Journal of Chromatography, 395 (1987) 307–315
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1104

TANDEM CAPILLARY GAS CHROMATOGRAPHY IN PESTICIDE RESIDUE ANALYSIS

L. G. M. Th. TUINSTRA*, W. A. TRAAG, A. J. VAN MUNSTEREN
State Institute for Quality Control of Agricultural Products, Bornesteeg 45, 6708 PD Wageningen (The Netherlands)
and
V. VAN HESE
Chrompack International BV, Middelburg (The Netherlands)

SUMMARY

Maximum residue levels of pesticides in food and feed are often below the 0.1 ppm level. On capillary columns, the maximum injection volumes are 5–10 μl in the splitless mode, although of course some (acceptable) overloading of the GC column occurs. Injection of larger volumes (up to 100 μl) is possible only when the solvent can be removed whilst at the same time the compounds of interest are concentrated in a small zone. The advantage of such a procedure is obvious: the limit of detection is improved by a factor of 10–20, so a concentration step can be omitted. A Chrompack MUSIC (MUltiple Switching Intelligent Controller) kit was connected with a Tracor 550 gas chromatograph with an electron-capture detector for chlorinated compounds. Data are presented that show that gel permeation chromatography on Bio-Beads SX-3 as a clean-up medium in combination with MUSIC allows the detection of sub-ppm levels of organochlorine pesticides in grains, fats and vegetables by splitless injection of a 100-μl sample on to the chromatographic column.

INTRODUCTION

Residue laboratories are often required to analyse food and feed samples containing less than 0.1 ppm of pesticides, which means that in small sample volumes sub-nanogram amounts have to be measured. The use of capillary columns has increased the reliability of the identification of compounds compared with the use of packed columns but, because of the restricted capacity of capillary columns, only small injection volumes (maximum 5 μl) are permitted and therefore the extract of the sample must be evaporated to a small volume.

This process is not only time consuming but may also result in losses of compounds, especially of lower and intermediate boiling compounds. This inconvenience can be overcome by injecting large volumes (100 μl) on to the capillary column by using on-column injection into a long retention gap[1], packed temperature programmed vaporizers[2,3] or tandem chromatography, based on flow switching[4]. The

0021-9673/87/$03.50

last principle is often used for small volumes, mostly by research laboratories, as these switching systems are often tailor-made. Commercially, very few manufacturers offer complete (expensive) systems, included with the gas chromatograph.

Recently, Chrompack (Middelburg, The Netherlands) introduced MUSIC (MUltiple Switching Intelligent Controller), a modular system that can be built into modern gas chromatographs[5]. The system consists of four components (see Fig. 1). (a) The cold trap, cooled with liquid carbon dioxide, and (b) a pneumatic controller (valve box), consisting of solenoid valves, needle valves and pressure and flow controllers, are mounted outside the oven. (c) The programmer unit (microprocessor) controls the MUSIC system. The part of the system that comes into contact with sample components is made entirely of fused silica, thus making the instrument well suited for the analysis of polar and/or labile compounds. Columns and connecting capillaries are coupled with (d) the column module (oven bracket). This column moduile is equipped with zero dead volume connectors. The fused-silica columns can be connected without tools.

With MUSIC, on-line separation is carried out on a pre-column (wide-bore fused-silica capillary column) with a high capacity. One method of proceeding (heart cutting) is as follows. Components of interest, eluting in a certain part of the chromatographic analysis, can be further separated by switching the direction of the flow in the column module (via valve V3) so that these compounds elute in the direction of the cold trap. After some time, all compounds are gathered in the trap. The flow direction in the column module is again switched and the trap is now heated quickly, resulting in an injection on to the narrow-bore fused-silica column with a high separation power. The cold trap between the two columns reduces the peak broadening originating from the pre-column.

