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RETENTION BEHAVIOUR OF SOME CLASSES OF PHARMACEUTICAL PRODUCTS ON CHEMICALLY MODIFIED THIN-LAYER CHROMATOGRAPHIC PLATES

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SUMMARY

In order to widen the application of chemically modified plate materials in the analysis of drug substances, the characteristics of such commercially available precoated polar and non-polar modified layers have been studied using a series of butyrophenones and benzimidazol-2-ones as model compounds, and normal as well as reversed-phase conditions. The influence of different types of salts, salt concentrations, organic modifier content, the use of ion-pair reagents and the influence of pH on the retention behaviour have been examined. The most suitable layers for reversed-phase thin-layer chromatography of the compounds investigated are the cyano-, RP-2 and RP-18W (completely wettable RP-18 layer) high-performance plates. Because of the low wettability with water-rich solvent mixtures, and hence a long development time and more diffuse spots, the RP-8 and RP-18 layers are less suitable. The high-performance amino-modified precoated plate, used under normal-phase conditions, is very suitable for chromatography of the alkaline compounds investigated.

INTRODUCTION

Up to a few years ago, most problems related to thin-layer chromatography (TLC) were solved in our laboratories by means of normal-phase chromatography on silica gel as the stationary phase. After the introduction of the first commercially available, chemically modified, plate materials in 1977-1978 (Whatman, Antec, Merck, Macherey-Nagel, etc.), and partly under the influence of high-performance liquid chromatography (HPLC) technology, in which reversed-phase packing materials were being used quite generally at that time, more TLC methods were systematically developed on chemically modified plate materials. At present in our laboratories the ratio of silica gel/chemically modified phase TLC applications is approximately 20:80.

TLC, using silica gel as a stationary phase, is still used quite frequently for pilot investigations and partial optimization of chromatographic parameters for preparative column chromatographic separations. Both for quality-control applications, in which minute percentages of starting materials and by-products have to be determined in a pharmaceutical substance, and for process-control purposes, in which measurements have to be performed within a wide concentration range, we initially opted for the KC_{18} plate material supplied by Whatman. Using this material, we were able to achieve a good compromise between the hR_F value, spot shape and running time by means of relatively simple eluent combinations, usually consisting of a 0.5–1 *M* salt solution (sodium chloride, ammonium acetate) and 1,4-dioxane, tetrahydrofuran, methanol or a mixture of these organic modifiers. In order further to improve the separation we later started to use high performance (HP) TLC plate materials (RP-18, Merck). However, when we introduced the smaller 10 cm × 10 cm HPTLC plates, a number of major advantages soon became apparent. In comparison to the normal quality TLC plates, separation times appeared to be shorter, spots were less diffuse and less solvent was consumed. Thus, we continued to use systematically the 10 cm × 10 cm TLC quality plates, as if they were HPTLC plates. This implied the application of smaller amounts, the use of shorter migration distances and development in smaller chambers. The results were very successful, since separations could usually be transferred quite easily from the 20 cm × 20 cm to the 10 cm × 10 cm plates. Rarely had the mobile phase composition to be modified, whilst the detection limit of the impurities relative to the main component remained almost constant. For a number of semiquantitative analyses, cutting up the 20 cm × 20 cm TLC plates was (and still is) a valuable alternative to the use of the more expensive HPTLC quality plates.

Nevertheless, at present we are still using real HPTLC plates most of the time, since a greater variety of plate types^{1–8} is available. Moreover, because of the finer surface structure of the layer, this type of plates permits a much better detection of smaller quantities of product by means of *in situ* scanning equipment (improved signal-to-noise ratio).

To acquire an insight into the retention behaviour of diverse product classes on the available modified plate materials, systematic research is required. For this purpose we have composed a number of series comprising our most frequently occurring product classes. Each series contains starting materials, isomeric compounds, analogous structures, decomposition products and by-products. Such a series thus represents a realistic model of a normal analytical problem. For product series we chose the butyrophenones and benzimidazol-2-ones. The composition of both series with the chemical structure of each component is given in Tables I and II.

EXPERIMENTAL

All experiments were carried out on HPTLC precoated plates from Merck (Darmstadt, F.R.G.). Plate types used: RP-2 F_{254s} (Art. 13726); RP-8 F_{254s} (Art. 13725); RP-18 F_{254s} (Art. 13724); NH_2 F_{254s} (Art. 15647); CN F_{254s} (Art. 16464); RP-18 WF_{254s} (Art. 13124). Due to visualization problems with certain products, 500 ng was used as a standard application aliquot.

A Camag twin-trough chamber was used for development, one trough containing 10 ml of the eluent. The water used for the preparation of the salt solutions was purified via reverse osmosis prior to use. All solvents and reagents used were of analytical grade quality or were purified by us prior to use. Reflection measurements were performed on a Camag TLC scanner II, linked with an HP 9816 computer (Camag software 1986, revision 7.01).

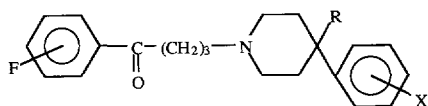
The major parameters investigated were: effect of the type of salt^{3,7} and its molar concentration on the retention behaviour and the spot shape; use of ion-pair reagents^{1,5}; pH effects (addition of inorganic acids/bases, or the use of buffer solutions); influence of choice of organic modifier⁶ on the selectivity.

The salts used were lithium chloride, ammonium chloride, ammonium acetate and sodium chloride (1, 0.5, 0.25, 0.1 and 0.05 *M*), the organic modifiers were methanol, acetone, 1,4-dioxane and 2-propanol and the ion-pair reagents were 1-butane-, 1-hexane- or 1-octanesulphonic acid sodium salt in buffered (pH 3.5) and non-buffered media (1, 0.5, 0.25 and 0.1 *M*).

