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NORMAL- AND REVERSED-PHASE LIQUID CHROMATOGRAPHY WITH ON-LINE ELECTRON-CAPTURE DETECTION

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SUMMARY

As a continuation of earlier work, the potential of high-performance liquid chromatography (LC) with on-line electron-capture detection (ECD) has been further explored. For normal-phase LC it is demonstrated that the use of toluene instead of hexane as main mobile phase component has distinct advantages because it allows the rapid elution of even fairly polar model compounds. For reversed-phase systems, on-line LC–ECD is shown to be feasible if miniaturized equipment (1 mm I.D. columns; flow-rates of *ca.* 50 μl min^{-1}) is used. Preliminary experiments indicate that reversed-phase LC combined with an on-line post-column extraction module may be an interesting alternative approach in reversed-phase LC–ECD.

INTRODUCTION

Today, it is generally acknowledged that the potential of high-performance liquid chromatography (LC) is fully comparable to that of gas chromatography (GC). A distinct drawback of the former technique is, however, that LC detectors have not yet reached the sensitivity level of GC detectors. Various workers have advocated the use of electron-capture detection (ECD) in LC, and their attempts have met with some success (see, *e.g.*, refs. 1–5). It can be said that, today, interfacing the LC column and the electron-capture detector no longer constitutes a real problem; with modern development being in the direction of smaller bore columns and lower flow-rates, neither does the handling, by the detector, of the flow of solvent emerging from the LC column. One major problem still exists, however: at present, polar mobile phases such as are necessary in reversed-phase LC, and even non-polar mobile phases containing a high proportion of a polar organic modifier, cannot be employed in LC–ECD.

This paper deals with the further exploration of the types of mobile phase compatible with ECD, in order to define more precisely the range of applicability of on-line LC–ECD.

EXPERIMENTAL

Methods

The conventional-scale LC system consisted of an Orlita (Giessen, F.R.G.) Model FE 034 sRC reciprocating pump, a Valco (Houston, TX, U.S.A.) six-port injection valve with a 25-μl loop, a 25 cm × 4.6 mm I.D. stainless-steel column pre-packed with 8-μm CP-Spher-Si or 10-μm LiChrosorb 10 NH$_2$ (Chrompack Nederland, Middelburg, The Netherlands), a Pye Unicam (Philips, Eindhoven, The Netherlands) ^{63}Ni electron-capture detector (for GC, mounted in the commercial interface/detector oven). The effluent from the LC column passed through a Valco tee-piece of zero dead volume, a suitable portion (30–50%) of the eluent being directed to the detector via the vaporization interface, which consisted essentially of a 75 cm × 0.25 mm I.D. capillary enclosed in a massive stainless-steel tube and kept at 250–300°C[4]. A stream of oxygen-free nitrogen purge gas was used for optimum LC-ECD operation, *viz.*, to suppress the background signal. The purge gas flow-rate varied from 15 ml min^{-1} with pure *n*-hexane as the mobile phase, through 50 ml min^{-1} for *n*-hexane–toluene (50:50) and pure toluene, to 100 ml min^{-1} for toluene containing 5% of acetonitrile.

For micro-LC, a Gilson (Villiers-le-Bel, France) Model 302 pump, which can deliver flow-rates between 5 μl min^{-1} and 5 ml min^{-1}, a Rheodyne (Berkeley, CA, U.S.A.) six-port valve with a 1-μl loop, a 10 cm × 1 mm I.D. glass-lined stainless-steel column home-packed with 5-μm Hypersil ODS and the same electron-capture detector as reported above were used. In this instance, the vaporization interface was a 30 cm × 0.25 mm I.D. capillary made of nickel tubing (Valco). It was mounted in a stainless-steel block similar to the above and kept at 300°C. The purge gas flow-rate was about 100 ml min^{-1}.

Materials

Reagents were of analytical-reagent grade unless indicated otherwise.