In addition to heart cutting, other well known techniques, such as pre-concen-

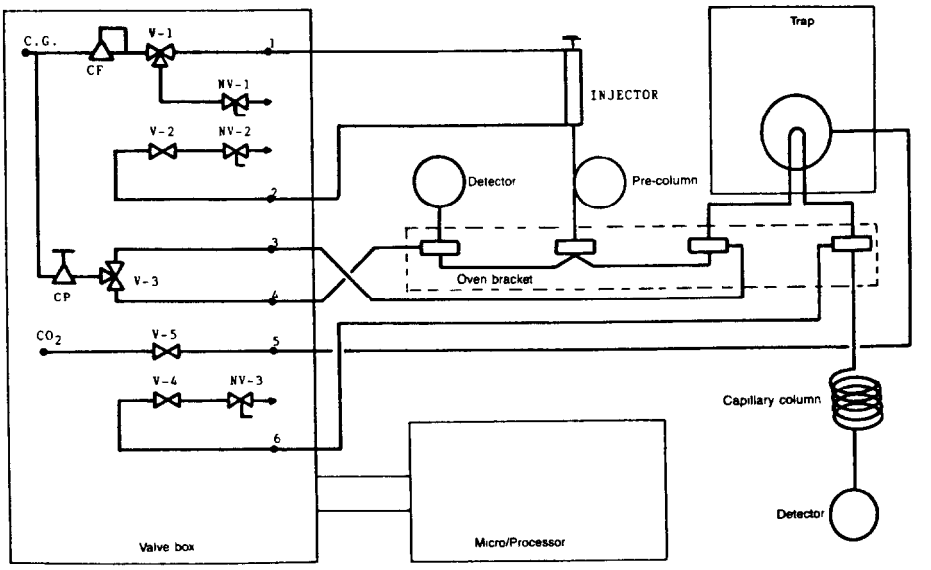

Fig. 1. Flow scheme of the MUSIC system.

tration, back-flushing and pre-separation, can be carried out with this system. Further, MUSIC can easily be transferred to another gas chromatograph linked to a mass spectrometer for particular applications.

As the possibility of injecting extra large volumes together with increased reliability of identification seemed very attractive to use, we decided to test the MUSIC system on organochlorine pesticides with regard to adsorption and linearity behaviour, peak broadening, maximum injection volume, reproducibility of injection and usefulness for real samples.

EXPERIMENTAL

Apparatus

A Tracor 550 gas chromatograph (Austin, TX, U.S.A.), equipped with two electron-capture detectors (^{63}Ni), was used in combination with the MUSIC system. Injections were performed at an oven temperature of 100°C into a wide-bore fused-silica capillary column (3.5 m × 0.5 mm I.D.) coated with CP-Sil-5CB, film thickness 5 μm (Chrompack).

The analytical column, also a fused-silica capillary (25 m × 0.22 mm I.D.) coated with CP-Sil-5CB, film thickness 0.2 μm (Chrompack), was connected with the capillary trap, using uncoated, deactivated fused silica. A constant flow of 10 ml/min of helium was used for the wide-bore column and a constant pressure of 1.1 bar of helium for the analytical column. Four minutes after splitless injection, the oven was heated at 20°C/min to 200°C.

After elution from the wide-bore pre-column, the components of interest were trapped in the cold trap, which was cooled to −70°C with liquid carbon dioxide. The trapped components were re-injected by heating the cold trap for a few seconds at 220°C.

Materials

The organochlorine compounds α-, β- and γ-HCH (hexachlorocyclohexane), β-heptachlor epoxide (1,4,5,6,7,8,8a-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-endomethyleneindane), *p,p'*-DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene], *p,p'*-DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], *p,p'*-TDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane], dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-5,8-dimethanonaphthalene), α-endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,5-benzodioxathiepin-3-oxide), β-endosulfan, hexabromobenzene, methoxychlor [1,1,1-trichloro-2,2-di(4-methoxyphenyl)ethane], 2,4,5,2',4',5'-hexachlorobiphenyl (PCB 153) and 2,3,4,2',4',5-hexachlorobiphenyl (PCB 138), dissolved in isooctane in appropiate concentrations, were used for standard experiments.

Practical samples, such as wheat and animal fat extracts, were obtained by extraction with ethyl acetate and cleaned up on an SX-3 gel permeation column[6].

The isolated fractions in ethyl acetate–cyclohexane were used for direct injection.

RESULTS AND DISCUSSION

Degradation

p,p'-DDT is suitable for testing the system for inertness, as it is easily degraded into *p,p'*-DDE and *p,p'*-TDE[7]. A 1-ng amount of *p,p'*-DDT was injected into the system (with MUSIC). The results (Fig. 2) indicate that not more than 1% of DDT was degraded into DDE and TDE. It must be stressed that the quality of the columns can strongly influence this degradation. From Fig. 2 it is clear that the total system, including MUSIC and the pre-column, is inert towards these types of compounds.