RESULTS AND DISCUSSION

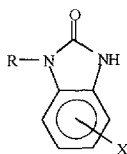
A detailed discussion of all experiments performed is obviously not possible. Therefore, we shall restrict ourselves to the most important observations.

TABLE I
COMPOSITION OF THE BUTYROPHENONE SERIES



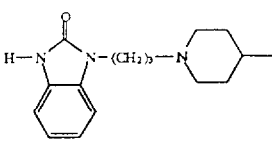
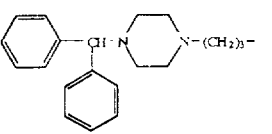
Compound	Research No.	Position of F atom	R	X
1	R 1616	4	OH	4-F
2	R 1625	4	OH	4-Cl
3	R 1658	4	OH	4-CH ₃
4	R 1838	4	OH	4-H
5	R 2498	4	OH	3-CF ₃
6	R 8719	3	OH	4-Cl
7	R 9298	4	OH	3-CF ₃
				4-Cl
8	R 11333	4	OH	4-Br
			-C- <i>n</i> -C ₉ H ₁₉	
9	R 13672	4	 O	4-Cl
			-C- <i>n</i> -C ₉ H ₁₉	
10	R 46541	4	 O	4-Br
			O	
11	R 65996	2	OH	3-CF ₃
12	R 72265	2	OH	4-CH ₃

TABLE II
COMPOSITION OF THE BENZIMIDAZOL-2-ONE SERIES



Compound	Research No.	R	X
1	R 4748		H
2	R 30507		H
3	R 29676		5-Cl
4	R 4730		H
5	R 4749		H
6	R 6238		H
7	R 6413		H
8	R 29764		5-Cl
9	R 33812		5-Cl
10	R 34009		5-Cl
11	R 34301		5-Cl

TABLE II (continued)

Compound	Research No.	R	X
12	R 34315		H
13	R 35443		H

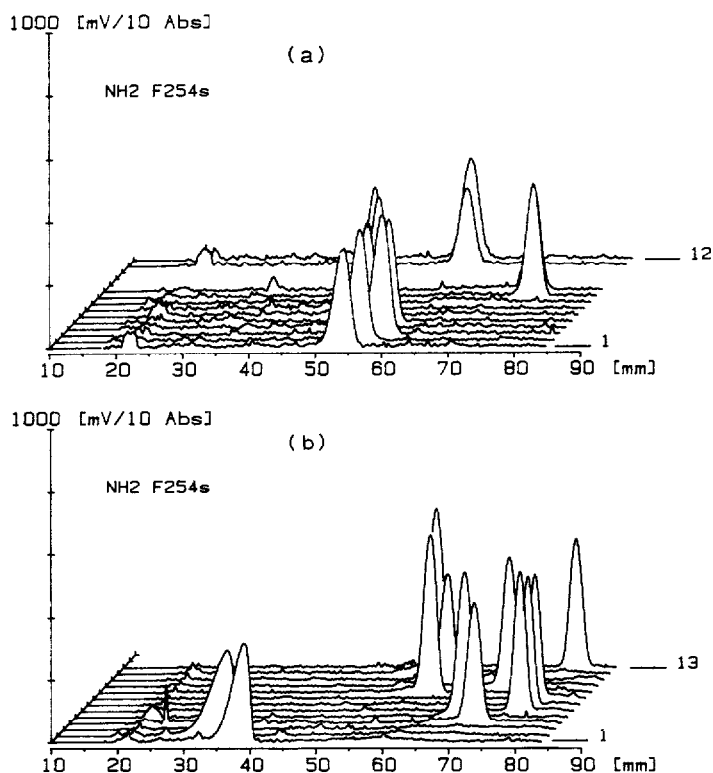


Fig. 1. Normal-phase chromatography on amino-modified HPTLC plates, NH₂ F_{254s}: (a) butyrophenones, mobile phase cyclohexane-1,4-dioxane (70:30, v/v); (b) benzimidazol-2-ones, mobile phase cyclohexane-2-propanol (70:30, v/v).

Amino plate

For both product classes none of the reversed-phase conditions tested (mixtures of water and organic modifiers in a ratio of 40:60, v/v) yielded good results on the NH_2 phase. The products were mostly eluted as "front-running" spots. Under normal-phase conditions (mixtures of cyclohexane and polar modifiers in different ratios), on the other hand, the retention is well controllable both for the butyrophenones and the benzimidazol-2-ones and a good spot shape is observed in virtually all cases (Fig. 1). In general, we have observed for our products that, in order to obtain a certain separation, the composition of the eluents on the NH_2 plate material is much more simple than that required for an equivalent result on silica gel as the stationary phase. Separations on an amino phase are also more reproducible than on silica gel. Due to the less polar character of the former plate type, the retention seems to be influenced to a smaller extent by the relative humidity.

In most cases, a smaller amount of the polar component is required, which obviously has a beneficial effect on the prevention of solvent separation or, in other words, multifront formation. The favourable results obtained under normal-phase conditions on this type of plate are presumably the consequence of the alkaline characteristics of the alkylamino groups present on the plate. The effect of the polar component in the eluent on the retention behaviour is illustrated in Fig. 2.