Toluene and HPLC-grade *n*-hexane (J. T. Baker Chemicals, Deventer, The Netherlands) were purified by treatment with a dispersion of 45% sodium in paraffin (Fluka, Buchs, Switzerland) and subsequent distillation. Acetonitrile (E. Merck, Darmstadt, F.R.G.) was distilled immediately prior to use; methanol (J. T. Baker) was used as received. Heptafluorobutyric anhydride (HFBA) was obtained from E. Merck and used for derivatization according to a published procedure[6] without further purification.

All compounds used as test solutes were commercially available analytical-reagent grade products. The technical polychlorinated biphenyl mixtures Aroclor 1254 and 1260 were purchased from Analabs (North Haven, CT, U.S.A.).

RESULTS AND DISCUSSION

Non-polar mobile phase

It has repeatedly been shown[1–4] that non-polar solvents such as *n*-hexane and isooctane can conveniently be used as mobile phases in adsorption LC with on-line ECD. As for the addition of organic modifiers, initial work by Chamberlain and Marlow[2] showed dioxane to be a good choice. In our laboratory, *n*-hexane contain-

ing 6% of dioxane and, more recently, 10–25% of this modifier have been used successfully for several separations[4,6,7]. The examples reported in the literature cited also emphasize the double advantage that derivatization can have: if carried out with a suitable electron-capture reagent, the less polar reaction product will be more easily eluted within the range of mobile phase compositions tolerated in LC–ECD, and simultaneously display increased sensitivity towards ECD.

In this study project, acetonitrile was thought to be another attractive modifier, as much lower percentages of acetonitrile compared with dioxane will be required to cause the same reduction in the capacity ratio of solutes of interest. For a number of test compounds it was indeed observed that n-hexane containing 2% of acetonitrile has the same effect as n-hexane with 10% of dioxane added. Unfortunately, however, acetonitrile is only sparingly soluble in n-hexane and, even with 2% of acetonitrile, mixing in an ultrasonic bath is required. Hence the use of acetonitrile as a modifier for n-hexane is not a promising alternative.

The results presented above demonstrate that, for adsorption LC–ECD, the "elution problem" has been solved with a fair measure of success. Still, it is of course highly desirable to find a solvent more polar than the alkanes that is compatible with ECD even if used as the main mobile phase component. A scrutiny of the literature on LC–ECD and several series of test experiments revealed only one possibility, viz., toluene. Toluene has been used in a single study[5] on the analysis of explosives by means of LC–ECD on, e.g., NH_2- and CN-bonded phases. Surprisingly, the authors

TABLE I

CAPACITY RATIOS AND DETECTION LIMITS OF SUBSTITUTED PHENOLS, CHLOROHY-DROXYBIPHENYLS AND NITROAROMATICS IN THE LC SYSTEM SILICA/HEXANE-TOLUENE–ACETONITRILE USING ELECTRON-CAPTURE DETECTION

Type	Compound	Capacity ratio* with n-hexane–toluene–acetonitrile			Approx. detection limit (pg)**
		50:50:0	0:100:0	0:95:5	
Phenols	2,4-Di-Cl	2.8	1.1	1.1	60
	2,3,4-Tri-Cl	3.0	1.1	1.2	5
	Penta-Cl	2.3	0.7	0.7	10
	4-NO_2		9.1	3.2	10
	2,4-Di-NO_2	4.8	1.7	0.7	35
Biphenyls	2-Cl-4-OH	3.2	1.1	0.8	70
	3,5-Cl-2-OH	1.5	0.8	0.5	15
	3,5-Cl-4-OH	2.0	1.0	0.8	20
	3,4',5-Tri-Cl-4-OH	2.0	0.9	0.6	10
	4,4'-Di-Cl-3,3'-di-OH		6.5	2.5	10
	3,3'-Di-Cl-4,4'-di-OH		3.0	1.4	10
	3,3',5,5'-Tetra-Cl-4,4'-di-OH	8.6	2.2	1.0	5
Nitroaromatics	4-Nitroaniline		7.5	2.8	10
	4-Nitrotoluene	1.5			65
	1,3-Dinitrobenzene	4.1	1.3		160
	1-Chloro-4-nitrobenzene	1.0			25
	1,5-Dinitronaphthalene	2.8	1.0		25

* $k' = (t_R - t_0)/t_0$.
** For toluene or toluene–acetonitrile (95:5) as mobile phase.

concerned did not compare the compatibility of toluene and alkanes with ECD. We have now further explored the potential of toluene in adsorption LC–ECD using polar compounds such as chlorophenols, hydroxylated chlorobiphenyls and nitroaromatics as test solutes.

