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### Use of various organic modifiers in thin-layer chromatography on chemically bonded phases

U. A. Th. BRINKMAN\* and G. DE VRIES

*Department of Analytical Chemistry, Free University, De Boelelaan 1083, 1081 HV Amsterdam (The Netherlands)*

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According to a recent review<sup>1</sup>, about 100 papers have been published on thin-layer chromatography on non-polar chemically bonded stationary phases (RP-TLC). In most of these papers, methanol and acetonitrile were the only organic modifiers used. Data on other organic solvents are occasionally included (see, *e.g.*, refs. 2-4), but systematic studies are still very scarce<sup>5</sup>. This is surprising as it is generally considered to be one of the advantages of thin-layer over column liquid chromatography that the selection of solvents suitable as mobile phase (constituents) is less restricted.

In order to remedy this situation, this paper reports the results of a short systematic study on the use of acetone, dioxane, isopropanol and tetrahydrofuran (THF) as organic modifiers in RP-TLC on five types of commercially available pre-coated thin-layer plates. The main characteristics of these plates are summarized in Table I.

#### EXPERIMENTAL

Commercially available chemical bonded RP-TLC plates obtained from five firms (Table I) were cut into rectangular plates of about 6.5 × 1.5 cm using the device described in ref. 6. After the application of spots using a fine-tipped melting-point capillary, ascending development was carried out in a saturated chromatographic chamber (Hellendahl staining jar) over a distance of 5.0 cm. Detection was accomplished by viewing the plates under 254-nm UV light (phthalate esters) or after irradiating the RP-TLC plate for 3-5 min with a medium-pressure mercury lamp (aminophenols).

The six phthalate esters and three isomeric aminophenols used as model compounds, and all solvents, were of normal analytical-reagent grade. Approximately 1% sample solutions in dioxane were used for spotting.

#### RESULTS AND DISCUSSION

For each of the five types of non-polar chemically bonded phases selected (Table I), four solvent systems were studied, *viz.*, acetone-water, dioxane-water,

TABLE I  
MAIN CHARACTERISTICS OF RP-TLC PRE-COATED PLATES

Manufacturer	Plate designation	Product number	Carbon chain	Particle diameter ( $\mu\text{m}$ )	Remarks
Antec, Bennwil, Switzerland	OPTi-UP C <sub>12</sub>	1028	12	10-20	80% 10-15 $\mu\text{m}$
Baker, Phillipsburg, NJ, U.S.A.	Si-C <sub>18</sub>	7013-4	18	—	
Macherey, Nagel & Co., Düren, G.F.R.	Nano SIL C18-50	811.064	18	5-10	"MN-50"; 50% silanized
Merck, Darmstadt, G.F.R.	RP-18 (TLC quality)	15423	18	11-12	Silanization not completely exhaustive
Whatman, Clifton, NJ, U.S.A.	KC <sub>18</sub>	4803-800	18	10-11	End-capped with C <sub>2</sub>

isopropanol-water and THF-water, both with and without the addition of 3% of sodium chloride (for a discussion of the relevance of the addition of salt to the mobile phase see, *e.g.*, refs. 5, 7 and 8). Data on migration rate *versus* mobile phase composition are presented in Fig. 1.

Relatively slow migration is observed for the isopropanol-water mixtures recommended for use in ref. 5; this is to be expected on the basis of the high viscosity of such mixtures. Higher migration rates are obtained for systems involving acetone, dioxane and THF. With these solvents, the results for each type of plate are rather similar in all instances except for RP-18-coated plates. For example, with the KC<sub>18</sub> and Si-C<sub>18</sub> plates, and in the absence of added sodium chloride, migration invariably is rapid (less than 20 min for a 5-cm run) for solvent mixtures containing up to about 40% of water. At higher water contents, migration suddenly becomes very slow and/or the pre-coated layers swell and flake off from the glass backing. These results are similar to those previously recorded for methanol- and acetonitrile-water mixtures (*cf.*, Fig. 4 in ref. 6, where no curve is drawn for less than 60% of organic modifier as migration then becomes intolerably slow). A similar situation is encountered with the MN-50 plates, but the limiting value is now much higher, *i.e.*, about 70% of water; again, this is in close agreement with earlier data for methanol and acetonitrile (*cf.*, Fig. 5 in ref. 6). Mutual similarity of the plots in Fig. 1 is very striking for the C<sub>12</sub> plates.