The low hR_F values observed for the benzimidazol-2-one derivatives, when acetone and dioxane are used as polar components in the mobile phase, are probably

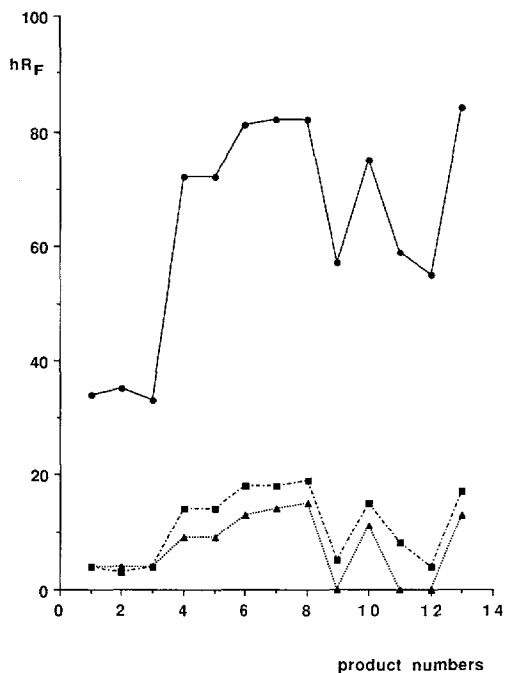


Fig. 2. Effect of the polar component in the mobile phase on the retention of benzimidazol-2-ones (see Table II for product numbers) on NH_2 F_{254s}. Mobile phases: \blacktriangle = cyclohexane-acetone (70:30, v/v); \blacksquare = cyclohexane-1,4-dioxane (70:30, v/v); \bullet = cyclohexane-2-propanol (70:30, v/v).

the result of strong interactions between the somewhat acidic secondary amide hydrogen of the benzimidazol-2-one and the alkaline alkylamino groups on the plate. Therefore, a stronger eluent will probably be required to displace these products.

Ion-pair reagents

On the plate types investigated (RP-2, CN and RP-18W), ion-pair reagents have a particularly positive effect on the spot shape for both product series (Fig. 3).

For the various ion-pair reagents applied, the results obtained in a buffered medium are comparable to those obtained in a non-buffered medium. This may presumably be ascribed to the pH value of the ion-pair reagent used. For the ion-pair reagent dissolved in buffer pH 3.5, as well as for the reagent dissolved in water, the pH values were always found to be approximately 4–5 units lower than the pK_a values of most of the products investigated, so that we can be sure that ion-pair formation occurs in both cases. When ion-pair reagents dissolved in buffer pH 3.5 are used, the spots formed are usually smaller than in experiments carried out under identical circumstances in the non-buffered medium.

For both product classes, the lowest hR_F values are obtained at a concentration of 0.1 *M* of the ion-pair reagents investigated, which may be regarded as a logical consequence of increasing dissociation of the reagent at lower concentrations, so that the chance of ion-pair formation increases. In general, however, the spot size increases with decreasing concentration of ion-pair reagent. At a concentration of 0.1 *M*, the spots usually become longer on the CN phase. Fig. 4 shows the effect of the different ion-pair reagents on the retention behaviour of the butyrophenones on an RP-2 plate. The hR_F values obtained under identical chromatographic conditions for a 0.25 *M* sodium chloride solution were used as a reference. This curve clearly demonstrates that the hydrophobicity of the ion pairs formed increases with increasing chain length of the reagent used.

In general, on the RP-2 and RP-18W material, the effect on the retention behaviour is rather small for butanesulphonic acid. The greatest differences from the reference curve were observed for octanesulphonic acid. As regards the spot shape, butanesulphonic acid yields the best results.

Owing to the presence of a polar functional group, the cyano plate has a more polar character than the RP-2 and RP-18W plate types. This is clearly visible: in contrast to the observations on the RP-2 and RP-18W material, the hR_F values measured for the reference solution (0.25 *M* NaCl) are lower for both product series than in those cases where an ion-pair reagent was applied. On the basis of this observation, we expect a decrease in the hR_F values in the order octyl, hexyl, butyl as a result of an increasing hydrophobicity of the ion pairs formed with increasing chain length of the ion-pair reagent used. This appeared to be the case for none of the product classes investigated. The highest retention was always observed for the ion pairs formed with octanesulphonic acid. Therefore, both the adsorptive and the distributive characteristics of the layer can be utilized on the cyano plate.

Effect of salts

As is generally known, the addition of inorganic salts to the aqueous phase, in reversed-phase TLC, considerably improves the spot shape. However, our experiments demonstrated that addition of inorganic salts to the mobile phase may also exert a number of other effects on the TLC behaviour of the compounds investigated.

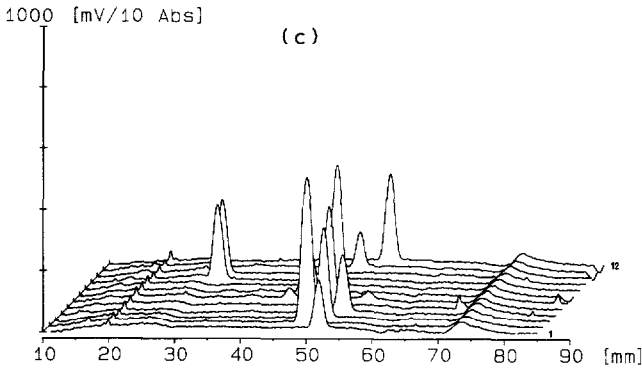
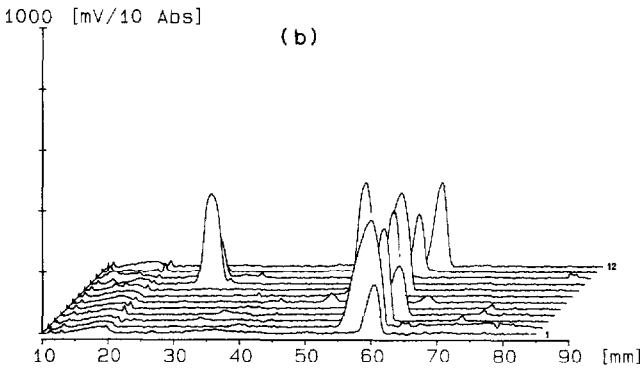
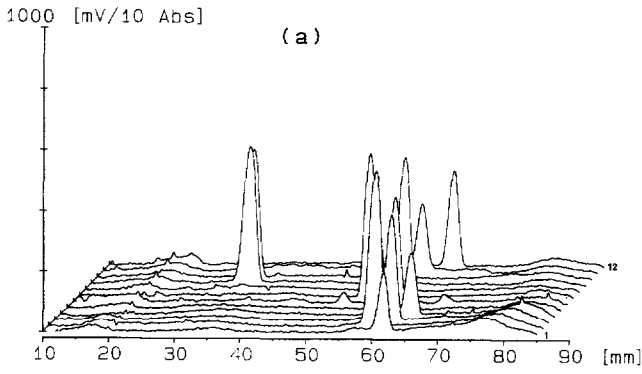


