Journal of Chromatography, 328 (1985) 93–100
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 674

SELECTION OF MOBILE PHASES FOR REVERSED-PHASE LIQUID CHROMATOGRAPHY WITH ON-LINE ELECTRON-CAPTURE DETECTION

F. A. MARIS*, R. B. GEERDINK and U. A. Th. BRINKMAN
Section of Environmental Chemistry, Department of Analytical Chemistry, Free University, De Boelelaan 1083, 1081 HV Amsterdam (The Netherlands)

(Received February 23rd, 1985)

SUMMARY

Reversed-phase column liquid chromatography with on-line electron-capture detection can conveniently be carried out if miniaturized equipment (1 mm I.D. columns) is used. As eluents, mixtures of methanol or dioxane and water are preferred to acetonitrile–water mixtures. With the former two modifiers, working at flow-rates of *ca.* 50 μl min^{-1} does not create any problems with mixtures containing between 0 and 50% water. At lower flow-rates, even pure water can be used as eluent. The addition to the mobile phase of small amounts of polar compounds such as phosphoric acid, can be tolerated. Examples of successful applications include the analysis of barbiturates, benzodiazepines, nitroaromatics, C_1–C_2 halogenated aliphatic hydrocarbons, and chlorophenols.

INTRODUCTION

In recent years, it has become evident that column liquid chromatography (LC) and gas chromatography (GC) are complementary rather than competitive techniques. It is a drawback of LC, however, that its detectors do not always match the selectivity, and sensitivity, of GC detectors. Repeated attempts have, therefore, been made to incorporate GC detection principles in LC instrumentation. The first successful combination of LC with on-line electron-capture detection (ECD) was reported[1] in 1974. It took several years before normal-phase LC–ECD with hexane or toluene (containing up to *ca.* 15% of dioxane as modifier) at a flow-rate of *ca.* 0.5 ml min^{-1} as eluent could be used[2–4] for routine operation. Detection limits for favourable analytes were shown to be in the 5–10 pg range.

In an early paper, Nota and Palombari[5] briefly mentioned that polar solvents such as methanol, acetone, and even water, can be used as eluents in LC–ECD, provided the flow-rate is sufficiently low (10 μl min^{-1}). In the next decade, however, no further work in this direction was published. Therefore, despite the successes mentioned in the previous paragraph, even about a year ago LC–ECD was still considered —and rightly so— a rather limited technique since it could not be used for the reversed-phase systems that are used in some 70% of all LC work done today.

0021-9673/85/$03.30

In 1984, first results were published[6] on the routine use of narrow-bore LC–ECD, utilizing 0.7 or 1.0 mm I.D. columns packed with C_{18}-modified silica, with methanol–water (85:15) at a flow-rate of 50 μl min^{-1} as eluent. Various mixtures of halogen-containing compounds were successfully separated, and for 2,2′,6,6′-tetrachlorobiphenyl a detection limit of 100 pg was reached.

This paper deals with the further exploration of the potential and limitations of reversed-phase LC–ECD. Attention is mainly devoted to the composition of the mobile phase and its influence on baseline noise. Several separations are shown to indicate the application range.

MATERIALS AND METHODS

Apparatus

The narrow-bore LC system consisted of a laboratory-made syringe pump, which can deliver flow-rates of between 10 μl min^{-1} and 1 ml min^{-1}, a laboratory-made micro injection valve with a 50- and a 500-nl loop, various laboratory-packed glass-lined stainless steel columns of 10–20 cm length and 1.0 mm I.D. packed with 5-μm LiChrosorb RP-18 (E. Merck, Darmstadt, F.R.G.), and a Pye Unicam (Philips, Eindhoven, The Netherlands) ^{63}Ni electron-capture detector. The effluent from the LC column was directed to the detector via a vaporization interface. Because clogging of the vaporization capillary occurred occasionally, especially in some of the studies on the addition of acids to the eluent, the design of the interface was slightly changed compared with the previous model. It essentially consists of a massive stainless-steel block through which a straight groove has been drilled, with a 20 cm × 0.25 mm I.D. nickel or stainless steel capillary inside. The block can easily be dismounted for rapid exchange of capillaries. A stream of oxygen-free nitrogen purge gas was used for optimum LC–ECD operation, to suppress the background signal, at a flow-rate of 100 ml min^{-1}. The temperature of the interface and detector was kept at 300°C and a detector current of $1 \cdot 10^{-10}$ A was used.