In the presence of sodium chloride, demixing occurs with acetone, dioxane and THF, but not isopropanol, at relatively high modifier concentrations. A real problem is encountered only with THF, however. Here, demixing is observed already above 45% THF, while development with THF-water mixtures without added salt is extremely slow below about 60% THF, at least on the KC<sub>18</sub> and Si-C<sub>18</sub> plates. It should further be noted that with the MN-50 pre-coated plates, the use of THF-water-3% sodium chloride solutions also poses problems with mixtures containing less than 45% THF, as development of the plates is always very slow (over 60 min for a 5-cm run), if it occurs at all. The combined data suggest that, as far as rapid and regular solvent migration is concerned, acetone-water mixtures should be the first choice, with dioxane-water second.

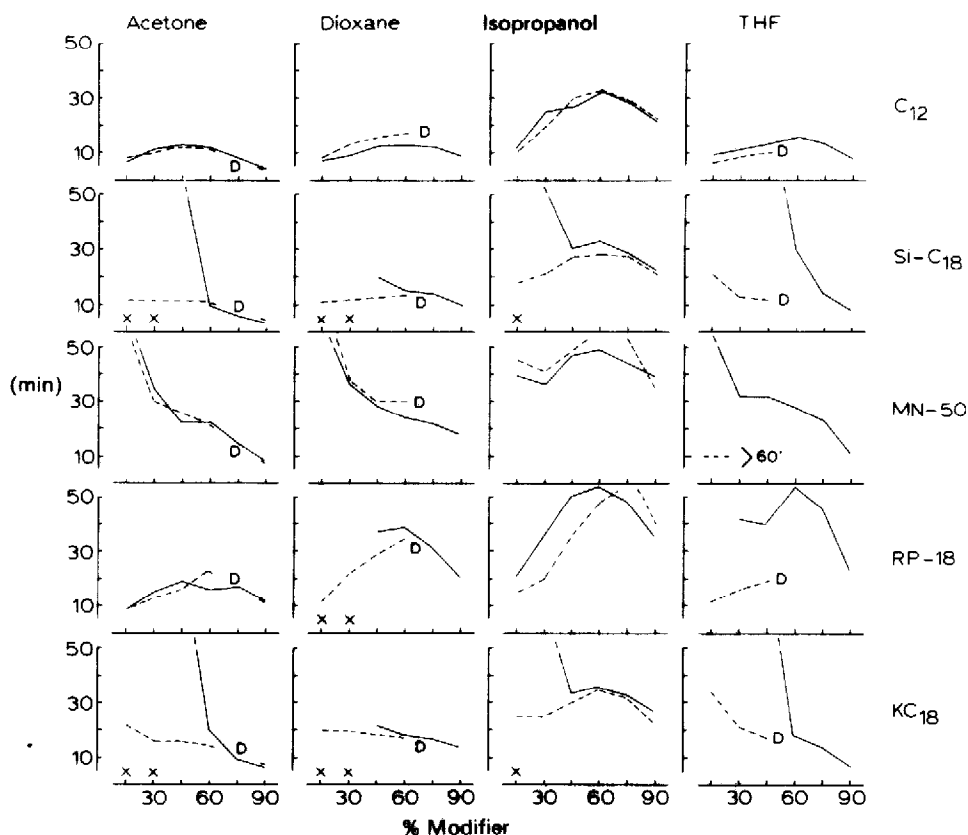


Fig. 1. Dependence of migration time, for a 5-cm run, on the organic modifier content of the mobile phase in the presence (-----) and absence (——) of sodium chloride for four organic solvents and five types of pre-coated RP-TLC plates. D, Demixing occurring in the presence of sodium chloride. Crosses indicate swelling of the thin layer and/or loosening of the layer from the glass backing in the absence of added salt. In the MN-50/THF-water-NaCl system, migration is invariably very slow (-----, > 60 min).