Linearity

A test mixture of organochlorine compounds was injected (5 μl in isooctane) in increasing concentrations into the analytical column, which, for this experiment, was mounted in the usual way for splitless injection. Results are shown for a few compounds in Table I, where the peak height (H) obtained for a given amount (M) of the compound is divided by that quantity (giving H/M) and, after normalization, tabulated against M. A linear relationship between H and M is obtained, when the value H/M is constant at any M. Over the tested mass range the results obtained with the MUSIC system (Table II) are comparable ($\pm$ 10%) to those obtained without MUSIC and pre-column, and are therefore acceptable.

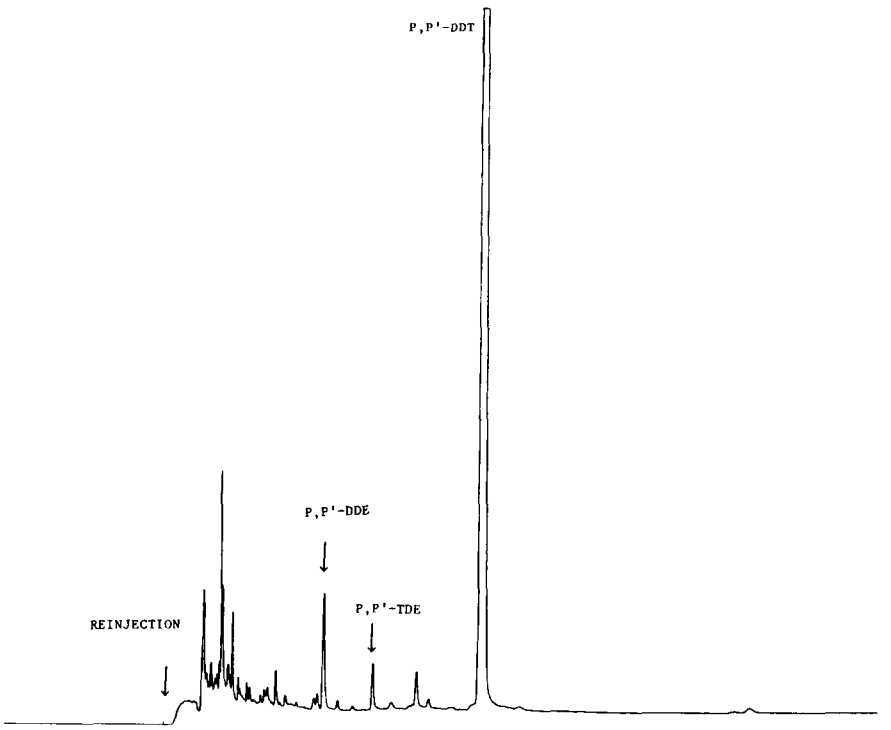

Fig. 2. Gas chromatogram of trapped and re-injected *p,p'*-DDT on the analytical column (1 ng of *p,p'*-DDT).

TABLE I

RELATIVE PEAK HEIGHT/MASS *VERSUS* MASS (*H/M*) FOR FOUR COMPOUNDS ANALYSED WITH SPLITLESS INJECTION (WITHOUT MUSIC) (MEAN RESPONSE PER COMPONENT = 100%)

*Mass injected**	*H/M (%)*			
	γ-HCH	*p,p'-DDT*	*PCB 153*	*PCB 138*
1F	87	80	94	90
2F	92	99	104	105
4F	96	101	107	103
8F	103	99	104	108
12F	104	104	98	98
16F	104	106	100	102
20F	105	103	96	98
24F	107	110	96	97

* F = 1.25 pg γ-HCH + 6.25 pg *p,p'*-DDT + 6.25 pg PCB 153 + 6.25 pg PCB 138.

TABLE II

RELATIVE PEAK HEIGHT/MASS *VERSUS* MASS (*H/M*) FOR FOUR COMPOUNDS ANALYSED WITH MUSIC (MEAN RESPONSE = 100%)

*Mass injected**	*H/M (%)*			
	γ-HCH	*p,p'-DDT*	*PCB 153*	*PCB 138*
1F	104	86	93	95
2F	104	86	101	99
4F	104	86	101	95
8F	100	97	103	99
12F	96	102	101	101
16F	102	110	101	102
20F	95	114	100	101
24F	94	118	99	107

* F as in Table I.

