × 0.1 mm i.d. retention gap to create the same pressure profile in the column as in a GC × GC run, and the same chromatographic conditions were applied. All relevant data are shown in Table 4. Next, second-dimension separation was tested under the same conditions as in the case of the DB-1 column. Only three second-dimension columns, 007-65HT, VF-23 and LC-50, were used, because

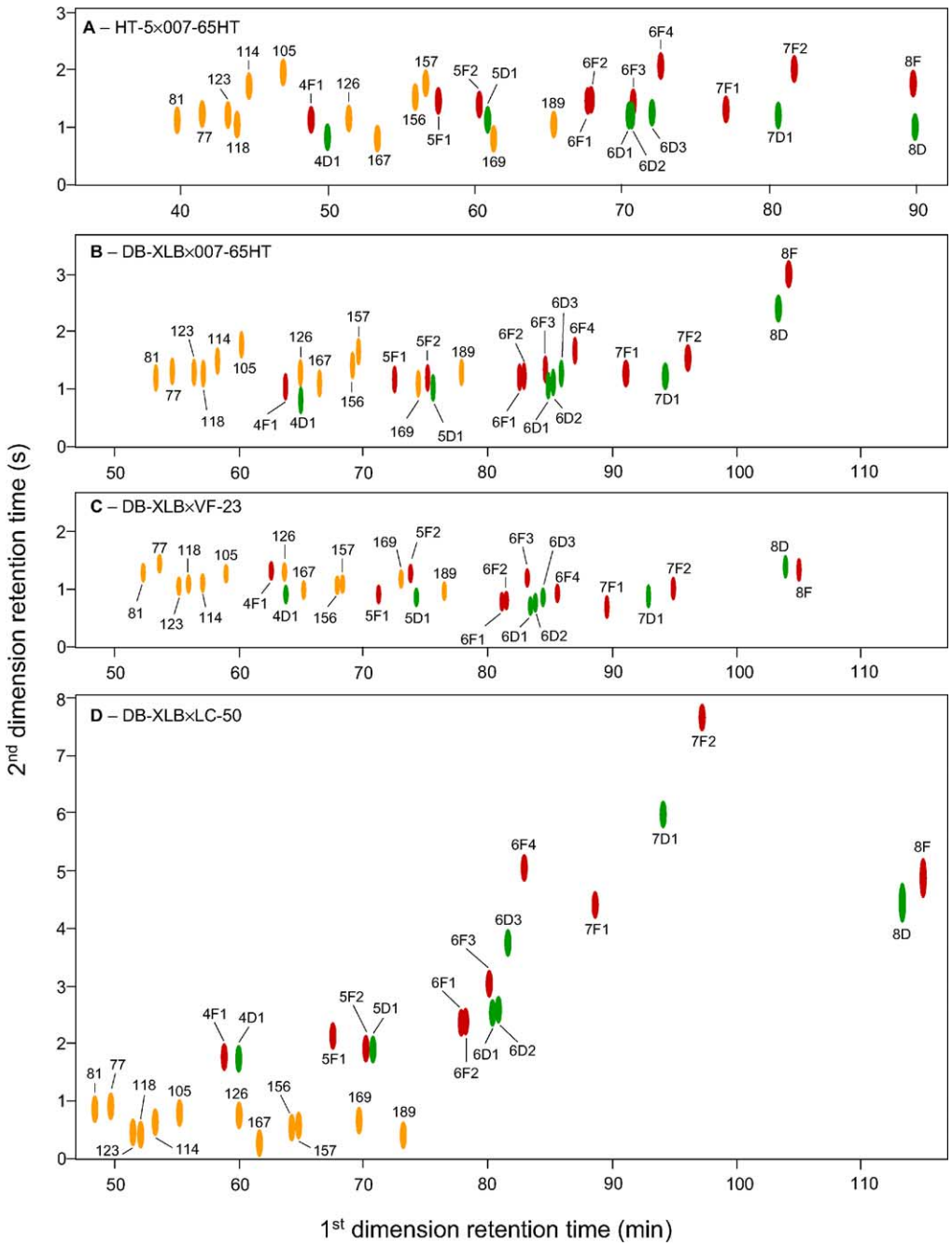

Fig. 3. GC × GC–μECD chromatograms of the mixture of 10 CDFs (red), 7 CDDs (green) and 12 dioxin-like CBs (orange) for column combinations as indicated in the frames. Temperature programme for both columns: 90 °C (2 min), at 30 °C/min to 170 °C (5 min), then at 1.5 °C/min to 290 °C (10 min). Final temperature for LC-50 was 270 °C, because of temperature limit. Inlet pressure for DB-XLB × LC-50, 39 p.s.i., for other combinations, 45 p.s.i. Modulation period, 8 s.

they showed the highest selectivity with DB-1 in the first dimension (Table 3). The experimental results are shown in Fig. 3.

