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Characterization of polychlorinated *n*-alkanes using comprehensive two-dimensional gas chromatography–electron-capture negative ionisation time-of-flight mass spectrometry

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Abstract

Comprehensive two-dimensional gas chromatography with electron-capture negative ionization time-of-flight mass spectrometry (GC × GC–ECNI-TOF-MS) is used to study the composition and characteristics of short-, medium- and long-chain polychlorinated *n*-alkane (PCA) mixtures. Distinct ordered structures, which enable the highlighting and interpretation of group and sub-group separations are observed when using a DB-1 × 007-65HT column combination. The analysis of a number of, mutually rather different, technical mixtures and 35 individual standard compounds provides information on the role of chlorine substitution (number of substituents as well as their position), the contribution of carbon versus chlorine atoms to analyte volatility, i.e. GC × GC behaviour, and the influence of the chain length of the carbon skeleton. Two dust samples are analyzed to illustrate the practical usefulness of the proposed procedure.

Keywords: GC × GC; Comprehensive two-dimensional gas chromatography; Polychlorinated alkanes; Chlorinated paraffins; ECNI-TOF-MS

1. Introduction

Polychlorinated *n*-alkanes (PCAs) are complex mixtures with a chlorination degree between 30 and 70%, and carbon chain lengths of C_{10}–C_{13} (short-chain PCAs), C_{14}–C_{17} (medium-chain PCAs) or >C_{17} (long-chain PCAs). They are produced by chlorination of an *n*-alkane feedstock using molecular chlorine at temperatures between 50 and 150 °C, at elevated pressures and/or in the presence of UV light [1]. PCAs are used as extreme-pressure additives in industrial cutting fluids, plasticizers and flame retardants for polyvinyl chloride (PVC) and other plastics (polyester, polyolefins, polystyrene) and rubbers (neoprene), and as additives in paints and sealants [1]. Since their introduction in 1932, the global consumption has increased to 300 000 t/year throughout the 1990s [1]. The presence of PCAs has been reported in both abiotic matrices such as water [2,3], sediments [4] and air [2,5], and biota such as fish [6,7], terrestrial mammals [6], and human milk [2]. The concentration range reported in, e.g. river water is 0.02–4 μg/l [2,3], and in fish liver 80–600 ng/g [7].

The analysis and monitoring of PCAs is of particular interest since they are classified as priority toxic substances under Canada's Environmental Protection Act and they are included on the list of priority hazardous substances [8,9] by the European Union and are also on the US Environmental Protection Agency (EPA) Risk Reduction List. Therefore, sensitive and selective analytical methods are required to enable exposure assessment.

The analysis of PCAs is a difficult task and today only semi-quantitative determination can be performed because of the complexity of the mixtures and the lack of quantification standards. PCAs are analysed by gas chromatography with electron-capture detection (GC–ECD) or coupled to low- or high-resolution mass spectrometry (GC–MS). Even if long narrow-bore capillary columns are used, GC fails by far to separate all individual congeners and isomers, and the

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chromatograms have a characteristic unresolved and broad profile which clearly indicates the presence of a very large number of co-eluting and/or partly overlapping peaks. In GC–ECD, the only way of reporting results is as a total [(short + medium + long)-chain] PCA concentration and, frequently, many interfering halogenated compounds present in the samples are included in that sum total. If MS detection in the electron ionisation (EI) mode is used, again it is possible only to report 'total PCA', with the additional drawback of lower sensitivity than with ECD detection. This is because EI leads to a strong fragmentation of the PCA compounds, with mass spectra giving no compound-specific information. The use of EI-MS/MS detection increases the selectivity and avoid matrix interferences on the determination of PCAs, allowing to improve the detectability to an acceptable level of, for example, ca. 1 ng of the C_{10}–C_{13} PCAs (55% Cl content) [7]. Nevertheless, a congener and homologue specific analysis is not possible. GC–MS with electron-capture negative ionization (ECNI) mode is the mostly applied method for the determination of PCAs. GC–ECNI-MS procedures [6,10,11] that monitor the not particularly characteristic *m/z* 70–73 ions, i.e. $Cl_2^{\bullet -}$ and HCl_2^-, also only enable reporting results as a total PCA concentration, with the additional problem that many other chlorine-containing hydrocarbons present in a sample or sample extract may fragment to yield the same ions. Other methods [3,12,13] are based on the monitoring of the $[M–Cl]^-$ or $[M + Cl]^-$ ions. Concentrations of individual classes – i.e. congeners with the same number of carbon and chlorine atoms – can now be determined. However, the results are usually too high due to 'mass leakage' or 'cross-over' problems among the PCA congeners. For example, mass *m/z* 327 can be due to $[C_{11}H_{18}{}^{37}Cl^{35}Cl_4]^-$ formed by loss of chlorine from a $C_{11}H_{18}Cl_6$ congener or due to $[C_{11}H_{20}{}^{35}Cl_5]^-$, the chlorine adduct of a $C_{11}H_{20}Cl_4$ congener. In addition, interferences from, e.g. toxaphene- and chlordane-related compounds, which all have molecular masses similar to short- and medium-chain PCAs and are difficult to separate during clean-up, further contribute to too high results. Today, such problems can be avoided by quantification based on monitoring characteristic negative ions produced by high-resolution ECNI-MS (resolution, ca. 12 000) [14]. Unfortunately, this approach is quite expensive, because of the high instrument cost. Moreover, repetitive injections are required because a large number of ions has to be monitored.

In this study, comprehensive two-dimensional gas chromatography (GC × GC), well known for its overall improved separation, was evaluated for the separation of PCA mixtures. A newly introduced ToF MS spectrometer operating in the ECNI mode was used to unravel the ordered structures observed in the GC × GC chromatograms.

2. Experimental

2.1. Samples and chemicals

PCA mixtures of C_{10}–C_{13} carbon chain length (chlorine contents, 51.5, 55.5 and 63%, w/w), C_{14}–C_{17} (chlorine contents, 42 and 57%, w/w), C_{18}–C_{20} (chlorine content, 36%, w/w), all with concentrations of 100 ng/μl in cyclohexane, and C_{10} mixtures (chlorine contents, 44.82, 55.00 and 65.02%, w/w), C_{11} mixture (chlorine content, 55.20%, w/w), C_{12} mixture (chlorine content, 55.00%, w/w) and C_{13} mixture (chlorine content, 55.03%, w/w), all with concentrations of 10 ng/μl in cychlohexane, were purchased from Dr. Ehrenstorfer (Augsburg, Germany). PCA-60 technical mixture was obtained from Dover Chemical (Dover, OH, USA). A solution with a concentration of 100 ng/μl was prepared in isooctane of nanograde quality (Promochem, Wesel, Germany). A standard mixture containing 35 PCA congeners was