Chemicals

Acetonitrile and dioxane (J. T. Baker, Deventer, The Netherlands) were distilled immediately prior to use. HPLC-grade methanol and water (J. T. Baker) were used as received. The compounds used as test substances and all other chemicals were of analytical-grade quality.

RESULTS AND DISCUSSION

In our earlier paper[6] on the use of methanol–water mixtures as eluent in narrow-bore LC with on-line ECD, it was noted that, in the reversed-phase LC experiments, baseline noise was considerably higher than in hexane- or toluene-based normal-phase LC work. Consequently, detection limits for favourable analytes were of the order of 100 rather than 5–10 pg.

In the present study, initial experiments showed that the high baseline noise is not caused by irregular, or incomplete, vaporization of the polar eluent, nor by rapid changes in the flow-rate. Further work revealed that degassing is the main problem. After the laboratory-made syringe pump has been filled with an eluent that

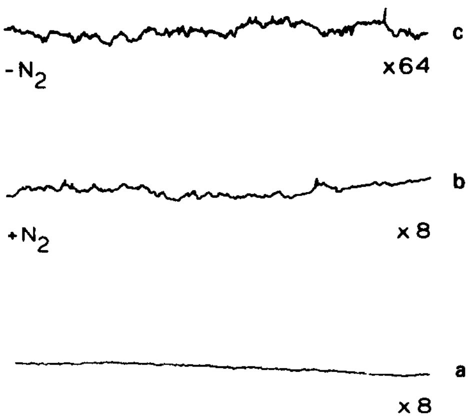

Fig. 1. Baseline noise levels for hexane at a flow-rate of 0.5 ml min^{-1} (a), and for methanol–water (80:20) at a flow-rate of 25 μl min^{-1} with (b) and without (c) additional degassing of the methanol–water mixture in the pump reservoir with nitrogen.

has been ultrasonically degassed, additional degassing of the eluent in the pump reservoir is required for optimum performance. To this end, a nitrogen gas purge is passed through the pump reservoir via a PTFE capillary for *ca.* 30 min. This suffices to reduce the baseline noise by about an order of magnitude, as is demonstrated in Fig. 1 for a methanol–water (80:20) mixture run at a flow-rate of 25 μl min^{-1}. The figure includes a typical baseline recorded in normal-phase LC with hexane as eluent, at 0.5 ml min^{-1}, using the same attenuation. Obviously, the 10–20-fold noise level difference referred to above can be reduced to a *ca.* four-fold difference by proper degassing.

In reversed-phase LC, methanol, acetonitrile and dioxane are well-known modifiers. In the present project, eluent mixtures containing each of these solvents and ranging in composition from pure organic solvent to organic solvent–water (50:50) were used for a prolonged time. The flow-rate was varied between *ca.* 10 and 150 μl min^{-1}, and two criteria were selected to assess the performance of the various eluents, *viz.* baseline noise and height of the baseline signal. The latter parameter has a semiquantitative rather than a real quantitative nature, hence one should realize that the electron-capture detector only works properly if a sufficient number of free electrons are present in the plasma around the ^{63}Ni foil. It is possible to increase this number by increasing the flow-rate of the nitrogen used as make-up gas. In our system, the maximum nitrogen flow-rate was 100 ml min^{-1}. When the flow of the organic–aqueous eluent becomes too high, not enough electrons remain in the plasma and, consequently, the signal from the constant-current detector is so high that measurements at low attenuation cannot be performed.