A comparison of the migration rates of the selected solvent systems on the various pre-coated RP-TLC plates clearly indicates that very rapid development is invariably obtained with the OPTi-UP C<sub>12</sub> plates (*cf.*, ref. 5). With the remaining four stationary phases, an unequivocal sequence cannot readily be given. The MN-50 plates, however, are seen to behave somewhat less satisfactorily than the RP-18, KC<sub>18</sub> and Si-C<sub>18</sub> plates.

Separation efficiency, obviously, is a more important parameter than migration rate. In this work, two series of compounds were selected to study this aspect, *viz.*, six phthalate esters (di-C<sub>2</sub>, -C<sub>4</sub>, -C<sub>5</sub>, -C<sub>8</sub>, -C<sub>10</sub> and -C<sub>13</sub>) and the three isomeric aminophenols. With the phthalate esters, the best separations are invariably obtained using mobile phases containing about 75% or organic modifier. Six well resolved spots show up for all pre-coated layers except OPTi-UP C<sub>12</sub>, and for all four organic solvent systems studied. With the C<sub>12</sub> plates, the separation leaves something to be desired, with the di-C<sub>4</sub> and di-C<sub>5</sub> esters generally being the pair most difficult to

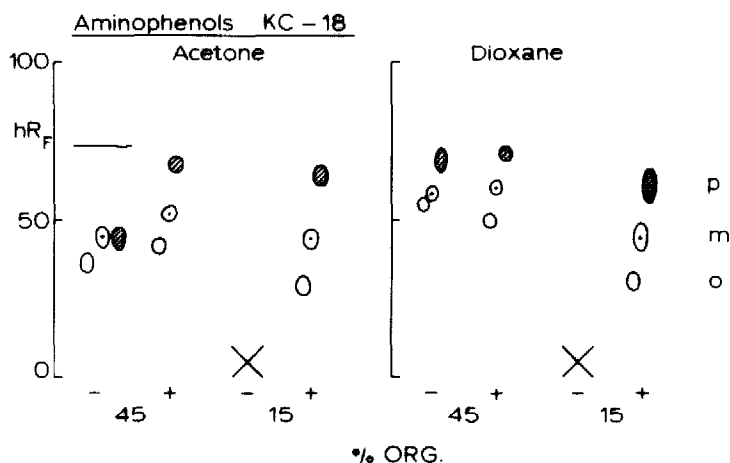


Fig. 2. RP-TLC, on  $KC_{18}$  plates, of *o*-, *m*- and *p*-aminophenol using acetone-water and dioxane-water mixtures, with (+) and without (-) added sodium chloride, as the mobile phase. Crosses indicate swelling of the thin layer and/or loosening of the layer from the glass backing. Note the solvent front position on the chromatogram at the extreme left, which indicates slow migration.

separate. The addition of 3% of sodium chloride to the mobile phase, studied with acetone, dioxane and isopropanol as organic modifiers, and with all types of pre-coated plates has a negligible effect on the  $hR_F$  values.