Table 1
List of individual PCA congeners used

Name	Code	Name	Code
1,2-Dichlorooctane[Ch]	C_8Cl_2	1,1,1,3-Tetrachloroundecane[Ch]	$C_{11}Cl_4$
1,2,7,8-Tetrachlorooctane[Ch]	C_8Cl_4	1,1,1,3,10,11-Hexachloroundecane[Ch]	$C_{11}Cl_6$
1,1,1,3-Tetrachlorooctane[Ch]	C_8Cl_4	1,1,1,3,9,11,11,11-Octachloroundecane[Ch]	$C_{11}Cl_8$
1,1,1,3,6,8,8,8-Octachlorooctane[Ch]	C_8Cl_8	1,2-Dichlorododecane[Ch]	$C_{12}Cl_2$
1,2-Dichlorononane[Ch]	C_9Cl_2	1,12-Dichlorododecane[Ch]	$C_{12}Cl_2$
1,2,8,9-Tetrachlorononane[Ch]	C_9Cl_4	1,1,1,3-Tetrachlorododecane[Ch]	$C_{12}Cl_4$
1,1,1,3-Tetrachlorononane[Ch]	C_9Cl_4	1,2,11,12-Tetrachlorododecane[Ch]	$C_{12}Cl_4$
1,1,1,3,8,9-Hexachlorononane[Ch]	C_9Cl_6	1,1,1,3,11,12-Hexachlorododecane[Ch]	$C_{12}Cl_6$
1,2-Dichlorodecane[Ch]	$C_{10}Cl_2$	1,1,1,3,10,12,12,12-Octachlorododecane[Ch]	$C_{12}Cl_8$
1,2,9,10-Tetrachlorodecane[Ch]	$C_{10}Cl_4$	1,2-Dichlorotridecane[Ch]	$C_{13}Cl_2$
1,1,1,3-Tetrachlorodecane[Ch]	$C_{10}Cl_4$	1,1,1,3-Tetrachlorotridecane[Ch]	$C_{13}Cl_4$
2,5,6,9-Tetrachlorodecane (3 isomers)[Eh]	$C_{10}Cl_4$	1,1,1,3,12,13-Hexachlorotridecane[Ch]	$C_{13}Cl_6$
1,2,5,6,9-Pentachlorodecane (2 isomers)[Eh]	$C_{10}Cl_5$	1,1,1,3,11,13,13,13-Octachlorotridecane[Ch]	$C_{13}Cl_8$
1,1,1,3,9,10-Hexachlorodecane [Ch]	$C_{10}Cl_6$	1,2-Dichlorotetradecane[Ch]	$C_{14}Cl_2$
1,2,5,6,9,10-Hexachlorodecane (3 isomers)[Eh]	$C_{10}Cl_6$	1,2,13,14-Tetrachlorotetradecane[Ch]	$C_{14}Cl_4$
1,1,1,3,8,10,10,10-Octachlorodecane[Ch]	$C_{10}Cl_8$	1,1,1,3-Tetrachlorotetradecane[Ch]	$C_{14}Cl_4$
1,2-Dichloroundecane[Ch]	$C_{11}Cl_2$	1,1,1,3,12,14,14,14-Octachlorotetradecane[Ch]	$C_{14}Cl_8$
1,2,10,11-Tetrachloroundecane[Ch]	$C_{11}Cl_4$		

Ch: Produced by Chiron (Trondheim, Norway); concentration, 1 μg/μl in isooctane. Eh: Produced by Dr. Ehrenstorfer; concentration, 10 ng/μl in cyclohexane.

prepared from the individual standard solutions of the PCA congeners listed in Table 1. The final congener concentration in this mixture was approx. 10 ng/μl. Each solution of standards and samples was spiked with CB 40 and BDE 127 as internal standards to a final concentration of 9 and 0.1 ng/μl, respectively, in order to check the retention-time stability.

Dust samples of household origin, collected in central part of Spain and in north-east part of Slovakia, were prepared according to a method validated for PCA and PBDE determination by GC–ECNI-MS. A brief summary is as follows. Two grams of dust were Soxhlet-extracted for 12 h with 160 ml of *n*-hexane–acetone (3:1, v/v) at 70 °C. After the addition of CB 112 and [$^{13}C_{12}$]BDE 209 as internal standards, the extract was concentrated on a rotary evaporator, and demineralized water (adjusted to pH 2) was added and the organic layer collected. The aqueous phase was extracted two more times with isooctane. The organic extracts were combined and concentrated in 2 ml of dichloromethane. The concentrate was cleaned by gel permeation chromatography over two Polymer Laboratories (Church Stretton, UK) gel columns (polystyrene–divinylbenzene; 300 mm × 25 mm; pore size, 10 μm) connected in series, using dichloromethane at 10 ml/min. The collected fraction (18–23 min) was concentrated under nitrogen, dissolved in isooctane and further purified by shaking with conc. sulphuric acid. Finally, the isooctane layer was concentrated under nitrogen to 2 ml (isooctane) and purified on a 2%-water deactivated silica column. Two fractions were obtained using 11 ml of isooctane and, next, 10 ml of diethyl ether–isooctane (15:85, v/v). The latter fraction, which contains the PBDEs and PCAs, was concentrated to 1 ml (isooctane).

2.2. GC × GC–μECD

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). The principles and operation of the KT2002 modulator are described in [15]. The hot air pulse duration was 200 ms, the hot jet temperature was 400 °C, and the modulation period 8 s. At the start of each run, the CO_2 flow was adjusted by means of 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 at a constant flow of 1.2 ml/min. A micro-ECD (Agilent) system was operated at 280 °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-microliter samples were injected manually into a split/splitless inlet port operated in the splitless mode at 280 °C with the split opening 2 min after injection. A 30 m × 0.25 mm × 0.25 μm DB-1 (100% methylpolysiloxane) fused-silica column purchased from J&W Scientific (Agilent) was used as first-dimension column. The six fused-silica columns used in the second dimension are listed in Table 2. The columns were coupled to each other via a 1.5 m × 0.1 mm I.D. uncoated fused-silica deactivated column (BGB Analytik, Aldiswil, Switzerland), which served as the modulator loop. Mini press-fits (Techrom, Purmerend, The Netherlands) were used for the connections. The temperature programme for both the first- and second-dimension columns was 90 °C (2 min), at 20 °C/min to 170 °C, then at 2 °C/min to 280 °C (5 min). HP Chemstation software (Agilent) was used to control the GC instruments and to acquire data. Raw data files were imported into HyperChrom software (ThermoElectron, Milan, Italy) used for GC × GC data processing, evaluation and visualization. Colour contour plots were produced by Transform software (Fortner Research, Sterling, VA, USA).

