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NARROW-BORE NORMAL PHASE LIQUID CHROMATOGRAPHY WITH ON-LINE ELECTRON-CAPTURE DETECTION

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SUMMARY

Miniaturization of an on-line liquid chromatography–electron-capture detection system has been achieved by using a suitable micropump, and a 40-μm I.D. fused-silica capillary as evaporation interface. The baseline stability is high and extra-column band broadening is comparable to that in conventional systems. The detection limits for electron-capture-sensitive compounds are of the order of 1 pg, and calibration plots are linear over three orders of magnitude. The system has successfully been used for gradient elution with hexane–toluene mixtures on an amino-bonded phase.

INTRODUCTION

In the past decade, the potential of column liquid chromatography (LC) has become equivalent to that of gas chromatography (GC). It is in many respects complementary to GC. However, the sensitivity and selectivity of detection are often considered a weak point of LC. One way to improve this situation is the on-line coupling of LC separation columns and GC detectors; on-line LC–mass spectrometry and LC–electron-capture detection (LC–ECD) have been notably successful[1,2].

The first promising results with a normal phase LC–ECD chromatography system were published over 10 years ago[3]. More recently, its potential has been considerably enhanced by, *e.g.*, the design of an improved interface[4], the use of up to about 15% of dioxane as organic modifier[5] and the use of toluene instead of hexane as mobile-phase component[6]. In all these systems, isocratic elution was used, and the detection limits for ECD-sensitive compounds such as chlorinated pesticides were typically 10–50 pg. In the past 2 years, the first results on reversed-phase LC–ECD have been published[6,7]; detection limits for favourable compounds again are 10–50 pg. However, narrow-bore (about 1 mm I.D.) columns have to be used for reversed-phase LC–ECD. In other words, the injection volumes are much smaller than in conventional (4.6 mm I.D.) LC and the concentration detection limits are correspondingly not as good.

A disadvantage of conventional (normal phase) LC–ECD is its relatively high

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operating cost, due to the fact that eluents prepared from high-quality solvents must be used in order to keep the baseline sufficiently low. Our recent experience with narrow-bore reversed-phase LC–ECD prompted us to test a narrow-bore normal phase LC–ECD system. It is well known that many problems in micro-LC are caused by extra-column band broadening due to insufficient optimization of, *e.g.*, post-column reactors or detectors. In the present study, special attention was devoted to the sensitivity of the system, and to the design of the interface (low dead volume, rapid evaporation of even high-boiling compounds) and to the introduction of gradient elution in LC–ECD.

MATERIALS AND METHODS

The LC system consisted of a MPLC Micropump (Brownlee, Santa Clara, CA, U.S.A.), a home-made injection valve with two loops (60 and 560 nl), a 20 cm × 0.7 mm I.D. GLT column home-packed with 5-μm Spherisorb-NH_2 (Merck, Darmstadt, F.R.G.) and a Pye Unicam ^{63}Ni electron-capture detector (Philips, Eindhoven, The Netherlands), which is identical with the constant-current detector used in Pye Unicam GC equipment. The interface consisted of a massive stainless-steel block through which a straight groove had been drilled, in which a 10 cm × 0.25 mm I.D. stainless-steel capillary was inserted. This capillary is coaxial with a 30 cm × 0.17 mm O.D. fused-silica capillary with an I.D. of 40 μm (Techmation, Utrecht, The Netherlands) which is coupled to the detector via a seal similar to that used in capillary GC–ECD. The fused-silica capillary was coupled to the column with a home-made PTFE ferrule. The interface and detector were kept at a temperature of 300°C, unless indicated otherwise. For optimum LC–ECD operation, a stream of oxygen-free nitrogen make-up gas was used at a flow-rate of 40 ml/min.

During the experiments with gradient elution, a mixer, comprising a 10 cm × 3.2 mm I.D. stainless-steel tube packed with 100-mesh glass beads, was inserted between the pump and the injection valve.

Nanograde hexane (Mallinckrodt, St. Louis, MO, U.S.A.) and analytical grade toluene (Baker, Deventer, The Netherlands) were purified by treatment with a dispersion of 45% sodium in paraffin (Fluka, Buchs, Switzerland) and subsequent distillation.

