Journal of Chromatography, 214 (1981) 209–216
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,955

Note

Comparison of C_n bonded silica gel thin-layer chromatographic plates: conditions for use and separations of some barbiturates*

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(Received April 22nd, 1981)

The advantages of a hydrolytically stable stationary phase, made with small (5–20 μm) silica gel particles chemically bonded with *n*-octyl (C_8) or *n*-octadecyl (C_{18}) groups, are well known in high-performance liquid chromatography (HPLC)[1]. At present, in most papers dealing with separations on columns, these types of bonded coatings are employed in a reversed-phase mode with aqueous–organic modifier eluents. The advantages of aqueous solvent systems include compatibility with biological samples. Also, highly polar compounds, previously separated mainly by means of ion-exchange chromatography, can be separated successfully by reversed-phase (RP) HPLC.

Similar advantages exist for reversed-phase thin-layer chromatography (RP-TLC) carried out on chemically modified silica gel-coated plates. However, there have been few TLC studies using this type of bonded coating material[2–5], although the performance of TLC with reversed-phase plates has been studied[6] and examples of analyses have been published[7]. The use of RP-TLC to assess hydrophobicity in studies of quantitative structure–activity relationships for drugs[8] and to correlate R_f values with the structures of barbiturates[9] have also been reported.

Another potentially useful application of bonded plates arises from their analogous behaviour to RP-HPLC on columns[10–12]. In fact, using RP-TLC, many eluting systems can be tested inexpensively and in a relatively short time, and these preliminary results may be easily adapted to RP-HPLC on columns.

As an extension of previous work[13] and in order to widen the application of RP-TLC in the analysis of drugs and/or biological materials, in this work we investigated the behaviour of some commercially available reversed-phase silica gel precoated plates, in connection with some properties of the eluent, *viz.*, the type of organic modifier, the percentage of water, the pH and the ionic strength. With a view to possible applications, the experimental conditions were chosen so as to shorten the development time as much as possible.

In addition, the separation of mixtures of barbituric acid derivatives is reported. Numerous papers on the separation of barbiturates by conventional TLC have been published[14–17]; nevertheless, the very short time of separation (about 15 min)

* Presented in part at "*3° Congresso Nazionale di Chimica Analitica*", *Siena, Italy, 1–4 October, 1980.*

0021-9673/81/0000–0000/$02.50

and the high reproducibility of the R_F values without any special experimental precaution seemed to be noteworthy.

EXPERIMENTAL

Compounds

Table I lists the barbiturates examined. Because of the hydrophobicity of RP-8 and RP-18 layers, methanol was selected as the solvent; 0.1–1 % methanolic solutions of samples were employed.

Thin-layer chromatography

Plates. Different types of chemically bonded silica gel pre-coated plates, all 5 × 5 cm and containing a fluorescent indicator, were tested: HPTLC F_{254} RP-8 and RP-18 (Merck, Darmstadt, G.F.R.); Stratocrom Si F_{254}–C_{18} W (made by Whatman for Farmitalia Carlo Erba, Milan, Italy); and RP OPTI-UP C_{12} (Antec, Bennwil, Switzerland) (for preliminary experiments only). No pre-treatment of the plates before use was performed.

Eluents. Mixtures containing from 10 to 90 % (v/v) of an organic modifier and water (or aqueous solutions) were employed. The organic modifiers were methanol, acetonitrile, isopropanol and tetrahydrofuran. Aqueous solutions were 0.05–1 *M* lithium chloride, 0.2 *N* hydrochloric acid, 0.1 *N* acetic acid, 0.1 *N* ammonia solution and 0.1 *N* sodium hydroxide. Buffer solutions were 0.05 *M* borax + 0.1 *N* sodium hydroxide (pH 10.32) and 0.1 *M* potassium dihydrogen orthophosphate + 0.1 *N* sodium hydroxide (pH 6.11).

Spotting and development. To achieve high resolutions, the spot area must be very small[18], and in this work it was usually about 2 mm. Portions of 0.2–0.3 μl of 0.1–1 % methanolic solutions of samples were applied 1 cm from the lower edge of the plate using a Hamilton syringe (1 μl) in connection with a micrometer. Rapid saturation of the developing chamber can be achieved by employing a glass jar, as small as is compatible with the size of the plate (10.5 cm high × 6.5 × 6.5 cm) and equipped with a ground-glass stopper. Standardized conditions for dipping the plates in the eluent were found to be very useful: a height of about 0.7 cm of eluent gave a short elution time without affecting the resolving power. The eluent was placed in the glass jar *ca.* 30 min prior to insertion of the plates. Ascending development was carried out at room temperature for 3.8 cm.

