Comprehensive two-dimensional gas chromatography—a powerful and versatile technique

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Comprehensive two-dimensional gas chromatography (GC × GC) was introduced about a decade ago and is, today, rapidly becoming a powerful and widely applicable technique for the characterisation and analysis of a variety of complex samples. GC × GC provides structurally ordered chromatograms in the 2D plane, and features an impressive peak capacity. Combining GC × GC and time-of-flight mass spectrometry (ToFMS), with its fast acquisition rates and excellent deconvolution potential, has been an important step forward which enables identification and identity confirmation of large numbers of target analytes and unknowns in one run. Up until now, some 150 papers have been published in this area. Many of these deal with applications in fields such as petrochemical, fish and food, environmental and air analysis. Polyhalogenated aromatics and other priority pollutants, flavours and fragrances, fatty acid methyl esters and essential oils, are among the classes of compounds which frequently receive attention. In summary, GC × GC is increasingly being recognised as the separation technique of choice in problem areas.

In the past few years, a novel separation technique—comprehensive two-dimensional gas chromatography or GC × GC—has attracted considerable attention in organic trace-level analysis. It is well known that, despite its high efficiency, conventional (*i.e.*, 1D) capillary GC cannot resolve all analytes of interest in, for example, mixtures of polychlorinated biphenyls, toxaphene or fatty acid methyl esters, typically encountered in real-life samples, or in cigarette smoke, air or petrochemical products. Earlier, this was one of the reasons for the development of multi-dimensional GC (GC–GC), a heart-cut technique with which one or a limited number of fractions of the first-column eluate is transferred to a second column with different characteristics, to obtain an improved separation. The main drawback is that the individual fractions have to be small in order not to lose the separation already obtained. This means that the technique is not useful for samples in which the analytes of interest are scattered throughout the first-dimension chromatogram or in which attention has to be devoted to unknowns more than to target analytes; it then becomes much too time consuming.

The interesting aspect of a comprehensive, as opposed to a heart-cut technique, here GC × GC *versus* GC–GC, is that now (i) the entire sample is subjected to two independent separations, and (ii) the 2D separation is completed in the run time of the first separation. That is, considerably more information on the sample constituents is provided, while the time of analysis has been seriously reduced. In addition, as will be discussed below, highly structured 2D chromatograms are often obtained which facilitate fingerprinting and provisional identification of unknowns. Since detailed discussions of the principles of GC × GC and instrument design have been published several times,[1–3] treatment of these topics in thevpresent review will be relatively brief. Most attention will be devoted to discussing a selected number of examples.

Principles and instrumentation

In most GC × GC studies, two independent types of separation are applied to a sample. The schematic of such a system is shown in Fig. 1A. The first-dimension separation, typically, is on a normal-bore, 10–30 m, non-polar column in the programmed-temperature mode. The interface between the two columns, called a modulator, isolates the first-column eluate into a very large number of adjacent small fractions (preferably *ca.* 0.25 of peak width to maintain resolution). The fractions are refocused into narrow pulses of about 0.01 s width, and launched into the second-dimension column. Most recent modulators are of the cryogenic-jet type, and use liquid carbon dioxide, or nitrogen for cooling: they are robust and user-friendly.[1,4,5] The operation of a dual-jet system is explained in Fig. 1B.

The second-dimension separation is generally of a (semi-)polar or shape-selective nature. The separation on this narrow-bore and short (0.5–1 m) column is extremely fast and takes only some 2–6 s; that is, it is performed under essentially isothermal conditions. The fast separation results in very narrow peaks with baseline widths of, typically, 0.1–0.6 s. Such peaks require detectors with a small internal volume, a short rise