RESULTS AND DISCUSSION

Micropump performance and detection limits

The stability of the solvent flow delivered by the Brownlee Micropump was tested by connecting the pump directly to the evaporation interface, without inserting either a damper or an analytical column in between. At flow-rates of 1–50 μl/min the baseline stability was excellent, even for prolonged periods. The noise level was *ca.* 1% of full-scale deflection at attenuation ×2 (detector current, 1×10^{-10} A), which is about four times better than observed in conventional LC–ECD. At a detector current of $7 \cdot 10^{-10}$ A, corresponding to half of the run-away current and, therefore, the recommended setting when using a Pye Unicam electron-capture detector in combination with GC, the noise level was two times higher. Insertion of the analytical column, packed with an amino-bonded stationary phase, between the pump and the

interface caused a further slight increase in the noise level. The signal-to-noise ratios and, thus, the sensitivity for some test compounds were slightly better at a detector current of $7 \cdot 10^{-10}$ A than at $1 \cdot 10^{-10}$ A.

The above experiments were performed with hexane as the eluent. For example, the determination of thirteen organochlorine pesticides is shown in Fig. 1. The detection limits vary from 200 fg for *p,p'*-DDE to 1 pg for the very late eluting dieldrin (signal-to-noise ratio, 3:1). The chromatogram obtained after an 800-fg injection of *p,p'*-DDE is shown in Fig. 2. Calibration plots were found to be linear over three orders of magnitude, *i.e.*, in the whole pg range tested.

The 0.2–1 pg detection limits recorded for the present narrow-bore (0.7 mm I.D.) LC system are about 50 times lower than those found earlier in conventional LC–ECD with a 4.6 mm I.D. column. Both types of system were operated under roughly comparable conditions, *i.e.*, with the flow-rate and the injection volume being about $(4.6/0.7)^2$ or 50-fold lower in the miniaturized version. The concentration detection limits are therefore the same in both systems. In other words, contrary to what is often observed in modern LC, miniaturization has *not* caused a loss in sensitivity.

Interface performance

High-boiling compounds. Earlier studies[3,8] showed that the Pye Unicam interface gave satisfactory results for compounds with not too high boiling points. Severe tailing occurred with, *e.g.*, decachlorobiphenyl (DCB). Highly chlorinated terphenyls and decabromobiphenyl (DBB) were not detected[4]. These problems were eliminated

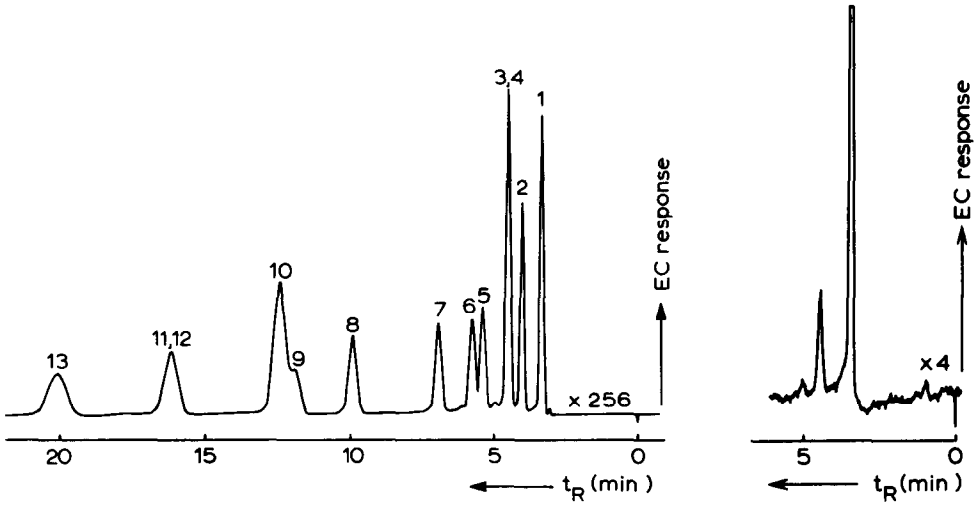

Fig. 1. Normal phase LC–ECD chromatogram of thirteen organochlorine pesticides in the system Spherisorb-NH_2/hexane. Column: 20 cm × 0.7 mm I.D. Flow-rate: 25 µl/min. Attenuation: × 256 at a detector current of $7 \cdot 10^{-10}$ A. Detector temperature: 300°C. Solutes: 1 = HCB; 2 = aldrin; 3 = *p,p'*-DDE; 4 = heptachlor; 5 = *o,p'*-DDE; 6 = *o,p'*-DDT; 8 = α-HCH; 9 = *p,p'*-DDD; 10 = endosulfan; 11 = γ-HCH; 12 = heptachlor epoxide; 13 = dieldrin. Volume injected; 60 nl. Amount injected; 100 pg of each compound.

