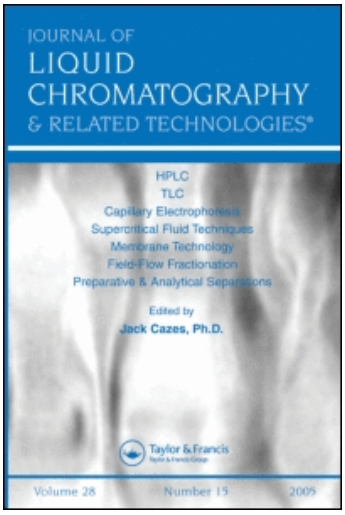


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Chromatographic Separation on Silica of Polar Aromatic Compounds II) Research of the Best Chromatographic System by Different Preloading in Thin Layer for a Transposition on Column

C. Guinchard[a]; J. D. Masson[a]; T. T. Truong[a]; M. Porthault[b]
[a] Laboratoire de Chimie Pharmaceutique 1, Place St Jacques, UER de Médecine et de Pharmacie, BESANCON, Cedex [b] Laboratoire de Chimie Analytique III, Université Claude Bernard Lyon, VILLEURBANNE, Cedex

To cite this Article Guinchard, C. , Masson, J. D. , Truong, T. T. and Porthault, M.(1982) 'Chromatographic Separation on Silica of Polar Aromatic Compounds II) Research of the Best Chromatographic System by Different Preloading in Thin Layer for a Transposition on Column', Journal of Liquid Chromatography & Related Technologies, 5: 6, 1123 — 1140
To link to this Article: DOI: 10.1080/01483918208067573
URL: http://dx.doi.org/10.1080/01483918208067573

JOURNAL OF LIQUID CHROMATOGRAPHY, 5(6), 1123-1140 (1982)

CHROMATOGRAPHIC SEPARATION ON SILICA OF POLAR AROMATIC COMPOUNDS

II) RESEARCH OF THE BEST CHROMATOGRAPHIC SYSTEM BY DIFFERENT PRELOADING IN THIN LAYER FOR A TRANSPOSITION ON COLUMN

C. GUINCHARD*, J.D. MASSON*, T.T.TRUONG*, M. PORTHAULT**

*Laboratoire de Chimie Pharmaceutique 1, Place St Jacques, UER de Médecine et de Pharmacie - 25030 BESANCON Cedex

**Laboratoire de Chimie Analytique III - Université Claude Bernard Lyon I - 43, bd du 11 novembre 1918 - 69622 VILLEURBANNE Cedex

ABSTRACT

A high performance thin layer chromatographic method for aromatic compounds separation with absorbant deactivation has been given in the precedent work. Our aim was to transpose the results obtain from HPTLC to HPLC. The two types of results were then related by a transposition coefficient, K_{tR} which interprets the geometrical variations of the two chromatographic methods. The values obtained for K_{tR} represent fairly well the linear relation between the retentions in thin layer chromatography and those in column chromatography. We have thus desmontrated the possibility of modifying adsorbent activity in the same manner in thin layer as in column chromatography by the fixation of chemical compounds, thus allowing the separation of relatively polar compounds.

1) INTRODUCTION

We showed that it is possible to make polar character substances migrate and to resolve them by thin layer chromatography on silica (Ref 1,2). This is produced by adsorbent activity modification, by preloading either with different relative moisture or with polar character entities (formid acid, diethylamine). In all chromatographic separations effectuated on thin layer, we used benzene as the migration solvent with relatively weak solvent strength. The obtained results on conventional plates (Ref 1,2) are confirmed in HPTLC with formic acid as the preloading compound. They led us to transpose the method in column HPLC, this technique is faster and more easily usable from a quantitative point of view. Moreover, this method helps to facilitate routine analysis.
Earlier works (Ref 3,4) showed that satisfactory results of transposition are obtained for a system of given phases providing that the separation mechanism is the same in thin layer and in column ; in this case the different retentions between the two techniques are due to the ratio of stationnary and mobil phases quantities. These differences are expressed by the transposition coefficient K_{tR} in the following relationship :

$$K'_{HPLC} = K_{tR} \left(\frac{1}{Rf_{HPTLC}} - 1 \right) \quad (1)$$

where $\left(\frac{1}{Rf_{HPTLC}} - 1 \right)$ corresponds to k'_{HPLC}

II) TRANSPOSITION CONDITIONS DETERMINATION

II-1 : Preliminary Experiments With The HPTLC Method

We chromatographied phenols and benzoic acids variously substituted on HPTLC silica plate "Merck" after modification of the activity, by formic acid according to the previously described method (Ref 2)
The obtained retentions are referred to as examples in figure 1-2 for benzoic acid for different deactivation rates. They show

