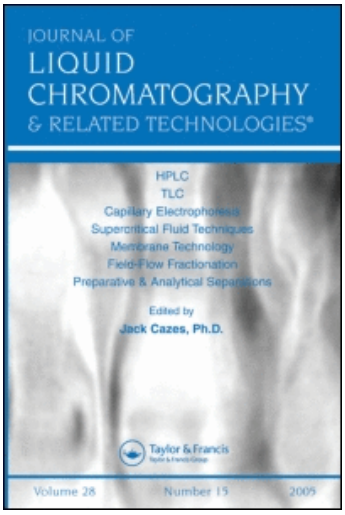


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# Chromatographic Analysis of Pharmaceutical Products of Toxicological Interest on Chemically Bonded Thin Layers Influence of the Organic Modifier Nature on Selectivity

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# CHROMATOGRAPHIC ANALYSIS OF PHARMACEUTICAL PRODUCTS OF TOXICOLOGICAL INTEREST ON CHEMICALLY BONDED THIN LAYERS INFLUENCE OF THE ORGANIC MODIFIER NATURE ON SELECTIVITY.

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## ABSTRACT

Properties and selectivities of the following organic modifiers were studied : $CH_3OH$ - $C_2H_5OH$ - Isopropanol - acetonitrile - T.H.F. in reversed phase high performance thin-layer chromatography (H.P.T.L.C.). With the tested solutes, ethanol appeared to be the most selective organic modifier whereas isopropanol the most interesting in HPTLC-HPLC data transfer. In every case , the best resolution was observed with mobile phases containing 40-50 % $H_2O$.

## INTRODUCTION

Chromatographic plates with chemically bonded apolar phases are now commercially available. These layers allow the analysis of numerous compounds that cannot be directly chromatographed on silicagel layers under normal conditions. In a very recent paper, Brinkman (1,2) made a summary of all the separations having been realized on such new plates. The properties and conditions of operating reversed-phase thin layers were also discussed by Siouffi (3), Kaiser (4) and all.

Our purpose, in this paper, is to study the role of the organic modifier (nature, concentration) present in the mobile phase. There-

fore, properties of various mobile phases : *water-alcohol, water-*acetonitrile and water-THF have been compared for the separation of pharmaceutical substances : barbiturates, benzodiazepines, methylxanthines.....

The influence of mobile phase composition on retention in High Performance Liquid Chromatography (HPLC) has been extensively studied in a great number of publications. In most cases, a logarithmic expression is found relating log k' (capacity factor) to the composition of mobile phase. In our previous articles (5,6), we noticed a similar relationship in reversed-phase thin layer chromatography (RPTLC) : straight lines were obtained when plotting $R_M$ = f (% MeOH or AcCN) either in classical RPTLC (5) or in ion-pair reversed phase thin layer chromatography (6). More recently, Irwin (7) and Dzido (8) have studied the influence of mobile phase composition in RPTLC in the separation of tricyclic neuroleptics and sulphonamides (7) and phenolic acids (8). Karger et al. investigated polar group selectivity in reversed phase liquid chromatography (9) ; they observed significant differences in group contribution with bonded phase coverage : the largest differences arising from MeOH-$H_2O$ mobile phases and the least from THF-$H_2O$.



## EXPERIMENTAL

### Thin Layer Chromatography

All ascending developments were carried out in glass ordinary tanks of the following dimensions : 13 x 13 x 5.5 cm (Chamagne, Lyon, France) ; the plate was introduced in the tank immediatly after pouring 10 ml of solvent. Spots of solutes were applied at 2 cm from the interior edge of the plate and developments were performed over a distance of 6 cm from the spotting line.

As all solutes were in aqueous media, it was necessary to wet the layer before spotting the solutes with a drop of methanol.(Hydrophobicyty of the chemical bonded phase). Visualisation was done

by viewing the plates under UV (254 nm or 366 nm) light or by submitting them to iodine vapors.

H.P.L.C.

The liquid chromatograph consisted of the following components Model 380 ALTEX pump (Touzart et Matignon, Vitry sur Seine, France) a damping system, a 70-10 rheodyne injection valve (Touzart et Matignon) and a LC-UV PYE UNICAM spectrophotometer. All measurements were made at room temperature.

All columns were of the same dimensions (10 cm x 4.6 mm i.d.) and packed according to a technique previously described (10).



Reagents - Materials

10 x 10 cm reversed-phase thin layer plates were obtained from Merck (Merck-Clevenot, Paris, France) : RP2, RP8 and RP18 (ref.13726, 13725, 13724). The same bonded phases were used as packings for the columns : LICHROSORB RP2, RP8 and RP18, 5 µm.

