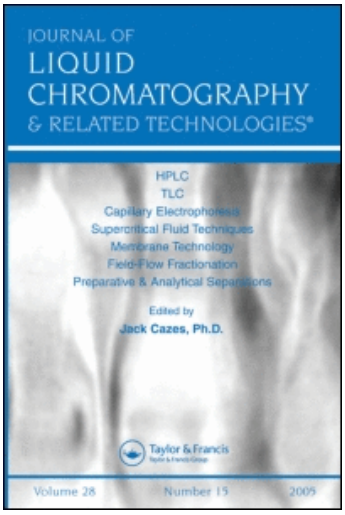


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# REVERSED-PHASE ION-PAIR THIN LAYER CHROMATOGRAPHY OF SOME ALKALOIDS

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# REVERSED-PHASE ION-PAIR THIN LAYER CHROMATOGRAPHY OF SOME ALKALOIDS

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## ABSTRACT

The retention behaviour of some alkaloids, as test samples, on reversed-phase pre-coated thin layer chromatography in the presence of different ion-pair reagents was investigated. Trifluoroacetic acid (TFA), pentafluoropropionic acid (PFPA), and heptaflluorobutyric acid (HFBA) were used as counter ions. The dependence of $R_F$ values on the eluent composition, the chain length of the ion-pair reagent and the ratio of two ion-pair reagents in the eluent is described. The retention values of the tested alkaloids after impregnation of the plate with the counter ion, were investigated.

## INTRODUCTION

Reversed-phase ion-pair chromatography is a well-known analytical method used for the separation of polar and charged substances by high performance liquid chromatography (HPLC). In this method, an organic salt, (the ion pair reagent) is added to the usually mainly aqueous eluent. The substances to be investigated form ion pairs with the ion-pair reagent, which are retained more strongly by the stationary phase. Tetra-alkyl ammonium compounds[1-3] have proved useful as ion-pair reagents for the separation of acids; alkyl sulphonates[4-6] and alkyl sulphates[7-8] have been successful for bases.

Perfluoroalkanoic acids are commonly used to supply pairing ions, especially in the separation of peptides and proteins and separation of aminoglycosides antibiotics.[12-14]

In thin-layer chromatography (TLC), the method has so far found only limited use. One of the first investigations was carried out by Lepri et al.[15-17] with silanized silica gel layers which were impregnated with sodium lauryl ether sulphate or triethanolamine dodecylbenzene sulphonate. This was followed by the work of Volkmann[18-20] and Gonnet[21] with HPTLC and TLC reversed phase pre-coated layer. Alkylsulphonates proved to be suitable ion-pair reagents for some alkaloids.[22] Hydro philic dyes were successfully separated with high selectivity on HPLC reversed-phase pre-coated plates using tetra-alkylammonium compounds.[21] A systematic study of the adsorption of different ion-pairing reagents on reversed-phase thin layers was done by Hadady[23-26] who used tetra methyl ammonium bromide (TMA), tetrabutyl ammonium bromide (TBA), trioctyl methyl ammonium chloride (TOMA), and cetyltrimethyl ammonium bromide (CTMA) as ion pairing reagents.

This paper describes the applicability of perfluorinated carboxylic acids as pairing-ion reagents for some alkaloids on reversed-phase pre-coated thin layer chromatography.



## EXPERIMENTAL

### Thin Layer Plates

TLC pre-coated RP-18 $F_{254}$ s plates from E. Merk (Darmstadt, G. F. R.) were used. Prior to use the pre-coated plates were activated by heating them at 130°C for 15 min.

For the impregnation technique, the thin-layer plates were impregnated with the mobile phase and dried for about 12 hours at room temperature. The chromatogram was developed with the same mobile phase used for impregnation.

### Mobile Phases

Different concentrations of methanol in mixture with citrate buffer of pH 1.5 were used. The solvents (100 mL) were placed together with paper liners into TLC tanks, which were then sealed and allowed to equilibrate for at least 30 min before use. The systems were run for about 10 cm from the base line, the solvent front was marked and the plates were air-dried. Not more than four plates were developed in each batch of solvent and each drug was chromatographed in duplicate in different batches of solvents.

Citrate buffer pH 1.5: 22 mL 0.1 M sodium citrate (21.008 gm citric acid monohydrate + 200 mL 1 N NaOH per liter) and 78 mL 0.1 N HCl are mixed together.

### Counter Ions

Trifluoroacetic acid (TFA), pentafluoropropionic acid (PFPA), and heptafluorobutyric acid (HFBA) were obtained from Fluka (Switzerland).

### Detection

All alkaloids were located by their response to short wavelength ultraviolet light (254 nm). After detection, the $R_F$ x 100 values were measured.