Fig. 3. Effect of ion-pair reagents on the spot shape of butyrophenones on: RP-18 WF_{2.54}. Mobile phases: (a) 1,4-dioxane-0.25 *M* sodium chloride (60:40, v/v); (b) 1,4-dioxane-buffer pH 3.5 (60:40, v/v); (c) 1,4-dioxane-0.25 *M* octanesulphonic acid sodium salt in buffer pH 3.5 (60:40, v/v).

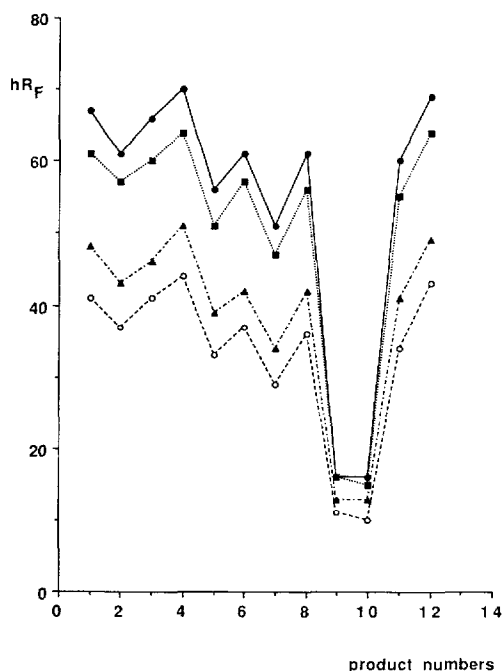


Fig. 4. Effect of ion-pair reagents on the retention behaviour of butyrophenones (see Table I for product numbers) on RP-2 F_{254s}. Mobile phases: 1,4-dioxane-aqueous phase (60:40, v/v); (●) 0.25 M sodium chloride, (■) 0.25 M sodium 1-butanedisulphonate-buffer pH 3.5, (▲) 0.25 M sodium 1-hexanedisulphonate-buffer pH 3.5, (○) 0.25 M sodium 1-octanedisulphonate-buffer pH 3.5.

Influence of the salt concentration on the retention behaviour and spot shape. Table III summarizes for the different plate types the influence of the salt concentration on the retention behaviour of the product classes investigated. Ammonium, lithium as well as sodium chloride yield good results as tailing reducers. For these salts,

TABLE III

BENZIMIDAZOL-2-ONES AND BUTYROPHENONES: INFLUENCE ON PRODUCT RETENTION OF THE SALT CONCENTRATION IN THE RANGE 0.05-1 M

—, Almost no effect, or only a very limited effect; +, distinct effect.

Salt	Plate type				
	RP-2	RP-8	RP-18	RP-18W	CN
Lithium chloride	}	—	—	—	—
Ammonium chloride					
Sodium chloride					
Ammonium acetate	+	+	+	+	+
Ammonium hydroxide	+	+	+	+	+

concentration changes within the range tested have only a very limited effect on the retention behaviour.

Ammonium acetate, on the other hand, shows a strongly deviating behaviour in comparison to the other salts. When the salt concentration is lowered, the retention of most compounds increases considerably (Fig. 5). A comparable effect is observed when dilute ammonia solutions are used as the aqueous phase. The fact that alkaline compounds are sometimes strongly retained on chemically modified phases can be explained by interactions between these compounds and residual acidic silanol groups in the packing material. In salt-containing mobile phases, the cation present will play a major role in suppressing silanol interactions. Although the phenomenon of "silanol effects" is still somewhat obscure, it is highly probable that the interaction between cations from the mobile phase, acidic silanol groups and protonated alkaline compounds occurs via an ion-exchange mechanism⁹:



For the product classes investigated, changes in salt concentration have little effect on the retention behaviour when lithium chloride, sodium chloride and ammonium chloride are used. It is likely that, with these salts, sufficient cations are present in the mobile phase, even at low concentrations, to shield the acidic silanol groups, or, in other words, to force eqn. 1 completely to the left.

When ammonium acetate solutions are used as the aqueous phase, it is clear that at low concentrations the alkaline compounds to be analyzed are preferably bound to the acidic silanol groups. Although the dissociation rate of a weak electrolyte increases when its concentration is lowered, there are, from an absolute point of view, more ions present in the solution at higher concentrations. As a result, upon increasing the salt concentrations the equilibrium in eqn. 1 will shift more and more to the left so that the retention due to silanol interactions decreases. The influence of ammonium hydroxide solutions on the retention behaviour of the compounds investigated can be explained in a similar fashion. Nevertheless, it should be noted that in such solutions most of the products tested are no longer present in a protonated form. In principle, however, these free bases are also able to bind to the acidic silanol groups without ion-exchange effects.

