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APPLICATIONS OF ON-LINE COUPLED LIQUID CHROMATOGRAPHY–GAS CHROMATOGRAPHY

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SUMMARY

On-line coupling of two efficient separation methods, liquid and gas chromatography (LC–GC), is proving to be a very powerful two-dimensional technique. The main reasons for adoption of this coupled method are simplification of sample preparation by minimizing the pre-separation and clean-up steps, efficient elimination of interfering components, better repeatability, improvement of quantitation, reduction of analysis times, more information about the sample components and automation. A survey of LC–GC applications is given.

SAMPLE TRANSFER THROUGH VAPORIZING INJECTION

The first on-line liquid chromatography–gas chromatography (LC–GC) interface based on a GC autosampler injector that was modified with a flow-through side-arm syringe was presented at the 1979 Pittsburgh Conference[1,2] and a paper describing the coupling and some applications was published 1 year later[3]. However, according to subsequent publications (Fig. 1), the method was not accepted even though the interface was commercially available and the technique was automated. Because conventional-size LC columns (4.6 mm I.D.) were used, only a small fraction of the LC peak could be transferred into the vaporizing injector of the GC instrument. The system was mainly suitable for the qualitative analysis of concentrated

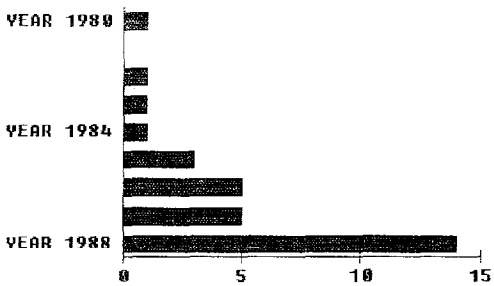

Fig. 1. LC–GC applications according to publication year.

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samples. The technique was applied to the analysis of atrazine in sorghum[3], *n*-alkanes and terpenoids in desert shrubs[4] and hydrocarbon group type in gasoline and diesel fuels[5].

The coupling was further automated in 1987 with pneumatic valves and applied to the analysis of folpet in hop samples. The transferred fractions were still small (2 μl)[6].

In order to reduce the volume of sample liquid introduced into the GC system, a bundled multi-capillary stream splitter placed between the LC and GC instruments has also been used[7,8]. An eluent splitter allows the interfacing of a conventional LC column with a GC system without the need for a retention gap. Multi-capillary splitters permit the simultaneous monitoring of the eluent for accurate zone sampling and transfer. However, the splitters decrease the sensitivity, because only a small volume of the sample is introduced into the GC system. The system was applied to the LC–GC analysis of a coal tar sample and LC–GC–mass spectrometry (MS) of a solvent-refined coal sample, with the three- and four-ring fractions being separated and identified[7–9].

TRANSFER OF THE WHOLE LC FRACTION INTO THE GC SYSTEM

The major problem connected with the direct transfer of an LC fraction into a GC system was the large volume of liquid, until the basis for a technique that allows the introduction of several hundred microlitres of liquid was developed[10,11]. The use of a retention gap pre-column was an important aspect[12].

The whole LC fraction (270 μl, 3 mm I.D. LC column) was directly transferred into a GC system for the first time in 1984[13]. The effluent was pumped through an on-column injector into a long retention gap (50 m) that was coupled to a separation column (30 m). The LC eluent spreads into an uncoated pre-column, which must be at least as long as the zone flooded with the solvent. This conventional retention gap technique was applied to the analysis of azulene in toothpaste. The transfer of the whole LC fraction guarantees more reliable qualitative and quantitative results. After 1984 the number of publications on LC–GC applications started to increase (Fig. 1).

It is possible to keep the volume of the LC effluent below 100 μl by using microbore LC columns and below 50 μl if efficient packed capillary columns are used. The sample capacity might then cause problems, especially with samples that contain large amounts of by-products. Chlorinated benzenes in fuel oil have been determined by on-line LC–GC using a 250-μm I.D. packed fused-silica capillary LC column[14].