Fig. 2. Normal phase LC–ECD chromatogram for 800 fg of *p,p'*-DDE (peak at 4.5 min). Conditions as in Fig. 1.

TABLE I

PEAK WIDTH AT 10% OF PEAK HEIGHT, $w_{0.1}$, ASYMMETRY FACTOR, B/A, SECOND MOMENT, $\bar{M}_2$, AND PEAK VARIANCE, σ^2, OF COMPOUNDS WITH A CAPACITY RATIO OF 0.7 IN THE SYSTEM SPHERISORB-NH_2/HEXANE (25 µl/min) USING ELECTRON-CAPTURE DETECTION

$k' = (t_R - t_0)/t_0$; T_{det} = temperature of interface and detector.

Compound	T_{det} (°C)	$w_{0.1}$ (s)	B/A	$\bar{M}_2$ (s^2)	σ^2 (s^2)
DDB	260	196	11	320	–
	300	54	2.6	270	–
	330	34	1.4	73	55
o,p'-DDE	300	24	1.1	34	32
	330	23	1.1	32	31
o,p'-DDT	300	25	1.1	36	35
	330	24	1.1	34	32

after a new interface with a more efficient heat transfer had been designed[4]. In that work, DBB turned out to be the most difficult compound. Measurements were done with conventional LC coupled to an UV detector and an electron-capture detector and the calculations were performed by using the law of additivity of variances. The effects of peak asymmetry were not considered.

In this study the peak observed for DBB was compared with those for two compounds with much lower boiling points, but having approximately the same retention ($k' = 0.7$) in the system Spherisorb-NH_2/hexane. For this purpose, *o,p'*-DDE and *o,p'*-DDT were selected. Because all three test compounds have the same chromatographic band broadening, the additional band broadening of DBB caused in the interface can be estimated by comparing its peak width with that of the other solutes. Secondly, it is well known that most peaks do not have the ideal gaussian shape, and that this leads to dramatic errors when calculations are made via simple variance measurements[9]. Therefore, the calculations were done using the second moment of the peaks, $\bar{M}_2$,

$$\bar{M}_2 = \frac{w_{0.1}^2}{1.76(B/A)^2 - 11.15(B/A) + 28} \quad (1)$$

where $w_{0.1}$ is the width of a peak at 10% of the peak height, and A and B are the widths of the front and rear sides of a peak at 10% of the peak height, respectively. For asymmetries of between 1.00 and 2.76, the relative error limits of $\bar{M}_2$ are smaller than 1.5%[10].

The results of the measurements, which were carried out in triplicate, are reported in Table I. For DBB, the interface plus detector temperature was varied from 260 to 330°C, which is about the maximum allowable, while it was varied from 300 to 330°C for the compounds with lower boiling points. It is seen that the interface temperature strongly influences the peak shape of DBB, while it has a negligible effect in the cases of *o,p'*-DDE and *o,p'*-DDT. Calculation via the $\bar{M}_2$ data of the additional band broadening of DBB due to its relatively slow evaporation results in

TABLE II

MELTING, M.P., AND BOILING, B.P., POINTS, PEAK WIDTHS AT 10% OF PEAK HEIGHT, ASYMMETRY FACTORS AND SECOND MOMENTS AFTER FLOW INJECTION IN THE SYSTEM SPHERISORB-NH_2/HEXANE (25 μl/min) USING ELECTRON-CAPTURE DETECTION AT 300°C

Compound	*M.P. (°C)*	*B.P. (°C)*	$w_{0.1}$ *(s)*	*B/A*	$\bar{M}_2(s^2)$
4-Nitrotoluene	54	238	9.6	1.7	6.6
4-Nitroaniline	149	332	17.2	3.3	28.3
2,4-Dinitrophenol	115	sublimes	8.8	2.0	6.0
2,4-Dichloroaniline	60	245	9.5	2.1	7.2
Pentachloroaniline	233		9.0	1.7	5.8
Pentachlorophenol	189	310	17.0	5.0	15.0
o,p'-DDE	89		9.2	2.1	6.8
o,p'-DDT	109	260	9.1	1.8	6.0
Decachlorobiphenyl	305	440	9.6	2.0	7.2
Decabromobiphenyl	385		16	5	15

a value of 38 s^2 (at a detector temperature of 330°C) as opposed to 21 s^2 if simple variance measurements are used. The extra band broadening of DBB in conventional LC was *ca.* 15 s^2, using variance measurements, at a detector temperature of 300°C.