Table IV summarizes for the butyrophenones and the benzimidazol-2-ones the influence of salt concentration on the spot shape. Ammonium, lithium and sodium chloride, up to a concentration of 0.25 *M*, usually result in a good spot shape on all plate types tested. On RP-8 and RP-18, the spots formed are usually more diffuse than on CN, RP-2 and RP-18W plate material.

Over the entire concentration range, the smallest spots are observed on RP-18W plates. For our products, salt solutions of 0.1 *M* result in problems with regard to spot shape in many cases. Elongation of the spots with decreasing salt concentration is manifested most clearly on the CN phase; spherical spots are observed on this plate type only at the highest concentrations (Fig. 6).

Ammonium acetate as a tailing reducer results in a good spot shape for both product series at higher salt concentrations (1–0.5 *M*). Ammonium hydroxide as the aqueous phase mostly yields, for the plate types tested (CN, RP-2, RP-18W), a very good spot shape at higher concentrations (1, 0.5, 0.25 *M*). However, for reasons of reproducibility, the use of dilute ammonia solutions is less suitable in practice.

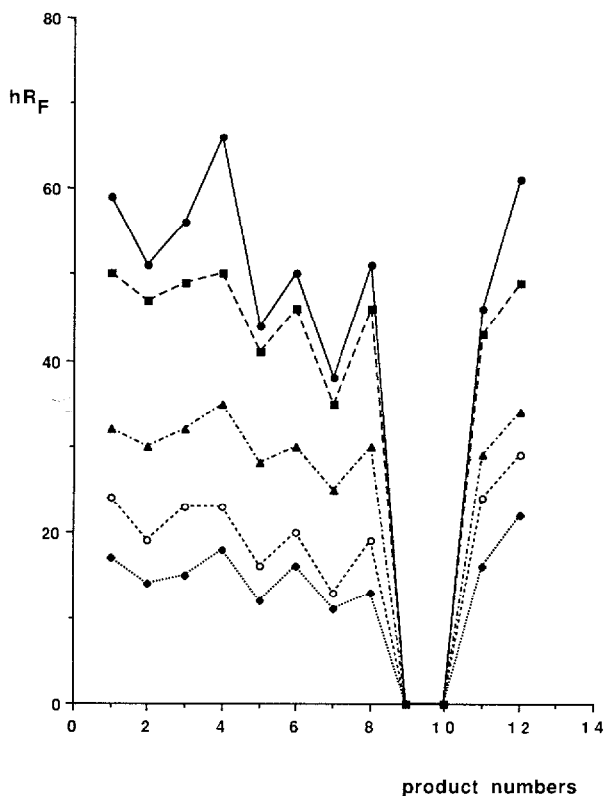


Fig. 5. Effect of ammonium acetate concentration on the retention behaviour of butyrophenones (Table I) on RP-18 F_{254s}. Mobile phases: 1,4-dioxane-aqueous ammonium acetate (60:40, v/v); (●) 1, (■) 0.5, (▲) 0.25, (○) 0.1, (◆) 0.05 M.

TABLE IV

BENZIMIDAZOL-2-ONES AND BUTYROPHENONES: INFLUENCE OF THE NATURE AND CONCENTRATION OF THE SALT SOLUTION USED ON THE SPOT SHAPE

+, Good spot shape; +(D), good spot shape, but rather diffuse spots; ++, round, well formed spots; +++, minute round spots; -, mostly poor spot shape (fronting, tailing, formation of stripes); -(E), distinctly elongated spots; NI, not investigated.

Salt	Concentration	Plate type				
		RP-2	RP-8	RP-18	RP-18 W	CN
Lithium chloride Ammonium chloride Sodium chloride	High	++	+(D)	+(D)	+++	+
	Low	-	-	-	++	-(E)
Ammonium acetate	High	++	+(D)	+(D)	+++	+
	Low	-	-	-	++	-(E)
Ammonium hydroxide	High	++	NI	NI	+++	+
	Low	+	NI	NI	++	-(E)

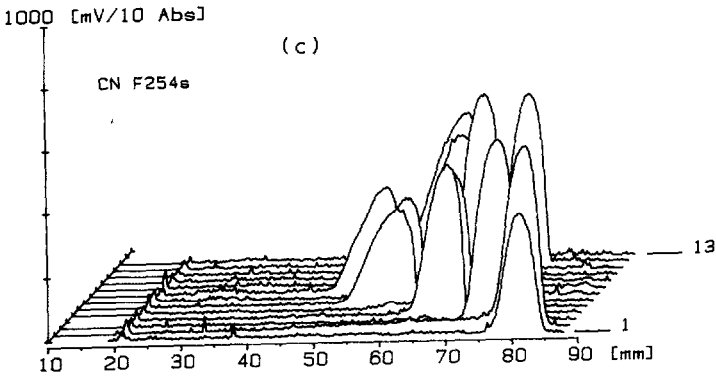
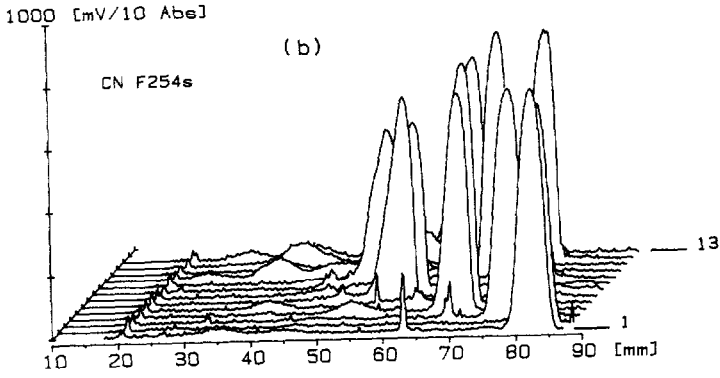
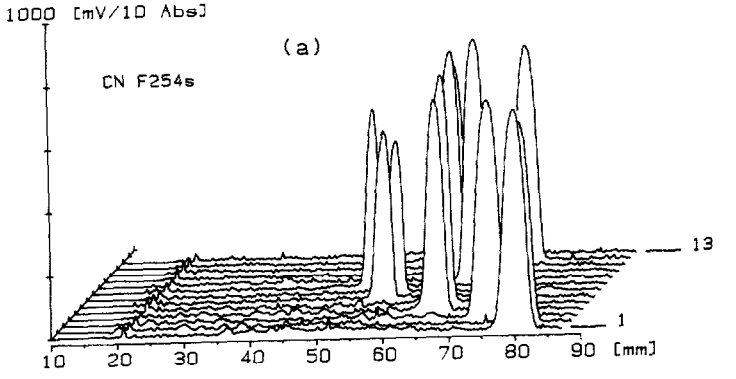