2.3. GC × GC–TOF-MS

The GC × GC–TOF-MS system was built from a Trace 2D (ThermoElectron) gas chromatograph coupled to a Tempus time-of-flight mass spectrometer (ThermoElectron, Austin, TX, USA). A 30 m × 0.25 mm × 0.25 μm DB-1 (100% methylpolysiloxane) fused-silica column purchased from J&W Scientific (Agilent) was used as first-dimension column. The 007-65HT column (for specifications, see Table 2) with dimensions of 1 m × 0.1 mm × 0.1 μm was used as second-dimension column. The front end of the second-dimension column was coupled directly to the first-dimension column and the back end to a 30 cm × 0.1 mm retention gap mounted in the GC–MS interface. Mini press-fits (Techrom) were used for the connections. Modulation was performed at the beginning of the second column with a modulation period of 6 s. Helium gas (Hoek Loos) with a purity of 99.999% was used as carrier gas at a constant flow of 1.2 ml/min. One-microliter samples were injected manually into a PTV inlet

Table 2
Second-dimension columns

Commercial code[a]	Stationary phase	Dimensions (m × mm × μm)
LC-50	50% Liquid crystalline-methylpolysiloxane	0.8 × 0.1 × 0.1
007-65HT	65% Phenyl-methylpolysiloxane	1.0 × 0.1 × 0.1
VF-23ms	Proprietary (high cyano containing polymer; with absolute cyano content 70–90%)	1.5 × 0.1 × 0.1
007–210	50% Trifluoropropyl-methylpolysiloxane	2.0 × 0.1 × 0.1
HT-8	8% Phenyl-methylpolysiloxane (carborane)	1.0 × 0.1 × 0.1
SupelcoWax-10	Polyethylene glycol	1.0 × 0.1 × 0.1

[a] LC-50 (J&K Environmental, Sydney, NS, CAN), 007-65 HT and 007-210 (Quadrex, New Haven, CT, USA), VF-23ms (Varian, Middelburg, The Netherlands), HT-8 (SGE International, Ringwood, Australia) and SupelcoWax-10 (Supelco, Bellefonte, PA, USA).

port operated in the constant-temperature splitless mode at 280 °C, with the split opening 2 min after injection. The mass spectrometer was tuned and calibrated in the electron ionisation (EI) and electron-capture negative ionisation (ECNI) mode using heptacosafluorotributylamine (Fluka Chemie, Buchs, Switzerland) as the reference gas according to the recommendations of the manufacturer. In the ECNI mode, methane (3.0 ml/min) was used as a moderate gas and the source temperature was 150 °C. In the EI mode, the source temperature was 200 °C. The mass range of 50–700 Da was acquired at a data acquisition rate of 40 Hz in both modes. The temperature of the GC–MS transfer line was 320 °C and the temperature programme for both columns was 65 °C (2 min), at 20 °C/min to 140 °C, then at 3 °C/min to 320 °C (5 min). Xcalibur software (ThermoElectron) was used to control the GC × GC–TOF-MS instrument and to acquire data. Raw data files were imported into HyperChrom software (ThermoElectron) used for GC × GC data processing, evaluation and visualization. Colour contour plots were produced by Transform software (Fortner Research).

3. Results and discussion

3.1. Column selection

In a parallel study, six column combinations were tested for the separation of 12 classes of organohalogenated compounds, with the mutual separation of these classes as the principal aim of the work [16]. One of these classes were the PCAs, represented by the technical mixture PCA-60, which consists of short-chain PCAs and is often used as quantification standard in PCA analysis. Extracting all relevant information on PCAs from that study and adding a 1D-GC chromatogram obtained under the same conditions, yields Fig. 1, which can serve as the foundation for our present research. The impressive gain in overall resolution created by using GC × GC instead of 1D-GC is observed for all column combinations tested. This is not unexpected: the PCA-60 mixture is so complex that even a very weak additional, i.e. second-dimension, selectivity will effect a noticeable improvement. In the present instance, this is exemplified by the 007-210 and HT-8 columns (Fig. 1B and C, respectively). At a first glance, it may seem that the DB-1 × VF-23ms set should be regarded as the best one: a very large part of the 2D plane is occupied, which underlines the orthogonality of the system (Fig. 1E). However, there actually is too much selectivity added here, which causes partial overlap of the various sub-structures (i.e. sub-groups) visible in the chromatogram. In other words, for a well-designed separation and characterization of the sub-groups present in PCA mixtures, the most promising column sets are comprised of DB-1 combined with SupelcoWax-10, LC-50 or 007-65HT (Fig. 1D, F and G, respectively). Further preliminary work revealed that the clearest group separations were achieved with DB-1 × 007-65HT, which was therefore used in all further work. An added advantage of this combination is that, in the paper on between-class separations quoted above [16], it was observed that this column set can also be recommended for the separation of PCAs, as a class, from many potentially interfering halogenated co-extractants.

3.2. GC × GC–TOF-MS of 35-congener mixture

In order to study the 2D retention characteristics of the individual PCAs, the 35-congener standard mixture was analysed by GC × GC–EI-TOF-MS. The EI ionisation mode was used because, due to its low response, the 1,12-$C_{12}Cl_2$ congener could not be detected in the ECNI mode even if a 1-mg/ml solution was injected. The GC × GC chromatogram shown as an apex plot in Fig. 2, reveals that compounds having the same chlorine substitution pattern but different carbon chain length are ordered as more or less parallel horizontal lines. This behaviour was observed for all five groups for which more than one congener is present in the mixture, i.e. 1,2-[C_8-C_{14}]Cl_2, 1,1,1,3-[C_8-C_{14}]Cl_4, 1,2,*x*-1,*x*-[C_8-C_{14}]Cl_4, 1,1,1,3,*y*-1,*y*-[C_9-C_{13}]Cl_6 and 1,1,1,3,*x*-2,*x*,*x*,*x*-[C_8-C_{14}]Cl_8, where $x = 8$–14 and $y = 9$–13. This is not an unexpected result because the compounds within each group have essentially the same polarity because their substitution pattern is the same and they only differ in their boiling points due to the different lengths of the carbon chain. On the other hand, there are obvious differences in polarity between the various groups. Compounds which have substituents on only one end of the carbon chain, i.e. 1,2-[C_8-C_{14}]Cl_2 and 1,1,1,3-[C_8-C_{14}]Cl_4, are less polar and, thus, have shorter second-dimension retention times than the compounds with chlorine substituents distributed over the entire length of the molecule. Further, Fig. 2 shows that 2,5,6,9-$C_{10}Cl_4$, 1,2,5,6,9-$C_{10}Cl_5$, 1,1,1,3,6,8,8,8-C_8Cl_8 and 1,2,5,6,9,10-$C_{10}Cl_6$ display two or three closely eluting peaks in the apex plot. This is due to the fact that PCAs can exist as a number of diastereoisomers. In the present GC × GC run, they are partly separated, sometimes in the first, and sometimes in the second dimension. For three of the congeners (cf. Table 1) it was stated by the manufacturer that they are present as a mixture of two or three diastereoisomers. For 1,1,1,3,6,8,8,8-C_8Cl_8, available from another manufacturer, no such information was supplied. Generally speaking, it will be clear that the presence of diastereoisomers will add to the complexity of chromatograms of PCAs as depicted in, e.g. Fig. 1.