Interface plus detector. For the evaluation of the general performance of the combination of the interface and detector, a flow-injection set-up was used, and second moments were calculated for the peaks of ten test solutes. The results obtained at an interface plus detector temperature of 300°C are reported in Table II. All measurements were made in triplicate and showed quite acceptable repeatability (relative S.D. of less than 5% in most cases). Apart from three exceptions (see below), the test solutes are seen to yield $\bar{M}^2$ values of 6–7 s^2, comparable with the band broadening reported earlier[4] for conventional LC–ECD (σ_t^2 = 7 s^2). In other words, the total contribution to the volume variance made by the present interface and detector is about 1 μl^2 at an eluent flow-rate of 25 μl/min. This is quite acceptable compared to the behaviour of other detectors for miniaturized LC which give contributions of 0.7–1.5 μl^2 at the quoted flow-rate[11,12].

As for the exceptionally high $\bar{M}_2$ values observed for 4-nitroaniline and pentachlorophenol (although the high peak asymmetry invalidates the use of eqn. 1), these obviously cannot be explained by evaporation problems due to high boiling points, as in the case of DBB (see previous section). Adsorption on the wall of the fused-silica capillary seems a more probable explanation, as is suggested by Fig. 6 in ref. 7 for pentachlorophenol, although this figure, admittedly, refers to a reversed-phase LC system. This problem is presently under investigation.

Gradient elution in LC–ECD

For the analysis of mixtures containing compounds which vary widely in polarity, gradient elution is often recommended. So far, no work has been published on the use of gradient elution for on-line LC–ECD. It was thought of interest to carry out a preliminary study with the Micropump, which with a dual syringe solvent-delivery system, should be capable of performing accurate and precise gradient analysis in conjunction with narrow-bore columns[12].

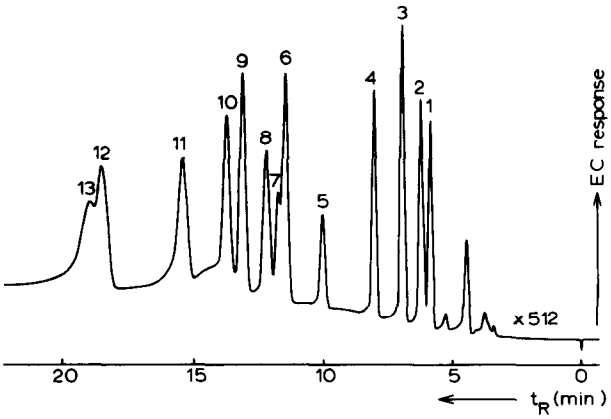

Fig. 3. Normal phase LC–ECD gradient separation of thirteen chloroanilines in the system Spherisorb-NH_2/hexane–toluene. Gradient (25 µl/min); 0 to 50% toluene in 10 min; subsequently isocratic for 15 min. Column: 20 cm × 0.7 mm I.D.; Attenuation: × 512 at a detector current of $7 \cdot 10^{-10}$ A. Detector temperature: 300°C. Anilines: 1 = 2,6-dichloro-; 2 = 2,4,6-trichloro-; 3 = 2,3,5,6-tetrachloro-; 4 = pentachloro-; 5 = 2,5-dichloro-; 6 = 2,4,5-trichloro-; 7 = 2,4-dichloro-; 8 = 2,3-dichloro-; 9 = 2,3,4,5-tetrachloro-; 10 = 2,3,4-trichloro-; 11 = 3,5-dichloro-; 12 = 2,3,5-trichloro-; 13 = 3,4-dichloro-. Volume injected: 60 nl. Amount injected: 0.2–100 ng per compound.