Results for capacity ratios and detection limits for seventeen compounds are presented in Table I. Chromatography was carried out in the conventional-scale system with silica as the stationary phase. It is evident that all three groups of compounds tested can be readily eluted from silica with one or more of the toluene-based mobile phases. This is a very favourable result because with *n*-hexane as the mobile phase, compounds such as 4-nitrophenol, the 3,5-dichloromonohydroxybiphenyls and 4-nitroaniline elute with capacity ratios of at least about 20. Similar results were obtained with a series of phenylurea herbicides (N^1-methyl-N^1-R-N^3-aryl ureas with R = alkyl or alkoxy; see, *e.g.*, ref. 7). These compounds and their direct derivatives with HFBA all elute with $k' < 10$ when using pure toluene and often also toluene–*n*-hexane (50:50) as the mobile phase. As regards speed of elution, in various cases this indicates a marked improvement over earlier results[7] with *n*-hexane containing 3–6% of dioxane.

For the rest, detection limits of 10–100 pg for almost all of the compounds in Table I and the various HFBA derivatives not only indicate satisfactory ECD sensitivity but also good baseline stability and a low noise level. Indeed, the noise level observed with pure toluene, of *ca.* 1% of full-scale deflection at attenuation × 8 (detection current, $1 \cdot 10^{-10}$ A), is similar to that recorded with *n*-hexane.

Experiments were also performed with an NH_2-bonded phase instead of silica, but using the same mobile phases. The retention was generally 2–6-fold higher on the chemically bonded phase, with the order of elution occasionally being different from that on silica. The detection limits as a rule were 10-fold higher than those reported for LC on silica in Table I. This can largely be explained as a combined effect of the differences in k' values and a higher noise level observed with the NH_2-bonded phase, *viz.*, 1% of full-scale deflection at attenuation × 32 (detection current, $1 \cdot 10^{-10}$ A).

The drugs flurazepam and diazepam were run in the system NH_2-bonded phase–toluene, with k' values of 1.6 and 3.7, respectively. Their detection limits were in the 100–200-pg range.

Polar mobile phases

The promising results obtained in the adsorption LC studies with toluene led us to explore an earlier suggestion[4,8] of inserting an extraction reactor between the outlet of the LC column and the detector inlet in order to extract the solutes of interest from a polar LC effluent into a less polar solvent compatible with ECD. For reasons of convenience, preliminary experiments were performed by shaking toluene or a toluene–*n*-hexane mixture with an equal volume of methanol–water (90:10) (exemplifying the mobile phase for reversed-phase LC) and using the non-polar phase, after separation of the layers, as the eluent for LC–ECD. As the actual mobile phase was therefore relatively nonpolar, both chemically bonded phases and bare silica could be used as stationary phases. The experiments were carried out in the conventional-scale LC system but the flow-rates were lowered to about 100 μl min^{-1}.