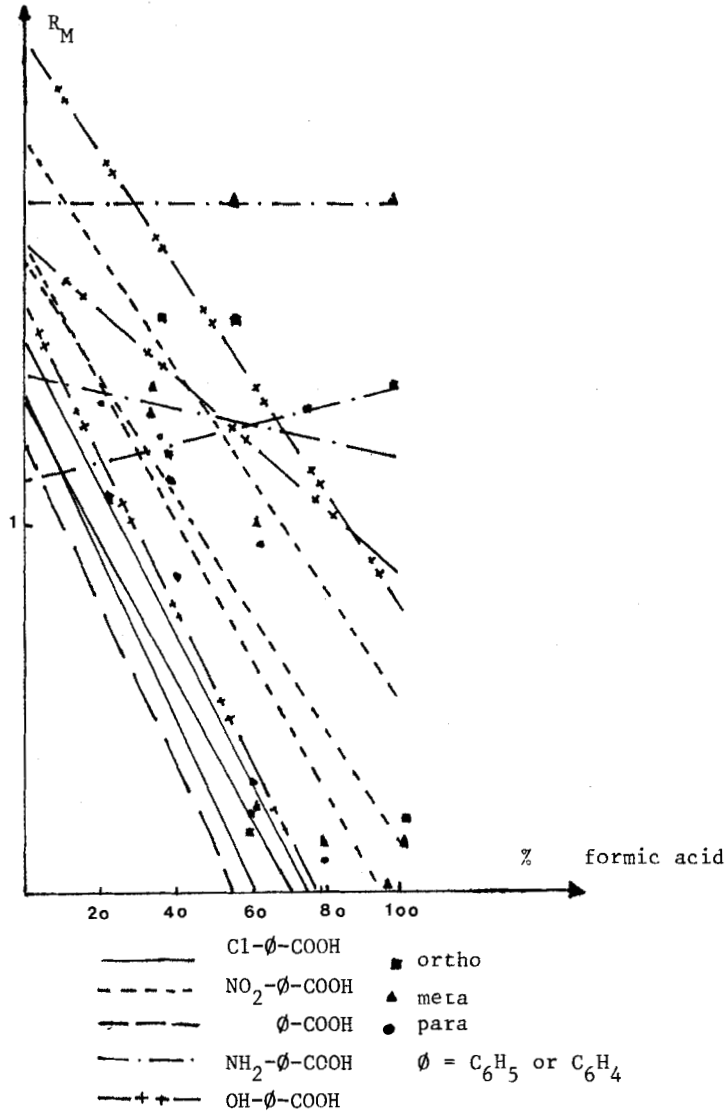


FIGURE 1

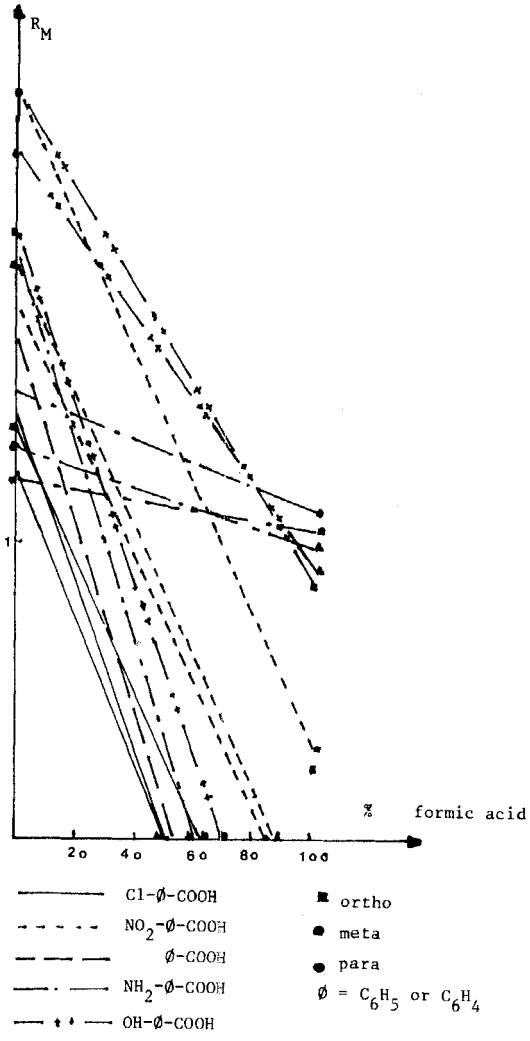


FIGURE 2

that the introduction of formic acid as deactivant in the vapor phase favours the migration of these compounds as a function of their polarity, whether we use water or diethyleneglycolmonoethyl-ether (DEGMEE) to prepare the formic acid solutions for preloading. At the same time, we know that to make a correct transposition we must choose in HPTLC an average Rf zone (Ref 3). Indeed for low Rf values, the compounds are relatively too tightly bound in these column, an on the other hand for too high Rf, they are eluted too quickly to be well resolved. In the case of deactivation with formic acid in DEGMEE solution, this happens for rates close to 50-60% of HCOOH by volume. For these percentages we showed that the separations follow an adsorption mechanism (Ref 8). So in this deactivation field we have used a certain percentage of deactivant on the silica in HPTLC that we must recover from the silica that was used in the column.