As regards the HT-5 column, Table 4 shows that all congeners with different TEF values were separated. When HT-5 was combined with the three selected second-dimension columns, the best result was obtained in the case of the 007-65HT column (Fig. 3A). Only five congeners now displayed partial co-elution—6F1/6F2 and 6D1/6D2/6F3. When combining the HT-5 column with either VF-23 or LC-50, six compounds co-eluted—6F1/6F2, 6D1/6D2 and 8F/8D (data not shown). All of this was an improvement compared with the best DB-1-based orthogonal separations (DB-1 × VF-23 and DB-1 × LC-50; Fig. 1), where two congeners were not resolved at all and six further compounds showed partial co-elution.

For the DB-XLB column, Table 4 shows that one pair of congeners with different TEF values, 4D1/126, was not resolved at all and two pairs, 6F1/6F2 and 6F3/6D1, were resolved only partly. However, when combining this column with any of the three second-dimension columns, all priority congeners were satisfactorily separated (Fig. 3B-D). Least separation ($^1R_S = 1.4$) was obtained for the same pair in all cases, 6F1/6F2.

In conclusion, the second aim of the study—the complete separation of all 29 priority congeners—has now also been achieved, *viz*. for all three combinations. In real-life applications, the separation of the target analytes from each other

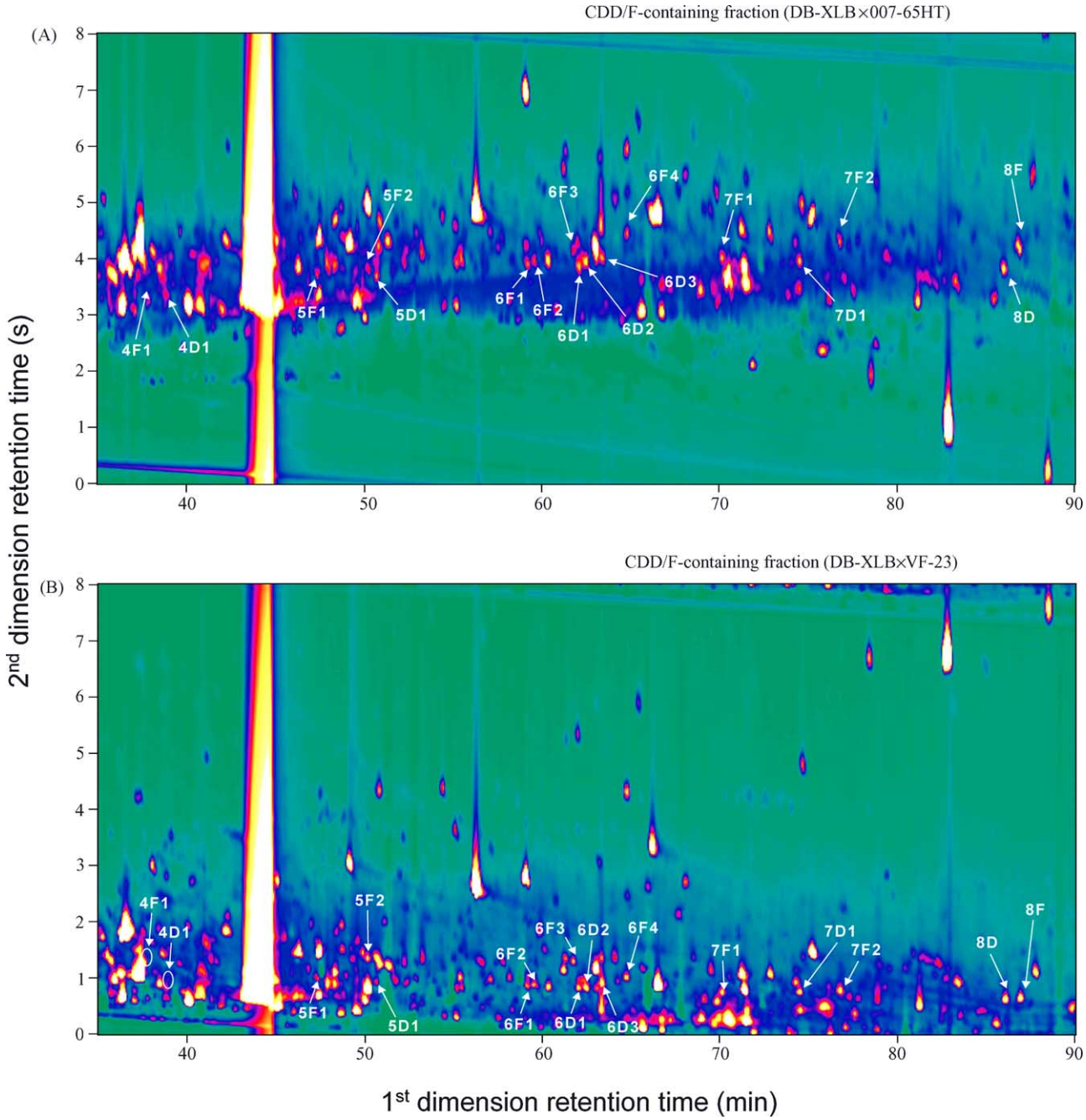

Fig. 4. GC × GC–μECD chromatograms of the CDD/F (A–C) and CB (D) fraction of a milk sample obtained with column combinations as indicated in the frames. Modulation period, 8 s. (A) Temperature programme, 90 °C (2 min), at 30 °C/min to 210 °C, then at 1.0 °C/min to 300 °C (5 min); constant flow, 1.1 ml/min. (B) Temperature programme, 90 °C (2 min), at 30 °C/min to 210 °C, then at 1.0 °C/min to 290 °C (15 min); constant flow, 1.1 ml/min. (C) Temperature programme, 90 °C (2 min), at 30 °C/min to 210 °C, then at 1.0 °C/min to 270 °C (25 min); ramped flow, 1.1 ml/min (49 min), in 0.5 min to 1.5 ml/min. (D) Temperature programme, 90 °C (2 min), at 30 °C/min to 180 °C, then at 1.5 °C/min to 261 °C, then at 20 °C/min to 270 °C (25 min); constant flow, 1.0 ml/min.