Most of the applications of coupled LC–GC have involved normal-phase LC. With reversed-phase LC, problems occur with very polar solvents (methanol and water) that do not wet the surface of the GC columns and that destroy very rapidly the deactivation of the pre-column. In 1985, a packed reversed-phase capillary LC column was used for the analysis of polychlorinated biphenyls by LC–GC[15]. The solvent was pure acetonitrile. Diazepam in urine has also been analysed by reversed-phase micro-LC–GC[16]. The mobile phase was methanol–water (80:20) containing 0.1% H_3PO_4, but the fraction volume was only 2 μl.

In addition to the conventional retention gap technique, partially concurrent solvent evaporation and fully concurrent solvent evaporation techniques have been reported for handling even larger sample volumes transferred from the LC into the

GC system with decreasing solvent evaporation times by using shorter retention gaps and an on-column interface[17]. Partially concurrent solvent evaporation has been applied to the group-type analysis of gasoline[18].

Fully concurrent solvent evaporation involves complete eluent evaporation during the transfer into the GC system. It eliminates restrictions on the volume of LC fractions transferred, but it is suitable only for the analysis of components eluted at relatively high column temperatures.

In 1986, a loop-type interface for concurrent solvent evaporation was introduced. It was applied to the analysis of raspberry ketone in a raspberry sauce[19]. LC–GC coupling via a loop-type interface is very convenient, as the only parameter to be selected is the column temperature during the introduction of the LC fraction. The conventional retention gap technique and also partially concurrent solvent evaporation techniques with an on-column interface need three parameters to be adjusted with respect to each other: carrier gas flow-rate, column temperature and the rate of eluent introduction.

Manual loop-type interfaces have been used in many applications, *e.g.*, in the analysis of wax esters in olive oil[20], polychlorinated biphenyls in fish[21], broxaterol in plasma and urine[22], diisooctyl phthalate in salad oil[23] and dicamba in tobacco[24]. The LC fraction volumes varied between 250 and 1250 μl.

We have used a loop-type interface with a ten-port rotating switching valve that makes it possible to cut two different fractions and analyse them independently. The interface has been modified slightly from our earlier system[25] (Fig. 2). Our coupling method has been applied to the determination of metals as diethyldithiocarbamate (DEDTC) chelates. A mixture of DEDTC chelates of Pd^{II}, Hg^{II}, Cu^{II}, Sn^{II}, Co^{III} and Fe^{III} was injected into the LC system with a silica column (2.1 mm I.D.). The 250-μl fraction containing Cu^{II}, Hg^{II} and Pd^{II} chelates was transferred into the GC system, where all three were successfully separated (Fig. 3). In Fig. 4, Se^{II}, Cu^{II} and Hg^{II} chelates were collected after high-performance liquid chromatographic (HPLC) sep-

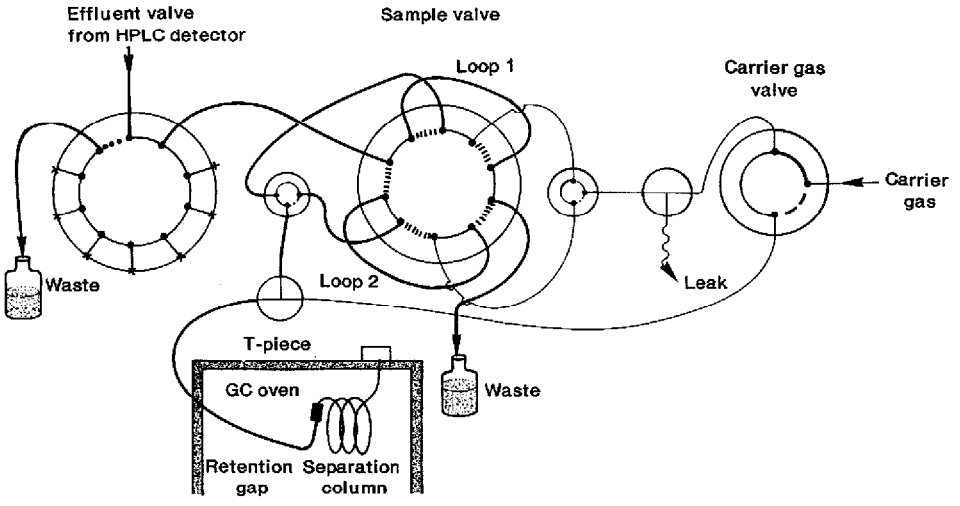

Fig. 2. Schematic diagram of a loop-type ten-port valve LC–GC interface.