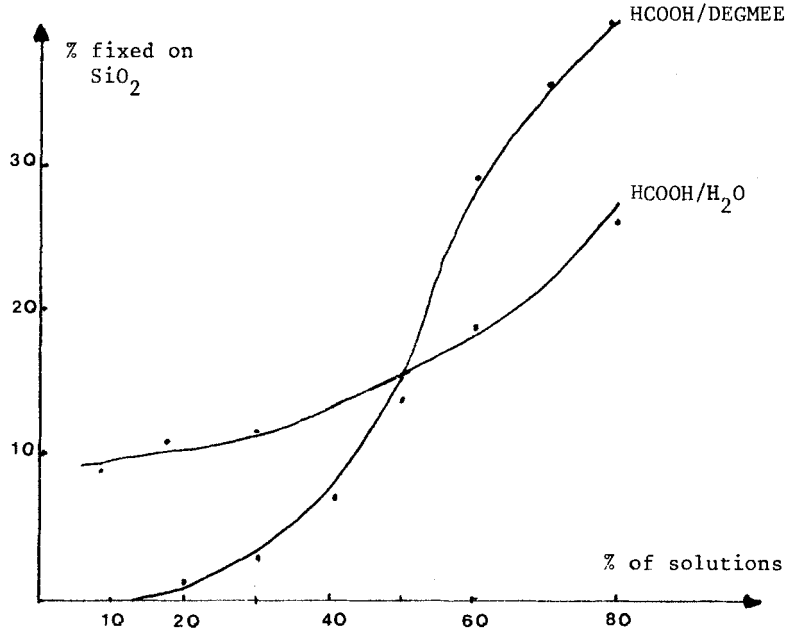


FIGURE 3

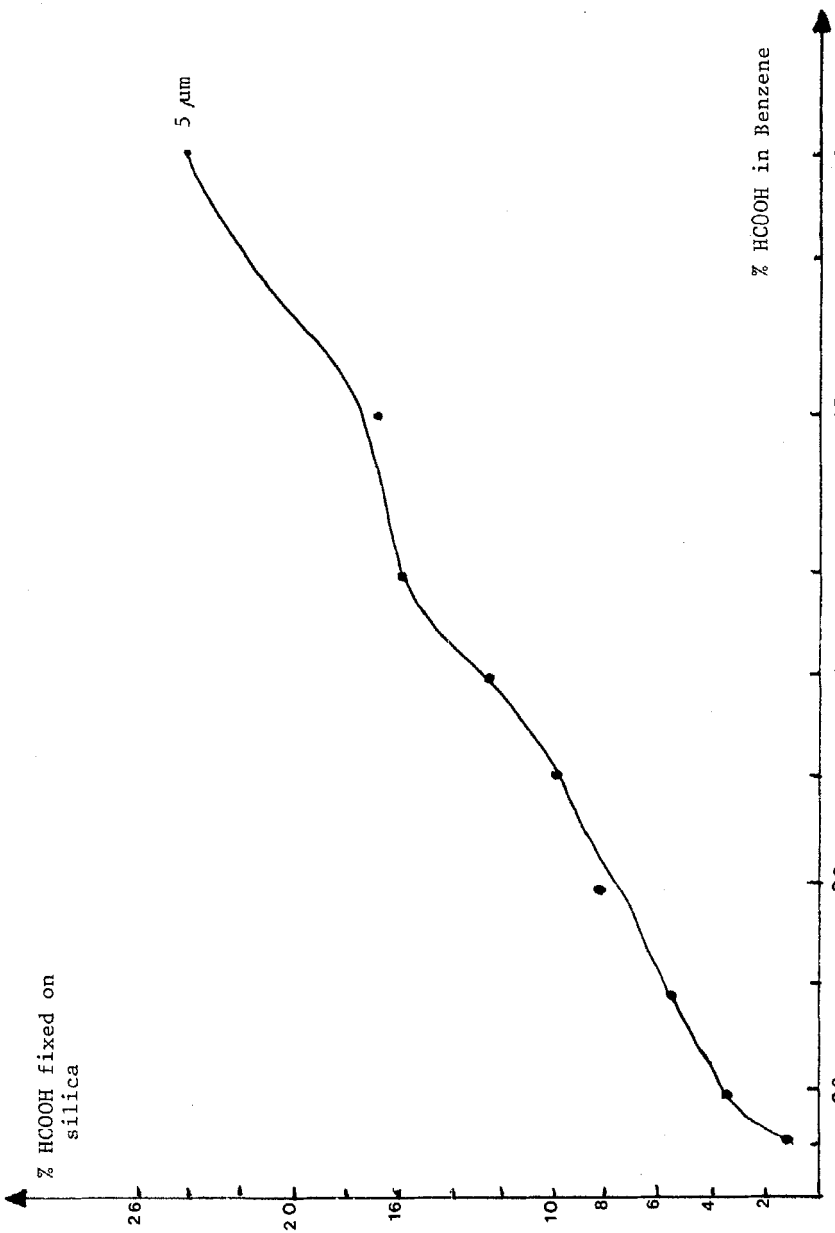


FIGURE 4

II-2 : Isotherm Determination of Deactivant Fixation on Stationary Phase

From precedent results, we are led to determine the deactivant amount previously fixed on thin layer silica by the vapor phase above preloading solution so as to recover the same condition of deactivation in column by passing through the mobile phase containing the deactivant compound.

Thus, two series of measurements were necessary : tracing at least a part of the deactivant fixation isotherm in HPTLC and in HPLC conditions.

For HPTLC, we obtained by weighing the isotherm represented on the figure 3. This shows that for preloading solutions with 50 or 60% vol/vol HCOOH in DEGMEE we have a level of deactivant fixation on thin layer respectively equal to 13,9 and 29,3% by weight.

For HPLC we used Lichrosorb 5 μ which offered the same characteristic as HPTLC silica.

After agitating ten minutes, particles of Lichrosorb mixed with different formic acid solutions in benzene, we separated the two phases. Then we dosed the formic acid remaining in benzenic phase by non aqueous protometry. In this way we deduced the formic acid adsorption isotherm, represented in figure 4.

According to isotherm 4, we see that a solution with 2% in volume of formic acid is sufficient to obtain a formic acid fixation equal to 23% in weight. Thus we can compare HPTLC and HPLC (23% is an intermediate value between the observed limit of satisfactory percentages in HPTLC 13,9 and 29,3%.

III) TRANSPOSITION

III-1 : Experimental Conditions

We used a HPLC apparatus which we built using a "Hibar Merck" column 25 cm x Ø 4 mm full of Lichrosorb Si 60,5 μ. The flow rate was 0,85 ml min^{-1}. The pressure loss was 90 bars. Elution patterns were monitored at 254 nm. First we equilibrated

the column by passing formic acid (2% solution in benzene), thus we obtained the required deactivation rate. Then we determined the retention time for a non retained naphtalene compound. Then we determined the retention times of a certain number of benzoic acids and phenols (t_R) with the benzene containing formic acid ; 2% in volume as mobile phase, hence we deduced the K'_{HPLC} values ($K'_{HPLC} = \frac{t_R - t_o}{t_o}$)

III-2 : Results

* Transposition validity

From these values of the pair of k'_{HPLC} and Rf_{HPTLC} we calculated the transposition coefficients k_{tR} with equation 1. We gathered the obtained results in the figures 5 and 6. They