Table I lists for several eluents the maximum flow-rates that can be handled by the electron-capture detector when it is working at attenuation ×32. In Fig. 2, baseline noise levels are shown for the three organic solvents, and their 50:50 mixtures with water, at the same flow-rate of 25 μl min^{-1} in all cases. The combined data show that acetonitrile is a distinctly less suitable modifier than are dioxane and methanol, with the latter solvent giving the best overall results. For the rest, it is somewhat surprising that, on the addition of water to dioxane or methanol, the noise level

TABLE I

COMPARISON OF ELUENT FLOW-RATES ACCEPTABLE FOR LC–ECD

Condition, maximum flow-rate in on-line LC–ECD at attenuation × 32, using maximum back-off with nitrogen as make-up gas at a flow-rate of 100 ml min^{-1}.

Eluent	*Flow-rate (μl min^{-1})*
Dioxane	100
Acetonitrile	12.5
Methanol	150
Water	50
Dioxane–water (50:50)	75
Acetonitrile–water (50:50)	25
Methanol–water (50:50)	100

decreases (Fig. 2). From experience gained so far, we tentatively conclude that minimum noise seems to be observed at *ca.* 20% water.

Finally, one should also note that on various occasions pure water has been used as eluent at flow-rates of up to 50 μl min^{-1} and for at least several hours, without any adverse effect on detection performance.

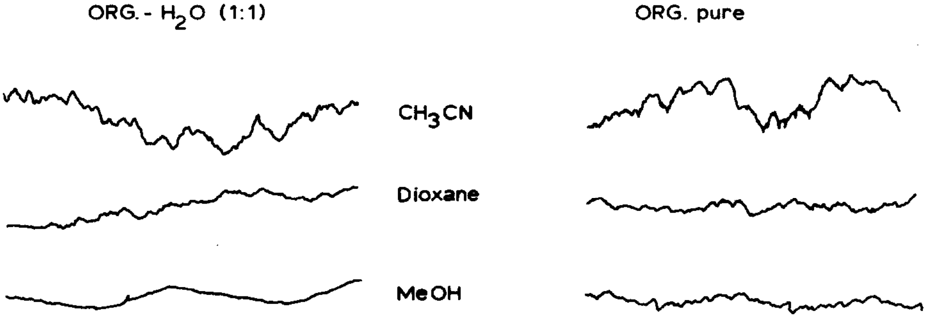

Fig. 2. Baseline noise levels (at attenuation × 64) for six eluents at a flow-rate of 25 μl min^{-1} after additional degassing in the pump reservoir with nitrogen. Eluents: acetonitrile, dioxane and methanol, as pure solvents (right) or 50:50 mixtures with water (left).

Applications

In the previous section, it has been shown that reversed-phase LC–ECD can be carried out over a wide range of mobile phase compositions. The following examples demonstrate that such a chromatographic system can serve a useful purpose in actual practice. For this preliminary work, standard solutions of the test solutes were used in all cases.

Fig. 3 shows the separation of three barbiturates, allo-, apro-, and butabarbital, on C_{18}-bonded silica with methanol–water (50:50) as the eluent. Under these conditions, the detection limit for, *e.g.*, allobarbital is *ca.* 40 pg. Comparable results were obtained for the separation of the benzodiazepines diazepam, desmethyldiazepam, clonazepam and nitrazepam in the same chromatographic system (detection limits, 20–60 pg).

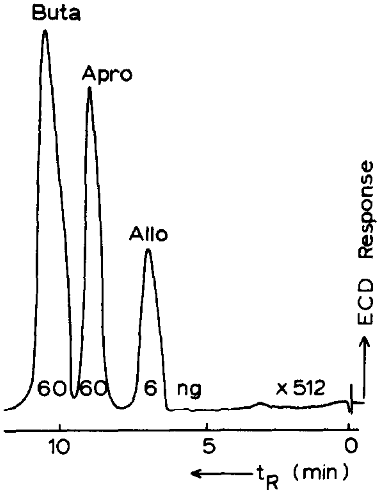

Fig. 3. Reversed-phase LC–ECD chromatogram of allo-, apro- and butabarbital (6, 60 and 60 ng, respectively) in the system LiChrosorb RP-18/methanol–water (50:50). Flow-rate, 25 μl min^{-1}; attenuation, ×512.