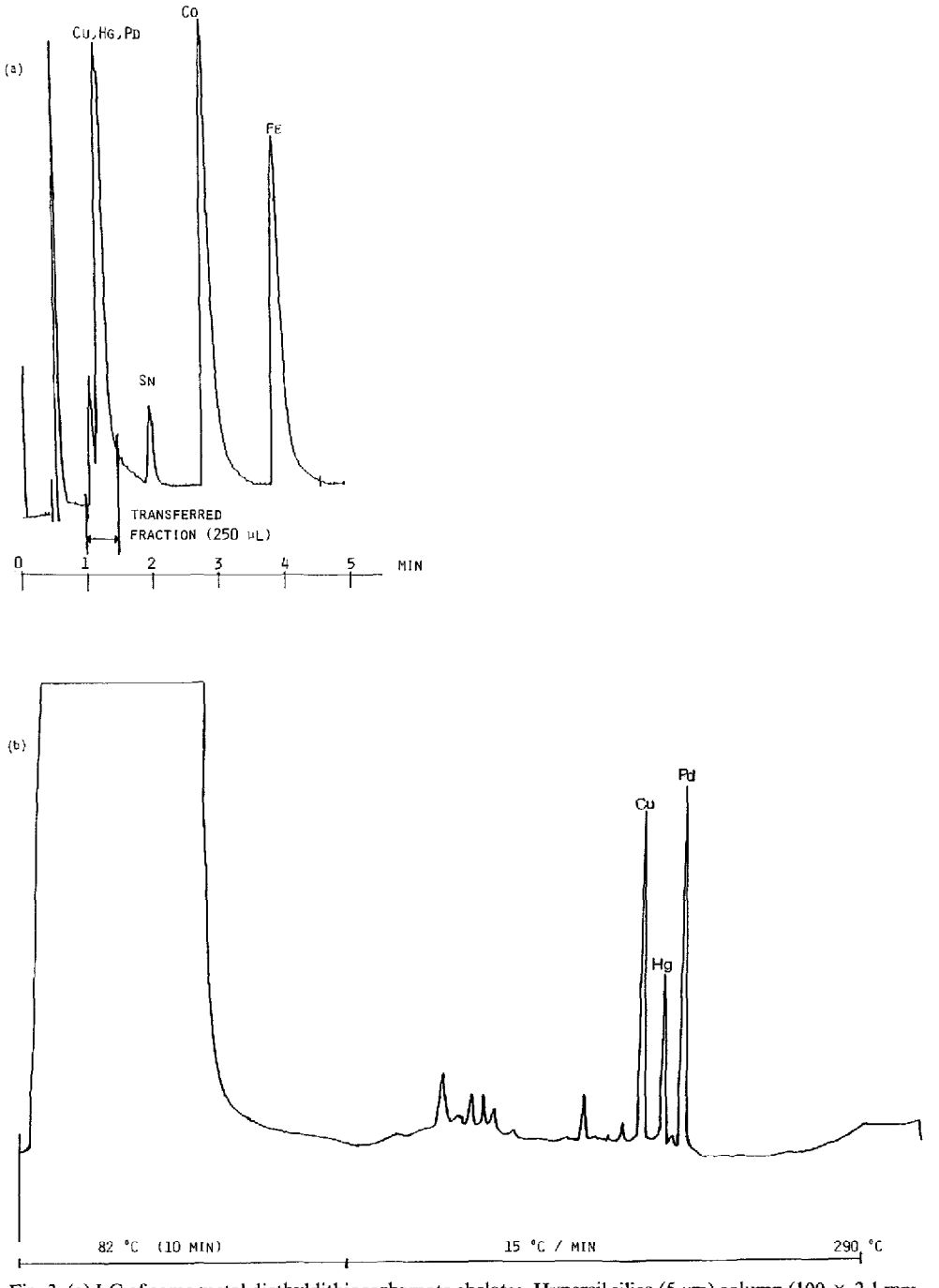

Fig. 3. (a) LC of some metal diethyldithiocarbamate chelates. Hypersil silica (5 μm) column (100 × 2.1 mm I.D.); eluent, hexane–dichloromethane (1:1, v/v). (b) GC of metal chelate fraction [$Cu(DEDTC)_2$, $Hg(DEDTC)_2$ and $Pd(DEDTC)_2$] after on-line LC separation. SE-54 (0.25 μm) column (5 m × 0.32 mm I.D.). Retention gap, 2 m × 0.53 mm I.D.