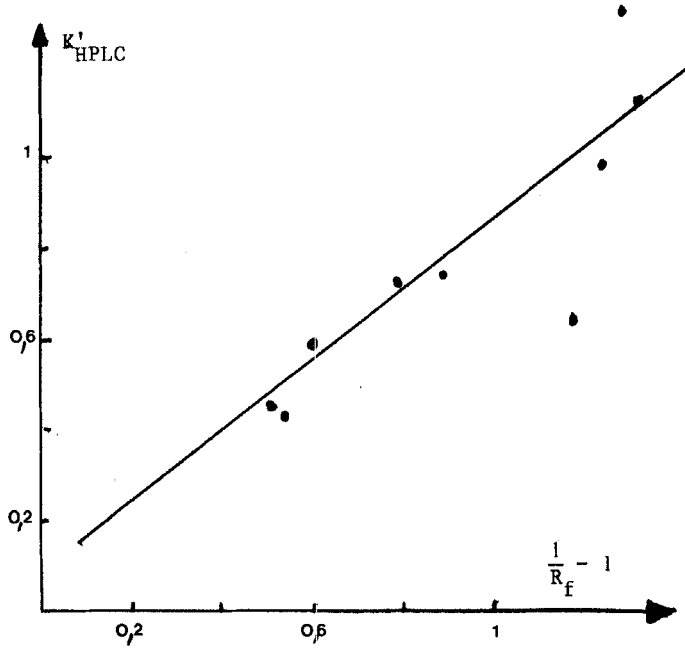


FIGURE 5

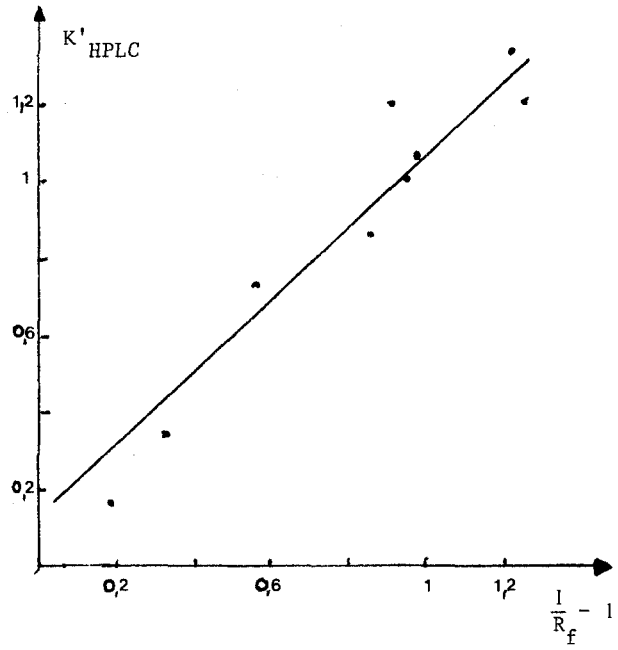


FIGURE 6

confirm the validity of the transposition relationship mentioned for the studied system. We note that the same compound possesses a retention time constant over time. This latter result proves the stability of equilibrium between adsorbent formic acid and solvent.

* Improvement in detection : Research of another system

The benzene used as initial solvent in HPTLC, then transposed in column does not allow the detection of the peaks with a good sensibility so we pursued this work using dichloromethan which has a greater solvent strength than that of benzene and which presents the advantage of absorbing little in UV at 254 nm unlike benzene (Ref 7). Then we easily adjusted the new chromatographic system in HPTLC, then in HPLC using the

previous method which had reveated itself as being satisfactory. We give the Rf and t_R for different deactivation rates of silica (figures 9,10 and table 11)

We see that dichloromethan which had a solvent strength slightly higher than benzene leads to slightly superior Rf in the same preloading conditions.

Furthermore it is easy to draw in HPLC, formic acid fixation isotherm CH_2Cl_2 solutions at different concentrations of deactivant as we did with the benzene. The results are given in figure 7.

These data allow to etablish the concentration of formic acid in mobil phase ; CH_2Cl_2 which corresponds to a same deactivation in HPLC and in HPTLC. For example a 5% HCOOH solution in