Fig. 4 shows the separation of one mononitro- and three dinitroaromatics on a C_{18}-bonded phase with methanol–water (80:20) as the eluent. The dinitro compounds show excellent sensitivity; detection limits are as low as 10 pg, which is the same value as found in normal-phase LC–ECD. Linearity was checked in this case and found to be satisfactory up to 10 ng. Another example deals with the separation

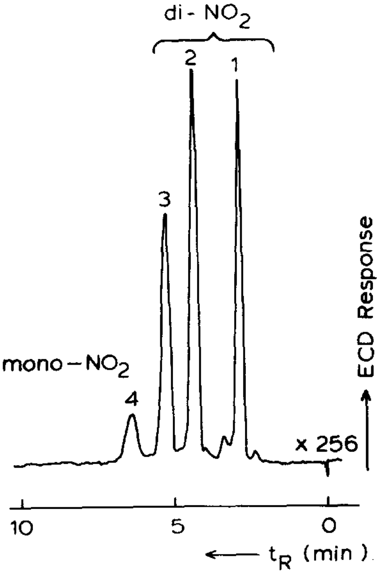

Fig. 4. Reversed-phase LC–ECD chromatogram of an injection of 6 ng of nitroaromatics in the system LiChrosorb RP-18/methanol–water (80:20). Flow-rate, 35 μl min^{-1}; attenuation, ×256. Solutes: 1 = 2,4-dinitrophenol; 2 = 2,4-dinitroaniline; 3 = 1,3-dinitrobenzene; 4 = 4-nitrotoluene.

of a number of halogenated aliphatic hydrocarbons (Fig. 5). The pure model compounds were extracted with water, centrifuged and, after separation of the aqueous layer, diluted with eluent; the dilute solutions were used for injections. With these volatile compounds, no attempt has as yet been made to determine detection limits. From earlier work[7], it can be calculated that in normal-phase LC–ECD these limits are *ca.* 20 pg.

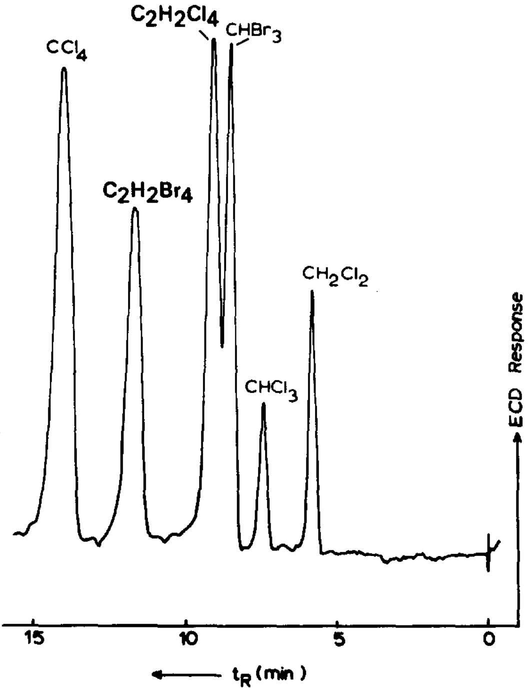

Fig. 5. Reversed-phase LC–ECD chromatogram of six halogenated hydrocarbons in the system LiChrosorb RP-18/methanol–water (70:30). Flow-rate, 40 μl min^{-1}; attenuation, ×256. Solutes (in order of increasing retention): dichloromethane; chloroform; bromoform; 1,1,2,2-tetrachloroethane; 1,1,2,2-tetrabromoethane; tetrachloromethane.