The mobile phases were made up by volume from analytical grade solvents and distilled, deionized water ; once the mobile phase has been prepared, it was degassed in an ultrasonic bath.

All drugs were of analytical or pharmacopoeial grade and were used without further purification.

## RESULTS AND DISCUSSION

Comparison of the different RP-HPTLC plates. Wettability of apolar bonded stationary phases.

The dependence of the time of development on the mobile phase composition is shown in Table I.

It can be seen that in these conditions, it is not possible to carry out separations when using mobile phases containing more than 60 % $H_2O$ on RP2 plates, 40 % on RP8 plates and 35 % on RP18 plates. This observation does not agree with Brinkman (2) who noticed the

TABLE I

Migration times for different RP-TLC plates.
- 6 cm run -

| Support / $CH_3OH-H_2O$ (V/V) | RP2 | RP8 | RP18 | KC18 |
|---|---|---|---|---|
| 100 - 0 | 22 *min* | 22 *min* | 20 *min* | 7 *min* |
| 90 - 10 | 30 *min* | 34 *min* | 37 *min* | 10 *min* |
| 80 - 20 | 47 *min* | 54 *min* | - | 14 *min* |
| 70 - 30 | 60 *min* | 1h 35 *min* | 2h 15 *min* | 20 *min* |
| 65 - 35 | - | - | 3h | 29 *min* |
| 60 - 40 | 1h 15 *min* | 3h 50 *min* | - | 47 *min* |
| 55 - 45 | - | 6h 30 *min* | - | - |
| 50 - 50 | 1h 55 *min* | - | - | - |
| 40 - 60 | 2h 20 *min* | - | - | - |
| 35 - 65 | 6h | - | - | - |



following order in the increase of time of run : RP18 < RP2 < RP8. but he studied only low percentages of water (% $H_2O \leq 20$ %) in the mobile phase.

Under certain conditions, it is possible to run chromatograms with mobile phases containing high percentages of water : using a chamber saturated with solvent vapor, the run time decreased considerably but in the same time, $R_F$ values decreased noticeably too. In table II, onecan see that the more pronounced the hydrophobic character of the layer, the slighter the decrease.

Using binary mixtures such as water-ethanol and water-THF, table III shows that in the first case, developments are longer than those carried out with methanol for mobile phases containing low percentages of water and become shorter for upper % $H_2O$ in mobile phases. A similar behavior can be observed in the case of $CH_3CN$ and THF.

TABLE II

$R_f$ values observed under various developing conditions. - 6 cm run - Solutes : (1) Thiogenal ; (2) butylbenzene, (3) dimethylaminoazobenzene.

| Plate | Mobile phase $CH_3OH$ - $H_2O$ | $R_f$ without solvent vapor | $R_f$ in saturated chamber | $R_{f(calc)} = f \times R_{f(obs)}$ * |
|---|---|---|---|---|
| RP2 | 85-15 | 1- 0.87<br>2- 0.79<br>3- 0.72 | 1- 0.59<br>2- 0.59<br>3- 0.50 | 1- 0.83<br>2- 0.83<br>3- 0.70 |
| RP8 | 90-10 | 1- 0.78<br>2- 0.80<br>3- 0.525 | 1- 0.625<br>2- 0.61<br>3- 0.42 | 1- 0.81<br>2- 0.79<br>3- 0.55 |
| RP18 | 90-10 | 1- 0.78<br>2- 0.80<br>3- 0.29 | 1- 0.73<br>2- 0.675<br>3- 0.27 | 1- 0.88<br>2- 0.81<br>3- 0.32 |

* f values taken from ref. 5

TABLE III

Developing time with different binary mixtures as mobile phases. - RP8 plate ; 8 cm run.

* RP8 plates were of a different batch than in Table I.

| X / X-$H_2O$ | $CH_3OH$ * | $C_2H_5OH$ | $CH_3CN$ | THF |
|---|---|---|---|---|
| 90-10 | 55 *min* | 1h 25 | 22 *min* | 45 *min* |
| 80-20 | 1h 20 | 2h 05 | 50 *min* | 1h 15 |
| 70-30 | 3h | 2h 50 | 2h 15 | 1h 35 |
| 60-40 | 6h 40 | 3h 25 | 2h 15 | 2h 15 |
| 50-50 | - | 5h 10 | 3h | 2h 30 |
| 100-0 | 30 *min* | 1h | 15 *min* | 25 *min* |

Dependence of retention and selectivity on the type of organic modifier in the mobile phase.