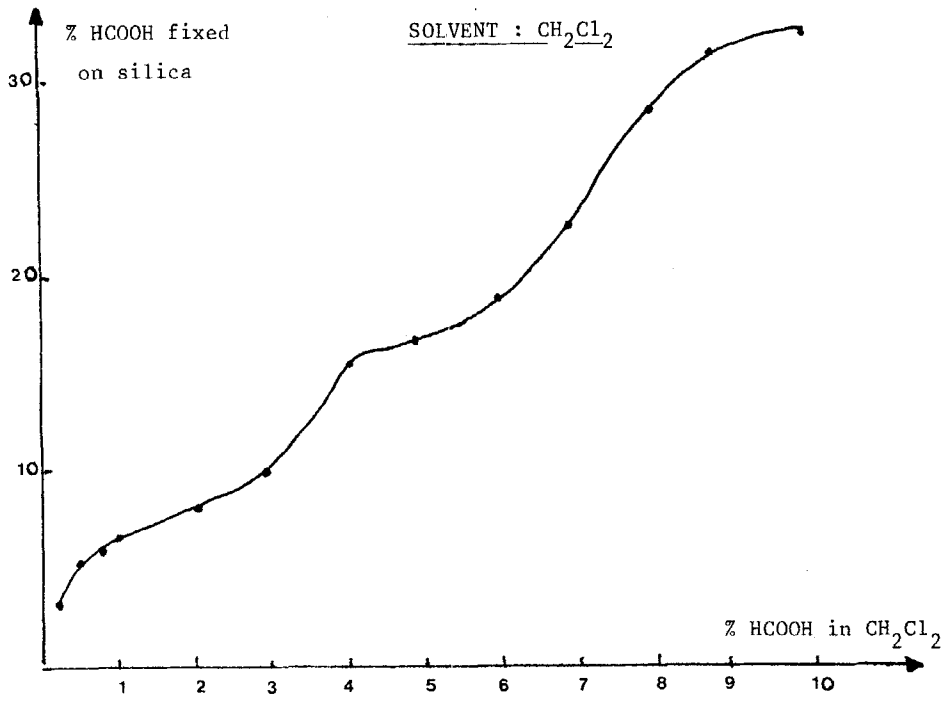


FIGURE 7

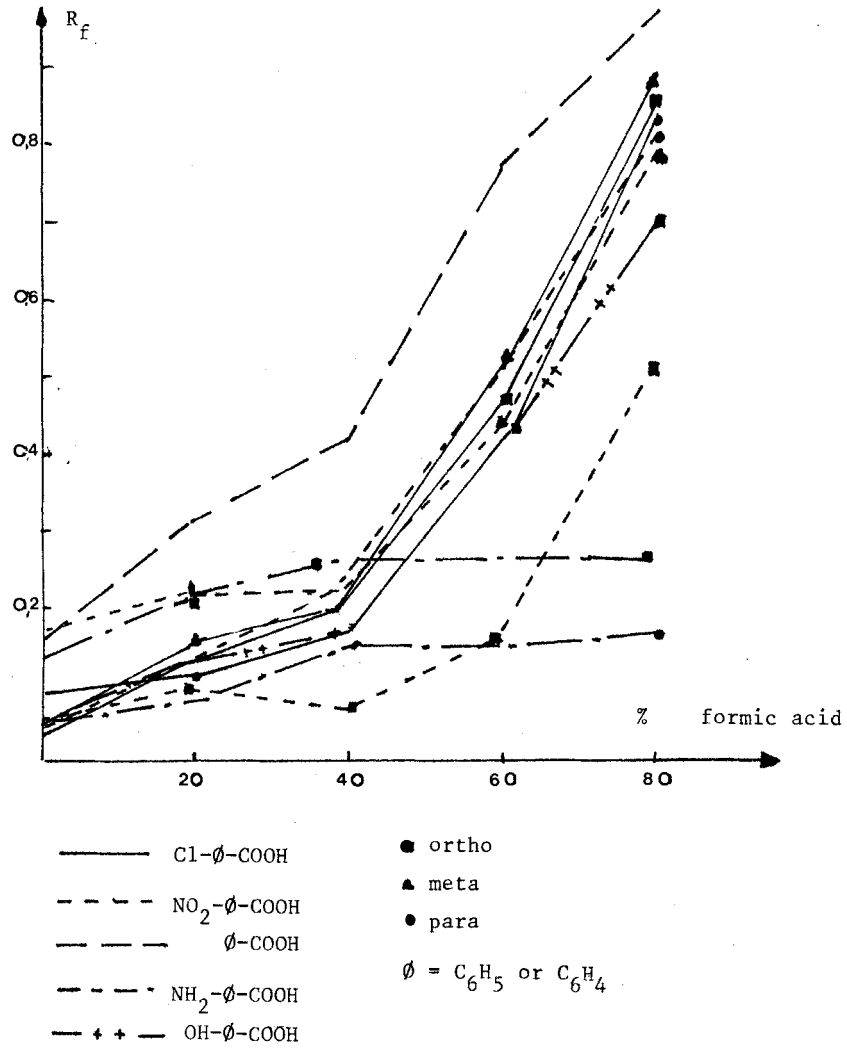


FIGURE 8

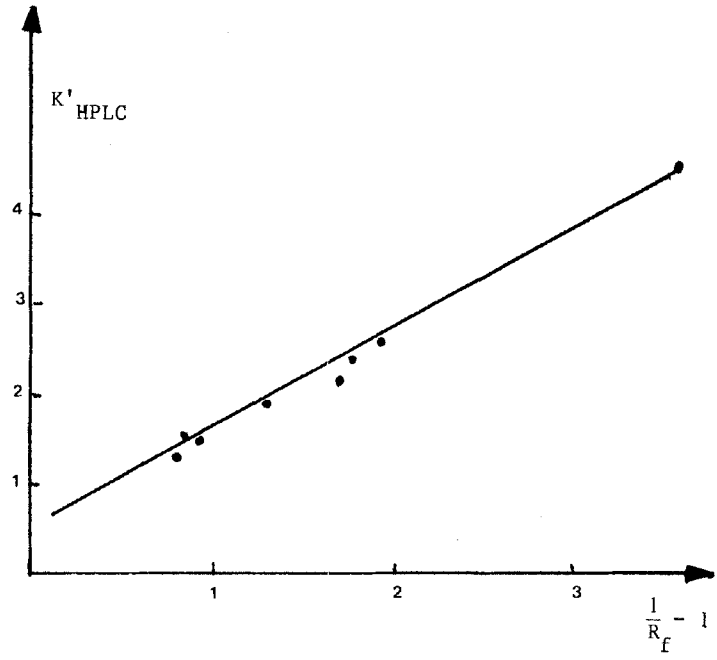


FIGURE 9

CH_2Cl_2 gives a fixation level of 18% in weight on the silica column. In HPTLC this is obtained with the preloading by a 53% solution by volume of formic acid in DEGMEE (figure 3).