A more complicated pattern emerges in the separation of the aminophenols. Here, elongated and ill-defined spot shapes are often encountered when using THF as the organic modifier; similar observations have previously been reported<sup>5</sup> for the separation of barbiturates. With the other organic solvents, the three isomers can be separated on all stationary phases, although under widely different conditions, and

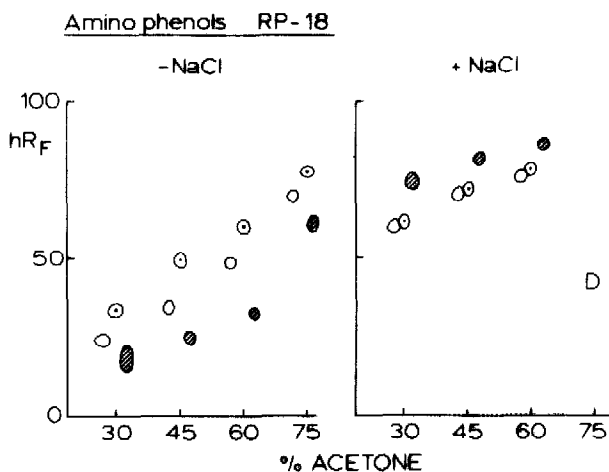


Fig. 3. RP-TLC, on TLC-quality RP-18 plates, of *o*-, *m*- and *p*-aminophenol using acetone-water mixtures, with (+) and without (-) added sodium chloride, as the mobile phase. D, Demixing. For symbols, see Fig. 2.

with the OPTi-UP C<sub>12</sub> plates again showing the least satisfactory results. The addition of sodium chloride to the mobile phase often improves the separation of the aminophenol spots to a considerable extent, as can be seen from the chromatograms on KC<sub>18</sub> plates shown in Fig. 2: the addition of salt affects spot resolution (development with 45% modifier) and allows plate development at very high water contents (15% modifier) where separation generally is best. The results for the Si-C<sub>18</sub> plates are virtually identical with those in Fig. 2, while the RP-18 pre-coated layers behave rather differently. With the latter type of plate, separations in the systems acetone-water and dioxane-water, both with 3% of sodium chloride, at best are very marginal. In the absence of salt, however, more satisfactory results are obtained, especially when using acetone as the organic modifier. Examples for the 30-75% acetone range are shown in Fig. 3. These also illustrate that the order of separation of the aminophenols in the absence of added salt (*para* < *ortho* < *meta*) is different from that in the presence of sodium chloride (*ortho* < *meta* < *para*). The latter sequence is that observed on all other stationary phases. Similar anomalous behaviour of the RP-18- and RP-8-coated TLC quality (but *not* the HPTLC quality) layers has earlier been reported<sup>6</sup> with methanol-water and acetonitrile-water mixtures as mobile phases. Lastly, on MN-50-type layers, the best results are obtained with either acetone-water (30:70) 3% sodium chloride or dioxane-water (30:70)-3% sodium chloride. These separations, however, are not as good as those recorded with the KC<sub>18</sub>, Si-C<sub>18</sub> and RP-18 layers and, in addition, are more time consuming.

#### CONCLUSION

On summarizing the above data and comparing them with results previously published for methanol-water and acetonitrile-water mobile phases, it is evident that acetone and, only slightly less so, dioxane are promising organic modifiers for use in RP-TLC, on account of (1) short time of development, (2) satisfactory separation efficiency and (3) relatively low toxicity compared with acetonitrile. As for the stationary phases, the KC<sub>18</sub> and Si-C<sub>18</sub> layers show the best performance, with sodium chloride having to be added for mobile phase water contents of over 40%. (It should be added that the data for the KC<sub>18</sub> and Si-C<sub>18</sub> plates consistently are so similar that one may suspect that these plates are obtained from the same source.) The RP-18-coated TLC plates are only slightly less efficient and have the advantage that, with acetone as modifier, addition of salt is unnecessary.

As regards separation efficiency, it should be borne in mind that only two classes of compounds have been studied and further work is necessary. Such work should also include RP-TLC on RP-8-coated TLC layers, which, in earlier studies, have shown good overall performance; their separation efficiency generally is closely similar to that of RP-18 pre-coated plates, while the development time is distinctly shorter.

#### ACKNOWLEDGEMENTS

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