To study the influence of organic modifier concentration and nature in RP.HPTLC, we have carried out separations of several drugs of toxicological interest. The tested solutes are the followings :

1 - Acetaminophen
2 - Theophylline
3 - Caffeine
4 - Phenobarbital
5 - Carbamazepine
6 - Diphenylhydantoïne
7 - Diazepam
8 - Ethosuccimide
9 - Barbital

Results are shown *in* Figure 1 with different mobile phases containing the same concentration of organic modifier (50 % v/v).

- With $CH_3OH$ as organic modifier, the observed $R_f$ range is lower than with all other modifiers ($0 < R_f < 0.5$).

It is interesting to point out the good separations observed with ethanol and isopropanol. In the case of isopropanol, $R_f$ values range from 0.3 to 0.8 and a good resolution can be noted. It has been previously shown (5) that the best $R_f$ range useful for RP.HPTLC - HPLC data transfer is 0.2 - 0.8. Further work in HPLC will be performed to confirm these observations.

- A great difference exists between n-propanol and isopropanol, retention *smaller with the latter.*

- The magnitude of $R_f$ is of the same order for $CH_3CN$, THF and $C_2H_5OH$ containing mobile phases but selectivity is very different from one to *the other.*

If we now examine the dependence of retention on mobile phase composition, we note in Figure 2 that retention increases with an increase of the water ratio of the mobile phase. Some exceptions are observed with low water contents of $CH_3CN$-$H_2O$ and $C_2H_5OH$-$H_2O$ (<20 %).

Plotting $R_f$ values against percentage of water in the mobile phase, *linear relationships can be noticed in the range* 20-60 % $H_2O$ (with ethanol as organic modifier). This range is *somewhat smaller when THF, $CH_3CN$ and $CH_3OH$ are used.*

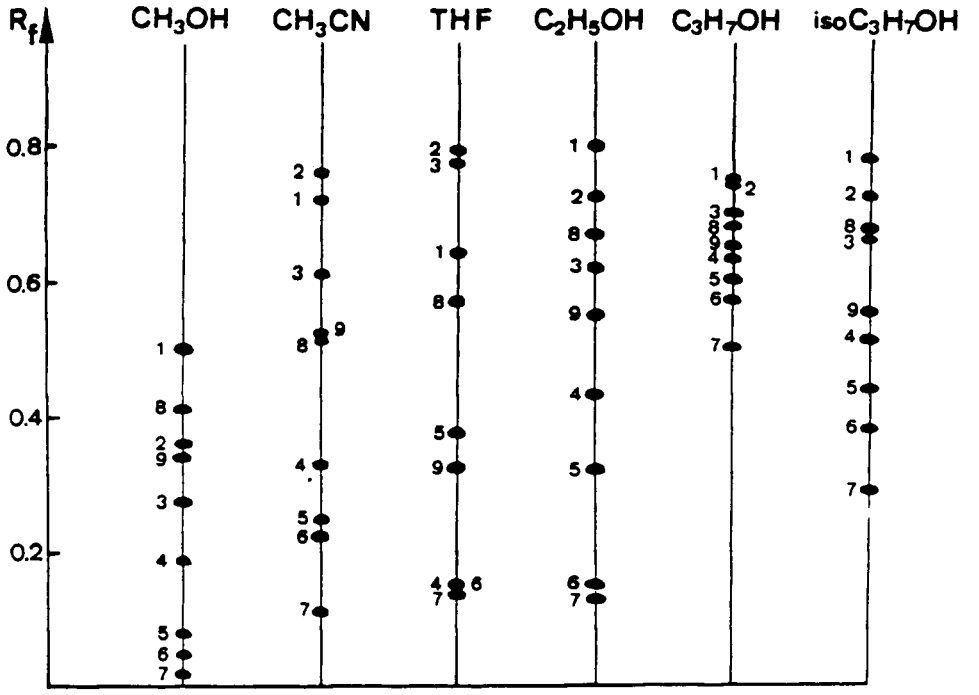


Figure 1 : $R_f$ values on LICHROSORB RP 8 HPTLC plate.
Solvent : organic modifier - water 50/50.
Solutes : 1 - Acetaminophen, 2 - Theophylline
3 - Caffeine, 4 - Phenobarbital,
5 - Carbamazepine, 6 - Diphenylhydantoïne,
7 - Diazepam, 8 - Ethosuccimide, 9 -Barbital.