TABLE III

PEAK WIDTH AT HALF-HEIGHT *VERSUS* INJECTION VOLUME FOR FOUR PESTICIDES ANALYSED WITH MUSIC

Injection volume (μl)	*Peak width at half-height*			
	α-HCH (0.2 ng)	*p,p'-DDT (0.2 ng)*	*PCB 153 (1 ng)*	*PCB 138 (2 ng)*
5	1.8	2.0	3.8	4.9
50	1.8	2.1	3.7	5.3
100	1.8	2.0	3.7	5.0
150	1.8	1.9	3.6	5.3
200	1.8	2.0	3.7	5.5
300	2.0	2.2	3.6	5.3
400	1.9	2.1	3.9	5.4

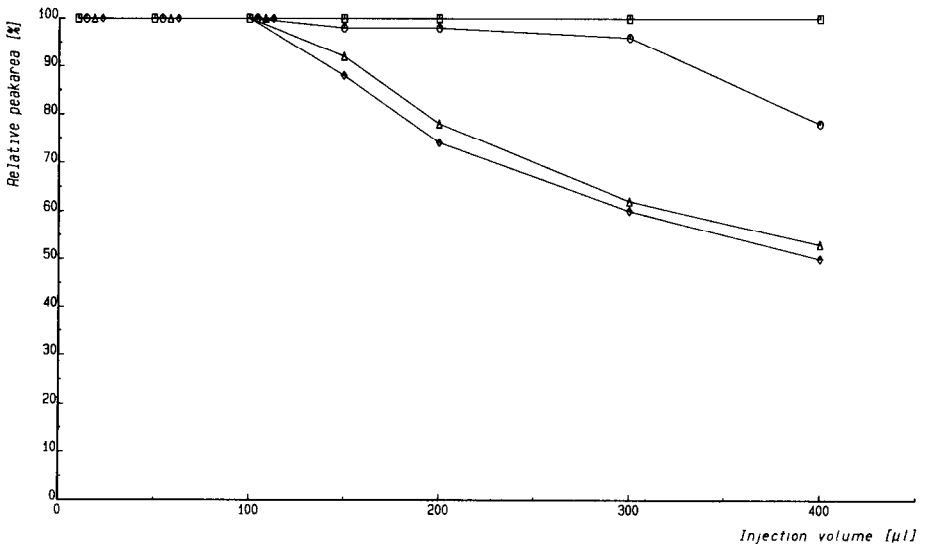

Fig. 3. Influence of injection volume on peak response for four compounds. □, *p,p'*-DDT; ○, β-heptachlor epoxide; △, γ-HCH; ◇, α-HCH.

Injection volume and reproducibility of injection

For several of the organochlorine compounds, different volumes (5–400 µl) were injected in such a way that the absolute amount injected for a given compound was constant over the tested range. From the chromatograms the response and peak width at half-height were measured.

The peak width (in seconds) shown in Table III was constant over the tested range for all compounds. For more volatile compounds (*e.g.*, α- and γ-HCH) the maximum volume without a decrease in response was *ca.* 150 µl. The manual injection speed was about 10 s per 100 µl. Injection of more than 150 µl caused losses of more volatile compounds (Fig. 3), probably as a result of back-flushing and a temporarily excessive pressure at the switching point in the column module, resulting in a partial

TABLE IV

REPEATABILITY OF ANALYSIS PERFORMED WITH SPLITLESS INJECTION AND WITH MUSIC

Component	*Coefficient of variation (%)*	
	Splitless injection (n = 13)	*MUSIC (n = 9)*
α-HCH (0.2 ng)	8.9	10.1
γ-HCH (0.2 ng)	8.4	6.8
p,p'-TDE (1 ng)	7.6	6.7
p,p'-DDT (1 ng)	7.0	7.6
p,p'-DDT (2 ng)	11.8	7.1