It will be clear that knowledge about the retention characteristics of the 35 individual PCAs does not suffice to interpret the ordered structure observed for PCA-60. Further work should involve PCA mixtures of constant chain length.

3.3. GC × GC–ECNI-TOF-MS of polychlorinated decanes

As a first test, the separation of polychlorinated decanes with a chlorine content of 55% (w/w) was studied. Fig. 3A shows a GC × GC–ECNI-TOF-MS extracted-ion

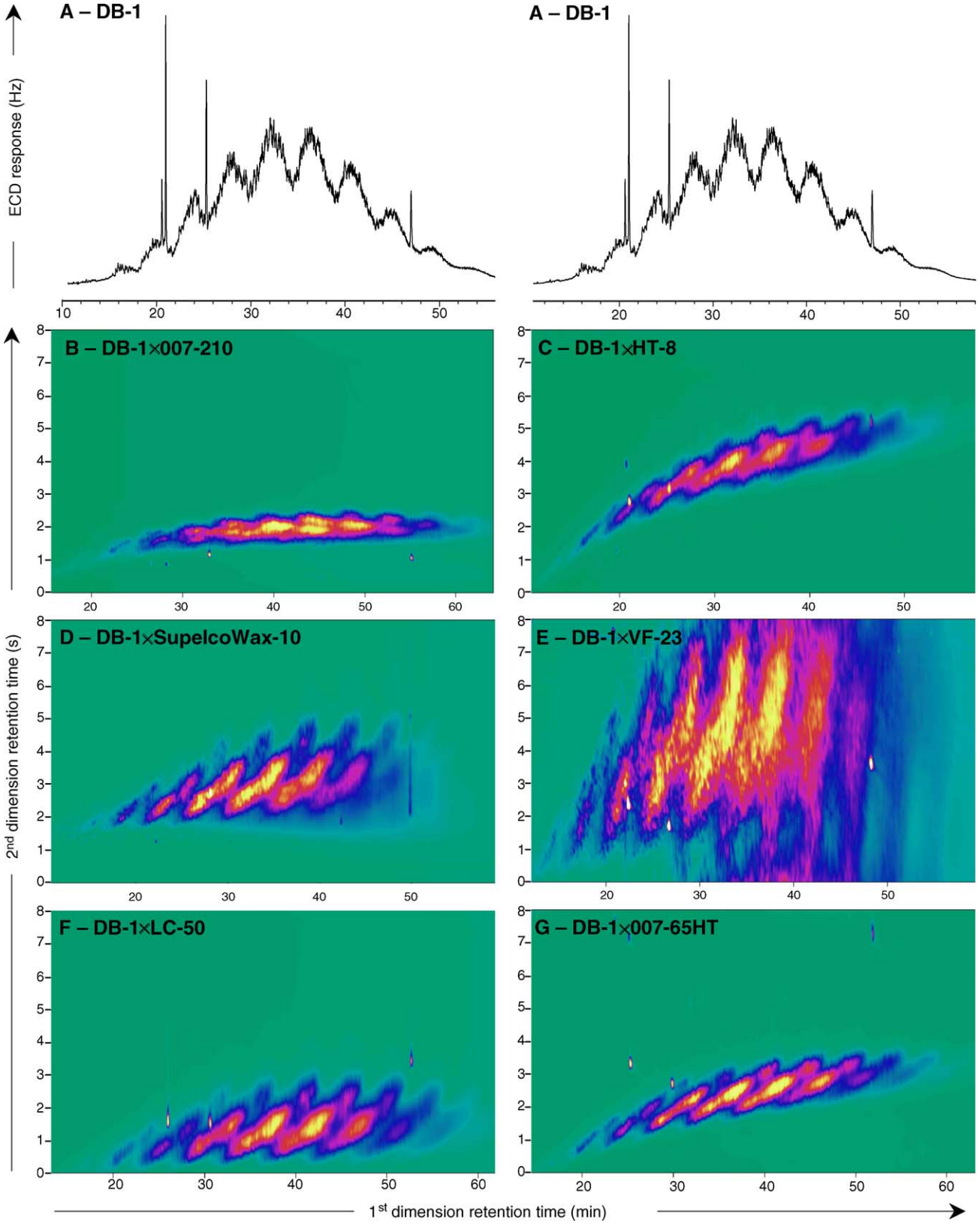

Fig. 1. (A) GC–ECD and (B–G) GC × GC–μECD chromatograms of PCA-60 on (A) DB-1 and (B–G) DB-1 in the first dimension and each of the six columns indicated in the second dimension. The three or four discrete peaks/spots visible in some of the frames are due to the added internal standards. For conditions, see Section 2.2.

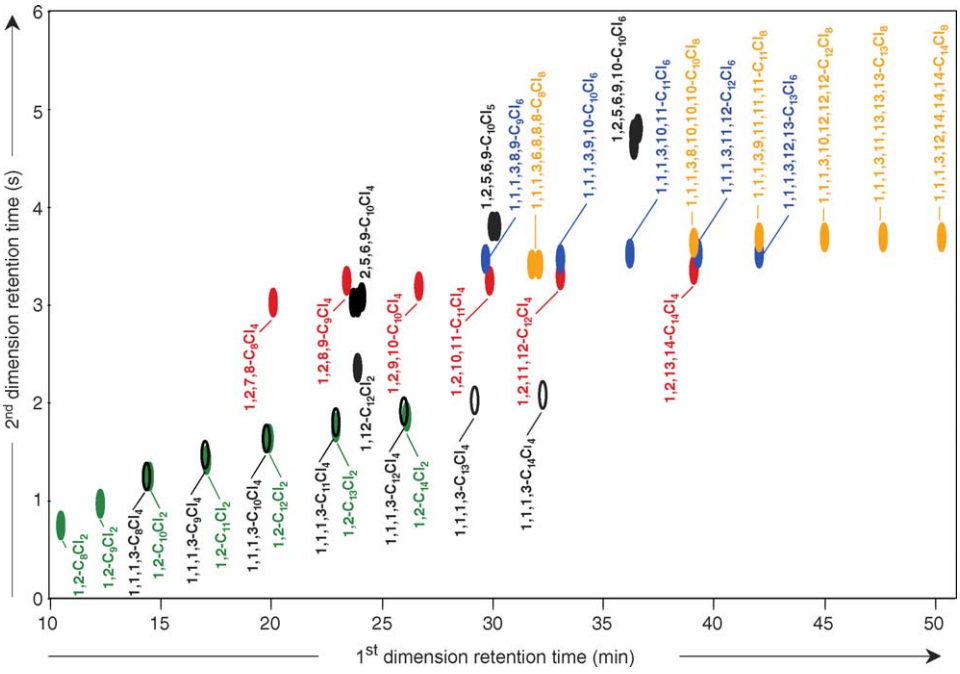

Fig. 2. GC × GC–EI-TOF-MS total-ion apex plot of 35 PCA congeners on DB-1 × 007-65HT column combination. For conditions, see Section 2.3.