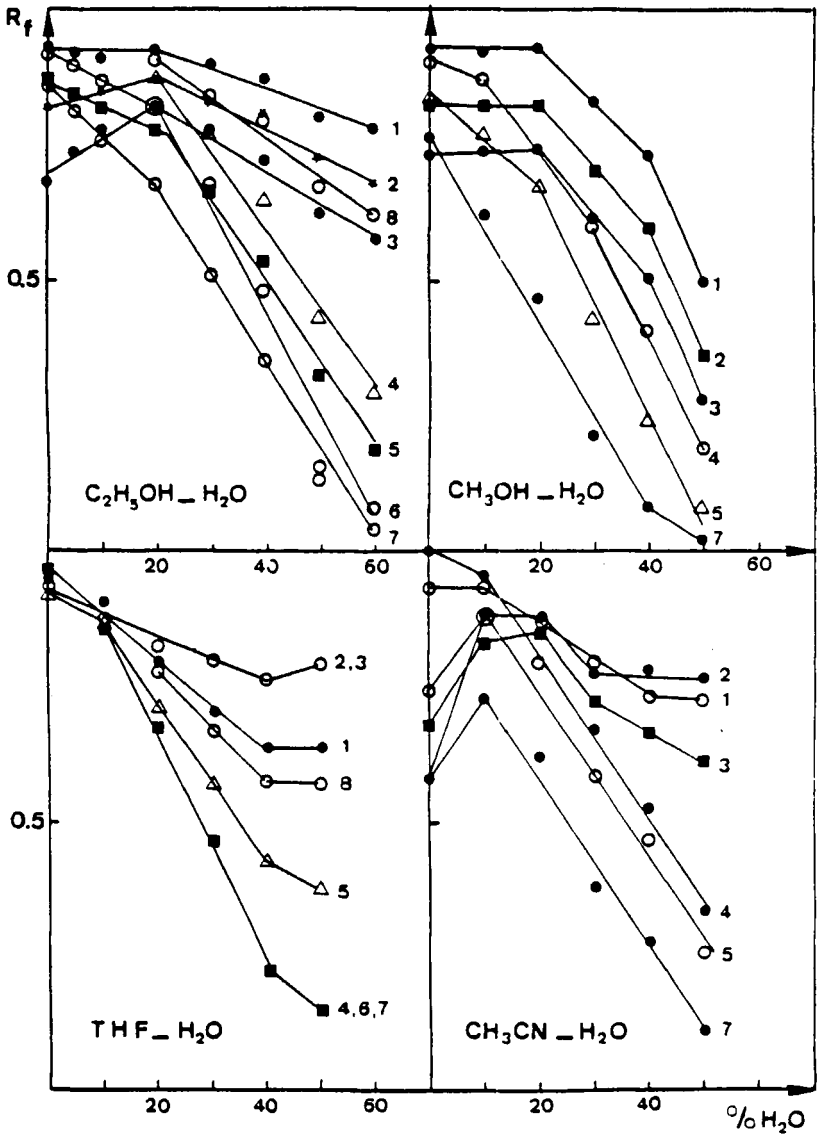


Figure 2 : Dependence of $R_f$ values on mobile phase composition.
Stationary phase : LICHROSORB RP 8.
Solutes : see figure 1.

The elution order of the tested solutes is not changed from one organic modifier to another for water content higher than 10-20 % in mobile phases. In the case of lower water contents, some inversions can be noticed.

- The best resolution is obtained for mobile phases containing 40-50 % water with all organic modifiers.

## Possibility of reversed-phase HPTLC-HPLC data transfer

In reversed-phase liquid chromatography, the most selective mobile phase for a given separation can be ajusted first in thin-layer chromatography (to save time and money). But in reversed-phase technique, possibilities of data transfer from HPTLC results to HPLC results are limited with high water contents of mobile phases.

In the separation of some drugs (Figure 3) on lichrosorb RP8 column, a mobile phase $CH_3OH$-$H_2O$ 50/50 is needed. In this case, the ajustment of mobile phase can't be done using HPTLC but Figure 4 shows that the nature of organic modifier or the nature of the reversed-phase packing can be studied first using chemically bonded reversed-phase thin-layers.

Transposition relationships $k'_{HPLC} = f(k'_{HPTLC})$ are illustrated in Figure 4 with RP8 and RP2 silicagels and $CH_3OH$-$H_2O$ and $CH_3CN$-$H_2O$ *as mobile phases*.