We reproduce (fig 11) a chromatogramm of the different solutes studied. Under such conditions we see that separation was satisfactory.

Having different experimental values of Rf_{HPTLC} and k'_{HPLC} we again verified in these conditions the transposition relation

$$k'_{HPLC} = K_{tR}\left(\frac{1}{Rf_{HPTLC}} - 1\right)$$

We give in table 2 the different values obtained for K_{tR}. The figures 9 and 10 illustrate well enough the linear relation between k'_{HPLC} and $\left(\frac{1}{Rf_{HPTLC}} - 1\right)$ expressed by the equation 1.

Moreover our preceding work in this domain (Ref 1,2) had allowed us to conclude that the principal mechanism of the chromatography with such preloading was adsorption (essentially for lower concentrations, less than 30% by weight of fixed formic acid on adsorbent then in our case. A limit equal to approximatively 30% correspounds to a monolayer which saturates silica.

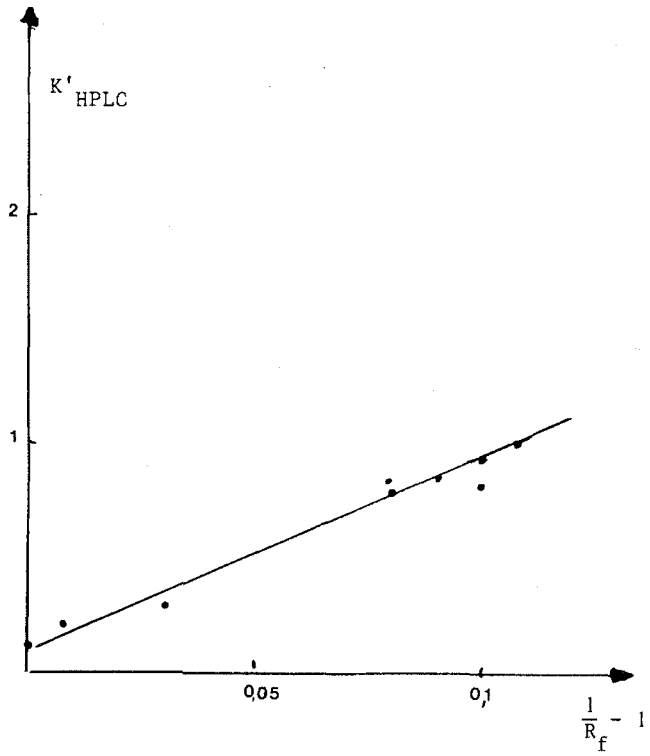


FIGURE 10

TABLE 1 : COEFFICIENTS OF TRANSPOSITION WITH CH_2Cl_2

COMPOUNDS	Rf	t_R	K'_{HPLC}	$\frac{1}{R_{fHPTLC}} - 1$	K_{t_R}
Ø-OH	0,641	23,70	1	0,56	1,78
2 Cl-Ø-OH	0,815	12,25	0,36	0,22	1,64
3 Cl-Ø-OH	0,584	20,85	1,31	0,71	1,83
4 Cl-Ø-OH	0,602	19,40	1,14	0,66	1,72
2 NO_2-Ø-OH	0,90	10,35	0,14	0,09	1,60
3 NO_2-Ø-OH	0,36	34,90	2,88	1,77	1,62
4 NO_2-Ø-OH	0,33	38,70	3,28	2,03	1,61
2 CH_3-Ø-OH	0,65	16,95	0,915	0,54	1,57
4 CH_3-Ø-OH	0,523	23,80	1,64	0,91	1,80

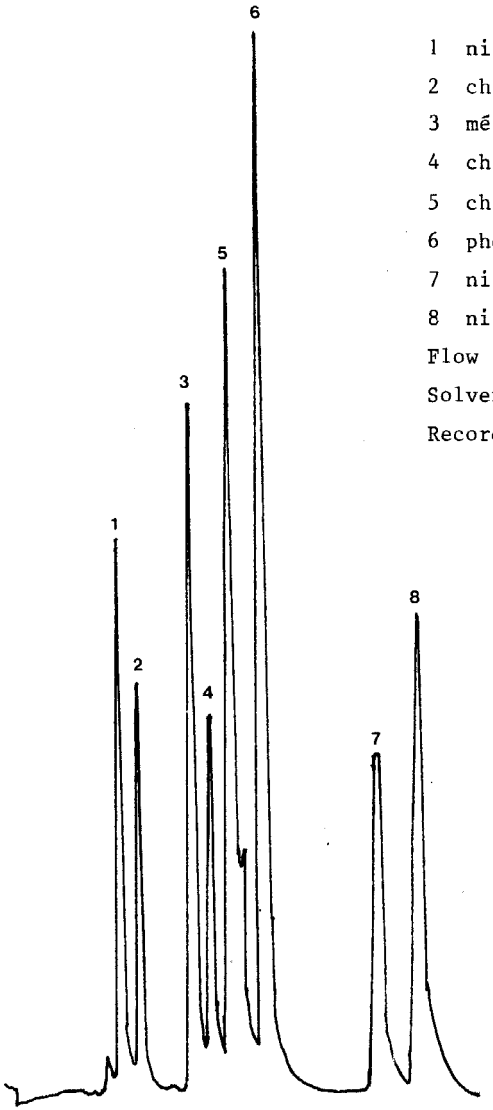
t_0 Naphtalene = 9 cm - Flow : 1 ml min^{-1} - Record : 180 mm min^{-1} - Sensibility of dect. : 0,5