chromatogram for *m/z* 70–73 which correspond to the unspecific $[Cl_2]^{\bullet-}$ and $[HCl_2]^-$ fragment ions. There is a distinct ordered structure. As with other classes of organohalogen compounds (see, e.g. [16]), such a separation can be expected to be based on the number of chlorine substituents. This was confirmed by visualizing the extracted-ion chromatograms for the mass ranges, *m/z* 209–211, 243–245, 276–283, 311–319, 345–355, 379–389, 413–423, 447–459, 481–493, 515–527 and 549–559, which correspond to the $[M–Cl]^-$ and $[M–HCl]^{\bullet-}$ clusters of polychlorinated decanes with 3–13 chlorine substituents. In most extracted-ion chromatograms, only one group of compounds was visible, which confirmed the suggested type of separation. However, for three mass ranges, i.e. those corresponding to penta- (*m/z* 276–283), hexa- (*m/z* 311–319) and hepta- (*m/z* 345–355) chlorinated decanes, more than one group showed up. As an example, Fig. 3B shows the results for the hexa-substituted compounds. Next to the dominant $C_{10}H_{16}Cl_6$ group, several minor groups and/or peaks are visible both below and above the main cluster. Close study of the mass spectra of the 'outliers' revealed that the peaks on the left-hand side can be attributed to pentachlorinated congeners with the $[M]^{\bullet-}$ ion in their mass spectra, while the peaks on the right-hand side are due to heptachlorinated congeners with the $[M–2HCl]^{\bullet-}$ ion in their spectra. Typical mass spectra are shown as inserts to Fig. 3B.

Fig. 3A also shows that each homologue band apparently comprises two or three sub-groups. Three-dimensional visualization, as shown for the hexachlorinated congeners in another insert of Fig. 3B (viewed from the bottom right-hand corner of the GC × GC chromatogram) displays a pattern which is remarkably similar to the profile of the same homologue group obtained by GC–HRMS of the PCA-60 technical mixture [14]. In order to study the position of individual congeners in the various bands of homologues, the 55% (w/w) polychlorinated decane mixture was spiked with the eight individual polychlorinated decanes available to us (cf. Table 1). In Fig. 3A, their positions are marked by black open circles. Three congeners, 2,5,6,9-$C_{10}Cl_4$, 1,2,5,6,9-$C_{10}Cl_5$ and 1,2,5,6,9,10-$C_{10}Cl_6$, show up well within the observed bands; this suggests that they are present in the technical mixture. Three other congeners, 1,1,1,3-$C_{10}Cl_4$, 1,1,1,3,9,10-$C_{10}Cl_6$ and 1,1,1,3,8,10,10,10-$C_{10}Cl_8$, are either absent from, or present at the trace level in, the technical mixture. This is not too surprising, since having three chlorine substituents bonded to the same carbon atom is a rather improbable outcome of an uncontrolled synthesis [1,17–19]. The positions of these three congeners in the GC × GC chromatogram are, however, rather unexpected, since they seem to be part of the band with one more chlorine atom. Obviously, the unusual 1,1,1-Cl_3 substitution pattern causes the simple 'number of chlorine substituents rule' to be violated. A somewhat similar observation can be made for 1,2,9,10-$C_{10}Cl_4$ which elutes contiguous to the penta-substituted rather than in the tetra-substituted band. Its presence in the mixture is, anyway, questionable, because low-chlorinated *n*-alkanes are more likely to have 1,3,5-type substitution due to steric effects [1]. Finally, the position of 1,2-$C_{10}Cl_2$ cannot be discussed with any confidence because no band of dichlorinated congeners was observed.

In summary, the above observations indicate that, if all – or most – of the theoretically possible congeners would be present in the mixture to be analysed, the iso-substitution bands would be rather broad and significant overlap with neighbouring bands would be observed. However, the present