aration (NH_2-bonded silica, 2.1 mm I.D. column) in the sample loop 1 (400 μl) and Pd^{II} and Co^{III} chelates in loop 2 (250 μl). The metal chelates were separated with good resolution by GC. LC serves as an excellent pre-separation method for metal chelates. The best results were obtained when the sample was flushed into the GC system with additional solvent. The use of coupled LC–GC for the determination of metals has been applied to water samples[26].

Most of the automated on-line LC–GC systems use a loop-type interface. Automated LC–GC has been used for, *e.g.*, the identification of two- to four-ring polycyclic aromatic compounds in diesel fuel. A ten-port valve interface and conventional retention gap technique were employed[27]. Rapid analysis for free sterols, esterified sterols and wax esters in oils and fats has been described[28]. This work was done on a prototype of the automated Carlo Erba LC–GC instrument. The volume of LC fraction transferred was 750 μl (2 mm I.D. silica column). A fully automated LC–GC system was also applied to the determination of free erythrodiol in olive oil. The LC fraction was as large as 850 μl, but the total analysis time was only 25 min[29]. In the last application, a solvent vapour exit was used to decrease the volume of solvent introduced into the GC system.

Recently, an LC–GC interface was described that allows the removal of the LC eluent by an early solvent vapour exit and simultaneous cold trapping of the solutes followed by splitless transfer of the solutes into the GC oven[30]. A pre-column was not used in the GC system. The method was applied to the determination of chlorinated pesticides in water.

From Fig. 1, it is seen that the number of publications on LC–GC applications increased considerably in 1988. Fig. 1 is based on the data in Table I. It can be seen that largest LC fractions are transferred by the loop-type interface. The applications can be grouped roughly into four areas: fuels, foodstuffs, environmental and medical samples. Fuel analyses have mainly been qualitative. Most of the samples analysed using on-line LC–GC were either fuels or foodstuffs. The division of applications according to the interface is not unambigious.

CONCLUSIONS

There are at least three approaches for coupling LC with GC: (1) modification of LC to meet the requirements of GC, that is, the use of microcolumns in LC; (2) modification of GC to "accept" large sample volumes; or (3) reduction of the sample liquid introduced into the GC system by using effluent splitters.

Micro-LC columns, especially packed capillary columns, permit high separation efficiencies. Because of the low flow-rate of the eluent, the LC fraction of interest can be introduced directly into the GC system. The saving in eluent costs is also an important aspect, in addition to short analysis times. The only drawback is the low sample capacity, especially with samples that contain large amounts of by-products. An important role of LC is to minimize clean-up and pre-separation steps before the final GC analysis. For trace analysis, conventional LC columns seem to be the best alternative.

It is possible to introduce large volumes of liquid into the GC system by using either on-column or loop-type interfaces. Long retention gaps used in conjunction with an on-column interface lengthen the analysis times and many parameters must