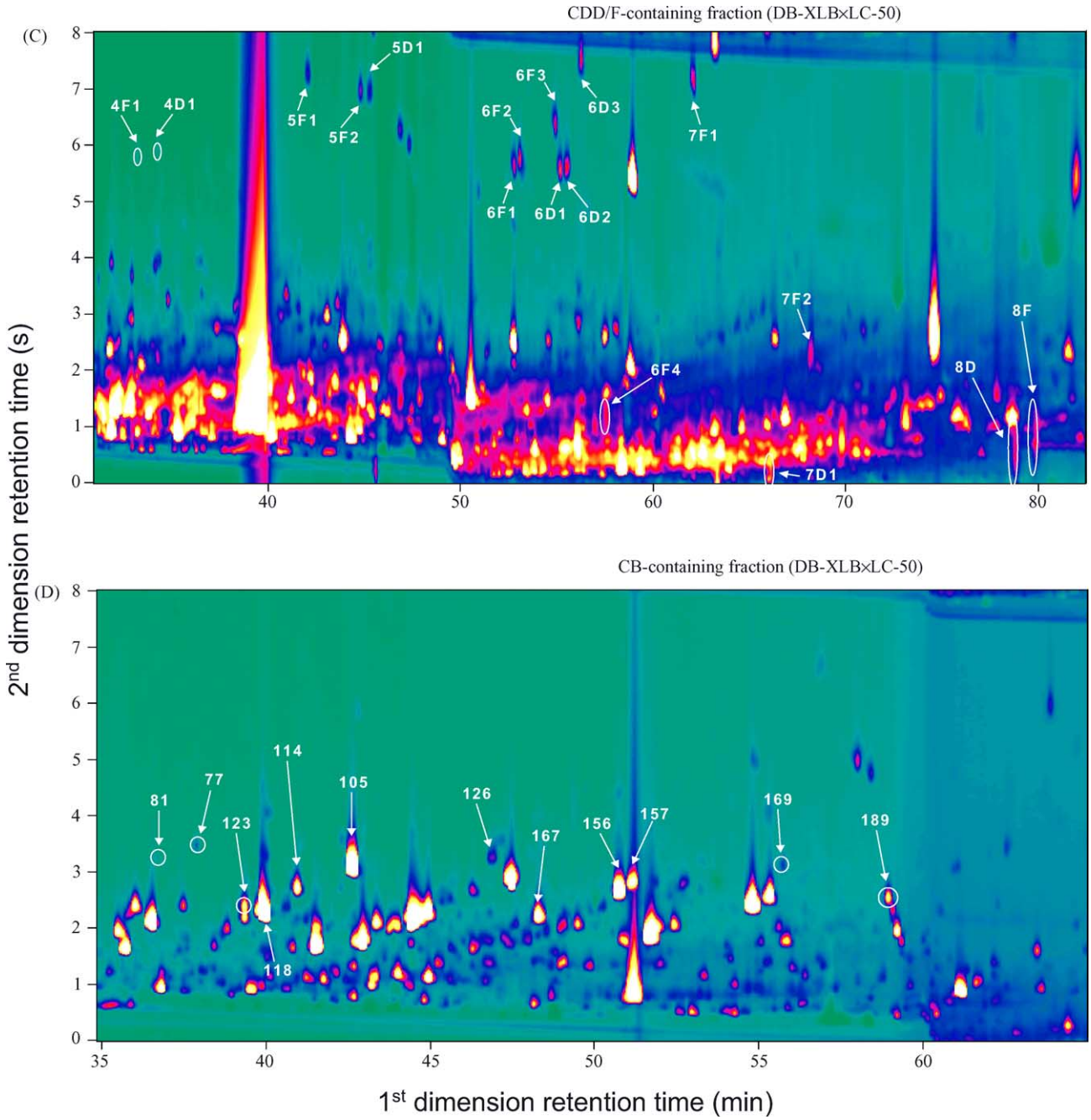

Fig. 4. (*Continued*).

is one goal. However, it is equally important to achieve the separation of the analytes from the matrix. This investigation is discussed in the next section.

3.3. Real-life applications

A milk extract spiked with the priority CDDs and CDFs (and containing incurred CBs) was fractionated into two fractions—one containing the mono- and non-*ortho* CBs and the other, the CDDs and CDFs—and analysed on the three column combinations selected in the previous section to evaluate their potential for real-life analysis. The chromatograms for the CDD/F and the dioxin-like CB fractions are shown in Fig. 4. As Fig. 4A and B show, 007-65HT and VF-23 failed to separate the target CDD/Fs from the matrix: essentially all analytes show up in the matrix band. While this is no serious problem when the analytes are relatively abundant, as e.g., 8D and 8F, it is extremely problematic for ultra-trace level analytes—such as, in this case, e.g., 4F1 and 4D1—and, also, of course for the more 'contaminated' regions.

For the DB-XLB × LC-50 combination, on the other hand, the excellent selectivity for dioxins and furans already

noted when discussing Fig. 1, is seen to provide the required extra selectivity (Fig. 4C). They are retained much stronger than the other sample constituents, which in most instances creates their efficient separation from the matrix. However, a drawback is that the retention of the hexa- to octa-substituted congeners is