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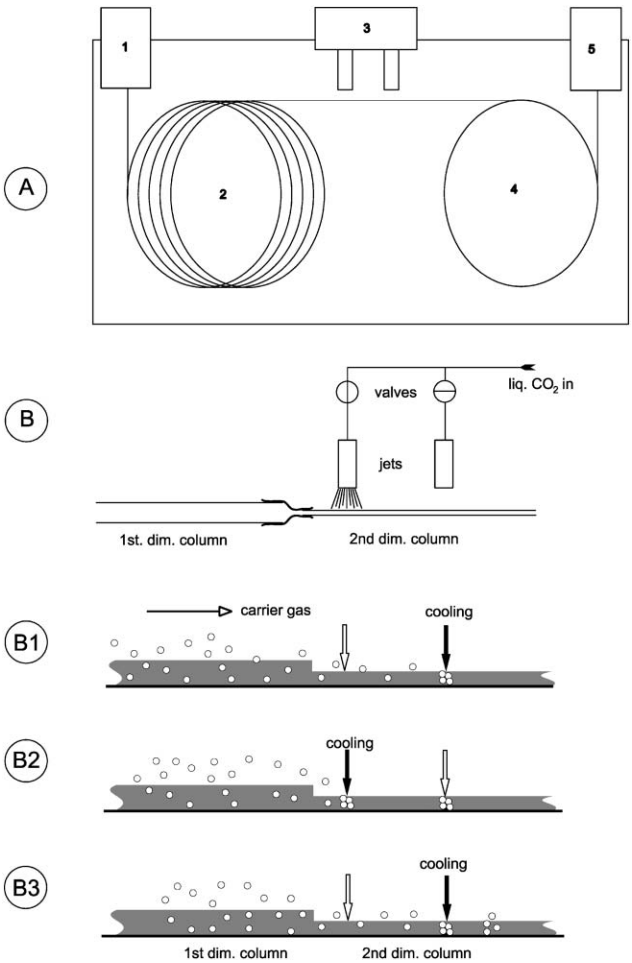

Fig. 1 (A) Schematic of GC × GC system: 1, injector; 2, first column; 3, modulator; 4, second column; 5, detector. (B) Schematic of dual-jet cryogenic modulator. (B1) Right-hand-side jet traps analytes eluting from first column; (B2) right-hand-side jet switched off, cold spot heats up rapidly and analyte pulse is released into second column; simultaneously, left-hand-side jet switched on to prevent leakage of first-column material; (B3) next modulation cycle is started.

time and a high data acquisition rate to ensure proper reconstruction of the second-dimension chromatograms (usually visualised as 2D colour or contour plots and, occasionally, as 3D plots). Initially, flame ionisation detectors (FID), with their small volumes and acquisition frequencies of 50–300 Hz, were the only detectors meeting these demands. Next, a micro electron-capture detector with an acquisition frequency of 50 Hz was added to the list and found to perform satisfactorily, despite some additional band broadening caused by its 150-μL volume.

The detectors mentioned above permit peak recognition, but provide no structural information. To achieve the latter goal, a mass spectrometer is indispensable. Up till now, only a ToF MS – which can be operated at high repetition rates of 5–30 kHz – can acquire the fifty or more mass spectra per second which are required for quantification. The rapidly increasing number of successful applications of GC × GC–ToF MS reported in the literature;[1,6,7,15] (also see Fig. 3 below, and the cover of this issue) vividly illustrate the potential of this hyphenated technique. It is interesting to add that, very recently, fast-scanning quadrupole mass spectrometers have also been shown to perform satisfactorily: with these instruments, a 33–50 Hz acquisition rate can be achieved for a scan range of 50–100 mass units. This may well become an interesting alternative for many applications for which a limited scan range is no problem.

Two element-selective detectors that have been used in GC × GC analysis are the atomic emission detector (AED; with a transfer line adaptation to eliminate the drawback of a mere 10 Hz frequency)[8] and the sulfur chemiluminescence detector (SCD).[9] In both instances, trace-level S-containing constituents of petrochemical samples could be identified with marked success.