Detection. After development, the wet plates were exposed to an ammonia atmosphere for 5 min and the spots of the barbiturates were located under ultraviolet light by quenching of fluorescence at 254 nm[19]. A KM-3 chromatogram spectrophotometer (Carl Zeiss, Oberkochen/Wurttemberg, G.F.R.), in the reflectance mode, was used to measure directly barbiturate spots after separation on RP-18 plates. The plates were scanned in a direction parallel to that of the solvent flow.

RESULTS AND DISCUSSION

Particular attention was paid to the chromatographic conditions in order to obtain a development time of less than 40 min in all the experiments and, at the same time, a high resolution. As mentioned under Experimental, development to a height

of about 4 cm was sufficient to obtain a good resolution of a mixture of four or five compounds having R_F values in a limited range (*e.g.*, all between 0.25 and 0.65).

Eluents and development time

In all the experiments described below, the barbiturates reported in Table I were studied. Figs. 1–3 show the dependence of the development time on the composition of the mobile phase (type of organic modifier and percentage of water) for some commercially available reversed-phase pre-coated plates. Four organic modifiers with increasing polarity and chosen from those most commonly used in HPLC were investigated as eluents.

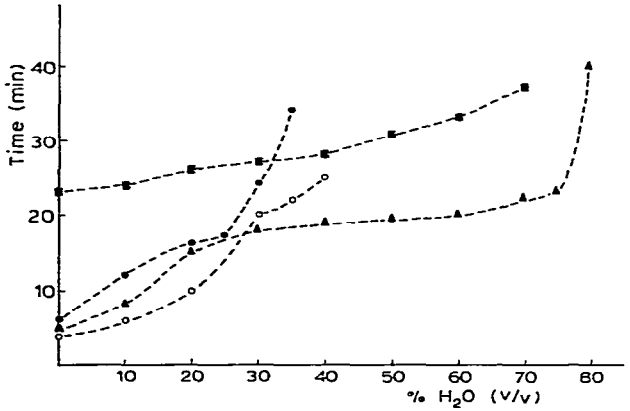

Fig. 1. Influence of the percentage of water in the eluent on the development time using HPTLC F_{254} RP-18 (Merck) silica gel pre-coated plates. Plates: 5 × 5 cm. Ascending development for 3.8 cm. ■, Isopropanol; ▲, tetrahydrofuran; ●, ethanol; ○, acetonitrile.

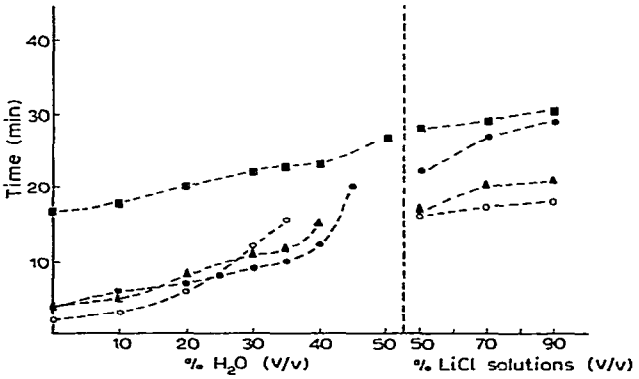

Fig. 2. Influence of the percentage of water in the eluent on the development time using Stratocrom Si F_{254}–C_{18} W (Carlo Erba) silica gel pre-coated plates. Chromatographic conditions and symbols as in Fig. 1.

It can be seen that the results depended on the type of plate used, and probably the binder material plays an important role. With Merck RP-18 plates (Fig. 1), when using methanol–water or acetonitrile–water mixtures as eluents, the elution time increased rapidly with increasing water content; 35–40% of water seems to be the upper

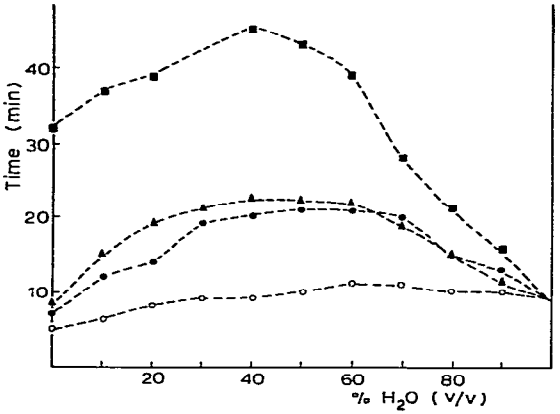

Fig. 3. Influence of the percentage of water in the eluent on the development time using RP OPTI-UP C_{12} (Antec) silica gel pre-coated plates. Chromatographic conditions and symbols as in Fig. 1.