Fig. 6.

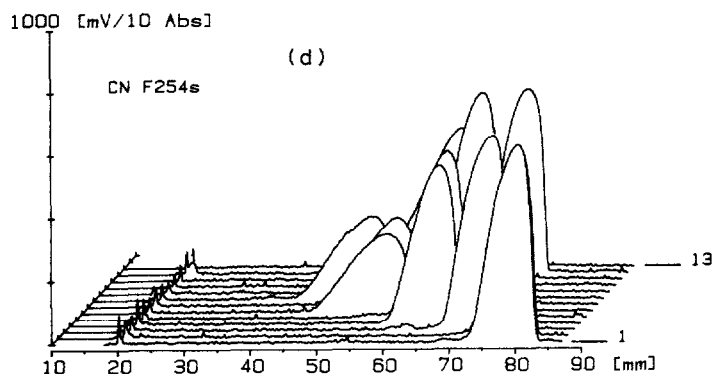


Fig. 6. Effect of inorganic salt concentration on the spot shape of benzimidazol-2-ones on CN F_{254s}. Mobile phases: 1,4-dioxane–aqueous ammonium chloride (60:40, v/v); (a) 1, (b) 0.5, (c) 0.1, (d) 0.05 M.

Effects on the spot shape which result from the combined effects of the kind of organic modifier and the nature and concentration of the salt solution used. For the product classes investigated, ammonium, lithium and sodium chloride in combination with the solvents tested (methanol, acetone, 2-propanol and 1,4-dioxane) result, at higher salt concentrations, in a good spot shape on all plate types. At salt concentrations of 0.1 M, problems with the spot shape are frequently encountered. A concentration of 0.05 M does not result in a good spot shape with any of the salt solution–organic modifier combinations. The spot shape remains relatively good at the lowest salt concentration only on the RP-18W plate.

Ammonium acetate, in combination with the solvents tested, results in a good spot shape for both product series at higher salt concentrations (1 and 0.5 M). In the more dilute solutions, fronting was observed on several plate types with methanol, 2-propanol and 1,4-dioxane as the organic modifiers. On the RP-18 plate, even stripes were seen over the entire migration distance. A good spot shape was obtained at lower salt concentrations only when acetone was used as the solvent.

Furthermore, according to the eluent composition, we often noted secondary fronts when using ammonium acetate. Moreover, the fact that the zone containing the salt rapidly turns yellow is often regarded as a nuisance, particularly for reflection measurements.

Ammonium hydroxide as an aqueous phase in combination with the various solvents investigated mostly resulted, for the plate types tested (CN, RP-2, RP-18W), in a very good spot shape at higher concentrations (1, 0.5, 0.25 M). Particularly on the CN phase, lower concentrations yield poorer results.

In general, 1,4-dioxane in combination with the different salt solutions furnishes the best spot shape and the most diffuse spots are observed with methanol as the organic modifier. An additional phenomenon for water–methanol mixtures is tailing of relatively apolar compounds (e.g., R 13672, a basic substance with a large hydrophobic moiety incorporated within the molecule), presumably a direct result of the poorer solubility of such products in polar solvents. Our experience shows that salt formation, as a result of which the solubility of these products in polar solvents increases, may solve this problem.

Correlation between the retention behaviour and the polarity of the plate type used.

For ammonium, lithium as well as for sodium chloride as tailing reducers, the retention behaviour for both product series may be correlated quite well with the polarity of the plate types tested. The lowest hR_F values are almost always found on RP-18W, the highest on RP-18. The hR_F values on RP-2 and RP-8 are situated, with a few exceptions, at predictable positions between these two extremes (Fig. 7).

The results obtained on the CN phase are somewhat more difficult to categorize, based only on the polarity of the material. As mentioned before, several mechanisms presumably determine the retention behaviour on this plate type.

As regards the selectivity, under identical chromatographic circumstances, there are no pronounced differences between the respective plate types.

When ammonium acetate is used the retention behaviour of the compounds investigated on the various plate types is less easy to predict, particularly for the benzimidazol-2-ones (Fig. 8). In this series the retention behaviour is so random that no sensible relationship can be established with the polarity of the plate type tested.

For dilute ammonium hydroxide solutions in combination with an organic modifier, the retention behaviour of the compounds investigated correlates well with the polarity of the plate types tested, with only a few exceptions (for example the polar secondary amines R 4748, R 30507, R 29676 which are strongly retained on RP-18W particularly due to additional silanol interactions on this material) (Fig. 9).