so high that they do not elute from the second column within the 8 s modulation time. That is, they show wrap-around and co-elution with other sample constituents sometimes occurs. For the majority of the hexa-substituted congeners (6F1, 6F2, 6F3, 6D1 and 6D2) this problem was solved by increasing the carrier gas flow from 1.1 to 1.5 ml/min just before their elution (at 49 min). However, the retention of two hexa- (6D3, 6F4), and the hepta- and octa-substituted congeners is so strong that they still show wrap-around and, consequently, some co-elution. Unfortunately, installing a second oven and keeping the second column at a higher temperature than the first-dimension column to speed up the elution of hexa- to octa-substituted congeners, is no generally valid solution. It is applicable only for the hexa congeners, since the maximum operating temperature of the LC-50 column (270 °C) is reached just during the elution of the hepta-substituted congeners, *viz.* at 66 min. Another option to reduce the second-dimension retention times is to use a shorter second column. A 0.6 m column was indeed tested, but the highest contributors to the TEQ value, 4F1 and 4D1, now showed insufficient separation from the matrix. The preferred solution is to use an LC-50 column with a smaller i.d., i.e., 0.10 instead of 0.18 mm. This would accelerate the second-dimension separation and, in addition, increase the separation efficiency. Unfortunately, such an LC-50 column is not commercially available today.