First attempts have been made to use reversed-phase LC systems involving eluents that contain small amounts of acids. So far, no extra problems have been encountered when using organic–aqueous eluent mixtures containing 0.1 *M* formic acid or acetic acid, 0.01–0.001 *M* phosphoric acid or 0.01 *M* triethylamine for periods ranging from some days to several months. Some increase of the noise level is observed, but baseline stability is satisfactory. Although acidified solvent mixtures can apparently be used freely, the analysis itself can well present problems. An example is shown in Fig. 6, where a mixture of chlorophenols is separated using methanol–0.001 *M* phosphoric acid (80:20) as the eluent. The rather strange peak shape of, especially, the more highly chlorinated phenols, suggests that some sort of breakdown occurs in the vaporization interface which, in this case, was constructed of nickel tubing. The distortion of the peaks was distinctly more serious when stainless steel

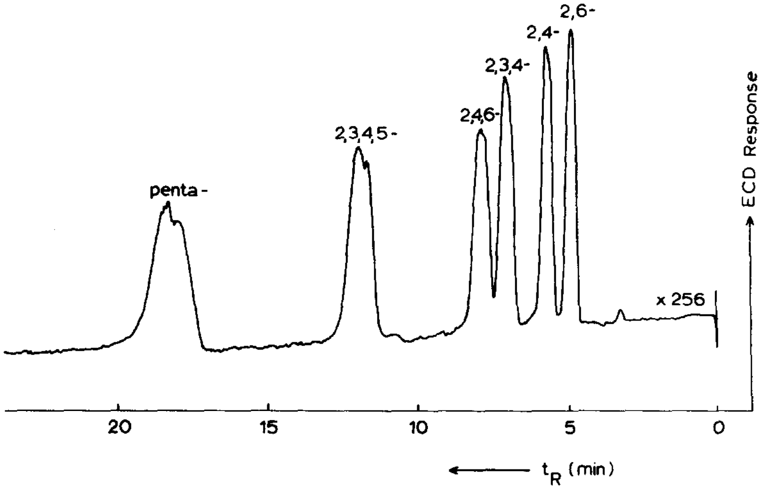

Fig. 6. Reversed-phase LC–ECD chromatogram of a mixture of di- (2,4 and 2,6), tri- (2,3,4 and 2,4,6), tetra- (2,3,4,5) and pentachlorophenols in the system LiChrosorb RP-18/methanol–0.001 *M* phosphoric acid (80:20). Flow-rate, 40 μl min^{-1}; attenuation, × 256; injected amounts, 20 ng of the dichlorophenols and 2 ng of the other compounds.

was used as construction material. No such problems were ever encountered[6] when, *e.g.*, pentachlorophenol was used in a normal-phase LC system such as silica–toluene. A systematic study is presently being carried out to evaluate the role of the construction material of the vaporization capillary, the nature of the acid added to the eluent, and of the analytes used as test compounds.

CONCLUSIONS

Several systems for reversed-phase narrow-bore LC with on-line ECD are kept running in our laboratory. One system has been in use for over a year without any special maintenance problems and, so far, no cleaning of the detector itself has been necessary. It is rather promising that methanol–water and dioxane–water mixtures containing up to at least 50% of water can be tolerated at flow-rates of, typically, 50 μl min^{-1}, and with 0.01–1 ng detection limits for various classes of ECD-sensitive compounds. One should realize, however, that even if the detection limits in reversed-phase and normal-phase LC–ECD are about the same, sensitivity (in terms of units of concentration) is *ca.* 10-fold better with the latter technique, because the injection volumes are *ca.* 10-fold different.

Current research is directed at the optimization of the vaporization interface, *i.e.* its size and construction material, in order to minimize band broadening and to extend the range of compounds that can be determined (*cf.* above). Another aspect of primary importance will be the application of the on-line reversed-phase LC–ECD technique to the analysis of real samples.

ACKNOWLEDGEMENT

We thank Pye Unicam (Cambridge, U.K.) for their financial support.

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 A. de Kok, R. B. Geerdink and U. A. Th. Brinkman, *J. Chromatogr.*, 252 (1982) 101.
4 I. S. Krull and D. Bushee, *Anal. Lett.*, 13A (1980) 1277.
5 G. Nota and A. Palombari, *J. Chromatogr.*, 62 (1971) 153.
6 U. A. Th. Brinkman, R. B. Geerdink and A. de Kok, *J. Chromatogr.*, 291 (1984) 195.
7 U. A. Th. Brinkman, P. M. Onel and G. de Vries, *J. Chromatogr.*, 171 (1979) 424.