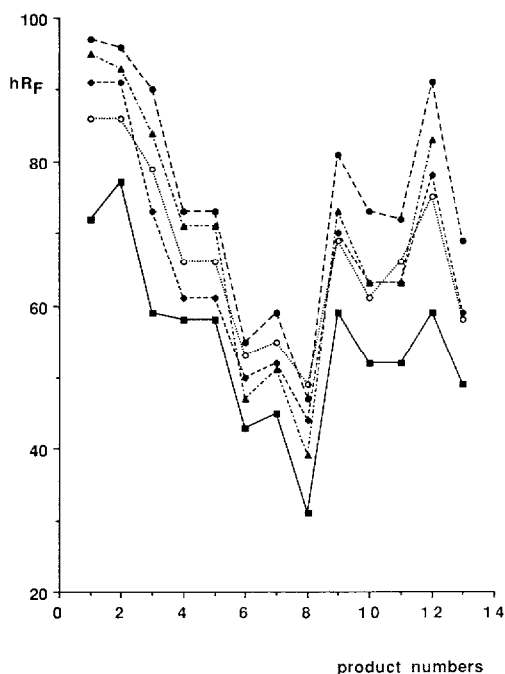


Fig. 7. Influence of the inorganic salt and different reversed-phase stationary phases on the retention of benzimidazol-2-ones (Table II). Plates: \circ = CN F_{254s} ; \blacklozenge = RP-2 F_{254s} ; \blacktriangle = RP-8 F_{254s} ; \bullet = RP-18 F_{254s} ; \blacksquare = RP-18 WF_{254s} . Mobile phase: 1,4-dioxane-1 M lithium chloride (60:40, v/v).

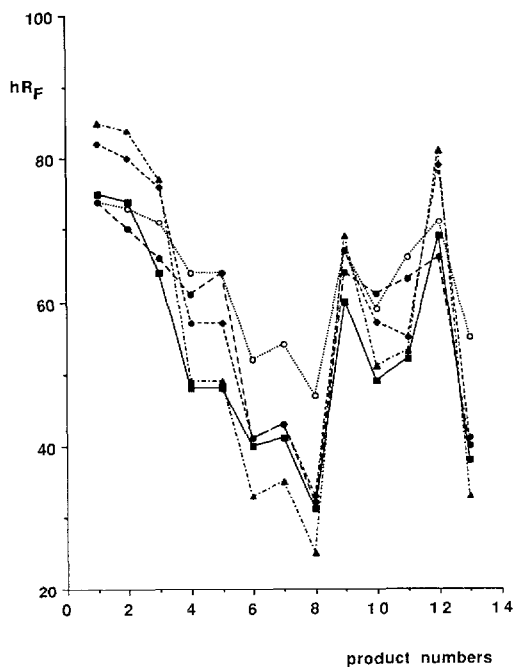


Fig. 8. Influence of ammonium acetate and different reversed-phase stationary phases on the retention of benzimidazol-2-ones (Table II). Plates: as in Fig. 7. Mobile phase: 1,4-dioxane-1 *M* ammonium acetate (60:40, v/v).

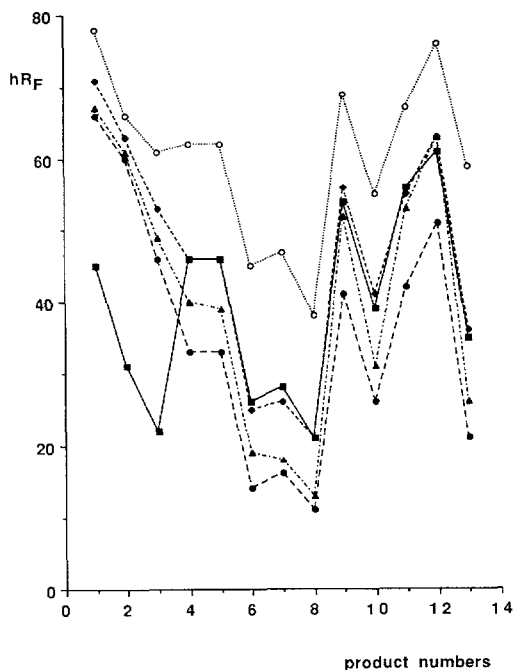


Fig. 9. Influence of ammonia and different reversed-phase stationary phases on the retention of benzimidazol-2-ones (Table II). Plates: as in Fig. 7. Mobile phase: 1,4-dioxane-1 *M* ammonia (60:40, v/v).

pH effect

pH control in the acidic range by the addition of hydrogen chloride to the chloride salt solutions used as part of the eluent has, for our products, no effect on the retention behaviour. This may be considered logical, since the pH values of the salt solutions used are usually sufficiently low to produce protonated forms of the substances to be subjected to chromatography.

Retention control in the alkaline range, on the other hand, is possible by using ammonium salts and pH control by means of concentrated ammonia or sodium hydroxide solutions. The influence on the retention behaviour by adding inorganic bases is certainly not a pure pH effect, since, for a neutral salt (0.25 M NaCl) practically no shifts in retention occur upon addition of sodium hydroxide or ammonia.

Ammonium chloride, on the other hand, adjusted to the desired pH with 10 M NaOH does show a certain shift of retention. The most sizeable effects on retention are observed for ammonium salts in combination with ammonia (Fig. 10).