Ø = C_6H_4 or C_6H_5

TABLE 11

COMPOUNDS	R_f	t_R	K'_{HPLC}	$\frac{1}{R_{fHPTLC}} - 1$	K_{t_R}
Ø-COOH	0,523	14,8	1,50	0,91	1,65
2 Cl-Ø-COOH	0,435	17,1	1,89	1,30	1,46
3 Cl-Ø-COOH	0,546	13,8	1,34	0,83	1,62
4 Cl-Ø-COOH	0,515	14,4	1,44	0,94	1,53
2 OH-Ø-COOH	0,369	18,4	2,10	1,71	1,22
2 NO_2-Ø-COOH	0,217	23,35	4,49	3,60	1,25
3 NO_2-Ø-COOH	0,515	21,05	2,57	1,94	1,32
4 NO_2-Ø-COOH	0,357	19,90	2,36	1,80	1,30

t_0 Naphtalene = 5,9 cm - Flow 1 ml min^{-1} - Record : 120 mm min^{-1} - Sensibility of dect. : 0,5



1 nitro-2-phenol
2 chloro-2-phenol
3 méthyl-2-phenol
4 chloro-4-phenol
5 chloro-3-phenol
6 phenol
7 nitro-3-phenol
8 nitro-4-phenol
Flow : 1ml-mn^{-1}
Solvent : CH_2Cl_2 with 0,5% HCOOH
Record speed : 30 mm-mn^{-1}

FIGURE 11

IV) CONCLUSION

In this work we show that it is possible to modify quantitatively and in a controlled manner the adsorbent activity by a chemical compound fixation both in HPLC and in thin layer chromatography. Thus we can bring about different separations of relatively polar compounds of to different chemical series in one run.

Indeed, depending on the solute which one wants to analyse it is possible to fix on the adsorbents either acidic or basic substances and to choose after previous study in thin layer chromatography, the best concentration in the preloading compound for an efficient and selective transposition in order to effectuate routine analysis in column HPLC.

In our particular case with the chosen system and solute used, the realization of deactivation in HPTLC and HPLC conditions appears to be relatively easy when working a field where the mechanism of separation seems to correspond to adsorption phenomenon.

If the verification of the transposition relation $k'_{HPLC} = K_{tR}\left(\frac{1}{Rf_{HPTLC}} - 1\right)$ is quite good in our conditions, it is necessary to be cautious, the knowledge of conditions for obtaining isoactivity between thin layer and column chromatography is most important to determine the conditions of a separation.

Thus in this study we were able to make polar compounds migrate in adsorption chromatography, polar compounds which are generally analysed in partition chromatography on low polar absorbents but rarely used silica (ref 6). The method that we adapted, after our study, with benzene allowed us to pass easily to dichloromethan.

REFERENCES

1 - GUINCHARD C., TRUONG T.T., MASSON J.D., PANOUSE J.J. Chromatographia, vol 8, n°10, (oct. 75), 584-586.

2 - GUINCHARD C., TRUONG T.T., MASSON J.D., PANOUSE J.J.
Chromatographia, vol 9, N°12, (dec. 1976), 627-629.

3 - COQ B., NICOLAS J.P., LAMOTTE A., PORTHAULT M.
J. of Chromatogr., 97, (1974), 137-150.

4 - VIRICEL M., LEMAR M.
J. of chromatogr., 116 (1976), 343-352).

5 - GUINCHARD C.
Thèse de Doctorat d'Etat ès Sciences, Université Claude Bernard Lyon 1, juillet 1980.

6 - SOCZEWINSKI E., MATYSIK G.
J. of chromatogr., 111, (1975), 7-19.

7 - SNYDER
"Principles of adsorption chromatography"
Inc, New York 1968, DEKKER M.

8 - GUINCHARD C., M. BAUD, J.J. PANOUSE, M. PORTHAULT
Chromatographic separation on silica of polar aromatic compounds
1) Study of some factors influenced by different conditions of preloading silica (deposed article)