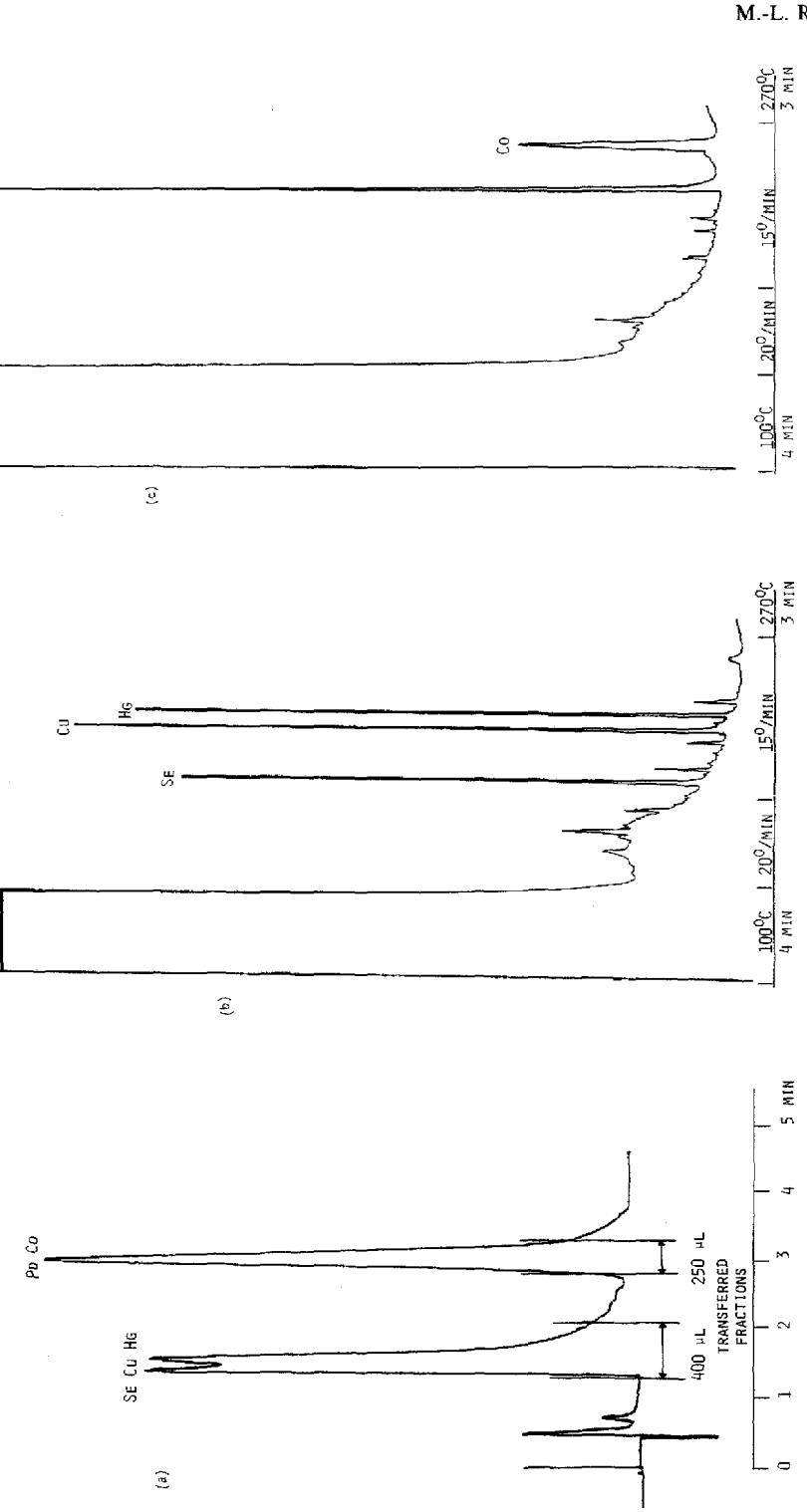

Fig. 4. (a) LC of Se^{II}, Cu^{II}, Hg^{II}, Pd^{II} and Co^{III} DEDTC chelates. Hypersil APS (5 µm) column (100 × 2.1 mm I.D.); eluent, hexane–dichloromethane (85:15, v/v). (b) GC of the first [$Se(DEDTC)_2$, $Cu(DEDTC)_2$ and $Hg(DEDTC)_2$] and (c) the second transfer [$Pd(DEDTC)_2$ and $Co(DEDTC)_3$] from the LC system.

TABLE I

LC–GC APPLICATIONS, VOLUME OF LC ELUENT TRANSFERRED TO THE GC SYSTEM AND THE INNER DIAMETER OF THE LC COLUMN INVOLVED, GROUPED ACCORDING TO THE INTERFACE