### Test Samples

The substances used were atropine sulphate, scopolamine hydrobromide, quinine dihydrochloride, papaverine hydrochloride, codeine phosphate, and strychnine nitrate. The appropriate amount and volume applied were 50 μg and 10 μL for each tested sample, except for quinine dihydrochloride and papaverine hydrochloride where the amount applied was 20 μg.

(**N.B.**: All the used chemicals and solvents were of analytical grade and were used without further purification.)

## RESULTS AND DISCUSSION

In reversed-phase chromatography, the $R_F$ values can be influenced by changes in methanol content of the eluent. This is also possible in presence of ion- pair reagents. Table (1-A) shows the relationship between the $R_F$ values of several nitrogen bases, and the methanol content of the eluent on RP- 18 $F_{254s}$ pre-coated TLC plates. It can be seen from Table (1-A) that $R_F$ values of the bases investigated are positively correlated to methanol content. On increasing methanol content in the eluent, the substance spots move further apart, so that the best selectivity is reached when the concentration of methanol in the eluent was 70%.

The behaviour of perfluorinated carboxylic acid as pairing ions was studied. The relationship between the $R_F$ values of the tested compounds and the concentration of trifluoroacetic acid (TFA) are given in Table (1-B). For all the tested alkaloids, we noticed different grades of reduction in $R_F$ values with increasing the concentration of TFA.

## Table 1

## Dependence of Retention on Methanol and Ion-Pair Concentrations

$R_F \times 100$ Values of Methanol/Citrate Buffer pH 1.5

A

| Test Samples | 80/20 | 70/30 | 60/40 | 50/50 | 40/60 | 30/70 |
|---|---|---|---|---|---|---|
| Atropine sulphate | 80 | 68 | 50 | 45 | 40 | 28 |
| Scopolamine hydrobromide | 78 | 63 | 44 | 34 | 31 | 22 |
| Quinine dihydrochloride | 55 | 40 | 28 | 22 | 18 | 11 |
| Papaverine hydrochloride | 53 | 48 | 27 | 24 | 18 | 10 |
| Codeine phosphate | 68 | 57 | 37 | 30 | 22 | 12 |
| Strychnine nitrate | 41 | 30 | 18 | 14 | 11 | 3 |

$R_F \times 100$ Values of Mobile Phases

| | B | | | | | | C | | | | | | D | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Test Samples | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 |
| Atropine sulphate | 64 | 62 | 56 | 57 | 55 | 55* | 62 | 58 | 55 | 51 | 51 | 50* | 62 | 58 | 55 | 51 | 51 | 50* |
| Scopolamine hydrobromide | 60 | 58 | 50 | 52 | 50 | 50* | 55 | 51 | 48 | 41 | 42 | 40* | 55 | 51 | 48 | 41 | 42 | 40* |
| Quinine dihydrochloride | 37 | 33 | 26 | 25 | 22 | 22 | 38 | 30 | 25 | 25 | 22 | 20 | 38 | 30 | 25 | 25 | 22 | 20 |
| Papaverine hydrochloride | 40 | 38 | 31 | 30 | 28 | 26* | 39 | 35 | 30 | 28 | 27 | 26 | 39 | 35 | 30 | 28 | 27 | 26 |
| Codeine phosphate | 55 | 52 | 46 | 44 | 42 | 40* | 53 | 50 | 42 | 40 | 38 | 38 | 53 | 50 | 42 | 40 | 38 | 38 |
| Strychnine nitrate | 31 | 25 | 20 | 20 | 18* | 17* | 28 | 25 | 22 | 20 | 18 | 16* | 28 | 25 | 22 | 20 | 18 | 16* |

1.- Methanol - Citrate buffer pH 1.5 70/30 + 0.002 M (B) TFA (C) PEPA (D) HEPA. 2.- Methanol - Citrate buffer pH 1.5 70/30 + 0.004 M (B) TFA (C) PEPA (D) HEPA. 3.- Methanol - Citrate buffer pH 1.5 70/30 + 0.006 (B) TFA (C) PEPA (D) HEPA. 4. - Methanol - Citrate buffer pH 1.5 70/30 + 0.008 M (B) TFA (C) PEPA (D) HEPA. 5. - Methanol - Citrate buffer pH 1.5 70/30 + 0.01M (B) TFA (C) PEPA (D) HEPA. 6. - Methanol - Citrate buffer pH 1.5 70/30 + 0.02 M (B) TFA (C) PEPA (D) HEPA. * Spots with tailing.