Applications

Selected examples of application areas of GC × GC are presented in Table 1. For more detailed information and access to the literature, one should consult

Table 1 Selected applications of GC × GC

Sample type	Analytes	References
Petrochemical products	Hydrocarbon groups	16
	S-containing compounds	17
	N-containing compounds	18
	Biomarkers	19
	Oxygenates	
Food, fish, fruits, wine	Flavours and fragrances	20
	PCBs (and enantiomers), PCDD/Fs	21
	Pesticides	
	FAMEs	
	Polyphenols	22
Air, breath, smoke	Volatiles (VOCs, aromatics)	23
	General characterisation	
Blood, urine	Drugs	
	PCBs, PCDD/Fs	24
	Pesticides	
Water, sediment, soil	PAHs, micro-contaminants	25
	BTEX, volatiles	
Plants	Essential oils	26
	Wound-induced volatiles	
Technical mixtures	Toxaphene, PCBs, polychlorinated paraffins	27

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references 1 and 10. Below, three typical applications are briefly discussed.

Mineral oil analysis

Mineral oil and petrochemical products were the target of most early GC × GC studies.[11,12] This is not too surprising because such samples combine several interesting features. They contain a very large number of individual homologues and isomers, but most of these belong to a limited number of classes of compounds, such as alkanes and alkenes, naphthenes (and their derivatives), and mono-, di- and tri-aromatics. In addition, in many instances emphasis is on a group-type characterisation only. Finally, detection and quantification can be performed by means of a fast FID. Prior to the introduction of GC × GC, procedures relied on dedicated, but highly complex, column-switching techniques. The simplification that can be achieved by using GC × GC is demonstrated in Fig. 2, which features the analysis of a light cycle oil.[11] The compound classes mentioned above all show up as clearly separated, distinct bands with a further, sometimes highly detailed, sub-division. The ordered structures enable rapid profiling (and quantification) and the total analysis takes slightly less than two hours.

Today, the GC × GC-based selective detection of S-containing compounds in petrochemical products is a topic that attracts much attention. Both ToFMS, SCD and AED detection have been used with marked success to separate the C_1–C_7 alkyl-substituted benzo- and dibenzothiophenes and benzonaphthothiophenes, and to unravel the complex composition of each of these classes.[1,8,9] One relevant example is presented in Fig. 3.

Polyhalogenated compounds

In many countries, polychlorinated biphenyls (PCBs or CBs) are routinely analysed in a variety of fish, fatty food and environmental samples. The focus is on the determination of the non-, and selected mono- and di-ortho CBs, which have TEF values similar to those of the priority polychlorinated dibenzo-*p*-dioxins and dibenzofuranes (PCDD/Fs). The complete separation of these priority compounds from each other and from the many other CBs and halogenated interferences present in technical mixtures and biota, by means of 1D-GC, has never been successful: adequate solutions require time-consuming heart-cut approaches or an LC pre-separation on a pyrenyl silica (PYE) column. What GC × GC can contribute here is most rewarding.[13,14] An example is displayed in Fig. 4, which shows the analysis of some ninety CBs in an extract of cod liver.[13] The separation of the priority CBs (yellow peak labels) is much improved, with the satisfactory resolution of the non-ortho CB 77 and the much more intense CB 136 as a relevant example. One also notices a clearly ordered structure: all CBs with the same number of chlorine substituents are seen to elute along the lines indicated in the figure. This facilitates the characterisation of CBs for which no standards are available (see asterisks). It was also found that, on each of the isochlorine lines, the number of ortho-chlorine substituents increases from top to bottom. Detection limits of selected CBs in GC × GC–μECD were found to be 10–20 fg, which is 3–5-fold better than in GC–μECD. This result, which is typical for GC × GC compared with conventional GC, can be explained by the re-focusing of the analyte-containing zones during modulation (*cf.* above).