System	*Sample analysed*	*Analytes*[a]	*Fraction volume (μl)*	*LC column I.D. (mm)*	*Ref.*
Automated LC–GC	Sorghum	Atrazine	8	4.0	3
	Desert shrub	*n*-Alkanes and terpenoids	9	4.6	4
	Gasoline and diesel fuel	Chemical classes	2	4.6	5
	Hop	Folpet	2	4.6	6
	Coal liquids	Chemical classes	0.1	–	7, 8
	Diesel exhaust particulates	PACs	150	1.0	31
	Turkish lignite	Chemical classes	150	1.0	32
	Kerosine and diesel fuel	Chemical classes	150	1.0	33
	Diesel fuel	PACs	150	1.0	27
	Urine	Heroin metabolites	500	2.0	34
	Oil, fat	Sterols	750	2.0	28
	Olive oil	Erythrodiol	850	3.0	29
On-column interface	Toothpaste	Azulene	270	3.0	13
	Petroleum	Chemical classes	100	2.0	18
	Bovine urine	Diethylstilbestrol	–	3.0	35
	Sediment	PCBs	180	0.7, 1.1	36
	Gasoline	PACs	2–3	0.32	37
	Urine	Diazepam	1–3	0.32	16
	Aqueous samples	Chlorinated pesticides and PCBs	100	1.1	38
Loop-type interface	Raspberry sauce	Raspberry ketone	450	2.0	19
	Fuel oil	Chlorinated benzenes	22	0.25	14
	Coal tar	PCBs	40	0.1, 0.3	15
	Olive oil	Wax esters	450	2.0	20
	Fish	PCBs	400	3.0	21
	Plasma and urine	Broxaterol	500	3.9	22
	Triglyceride	Di(2-ethylhexyl)phthalate	1200	4.6	23
	Dicamba	Tobacco	250	2.0	24
	Plasma	I-Moprolol β-blocker	500	4.0	39
Others	Coal	Aromatics	10	1.0	7–9
	Aqueous samples	Chlorinated pesticides	–	–	30

[a] PACs = polycyclic aromatic compounds; PCBs = polychlorinated biphenyls.

be adjusted with conventional retention gap and partially concurrent solvent evaporation techniques. A loop-type interface is fairly simple and easily automated for both conventional retention gap and concurrent solvent evaporation techniques. For the latter, only one parameter, the temperature of the oven during the introduction of the sample, needs to be adjusted. For eliminating contamination, very pure solvents are necessary and flushing of the sample loop with solvent may be needed. Loop-type interfaces are applied in the analysis of compounds eluted more than about 50°C

above the column temperature during sample introduction, but by using co-solvent trapping early peaks can also be analysed[40].

The introduction of the whole LC fraction into the GC system gives the most reliable results. Removal of LC solvent vapour by a vapour exit accelerates transfer and also minimizes problems that large sample volumes might cause for GC detectors.

The applications can be grouped into four areas; fuels, foodstuffs, environmental and medical samples. Polymers seem to be a new area for LC–(pyrolysis) GC[41]. Most of the applications have been qualitative and judgements regarding the significance of the results (precision, accuracy, repeatability, reproducibility, detection limit, determination limit, etc.) have not been common.

The coupling of reversed-phase LC with GC needs further development, although many useful attempts have been carried out.

The use of diode-array detectors in LC would improve the reliability of cutting LC fractions and LC–GC–MS, LC–GC–Fourier transform IR spectrometry and LC–multi-dimensional GC would provide more information about sample components.

It is evident that full automation of on-line LC–GC improves the repeatability of the analyses. An automated system is fast and easy to use. The availability of fully automated commercial instruments is probably the best way to increase the potential of this two-dimensional chromatographic system and to widen the areas of application.