The difference in effect observed upon addition of an inorganic base to a sodium chloride or ammonium chloride solution may perhaps be explained by changes in the ionic composition of the aqueous phase. When a sodium chloride solution is used as the aqueous phase the addition of small amounts of sodium hydroxide or ammonia will hardly have any effect on the ionic equilibrium. However, with the acidic salt ammonium chloride there is an effect. The pronounced interference with the retention behaviour, when ammonium chloride–ammonia is used, is probably to be ascribed to

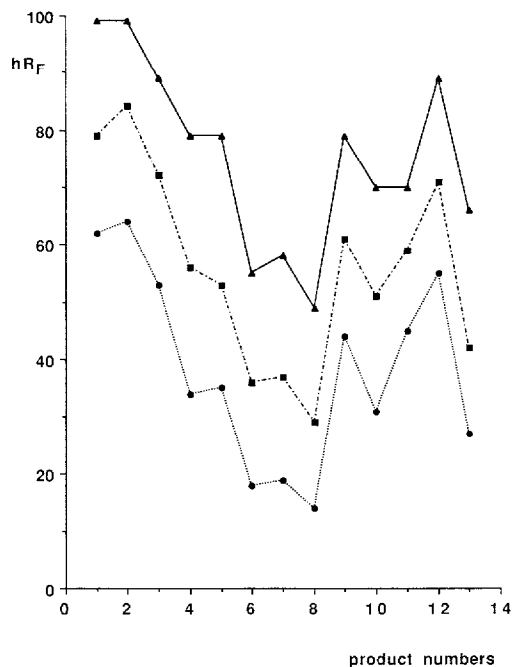


Fig. 10. pH effect on the retention of benzimidazol-2-ones (Table II). Plate: RP-2 F₂₅₄₈. Mobile phases: ▲ = 1,4-dioxane–0.25 M ammonium chloride (60:40, v/v); ■ = addition of sodium hydroxide to pH 10; ● = addition of ammonia to pH 10.

the aforementioned strong effect on the retention behaviour upon use of dilute ammonium hydroxide solutions.

An interesting observation is that ammonium chloride solutions, adjusted with NaOH, have an additional effect on the selectivity compared with identical solutions adjusted with ammonia (Fig. 11). At pH 8–9 or 10, no separation of the three analogous compounds R 29676, R 30507 and R 4748 was obtained on any of the plate types investigated (RP-2, CN, RP-18W), when ammonium chloride solutions were used which had been adjusted with sodium hydroxide. For ammonium chloride solutions whose pH had been adjusted with ammonia, there was, at pH 10, a baseline separation for these three components, on RP-18W as well as on the cyano plate. For the product series investigated, pH control by means of ammonia hence has a more pronounced effect on the selectivity than a pH adjusted with sodium hydroxide. However, similar tests on other product series from the benzimidazol-2-one group have demonstrated that the selectivity is sometimes influenced more by pH control with sodium hydroxide than by the addition of ammonia. Therefore, the example shown should certainly not be considered as a general rule.

CONCLUSIONS

It is possible to summarize the results of our investigations as follows.

For our alkaline compounds, cyano, RP-18W and RP-2 are the most interesting plate materials under reversed-phase conditions; RP-18W generally yields the best spot shape.

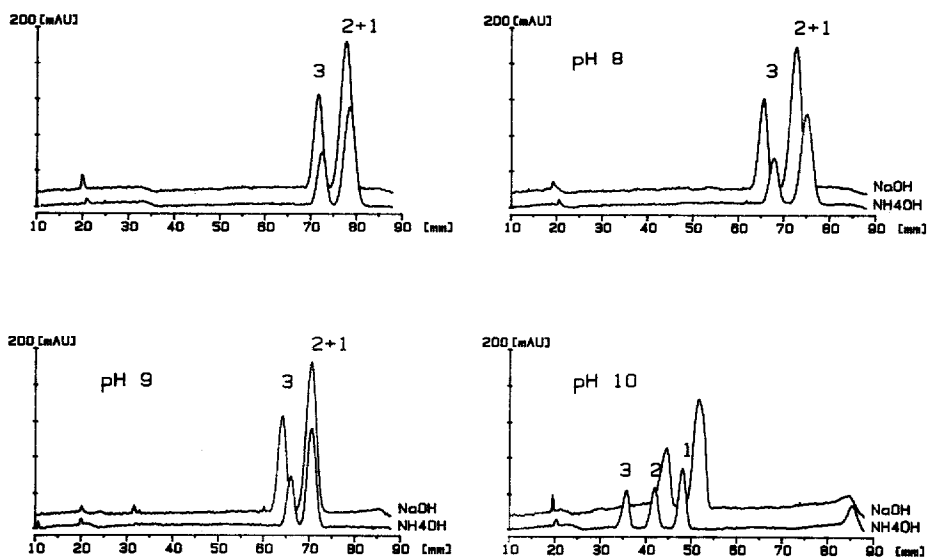


Fig. 11. Effect of pH control with ammonia or sodium hydroxide on the selectivity for benzimidazol-2-ones: 1 = R 29676; 2 = R 30507; 3 = R 4748. Plate: RP-18 WF_{254s}. Mobile phase: 1,4-dioxane–0.25 M ammonium chloride (60:40, v/v) adjusted to different pH values with ammonia or sodium hydroxide.

The amino phase, when used under normal-phase conditions, is also very suitable for our applications.

Improvement of the spot shape may be achieved by adding salts in which there is little difference in retention behaviour, if any, when using ammonium, lithium or sodium chloride. For these salts, concentration changes have only a limited effect on retention behaviour. The spot size usually increases with decreasing salt concentration. Ammonium acetate shows a behaviour which is totally different from those of the other salts. For this salt the retention of most compounds increases considerably with decreasing salt concentration, and no sensible relationship can be established with the polarity of the plate types investigated.

Retention can be strongly influenced by adding ammonia, particularly if this occurs in combination with an ammonium salt.

When using ion-pair reagents, a distinct improvement of the spot shape is achieved, and the selectivity can also be influenced.

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