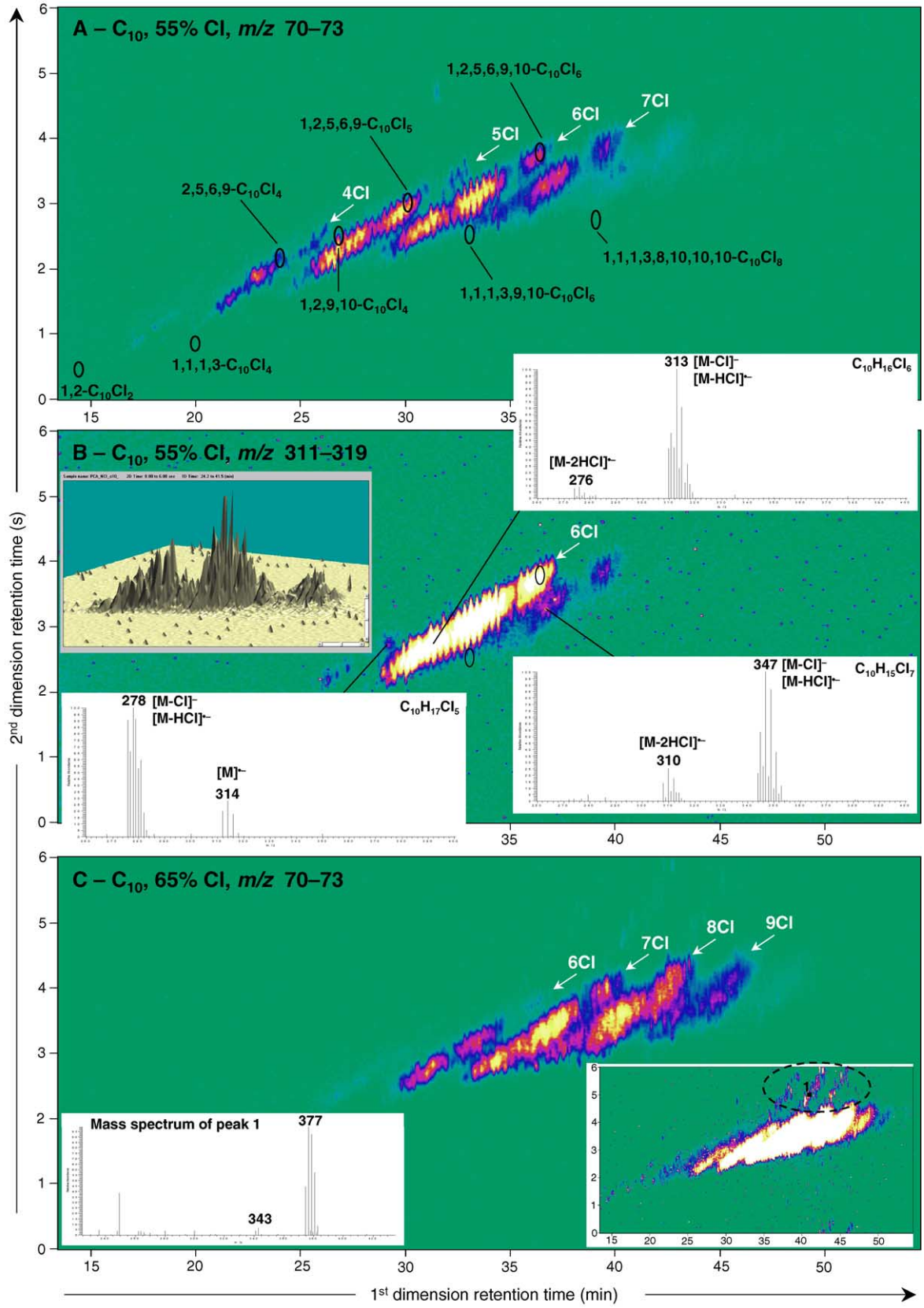

Fig. 3. GC × GC–ECNI-TOF-MS extracted-ion chromatograms of polychlorinated decanes, (A) 55% (w/w) *m/z* 70–73, (B) 55% (w/w) *m/z* 311–319, (C) 65% (w/w) *m/z* 70–73. Inserts of Fig. 3B show its 3D presentation and averaged mass spectra of selected peaks. Inserts of Fig. 3C show its zoom-out visualization and mass spectrum of selected peak. For conditions, see Section 2.3.

ECNI-TOF-MS-based evaluation shows that for the technical mixture produced by uncontrolled synthesis, this is no serious problem. For the much more limited number of (principal) compounds then formed, GC × GC separation based on the number of chlorine substituents works well—that is, band overlap is limited.

In order to study the role of the chlorine content, next to the polychlorinated decanes with 55% (w/w) of chlorine, the mixtures containing 45 and 65% (w/w) were analysed. Ordered structures were again obtained, with that for the 45% (w/w) mixture being closely similar to the chromatogram of Fig. 3A. This may well reflect the fact that with ECNI-ToF MS detection, the mono-, di- and trichlorinated congeners have very low responses and their increased presence in the 45% (w/w) mixture will, consequently, not show up in the chromatogram. On the other hand, the profile found for the 65% (w/w) mixture, which is shown in Fig. 3C, is seen to be considerably shifted to higher retention times (in both dimensions): hexa- through nano- rather than tetra- through hepta-substituted congeners now are dominant. The fact that the bands of the low-chlorinated congeners are quite narrow, but become broader when the number of substituents

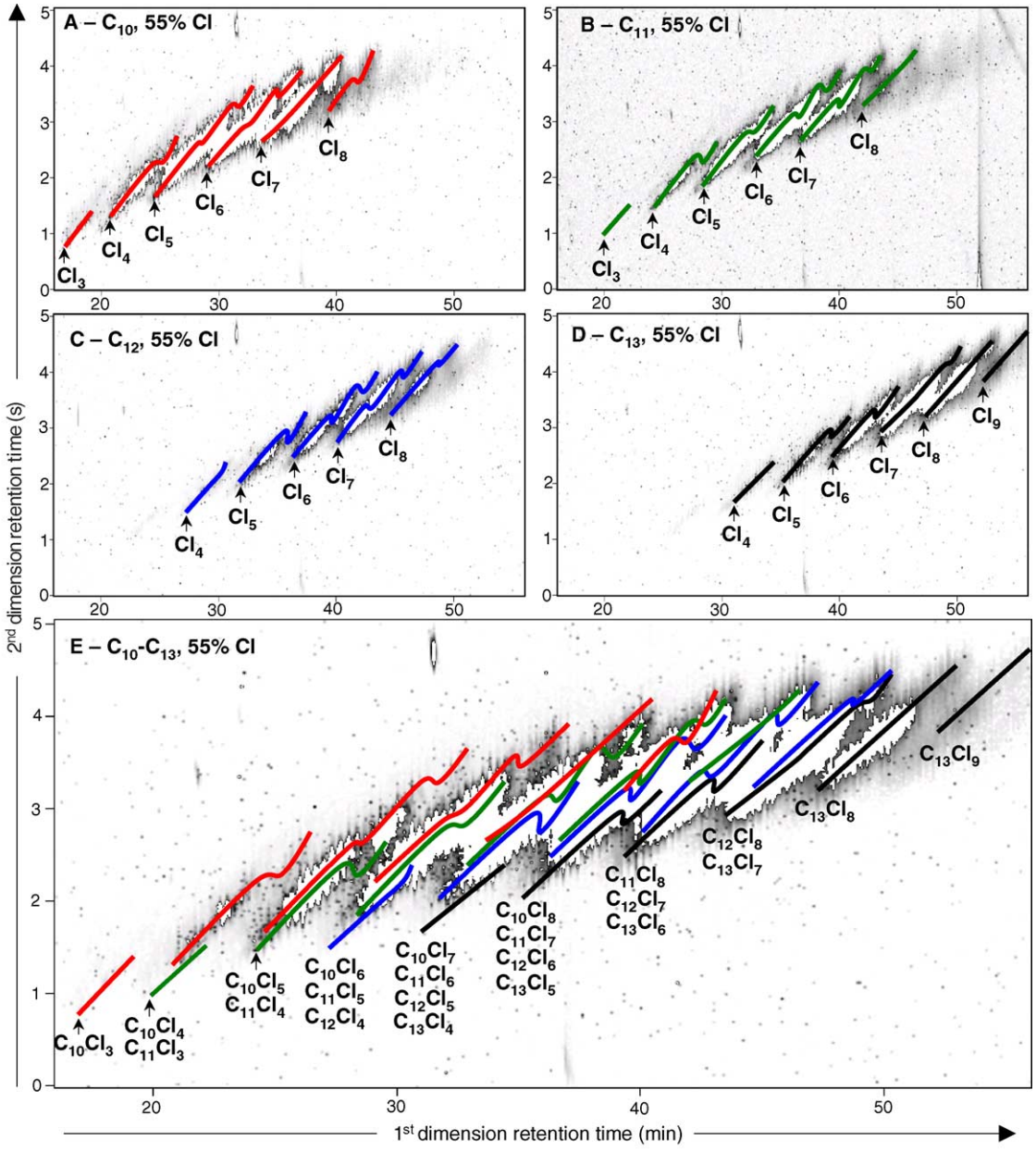

Fig. 4. GC × GC–ECNI-TOF-MS chromatograms of polychlorinated (A) decanes, (B) undecanes, (C) dodecanes, (D) tridecanes and (E) C_{10}–C_{13} technical mixture, all with 55% (w/w) Cl content, obtained on DB-1 × 007-65HT column combination. Lines indicate the positions of apices within the bands. For conditions, see Section 2.3.

increases, probably reflects the higher number of congeners and/or diastereoisomers that is formed.

One final observation regarding the 65% (w/w) polychlorinated decane mixture is that, upon zooming out, next to the ordered structures of Fig. 3C, another cluster of compounds comprised of three sub-groups was observed. They display higher second-dimension retention times and are indicated by a circle in the insert of Fig. 3C. The mass spectrum of one of the peaks from the central sub-group is shown as an insert. Isotope ratios in the *m/z* 377 cluster indicate the presence of seven chlorine substituents, but one should keep in mind that this cluster probably is due to the $[M–Cl]^-$ ion. The three sub-groups mutually differ by one chlorine atom, but we have so far not been able to identify them.