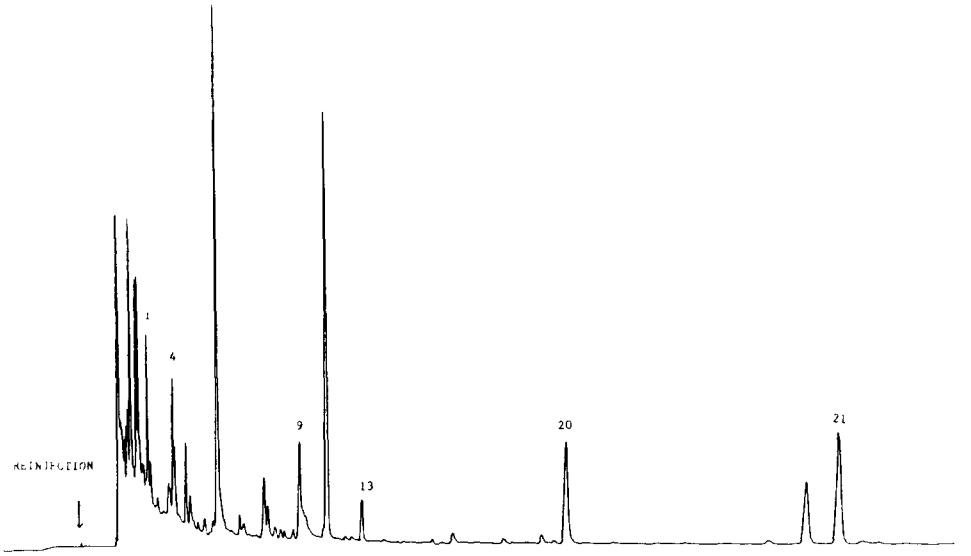

Fig. 4. Chromatogram of a wheat sample extract, after GPC clean-up, with MUSIC and without extra concentration. Injection volume, 100 μl = 0.85 mg of sample. Peaks: 4 = γ-HCH (0.02 mg/kg); 9 = α-endosulfan (0.04 mg/kg); 13 = β-endosulfan (0.04 mg/kg); 1, 20, 21 = internal standards (3-monochlorobiphenyl, hexabromobenzene and 2,3,4,5,6,2',3',5'-octachlorobiphenyl).

transport of compounds in the direction of the trap and analytical column. The latter can be decreased by reducing the injection speed or increasing the pre-column length.

The repeatability of the system was compared with a system lacking MUSIC and a pre-column, by repeated automatic injection of 5 μl of organochlorine com-

TABLE V

DETECTION LIMITS WITH AND WITHOUT MUSIC FOR ORGANOCHLORINE PESTICIDES IN FAT IN RELATION TO ACTION LEVELS

Action level = 50% of maximum residue limit.

Component	*Action level (mg/kg)*	*Detection limit (mg/kg)*	
		Without MUSIC	*With MUSIC*
HCB	0.1	0.01	0.002
α.HCH	0.15	0.01	0.002
β-HCH	0.05	0.02	0.004
γ-HCH	1	0.01	0.002
Heptachlorepoxide	0.1	0.02	0.004
Dieldrin	0.1	0.02	0.004
Endrin	0.04	0.04	0.008
DDT (total)	0.5	0.1	0.02
γ-Chlorodane	0.02	0.02	0.004
2,4,5,2',4',5'-Hexachlorobiphenyl	0.03	0.03	0.006
2,3,4,2',4',5'-Hexachlorobiphenyl	0.03	0.03	0.006