limit if an elution time longer than 40 min is to be avoided. Although under our experimental conditions a higher percentage of water than previously reported[2,7] may be employed, the range of possibilities was always limited. Shorter elution times were obtained with Carlo Erba plates by employing the same eluent mixtures (Fig. 2), but the limits in use seemed to be about the same: the silica gel layer became detached from the glass support when eluents with water content higher than 50% were used. Nevertheless, it is interesting that this damage to the layers could be avoided by replacing the water in the eluent with lithium chloride solutions of increasing concentration: 0.1 *M* with 50% of methanol or acetonitrile and up to 1 *M* with 10% of methanol or acetonitrile. The elution time always remained below 30 min (Fig. 2).

The same experiments were carried out on Merck RP-8 plates. For both methanol–water and acetonitrile–water eluents our results were in agreement with those of Siouffi *et al.*[7]; the development time was shorter for RP-8 than RP-18 plates.

As eluents containing more than 50% of water are often employed in HPLC on columns, other organic modifiers were tested. Good results were achieved by employing Merck RP-8 or RP-18 plates and isopropanol– or tetrahydrofuran–water as eluent. Up to 70–80% of water can be used (Fig. 1). This result was interesting because of the different properties of tetrahydrofuran compared with the other solvents tested[20]. With the Carlo Erba plates, the addition of lithium chloride is always necessary if isopropanol or tetrahydrofuran containing more than 50% of water must be employed (Fig. 2). However, tetrahydrofuran did not seem to be a good eluent for this type of plate, because of the elongated and irregular shapes of the spots.

Only preliminary experiments were carried out on OPTI C-12 plates. So far as the amount of water in the eluent is concerned, OPTI C_{12} plates behave differently (Fig. 3) from those described above. For all the four organic modifiers tested, any percentage of water could be used. The elution time was always very short, with a maximum and a subsequent decrease with increasing water percentage. This difference in behaviour could be ascribed to the capacity of this type of layer to absorb water. The sequence of elution time, however, agrees with that found for C_{18} and C_8 plates; isopropanol–water mixtures showed the longest elution times. Although com-

plete results cannot be given for OPTI C_{12} plates, their behaviour seemed to be interesting. The very short elution time and the possibility of utilizing any ratio between the organic modifier and the water in the eluent (Fig. 3) are very advantageous properties.

pH of eluent. No damage to any of the four types of plates was observed on employing strongly acidic or basic eluent mixtures [*e.g.*, methanol–0.2 *N* hydrochloric acid (6:4) or methanol–1 *N* sodium hydroxide (6:4)]. Probably this favourable result depended on the short development time. It is important to note that with respect to HPLC on columns, the range of pH of the eluents widened, mainly on the basic side.

R_F values

The Merck and Carlo Erba plates gave good reproducibility of the R_F values (relative standard deviation of R_F values = 0.02). In addition, the R_F values of the eight barbiturates under examination obtained on the two different types of RP-18 plates compared very well. No significant difference in the R_F values were found on employing as eluents mixtures containing a constant ratio of methanol or acetonitrile and lithium chloride solution of increasing concentration (0.1–1 *M*).

In general, two practical advantages were found by working with reversed-phase plates rather than conventional plates: first, no particular attention need be paid to the operating conditions with respect to the effect of ambient moisture on the layer, and second, there is the possibility in many instances of reusing the plates after washing them. Ascending chromatography with methanol as eluent gave satisfactory results and no change in performance was noted.

Separation of barbiturates

Table I gives R_F values for some barbiturates obtained with Merck and Carlo Erba RP-18 plates. To investigate the effect of the eluent on the R_F values, various mixtures of each of the four organic modifiers and water, in different ratios, were tested. In all instances the R_F values of each of the eight barbiturates decreased as the amount of water in the eluent increased and the sequence of the R_F values was the same for whatever organic modifier. Mixtures of organic modifier and aqueous acetic acid or ammonia, phosphate or borax buffer were also tested. A difference in R_F values occurred on working at basic or acidic pH (Table I). This difference probably depends on the predominance of the keto or enol form of the barbituric acid derivatives, the pK_a values being reported for some of these compounds in the range 7—8 (ref. 21).

The best separations were achieved by employing methanol and phosphate or borax buffer in the ratio 3.5:2 or isopropanol and the same buffer (1:2) (Figs. 4 and 5). A shorter elution time (about 15 min) was obtained by employing Carlo Erba plates and a better resolution by using the Merck plates.