For example, the separation of thirteen chloroanilines is shown in Fig. 3. Taking into account a 10-min delay caused by the mixer described in Materials and Methods, immediately after injection of the sample a linear gradient from pure hexane to hexane–toluene (50:50) at a total flow-rate of 25 µl/min was started. Subsequently, the system was operated under isocratic conditions for 15 min. Because of the difference in response of the detector to toluene and hexane, the baseline increased to 10% of full-scale deflection at an attenuation of × 512 (detector current, $7 \cdot 10^{-10}$ A) during the 10-min linear gradient. On the one hand, this allows an easy monitoring of the gradient profile; on the other hand, unless the rise of the baseline is compensated for, either electronically or by flow programming of the make-up gas, the lowest attenuator setting that can be used is × 128. This of course detracts from the sensitivity of the analysis. It is important to note that the rise in baseline is caused by the increasing proportion of toluene in the eluent and, thus, the increasing amount of toluene directed to the detector. It is evident, therefore, that such a gradient cannot be used in conventional LC–ECD, unless one of the compensation techniques is adopted.

The chloroaniline separation was mainly selected as an interesting example of multicomponent analysis. Still, it is interesting that the retention on the amino-bonded phase is determined by both the number of chloro substituents and, even more so, their position. As regards the latter, substitution closer to the amino group is seen to decrease retention (steric hindrance), the best example being provided by 2,6-dichloroaniline (eluted first) *versus* 3,4-dichloroaniline (eluted last).

The detection limits for the various chloroanilines depend rather strongly on the number and position of the substituents (as is also the case for, *e.g.*, polychlorinated biphenyls), and on their retention. Under isocratic conditions and with hexane–toluene mixtures as eluents, the detection limits varied from 10 pg for di-

chloroanilines to 0.2 pg for pentachloroaniline. Obviously, in terms of sensitivity, electron-capture detection can easily compete with other modern modes of detection such as post-column derivatization with fluorescence monitoring and electrochemical detection.

CONCLUSIONS

Miniaturization of a normal phase LC–ECD system has been accomplished successfully. An important aspect is the miniaturization of the interface which now houses a 40-μm I.D. fused-silica capillary. Its contribution (inclusive of the detector) to band broadening has a volume variance of about 1 μl^2 at an eluent flow-rate of 25 μl/min, which is quite acceptable. The heat transfer to this fused-silica capillary is efficient, and at an interface temperature of 300–330°C, evaporation problems only occur with very high-boiling compounds, such as decabromobiphenyl.

For favourable compounds the detection limits are of the order of 0.2–1 pg, and calibration plots are linear over three orders of magnitude. Compared to conventional normal phase LC–ECD, the minimum detectable amounts are about 50 times lower. This is partly due to a 2–4 fold decrease in noise level, but the main improvement (10–20 fold) is caused by the fact that the total flow of gas (nitrogen plus eluent) through the detector is much lower in the narrow-bore as compared to the conventional system used by us. From the results we conclude that the electron-capture detector for normal phase LC behaves as a concentration-sensitive detector.

The possibility of gradient elution is demonstrated by the analysis of thirteen chloroanilines using a hexane–toluene gradient. For sensitive detection, compensation for the rise of the baseline is necessary.

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REFERENCES

1 D. M. Desiderio and G. H. Fridland, *J. Liq. Chromatogr.*, 7 (1984) 317.
2 I. S. Krull, E. A. Davis, C. Santasania, S. Kraus, A. Basch and Y. Bamberger, *Anal. Lett.*, 13A (1980) 1277.
3 F. W. Willmott and R. J. Dolphin, *J. Chromatogr. Sci.*, 12 (1974) 695.
4 A. de Kok, R. B. Geerdink and U. A. Th. Brinkman, *J. Chromatogr.*, 252 (1982) 101.
5 U. A. Th. Brinkman, A. de Kok and R. B. Geerdink, *J. Chromatogr.*, 283 (1984) 113.
6 U. A. Th. Brinkman, R. B. Geerdink and A. de Kok, *J. Chromatogr.*, 291 (1984) 195.
7 F. A. Maris, R. B. Geerdink and U. A. Th. Brinkman, *J. Chromatogr.*, 328 (1985) 93.
8 R. J. Dolphin, F. W. Willmott, A. D. Mills and L. P. J. Hoogeveen, *J. Chromatogr.* , 122 (1976) 259.
9 J. J. Kirkland, W. W. Yau, H. J. Stoklosa and C. H. Dilks, *J. Chromatogr. Sci.*, 15 (1977) 303.
10 J. P. Foley and J. G. Dorsey, *Anal. Chem.*, 55 (1983) 730.
11 R. P. W. Scott and C. F. Simpson, *J. Chromatogr. Sci.*, 20 (1982) 62.
12 H. Schwartz and R. G. Brownlee, *Am. Lab. (Fairfield, Conn.)*, 16 (1984) 43.