For the CB-containing fraction, the situation was found to be similar to the above. Again, the LC-50 column provided much better selectivity for, in this case, the dioxin-like CBs than the other two second-dimension columns tested. However, in this instance the situation is less critical than with the dioxins and furans, mainly because of the higher concentrations of dioxin-like CBs in real-life samples. Fig. 4D also shows that there is now no problem with wrap-around of the target analytes on the LC-50 column, since the mono- and non-*ortho*-CBs are retained less strongly than are the CDD/Fs. Since the CBs were incurred and were not added as spikes, there are huge differences in spot intensities, especially between the non- and mono-*ortho*-CBs.

3.4. Quantitative performance

The results presented in the previous section enable the conclusion that DB-XLB × LC-50 is the most powerful column combination presently available. It was therefore used to assess the potential of GC × GC–μECD for quantitative analysis by measuring relevant performance data (Table 5). Repeatability was calculated as the relative standard deviation (R.S.D.) of 10 consecutive injections of a 50-fold diluted standard solution. The R.S.D.s for the CBs varied from 1.5 to 4.0% and for the CDD/Fs from 3.0 to 6.5%. Even for the two partially co-eluting congeners, 6F1 and 6F2, the values were in this range, but integration was, admittedly, somewhat difficult. Linearity, expressed as the correlation coefficient of linear regression, r^2, was calculated from measurements of five solutions prepared by diluting the standard mixture 2:5, 1:10, 1:50, 1:200 and 1:1000 with isooctane. Table 5 shows that the results are gratifying, with all but four (0.9980–0.9988) correlation coefficients being higher than 0.9995. The limits of detection (LODs) were calculated at a signal-to-noise ratio of 3:1. As can be seen from Table 5, LODs of 30–70 fg can be generally expected for the dioxin-like CBs and LODs of 40–150 fg for the priority dioxins and furans.

Table 5
Quantitative performance data for the 29 priority analytes in GC × GC-μECD on the DB-XLB × LC-50 column combination

Compound	R.S.D. (%)[a]	r^{2}[b]	LOD (fg)
CB 81	2.5	0.9998	60
CB 77	2.5	0.9997	70
CB 123	3.0	0.9999	40
CB 118	4.0	0.9999	40
CB 114	1.5	1.0000	30
CB 105	1.5	1.0000	30
2,3,7,8-TCDF	5.5	0.9981	70
2,3,7,8-TCDD	5.5	0.9988	90
CB 126	3.0	0.9999	60
CB 167	1.5	0.9997	50
CB 156	2.0	1.0000	30
CB 157	1.5	1.0000	40
1,2,3,7,8-PeCDF	3.5	0.9998	70
CB 169	2.5	1.0000	50
2,3,4,7,8-PeCDF	3.5	0.9995	70
l,2,3,7,8-PeCDD	3.5	1.0000	70
CB 189	1.5	0.9998	40
1,2,3,4,7,8-IIxCDF	6.5	0.9999	40
1,2,3,6,7,8-HxCDF	6.5	0.9996	50
2,3,4,6,7,8-IIxCDF	6.5	0.9996	40
l,2,3,4,7,8-HxCDD	5.5	1.0000	60
l,2,3,6,7,8-HxCDD	5.5	0.9999	50
1,2,3,7,8,9-HxCDD	5.0	1.0000	40
l,2,3,7,8,9-HxCDF	4.5	0.9999	50
l,2,3,4,6,7,8-HpCDF	5.0	0.9999	70
1,2,3,4,6,7,8-HpCDD	4.0	0.9999	70
1,2,3,4,7,8,9-HpCDF	4.5	0.9999	80
OCDD	6.0	0.9980	150
OCDF	6.0	0.9980	150

[a] For 10 injections of 50-fold diluted standard solution.
[b] Linear model; five calibration levels, (2:5, 1:10, 1:50, 1:200 and 1:1000)-fold diluted standard solution; one injection per calibration level.