For the possible use of these results in the screening of biological samples, it is interesting to note that the long-acting phenobarbital (compound 2) was separated from the short- to intermediate-acting amobarbital and secobarbital (compounds 7 and 8, respectively). In addition, as reported for conventional TLC[22], the speed of action of barbiturates seemed to be related to their R_F values: in our work, the faster acting barbiturates had lower R_F values than the slower acting compounds.

By means of the experiments described, 0.7 μg of phenobarbital and 0.2–0.3 μg of the other barbiturates can be detected.

TABLE I

R_F VALUES FOR BARBITURATES

Eluents: 1 = methanol–0.1 *M* acetic acid (3.5:2); 2 = methanol–phosphate buffer (3.5:2); 3 = methanol–0.1 *M* ammonia solution (3.5:2); 4 = isopropanol–borax buffer (1:2).

No.	*Barbituric acid*	*Trivial name*	*Proprietary name*	R_F *value*			
				Eluent 1	*Eluent 2*	*Eluent 3*	*Eluent 4*
1	5,5-Diethyl-	Barbital	Veronal	0.53	0.59	0.72	0.58
2	5-Phenyl-5-ethyl-	Phenobarbital	Luminal	0.41	0.50	0.63	0.40
3	5,5-Diallyl-	Diallylbarbital	Dial	0.43	0.51	0.60	0.41
4	5-(1-Cyclohexenyl)--5-ethyl-	Cyclobarbital	Phanodorm	0.32	0.38	0.52	0.31
5	5-Allyl-5-isobutyl-	Itobarbital	Sandoptal	0.33	0.36	0.48	0.30
6	5-(1-Cycloheptenyl)--5-ethyl-	Heptabarbital	Medomin	0.25	0.31	0.42	0.26
7	5-Ethyl-5-(3-methylbutyl)-	Amobarbital	Amital	0.23	0.28	0.39	0.20
8	5-Allyl-5-(1-methylbutyl)-	Secobarbital	Seconal	0.22	0.26	0.35	0.18

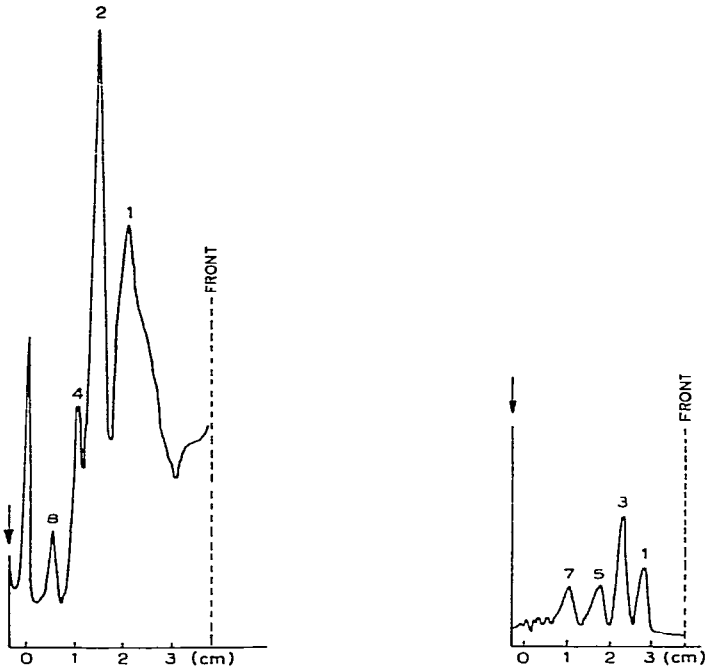

Fig. 4. Scan of some barbiturates separated on chemically bonded silica gel pre-coated plates (RP-18). Eluent: isopropanol–phosphate buffer (1:2, v/v). Ascending development to a height of 3.8 cm. Wavelength, 254 nm; slit width, 3.5 mm; v_p = 50 mm min^{-1}; v_c = 60 mm min^{-1}. Compounds: 1 = barbital; 2 = phenobarbital; 4 = cyclobarbital; 8 = secobarbital. v_p = plate travel; v_c = recorder chart speed.

Fig. 5. Scan of some barbiturates separated on chemically bonded silica gel pre-coated plates (RP-18). Eluent: methanol–borax buffer (3.5:2, v/v). Ascending development to a height of 3.8 cm. Wavelength, 254 nm; slit width, 0.5 mm; slit length, 3.5 mm; v_p = 100 mm min^{-1}; v_c = 100 mm min^{-1}. Compounds: 1 = barbital; 3 = diallylbarbital; 5 = itobarbital; 7 = amobarbital.

ACKNOWLEDGEMENTS

The authors thank Sig. Francesco Federici for his valuable technical assistance and Carlo Erba for the gift of Carlo Erba C-18 and Antec C_{12} plates.

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