Recent studies show that ordered structures become especially important when standards are not readily available. One example is toxaphene, an extremely complex mixture of polychlorinated bornanes, bornenes, *etc*. Whilst conventional GC procedures do not permit any useful conclusion regarding the overall

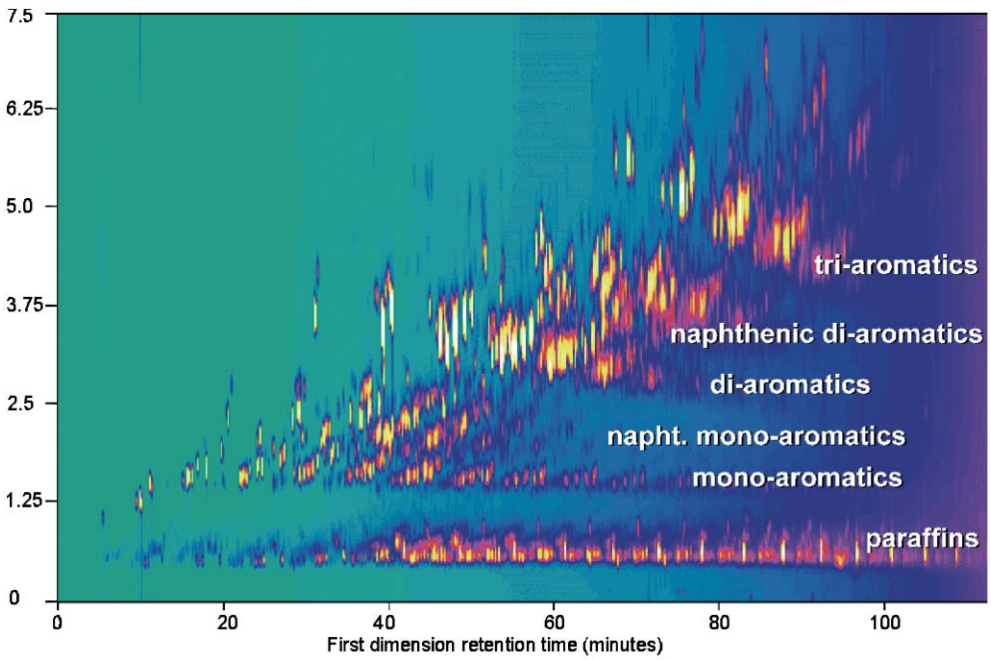

Fig. 2 GC × GC–FID of a light cycle oil on DB-1 × OV-1701 column combination. For explanation, see text.[11]