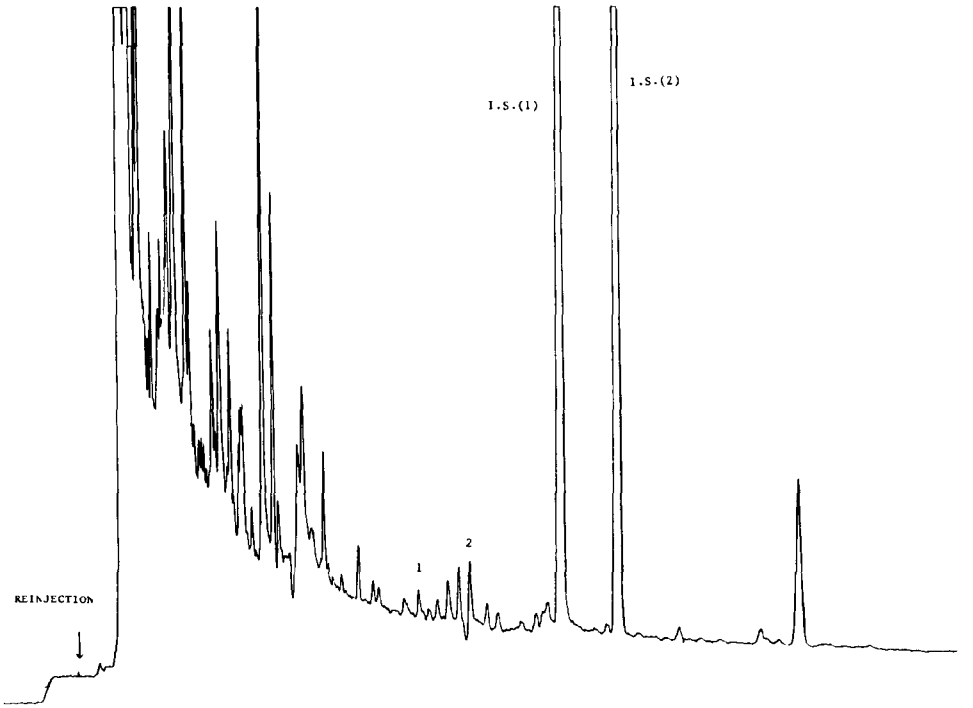

Fig. 5. Chromatogram of a fat sample extract, after GPC clean-up, with MUSIC and without extra concentration. Injection volume, 50 μl = 0.5 mg of fat. Peaks: 1 = PCB 153 (7 μg/kg); 2 = PCB 138 (14 μg/kg); I.S.(1) = internal standard (hexabromobenzene); I.S.(2) = internal standard (methoxychlor).

pounds in isooctane. No correction was made for internal standards. In Table IV the coefficients of variation are given for several compounds. No significant differences were observed when the MUSIC system was used.

Practical samples

Gel permeation chromatography (GPC) is a popular clean-up procedure in residue laboratories[8]. We prefer to use ethyl acetate extraction[6], concentration to one tenth of the volume and then GPC clean-up. When analysing wheat, the fraction thus obtained contained 0.85 mg of sample in 20 ml of eluent (ethyl acetate–cyclohexane). As the detection limit for a number of pesticides in wheat should be below 0.01 ppm (the tolerance is often 0.01 ppm), the fraction obtained must normally be further concentrated.

Fig. 4 shows a chromatogram obtained after injection of 100 μl of an unconcentrated extract of a wheat sample using the MUSIC system. The quantitative results are in agreement with those obtained without MUSIC and the pre-column, after concentration of the end fraction.

GPC was also used for analysing animal fats for organochlorine pesticides and chlorobiphenyls. Table V indicates the levels that are detectable when no further concentration is carried out. In relation to the action levels (50% of maximum residue

limit), it is necessary to concentrate the end extract of four compounds to at least one fifth of its volume. Using the MUSIC system, the desired detection limit can easily be obtained by injecting 50 μl of sample extract. Fig. 5 shows a chromatogram of a fat sample.

CONCLUSION

It has been demonstrated that the MUSIC system did not influence the detector linearity or solute adsorption when inactive fused-silica capillary columns were used. Large-volume injection (up to 400 μl) is possible without peak broadening. It could be shown by using practical samples (wheat, fats) that the detection limit could be improved without concentrating the final extract.

REFERENCES

1 K. Grob, Jr., D. Fröhlich, B. Schilling, H. P. Neukom and P. Nägeli, *J. Chromatogr.*, 295 (1984) 55.
2 J. V. Hinshaw and W. Seferovic, *6th International Symposium on Capillary Chromatography, 14–16th May, 1985, Riva del Garda*, Hüthig, Heidelberg, 1985, pp. 213–225.
3 W. Vogt, K. Jacob, A. B. Ohnesorge and H. W. Obwexer, *J. Chromatogr.*, 186 (1979) 197.
4 D. R. Deans, *Chromatography*, 1 (1967) 28.
5 *Chrompack News Special*, 85-3 (1985) 1.
6 L. G. M. Th. Tuinstra, A. H. Roos and F. Nab, *Anal. Chim Acta*, to be published.
7 L. G. M. Th. Tuinstra and W. A. Traag, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 723.
8 W. Specht, M. Tillkes, *Fresenius Z. Anal. Chem.*, 322 (1985) 443.