For example when we used low concentrations of TFA (0.002 M - 0.006 M) a marked decrease in $R_F$ values was noticed in all the tested alkaloids. On the other hand, when higher concentrations of TFA were used (0.008 M-0.02 M) a slight decrease of $R_F$ values was noticed in case of quinine, papaverine, codeine, and strychnine, while, for atropine and scopolamine no such changes were seen. Tailing was observed for some alkaloids in high concentrations of TFA. When pentafluoropropionic acid PFPA was used as ion-pairing agent, the $R_F$ values of all tested samples has been shown to be dependent on the concentration of PFPA but to a lesser extent than these obtained by TFA as shown in Table (1-C).

At low concentrations of PFPA (0.002 M-0.006 M) marked decrease in $R_F$ values was noticed while in higher concentrations (0.008 M-0.02 M) two different cases were seen: the first was a slight decrease of $R_F$ values in quinine, codeine, and strychnine, while the second case was no changes in atropine, scopolamine, and papaverine similar to what have been noticed in case of TFA experiments. Tailing was observed for some tested samples in higher concentrations of PFPA.

The $R_F$ values reached plateau when the concentration of heptafluorobutyric acid HFBA was higher than 0.006 M for nearly all the tested alkaloids and tailing was observed in those high concentrations Table (1-D). In this study, from the previous results, we conclude that the $R_F$ values decrease with increasing the chain length of the ion-pair reagents used.

To test the selectivity of the used ion pair, we used the impregnation technique.[27-30] Impregnation of the plate with the mobile phase including the ion-pair causes a decrease in the $R_F$ values in all the tested compounds when compared with $R_F$ data observed without impregnation in similar conditions (Table 2).

In most instances it is sufficient to add one ion-pair reagent to the eluent in sufficient amount to achieve a desired $R_F$ value. However, it is also possible to influence the selectivity of the chromatographic system in a particular direction by combining two ion-pair reagents in certain ratio.[22,31,32] To investigate this idea, TFA, PFPA and HFBA were used in different mixtures as shown in Table 3.

These results demonstrate that it is possible, by using a suitable mixture if ion-pair reagents with different alkyl chain length, to obtain $R_F$ values identical with these which would be obtained with a pure ion-pair reagent whose chain length lies between these of mixed ion-pair reagents. For example with the combination of TFA- HFBA (50 : 50), the $R_F$ values obtained for all the tested alkaloids were almost identical to those obtained with pure PFPA at the same concentration.

**Table 2**

**Retention Values After Impregnation of the Plate with the Ion-Pair**

| Test Samples | $R_F \times 100$ Values 1 | 2 | 3 |
|---|---|---|---|
| Atropine Sulphate | 60 | 55 | 53* |
| Scopolamine hydrobromide | 55 | 48 | 49 |
| Quinine dihydrochloride | 30 | 28 | 28 |
| Papaverine hydrochloride | 35 | 32 | 23 |
| Codeine phosphate | 50 | 47 | 37 |
| Strychnine nitrate | 24 | 22 | 16* |

1. - Methanol - Citrate buffer pH 1.5 70/30, + 0.004 M TFA. 2. - Methanol-Citrate buffer pH 1.5 70/30 ,+ 0.004 M PFPA. 3. - Methanol-Citrate buffer pH 1.5 70/30, + 0.004 M HFBA. * Spots with tailing.



**Table 3**

**Dependence of $R_F \times 100$ Values and Ion-Pair Mixture**

| Test Samples | Mixture of Ion-Pair Reagents TFA-PEPA (50% : 50%) | TFA-HFPA (50% : 50%) | PFPA-HFBA (50% : 50%) |
|---|---|---|---|
| Atropine Sulphate | 60 | 57 | 55 |
| Scopolamine Hydrobromide | 54 | 50 | 49 |
| Quinine Dihbydrochloride | 31 | 29 | 31 |
| Papaverine Hydrochloride | 36 | 33 | 30 |
| Codeine Phosphate | 50 | 48 | 43 |
| Strychnine Nitrate | 26 | 24 | 21 |

1. - Methanol - Citrate buffer pH 1.5 70/30 + (0.004 M TFA + 0.004 M PFPA 50 : 50). 2. - Methanol - Citrate buffer pH 1.5 70/30 + (0.004 M TFA + 0.004 M HFBA 50 : 50). 3. - Methanol - Citrate buffer pH 1.5 70/30 + (0.004 M PFPA + 0.004 M HFBA 50 : 50).

## CONCLUSION

The results show that perfluorinated carboxylic acids can be used conveniently as pairing ions in ion-pair reversed-phase thin layer chromatography. The chromatographic conditions can be easily adjusted to suit a given separation by varying the solvent composition and changing the nature and the concentration of the pairing ion.

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