Satisfactory results were obtained with the above-mentioned mobile phases with, for example, a dihydroxydichlorobiphenyl on a silica column, a number of di-
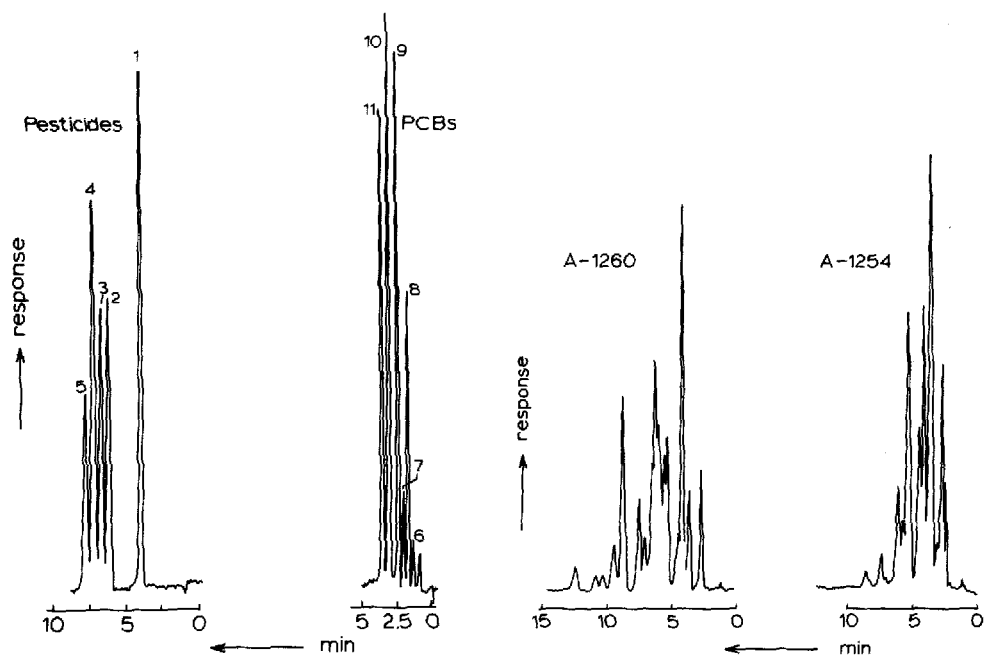
Fig. 1. Reversed-phase LC-ECD traces for five pesticides and six polychlorinated biphenyls (PCBs) in the system Hypersil ODS/methanol–water (85:15). Flow-rate, 50 μl min^{-1}. Attenuation, $\times 512$ (pesticides) or $\times 256$ (PCBs) at a detector current of $1 \cdot 10^{-10}$ A; oven temperature, 300°C. Pesticides: 1 = heptachlor epoxide; 2 = heptachlor; 3 = o,p'-DDE; 4 = p,p'-DDT; 5 = o,p'-DDT. PCBs: 6 = 4-monochloro-; 7 = 2,6,2',6'-tetrachloro-; 8 = 2,2'-dichloro-; 9 = 4,4'-dichloro-; 10 = 2,4,4'-trichloro-; 11 = 2,4,5-trichlorobiphenyl. Volume injected, 1 μl; amounts injected, 1–10 ng per compound.

Fig. 2. Reversed-phase LC-ECD traces of the technical PCB mixtures Aroclor 1254 and Aroclor 1260 in the system Hypersil ODS/methanol–water (85:15). Flow-rate, 50 μl min^{-1}. Attenuation, $\times 512$ at a detector current of $1 \cdot 10^{-10}$ A; oven temperature, 300°C. Volume injected, 1 μl; amounts injected, 10 ng of each mixture.

to penta-substituted chlorophenols on an NH$_2$- and a C$_2$-bonded phase and several phenylurea herbicides on a C$_{18}$-bonded phase. The emphasis in these cases should of course *not* be on the chromatography of the test solutes on the various columns, but rather on the fact that the use of a toluene-*n*-hexane mixture shaken with methanol–water can be tolerated in LC-ECD.