3.4. GC × GC–ECNI-TOF-MS of polychlorinated C_{10}–C_{13} n-alkanes

Technical mixtures of short-chain PCAs are composed of polychlorinated decanes, undecanes, dodecanes and tridecanes. In Fig. 4, the GC × GC chromatograms of each of the four single-chain mixtures are compared with each other (Fig. 4A–D) and with a short-chain (C_{10}–C_{13}) technical mixture (Fig. 4E). The chlorine content of all these mixtures was 55% (w/w). There is little need to emphasize again the general ordering on the basis of the number of chlorine substituents per molecule. Instead, the main observation should be that, with increasing length of the carbon skeleton, boiling points become higher and first-dimension retention times, consequently, increase. For the reader's convenience, in Fig. 4A–D the apices within each band have been connected by coloured lines. Some of these are immediately seen not to be straight, which indicates further sub-structuring as a consequence of different substitution pattern within the homologue group. Overlay of the chromatograms of the individual single-chain mixtures with the short-chain (C_{10}–C_{13}) technical mixture showed that the contribution to volatility of one carbon is approximately equal to the contribution of one chlorine. Consequently, compounds having the same number of (carbon + chlorine) atoms show up on the same diagonal line—for example, $C_{10}Cl_8$ is on the same line as are $C_{11}Cl_7$, $C_{12}Cl_6$ and $C_{13}Cl_5$. Detailed information on all bands of short-chain PCAs can be read from Fig. 4E. The position of the various compounds on each diagonal depends on the number of carbon atoms: the compounds with longer carbon chains have lower second-dimension retention times. This carbon-chain-length selectivity helps to create a distinct separation of compounds which differ by at least three carbons; that is C_{10} and C_{13} compounds show no overlap in Fig. 4E.

As mentioned in Section 1, the determination of individual homologue classes – i.e. congeners with the same number of carbon and of chlorine atoms – by GC–ECNI-LRMS often causes too high results due to 'mass-leakage' or 'cross-over' problems among the PCA congeners. The present GC × GC separation solves all such problems except for one, which is due to interferences among compounds with the same number of (carbon + chlorine) atoms. To quote an example, *m/z* 361, which is due to $[M–Cl]^-$ fragment ion of $C_{11}H_{17}Cl_7$, will interfere with the $[M]^{\bullet -}$ cluster of $C_{13}H_{23}Cl_5$. Probably, this problem can be solved by using dichloromethane as reagent gas because, as shown by Zencak et al. [7], $[M + Cl]^-$ ions are then predominantly formed for all congeners. This approach would also have a positive effect on the determination of low-chlorinated congeners, because the number of chlorine substituents has only a limited effect on analyte detectability in this mode of NCI-MS.

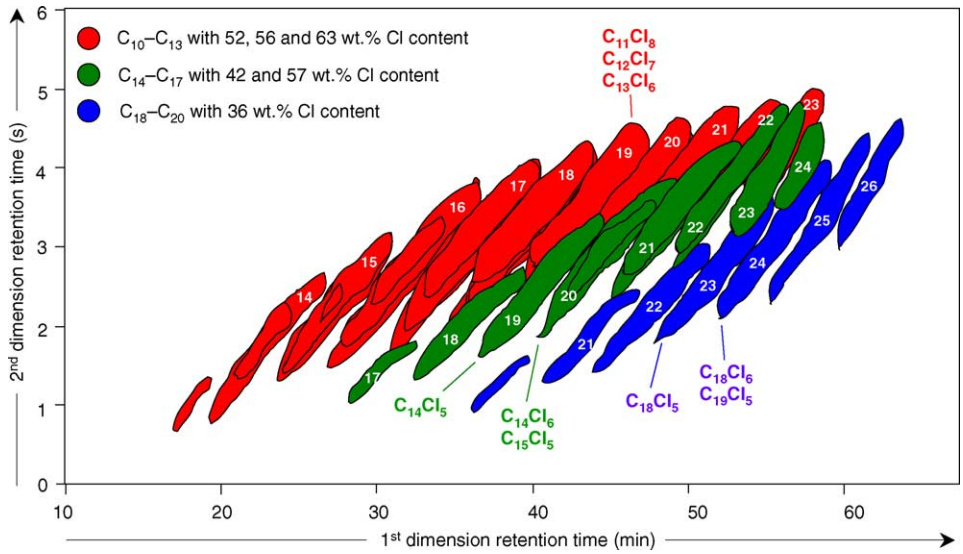

Fig. 5. Overlay of GC × GC–ECNI-TOF-MS chromatograms of (red) short- (C_{10}–C_{13}), (green) medium- (C_{14}–C_{17}) and (blue) long- (C_{18}–C_{20}) chain PCA mixtures with different chlorine content, obtained on DB-1 × 007-65HT column combination. Different chlorine contents are indicated by additional contours. White numbers indicate number of (carbon + chlorine) atoms of the compounds present in the bands. For conditions, see Section 2.3.

3.5. GC × GC–ECNI-TOF-MS of short-, medium- and long-chain PCA mixtures

Next to the short-chain PCAs, which have been discussed in some detail above, there are also medium-chain (C_{14}–C_{17}) and long-chain (>C_{17}) PCAs. The latter two classes are less well-known and, to the best of our knowledge, no individual mixtures representing a single carbon chain length are available. Our study, therefore, necessarily had to be brief and was restricted to a comparison of the GC × GC behaviour of short-chain PCAs of 52, 56 and 63% (w/w) chlorine, medium-chain PCAs of 42 and 52% (w/w) chlorine, and long-chain PCAs of 36% (w/w) chlorine. Overlaid chromatograms of all of these are shown in Fig. 5. As with short-chain PCAs, separation of congeners into bands with the same number of (carbon + chlorine) atoms – shown in white in the fig-