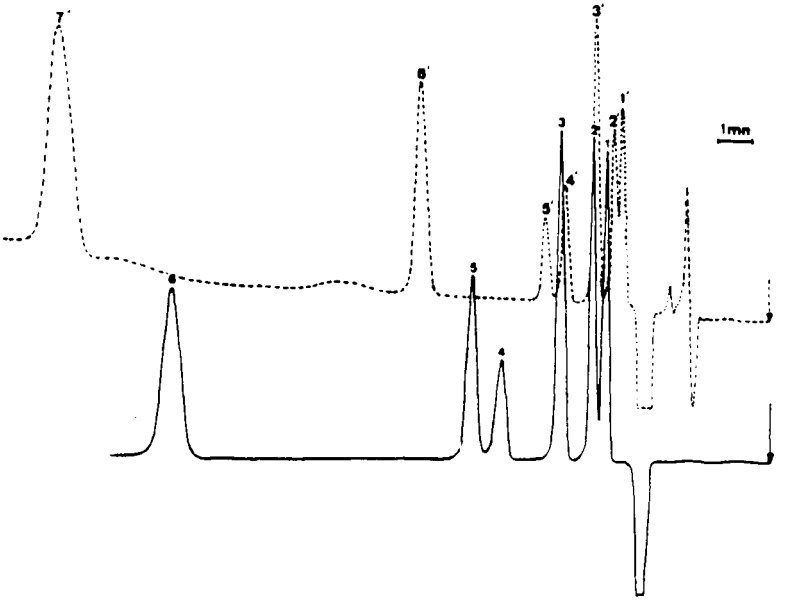


Figure 3 : Column chromatogramm of the tested solutes.
Column : 15 cm x 4.6 mm. I.D. LICHROSORB RP8 5 µm.
Mobile phase : ——— MeOH-$H_2O$ 40/60
------ MeOH-$H_2O$ 50/50
Flow-rate : 0.5 ml/mn.
UV detector : 210 nm.
Solutes : 1 - Theobromine, 2 - Acetaminophen,
3 - Theophylline, 4 - Caffeine, 5 - Barbital,
6 - Phenobarbital, 7 - Carbamazepine

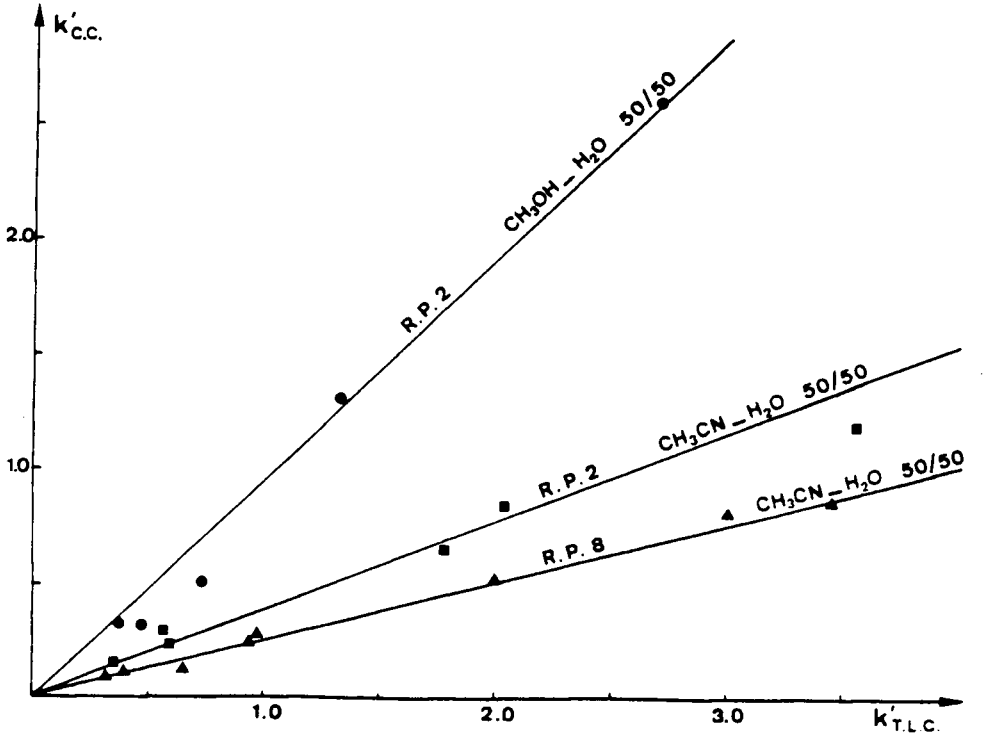


Figure 4 : Plots of $k'_{column}$ values against $k'_{HPTLC}$ values.

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