4. Conclusions

Comprehensive two-dimensional GC under orthogonal conditions—which are preferred because of the identification potential of ordered structures—provides a complete separation of all seventeen 2,3,7,8-substituted CDD/Fs and

12 dioxin-like CBs having different TEF values if a DB-1 column is combined with a second-dimension VF-23 or LC-50 column. For a complete separation of all 29 priority compounds from each other, orthogonality has to be (partly) sacrificed: combination of a DB-XLB column with either a 007-65HT, VF-23 or LC-50 second-dimension column yields the desired result. First work on real-life analysis indicates that, from the three combinations, DB-XLB × LC-50 is to be preferred because of the good separation of the target analytes from matrix constituents. In this context, it is highly desirable to have a 0.10 mm instead of the present 0.18 mm i.d. LC-50 column available, which will speed up the separation and avoid wrap-around of late eluted congeners.

The present result, combined with the satisfactory quantitative performance data obtained so far (linearity, repeatability, detectability) make GC × GC–μECD a promising technique for congener specific CDD/F and dioxin-like CB (screening) analysis. In subsequent studies, a larger number of different types of sample will be analysed and quantification data obtained by GC × GC–μECD and GC–high-resolution MS will be compared. In addition, attention will be devoted to possible co-elution of the present set of priority compounds and related classes of micro-contaminants such as polychlorinated naphthalenes and polychlorinated diphenyl ethers.

Acknowledgements

The authors gratefully acknowledge financial support by the European Commission under the projects DIAC (contract GRD2-2000-30037) and DIFFERENCE (contract G6D-2001-40002) and the help of Dr. J. Diaz-Ferrero (Institut Quimic de Sarria, Barcelona, Spain) in preparing the sample extracts.

References

[1] P. Patnaik, A Comprehensive Guide to the Hazardous Properties of Chemical Substances, second ed., Wiley, New York, USA, 1999, p. 304 (Chapter 15).
[2] A. Schecter, M. Pavuk, R. Malisch, J.J. Ryan, J. Toxicol. Environ. Health A 65 (2003) 1391.
[3] J. Knutzen, B. Bjerkeng, K. Naes, M. Schlabach, Chemosphere 52 (2003) 745.
[4] E. Eljarrat, J. Caixach, J. Rivera, Chemosphere 51 (2003) 595.
[5] D. Santillo, A. Fernandes, R. Stringer, R. Alcock, M. Rose, S. White, K. Jones, P. Johnston, Food Addit. Contam. 20 (2003) 281.
[6] M. Schuhmacher, M. Rodriguez-Larena, J. Diaz-Ferrero, J.L. Domingo, Chemosphere 48 (2002) 187.
[7] Council Regulation (EC) No. 2375/2001, amending Commission Regulation (EC) No. 466/2001, setting maximum levels for certain contaminants in foodstuffs, Official J. Euro. Commun. L 321 (2001) 1.
[8] J. Phillips, J. Beens, J. Chromatogr. A 856 (1999) 331.
[9] J. Dallüge, J. Beens, U.A.Th. Brinkman, J. Chromatogr. A 1000 (2003) 69.
[10] H.-J. de Geus, I. Aidos, J. de Boer, J.B. Luten, U.A.Th. Brinkman, J. Chromatogr. A 910 (2001) 95.
[11] J. Beens, R. Tijssen, J. Blomberg, J. Chromatogr. A 822 (1998) 233.
[12] M. Harju, C. Danielsson, P. Haglund, J. Chromatogr. A 1019 (2003) 111.
[13] P. Korytár, L.L.P. van Stee, P.E.G. Leonards, J. de Boer, U.A.Th. Brinkman, J. Chromatogr. A 994 (2003) 179.
[14] P. Korytár, P.E.G. Leonards, J. de Boer, U.A.Th. Brinkman, J. Chromatogr. A 958 (2002) 203.
[15] J. Diaz-Ferrero, Barcelona, Spain, personal communication.
[16] Technical Note KT030606-1, Zoex (http://www.zoex.com).
[17] K. Olie, P.C. Slot, H. Wever, Chemosphere 19 (1989) 103.
[18] P. Haglund, M. Harju, C. Danielsson, P.J. Marriott, J. Chromatogr. A 962 (2002) 127.