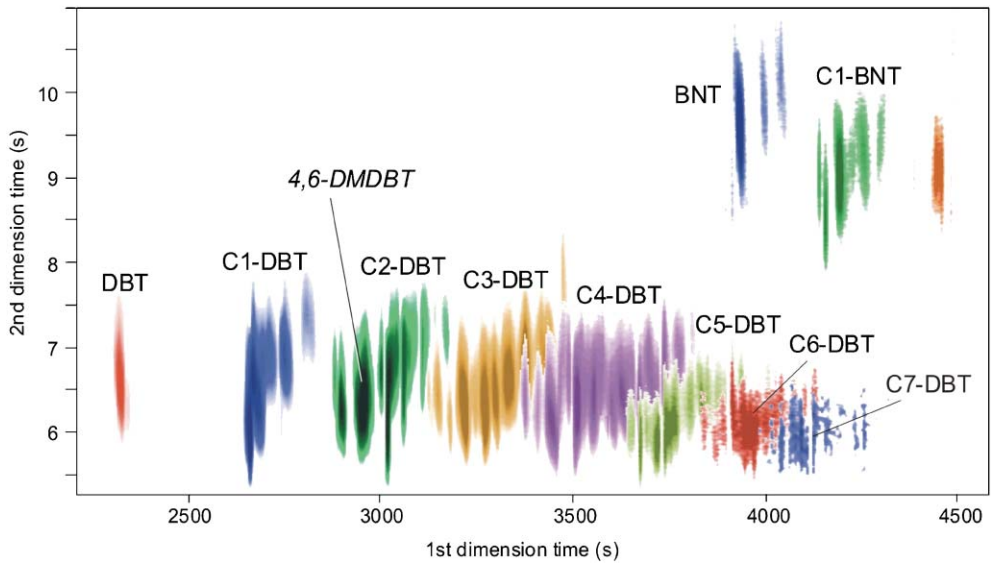

Fig. 3 Detail of GC × GC-ToF MS of a light catalytically cracked oil on a DB-1 × BPX-50 column combination, showing extracted-ion chromatograms (molecular ions) of alkyl-substituted dibenzothiophenes (DBT) and benzonaphthothiophenes (BNT). *4,6-DMDBT* is the highly refractory 4,6-dimethyl-DBT.[1]

composition, GC × GC with μECD and ToFMS detection to be made convincingly, they showed the presence of large numbers of, primarily, Cl_6–Cl_{10}-substituted bornanes which, also in this instance, eluted as separate bands in the 2D chromatogram. The improved separation enabled the recognition of over 1000 individual compounds.[13]

Cigarette smoke

The problems that are encountered when subjecting highly complex samples to GC × GC–ToFMS are vividly illustrated by the analysis of cigarette smoke (see figure on front cover of this issue, which shows the first-dimension 500–2600 s time window as a total ion current (TIC) contour plot).[15] One way to indicate the potential of the technique is by targeting attention on the small segment of the 2D plane with a first-dimension retention time of 583 s and a second-dimension retention time range of 0.25–1.75 s: eighteen peaks showed up in this tiny segment but, even in this crowded zone, no less than nine of these

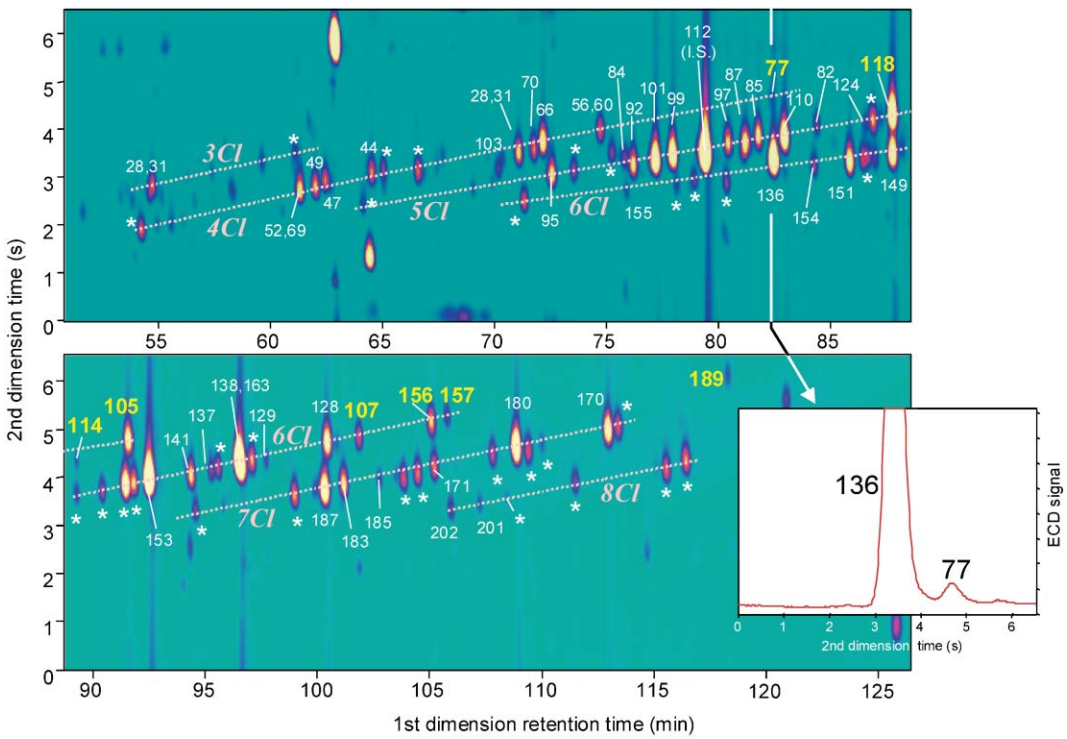

Fig. 4 GC × GC–μECD of CBs in a cod liver extract, using a HP-1 × HT-8 column combination. For explanation, see text.[13]