In some of the initial experiments the separation of the layers was sluggish so that the resulting "mobile phase" still contained water–methanol droplets. Even in such instances, however, the baseline stability of the detector was remarkably good. This observation led us to test a further possibility, *i.e.*, the direct introduction of a methanol–water LC effluent into the vaporization interface and, thus, the detector. Micro-LC equipment was now used, with flow-rates of typically 50 μl min^{-1}, the arbitrarily selected mobile phase being methanol–water (85:15). As the first experiments showed that, at 300°C, this mobile phase almost instantaneously attacks stainless steel, nickel tubing was employed in the vaporization interface. For reasons of convenience, a 30-cm straight capillary instead of the earlier 75-cm coiled capillary was used, and no attempt was made to optimize interface performance.
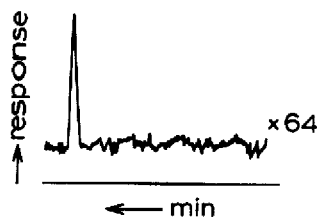
Fig. 3. Reversed-phase LC–ECD trace of an injection of 250 pg of 2,6,2′,6′-tetrachlorobiphenyl. Conditions as in Fig. 1.

Typical results are presented in Figs. 1 and 2. These show the separation of a number of organochlorine pesticides, several individual polychlorinated biphenyls and two technical mixtures of polychlorinated biphenyls. Obviously, LC–ECD can even be used under reversed-phase conditions and without a serious decrease in sensitivity, as is demonstrated in Fig. 3. The detection limit for 2,6,2′,6′-tetrachlorobiphenyl was calculated to be about 100 pg, and a linear calibration graph was obtained for this compound over a concentration range of 2–3 orders of magnitude.

CONCLUSION

In the literature LC–ECD is limited to chromatography in the adsorption mode using, with a single exception[5], *n*-hexane- or isooctane-based mobile phases. It has now been shown that replacing these alkanes with toluene offers considerable advantages. Toluene, which is a rather uncommon solvent in LC because of its unfavourable UV cut-off (285 nm), is more polar than the alkanes and therefore allows the rapid elution of many chlorophenols, chlorohydroxybiphenyls and other compounds of similar, high polarity. The baseline noise is low and satisfactory detection limits can be reached, the 10–100-pg values for a number of nitroaromatics comparing favourably with detection limits of about 1 ng reported in the literature[9].

Even more important, this work demonstrates that reversed-phase LC can, in principle, be combined with ECD. The main attraction of the on-line approach with miniaturized equipment is simplicity and the first results indicate that no serious problems seem to exist with hydrophobic analytes. Problems, however, may well turn out to be unsurmountable when more polar compounds are tested and buffers, ion-pairing agents, etc., have to be added to the mobile phase. In such situations, the use of an extraction module inserted between the outlet of the LC column and the detector inlet is a promising alternative: the relatively hydrophobic trace constituents of interest are transferred to a suitable organic phase, while the polar (in)organic ions remain in the aqueous layer. The admittedly few and still off-line experiments reported in this paper urge one also to pursue this line of approach in the further exploration of reversed-phase LC–ECD.

REFERENCES

1 F. W. Willmott and R. J. Dolphin, *J. Chromatogr. Sci.*, 12 (1974) 695.
2 A. T. Chamberlain and J. S. Marlow, *J. Chromatogr. Sci.*, 15 (1977) 29.
3 U. A. Th. Brinkman, P. M. Onel and G. de Vries, *J. Chromatogr.*, 171 (1979) 424.
4 A. de Kok, R. B. Geerdink and U. A. Th. Brinkman, *J. Chromatogr.*, 252 (1982) 101.
5 I. S. Krull, E. A. Davis, C. Santasania, S. Kraus, A. Bosch and Y. Bamberger, *Anal. Lett.*, 14A (1981) 1363.
6 U. A. Th. Brinkman, A. de Kok and R. B. Geerdink, *J. Chromatogr.*, 283 (1984) 113.
7 A. de Kok, R. B. Geerdink and U. A. Th. Brinkman, *Chromatographia*, 16 (1982) 237.
8 U. A. Th. Brinkman, in A. Zlatkis and C. F. Poole (Editors), *Electron Capture, Theory and Practice in Chromatography*, Elsevier, Amsterdam, 1981, Ch. 16.
9 I. S. Krull and D. Bushee, *Anal. Lett.*, 13A (1980) 1277.