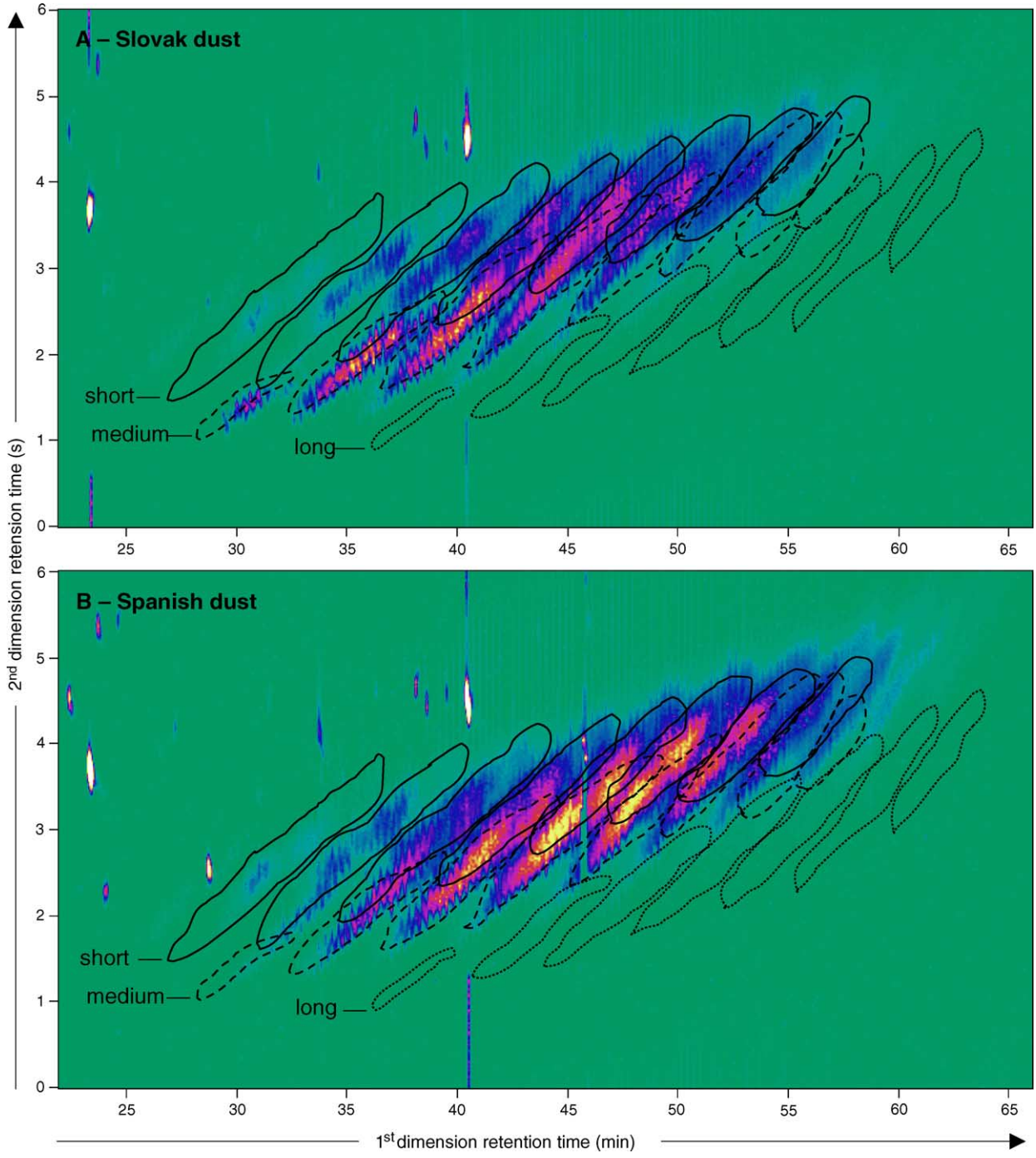

Fig. 6. GC × GC–ECNI-TOF-MS extracted-ion chromatograms (*m/z* 70–73) of dust extracts collected in (A) a Slovak and (B) a Spanish household, with the positions of short-, medium- and long-chain PCAs indicated. For conditions, see Section 2.3.

ure – was confirmed by studying mass spectra also for the medium- and long-chain PCAs. One interesting observation is that the longer-carbon-chain mixtures have lower second-dimension retention times. As a consequence, there is a rather clear separation of the three classes of compounds. As the figure shows, the mutual separation is especially rewarding for the lower chlorinated (groups of) congeners eluting at a first-dimension retention time of ca. 40 min or less. Another observation is that the (carbon + chlorine)-based ordering is seen to hold through a summed number of 26, i.e. over the entire range. As for the composition of the bands, some examples are given in Fig. 5. Generally speaking, in earlier eluting bands, a single group of compounds seems to predominate while two (or even three) groups show up in later eluting bands.

An obvious conclusion is that, for real-life samples containing PCAs from more than one class, a comprehensive GC separation has to be applied in order to elicit at least some information concerning the classes, and their sub-classes, present in the mixture.

3.6. Application

Two extracts of dust, collected in a Slovak and a Spanish household, were analysed to demonstrate the practicability of the present approach. GC × GC–ECNI-TOF-MS extracted-ion chromatograms (*m*/*z* 70–73) overlaid with the chromatograms of the short-, medium- and long-chain PCAs discussed in the previous section, are shown in Fig. 6. One clear observation is that the two samples do not contain any long-chain PCAs. For the rest, the GC × GC patterns of the short- and medium-chain PCAs present in the two samples are significantly different. In the dust sample from Slovakia (Fig. 6A), the most intense bands are at lower first-dimension, and also second-dimension, retention times than in the dust of Spanish origin (Fig. 6B). In addition, the bands in the Slovak sample are narrower and there is, also, a better separation within the bands in the second dimension. On the basis of the earlier discussions on PCA retention behaviour, one can conclude that the Slovak sample contains more medium- than short-chain PCAs and that these medium-chain PCAs have a relatively low chlorination degree. The Spanish dust sample, on the other hand, contains more short- than medium-chain PCAs and the short-chain PCAs have a higher degree of chlorination.

4. Conclusions

The combined information of the present paper demonstrates that GC × GC with ECNI-TOF-MS detection is a powerful tool for the study of the notoriously complex PCA mixtures. From among the six column combination tested, DB-1 × 007-65HT was found to be the best choice because it provides most information in terms of ordered structures, i.e. of group and sub-group separation.

Not unexpectedly, the separation of PCA congeners with the same chain length is based on the number of chlorine substituents. This creates sufficient resolution for the major constituents of the available PCA technical mixtures. If, however, a (much) larger number of the theoretically possible congeners would be present, the iso-substitution bands would become rather broad and there would be significant overlap of neighbouring bands. Since the number of individual standard PCAs is, at present, limited, some indication of the influence of the Cl-substitution pattern of the PCAs on their GC × GC behaviour can be given but, as yet, not a full explanation.

When mixtures of PCAs of varying chain length are analysed, ordered structures are observed which comprise compounds having the same number of carbon-plus-chlorine atoms. In other words, the contribution to volatility of a carbon atom is, to a first approximation, similar to that of a chlorine atom. With the present DB-1 × 007-65HT column combination, second-dimension selectivity effects a distinct separation of PCAs which differ by at least three carbon atoms in their chain length. This enables to partly distinguish short-, medium- and long-chain PCAs—an aspect of interest for the characterization of real-life samples, as is demonstrated for two, mutually rather different, household dusts.

In summary, the information on GC × GC analysis of PCA mixtures as presented in this paper is still limited and resolution, although distinctly superior to that of 1D-GC, is far from complete. However, one can safely conclude that this technique, preferably combined with ECNI-TOF-MS detection, will rapidly become indispensable for all profiling, pattern-recognition and – in a next step – quantification studies of PCA-containing environmental samples.

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