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REFERENCES

1 S. P. Cram, A. C. Brown, E. Freitas, R. E. Majors and E. L. Johnson, *Abstracts of Papers, Pittsburg Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, OH, 1979*, abstract 115.
2 R. E. Majors, E. L. Johnson, S. P. Cram, A. C. Brown and E. Freitas, *Abstracts of Papers, Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, OH, 1979*, abstract 116.
3 R. E. Majors, *J. Chromatogr. Sci.*, 18 (1980) 571.
4 D. J. Lutzbetak and J. J. Hoffmann, *J. Chromatogr. Sci.*, 20 (1982) 132.
5 J. A. Apffel and H. McNair, *J. Chromatogr.*, 279 (1983) 139.
6 K. A. Ramsteiner, *J. Chromatogr.*, 393 (1987) 123.
7 T. V. Raglione and R. A. Hartwick, *Anal. Chem.*, 58 (1986) 2680.
8 T. V. Raglione, J. A. Troskosky and R. A. Hartwick, *J. Chromatogr.*, 409 (1987) 205.
9 T. V. Raglione, J. A. Troskosky and R. A. Hartwick, *J. Chromatogr.*, 409 (1987) 213.
10 K. Grob and B. Schilling, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 531.
11 K. Grob, Jr., and H. P. Neukom, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 405.
12 K. Grob, Jr., G. Karrer and M.-L. Riekkola, *J. Chromatogr.*, 334 (1985) 129.
13 K. Grob, Jr., D. Fröhlich, B. Schilling, H. P. Neukom and P. Nägeli, *J. Chromatogr.*, 295 (1984) 55.
14 H. J. Cortes, B. E. Richter, C. D. Pfeiffer and D. E. Jensen, *J. Chromatogr.*, 349 (1985) 55.
15 H. J. Cortes, C. D. Pfeiffer and B. E. Richter, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 469.
16 D. Duquet, C. Dewaele, M. Verzele and S. McKinley, in P. Sandra (Editor), *Proceedings of 9th International Symposium on Capillary Chromatography*, Hüthig, Heidelberg, 1988, p. 450.
17 K. Grob, Jr. and B. Schilling, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 726.

18 F. Munari, A. Trisciani, G. Mapelli, S. Trestianu, K. Grob, Jr. and J. M. Colin, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 601.
19 K. Grob, Jr. and J.-M. Stoll, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 518.
20 K. Grob, Jr. and T. Läubli, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 593.
21 K. Grob, Jr., E. Müller and W. Meier, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 416.
22 Y. Gianesello, L. Bolzani, E. Brenn and A. Gazzaniga, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 99.
23 B. Pacciarelli, E. Müller, R. Schneider, K. Grob, W. Steiner and D. Fröhlich, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 135.
24 V. M. A. Häkkinen and K. Grob, Jr., *J. Chromatogr.*, in press.
25 V. M. A. Häkkinen, M. M. Virolainen and M.-L. Riekkola, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 214.
26 M.-L. Riekkola, P. Lukkari, J. Hannuksela, M. Virolainen, V. M. A. Häkkinen and M.-L. Mattinen, in preparation.
27 I. L. Davies, K. D. Bartle, P. T. Williams and G. E. Andrews, *Anal. Chem.*, 60 (1988) 204.
28 K. Grob, M. Lanfranchi and C. Mariani, *J. Chromatogr.*, 471 (1989) 397.
29 K. Grob, M. Biedermann and Th. Läubli, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 12 (1989) 49.
30 T. Noy, E. Weiss, T. Herps, H. Van Cruchten and J. Rijks, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 181.
31 I. L. Davies, M. W. Raynor, P. T. Williams, G. E. Andrews and K. D. Bartle, *Anal. Chem.*, 59 (1987) 2579.
32 I. L. Davies, K. D. Bartle, M. W. Raynor, D. J. Urwin, M. Tolay and E. Ekinci, in P. Sandra (Editor), *Proceedings of 9th International Symposium on Capillary Chromatography*, Hüthig, Heidelberg, 1988, p. 438.
33 I. L. Davies, K. D. Bartle, G. E. Andrews and P. T. Williams, *J. Chromatogr. Sci.*, 26 (1988) 125.
34 F. Munari and K. Grob, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 172.
35 K. Grob, Jr., H. P. Neukom and R. Etter, *J. Chromatogr.*, 357 (1986) 416.
36 F. A. Maris, E. Noroozian, R. R. Otten, R. C. J. M. van Dijck, G. J. de Jong and U. A. T. Brinkman, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 197.
37 D. Duquet, C. Dewaele and M. Verzele, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 252.
38 E. Noroozian, F. A. Maris, M. W. F. Nielen, R. W. Frei, G. J. de Jong and U. A. T. Brinkman, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 17.
39 E. Brenn, G. Figini, V. Gianesello and A. Gazzaniga, *J. Chromatogr.*, 473 (1989) 343.
40 K. Grob and E. Müller, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 388, 560.
41 H. J. Cortes, paper presented at *the International Symposium on Coupled Column Separations, Uppsala, Sweden, October 26–27, 1988.*