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could be identified by the ToFMS software. Another approach is to focus attention on the total number of peaks detected in the raw GC × GC chromatogram. The peak table was found to contain more than 30 000 entries! By means of automated data processing and by primarily relying on sufficiently high mass spectral match factors, typically over 800 for 'similarity' and over 900 for 'reverse', more than 1500 peaks (corresponding with some 500 compounds because of the usual 2–4 modulations per peak) could be satisfactorily identified. However, for a far larger number of peaks, *i.e.*, some 9000, identification was not up to standard. In such cases, additional information was required, *e.g.*, by using linear retention indices (LRI) for the first dimension or group-type (peak apices) information. Both approaches were found to be rewarding. To quote two examples, by using literature RIs in both dimensions and some chemical logic, over 600 peaks (150 compounds) from the total group of 10 500 peaks could be identified. Apex plots were highly successful in revealing the presence of large numbers of, for example, both 1H-alkyl- and 1-alkylpyrroles (alkyl: C_1–C_4), and also alkylpyridines and alkanenitriles.

The main message from this, and other similar, studies is that already now, GC × GC–ToFMS provides a separation and identification power unequalled in the field of chromatography. At the same time, the huge amount of information made available in each individual analysis (that is, each 60–90 min!) indicates that further developments aimed at seriously reducing the data-processing time are urgently required.

Conclusions and trends

Today, the principles and analytical strategies of GC × GC are well understood. Proper instrumentation is commercially available, inclusive of several robust and user-friendly cryogenic modulators and various detectors, notably a time-of-flight or fast-quadrupole mass spectrometer. In addition, replacing 1D-GC by GC × GC creates no problems in terms of sample treatment: because the first-dimension column of the latter technique is usually the same one as is used in high-resolution 1D-GC, existing preparation and injection techniques can be adopted without any modification. Optimisation of the column combinations is attracting increasing interest, specifically because there are indications that, with (highly) polar compounds, a 'reverse', *i.e.*, a polar × non-polar, column combination performs much better than the conventional non-polar × polar set-up. First studies in the area of food analysis, where polar analytes are of primary importance, demonstrate that, somewhat unexpectedly, ordered structures also show up with the reverse combinations; this will make the alternative approach even more attractive.

Analytical performance data in GC × GC, not discussed in this review, have been found to be fully satisfactory by several groups of workers. This holds true for retention time repeatability in both dimensions, improved (*cf.* above) analyte detectability and, also, for quantification, even though the summing of several modulations per analyte tends to make the procedure somewhat time-consuming. This is an area in which on-going software developments should make life easier for the analytical chemist.

Finally, it is the rapidly increasing number of applications, in fields as diverse as those included in Table 1, and reported by a continuously growing number of groups of workers, which demonstrates most convincingly that comprehensive two-dimensional gas chromatography is here to stay: resolving the disturbing background of state-of-the-art 1D-GC chromatograms, discovering the unexpected complexity of 'routine' samples, and easily finding up to a thousand (frequently largely unknown) peaks in many GC × GC chromatograms is both a challenge and a source of satisfaction.

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References

1 J. Dallüge, J. Beens and U. A. Th. Brinkman, *J. Chromatogr. A*, 2003, **1000**, 69.
2 P. J. Marriott, P. D. Morrison, R. A. Shellie, M. S. Dunn, E. Sari and D. Ryan, *LC-GC Eur.*, 2003, **16**, 23.
3 J. B. Phillips and J. Beens, *J. Chromatogr. A*, 1999, **856**, 331.
4 E. M. Kristenson, P. Korytár, C. Danielsson, M. Kallio, R. J. J. Vreuls, J. Beens and U. A. Th. Brinkman, *J. Chromatogr. A*, 2003, **1019**, 65.
5 R. M. Kinghorn and P. J. Marriott, *J. High Resolut. Chromatogr.*, 1998, **21**, 620.
6 M. Adahchour, L. L. P. van Stee, J. Beens, R. J. J. Vreuls, M. A. Batenburg and U. A. Th. Brinkman, *J. Chromatogr. A*, 2003, **1019**, 157.
7 M. M. van Deursen, J. Beens, J. Reijenga, P. Lipman, C. Cramers and J. Blomberg, *J. High Resolut. Chromatogr.*, 2000, **23**, 507.
8 L. L. P. van Stee, J. Beens, R. J. J. Vreuls and U. A. Th. Brinkman, *J. Chromatogr. A*, 2003, **1019**, 101.
9 F. C.-Y. Wang, W. K. Robbins, F. P. DiSanzo and F. C. McElroy, *J. Chromatogr. Sci.*, 2003, **41**, 519.
10 U. A. Th. Brinkman, M. Adahchour, J. Beens and P. Korytár, *Trends Anal. Chem.*, submitted for publication.
11 J. Blomberg, P. J. Schoenmakers and U. A. Th. Brinkman, *J. Chromatogr. A*, 2002, **972**, 137.
12 G. S. Frysinger and R. B. Gaines, *J. Sep. Sci.*, 2001, **24**, 87.
13 P. Korytár, P. E. G. Leonards, J. de Boer and U. A. Th. Brinkman, *J. Chromatogr. A*, 2002, **958**, 203.
14 M. Harju and P. Haglund, *J. Microcolumn Sep.*, 2001, **13**, 300.
15 J. Dallüge, L. L. P. van Stee, X. Xu, J. Williams, J. Beens, R. J. J. Vreuls and U. A. Th. Brinkman, *J. Chromatogr. A*, 2002, **974**, 16.
16 J. Blomberg, P. J. Schoenmakers and U. A. Th. Brinkman, *J. Chromatogr. A*, 2002, **972**, 137–173.
17 R. Hua, Y. Li, W. Liu, J. Zheng, H. Wei, J. Wang, X. Lu, H. Kong and G. Xu, *J. Chromatogr. A*, 2003, **1019**, 101–110.
18 F. C.-Y. Wang, *Presented at the First Symposium on Comprehensive Two-dimensional Gas Chromatography*, Volendam, The Netherlands, March, 2003.
19 G. S. Frysinger and R. B. Gaines, *J. Sep. Sci.*, 2001, **24**, 87–91.
20 R. Shellie and P. J. Marriott, *Anal. Chem.*, 2002, **74**, 5426–5430.
21 M. Harju, C. Danielson and P. J. Haglund, *Organo Halogen Compounds*, 2003, **60**, 395–398.
22 Y. Shao, P. Marriott and H. Hügel, *Chromatographia Suppl.*, 2003, **57**, 349–353.
23 J. V. Seeley, F. J. Kramp, K. S. Sharpe and S. K. Seeley, *J. Sep. Sci.*, 2002, **25**, 53–59.
24 J.-M. D. Dimandja, J. Grainger, D. G. Patterson, W. E. Turner and L. L. Needham, *J. Expo. Anal. Env. Epid.*, 2000, **10**, 761–768.
25 P. J. Marriott, P. Haglund and R. C. Y. Ong, *Clin. Chim. Acta*, 2002, **8027**, 1–19.
26 R. Shellie, L. Mondello, P. Marriott and G. Dugo, *J. Chromatogr. A*, 2002, **970**, 225–234.
27 P. Korytár, P. E. G. Leonards, J. de Boer and U. A. Th. Brinkman, *J. Chromatogr. A*, 2002, **958**, 203–218.