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REVERSED-PHASE HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY OF MEBEVERINE HYDROCHLORIDE AND RELATED COMPOUNDS

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SUMMARY

The chromatographic behaviour of mebeverine hydrochloride, a musculotropic spasmolytic agent, and some related compounds, *i.e.* degradation compounds and intermediates from the synthesis, on reversed-phase pre-coated high-performance thin-layer plates is described.

The retention and selectivity on different commercially available layers are studied in connection with different eluent properties. The developed method is applied to the quantitative determination of mebeverine hydrochloride. Excellent selectivity and good reproducibility are obtained, and the method provides a combined test of assay, purity and stability.

INTRODUCTION

Mebeverine hydrochloride (I), 3,4-dimethoxybenzoic acid 4-{ethyl-[2-(4-methoxyphenyl)-1-methylethyl]amino}butyl ester hydrochloride, a musculotropic spasmolytic agent, is frequently used in medicinal practice. Several papers have been published concerning its mode of action[1–2] and the activity[3–6], but there is little published work concerning the quantification of mebeverine hydrochloride. Two papers describe a chromatographic system[7,8]: both methods are screening tests for a few hundreds of compounds, more useful for identification than determination. Recently we described a high-performance liquid chromatographic determination of I[9]. As an exctension of this work and to demonstrate the possibilities of reversed-phase high-performance thin-layer chromatography (HPTLC) in the analysis of drugs (stability-testing, purity control and assay), a direct densitometric method on an RP-8 layer, suitable for the stability-indicating determination of I in Tablets, is described.

The specificity of the chromatographic system was evaluated by chromatographing a mixture of I, some degradation compounds (veratric acid (II) and 4-{ethyl-[2-(4-methoxyphenyl)-1-methyl-ethyl]amino}-1-butanol (III) and two intermediate compounds from the synthesis[10], 4-iodobutyl veratrate (IV) and N-[ethyl-(4-methoxyphenyl)isopropyl]amine (V). Papaverine hydrochloride (VI) is used as the

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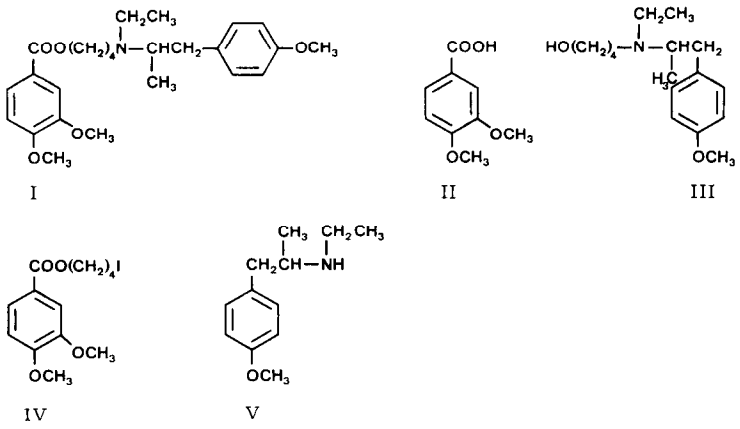

internal standard. Methyl veratrate (VII), also present in the mixture, is a result of the esterification of II with the methanol used as solvent.

The chromatographic behaviour of this complex solute mixture was investigated on different commercially available reversed-phase pre-coated HPTLC plates (different alkyl chain length and different degree of alkylation) in connection with different eluent properties (ammonia concentration, pH, water content, salt concentration and type and concentration of organic modifier). Some details concerning the influence of some of these separation variables on the development time are included.

EXPERIMENTAL

Reagents and solvents

Mebeverine hydrochloride, 4-iodobutyl veratrate and N-[ethyl-(4-methoxyphenyl)isopropyl]amine were kindly supplied by the Laboratoires Pharmaceutiques R. H. Trenker S.A. Brussels, Belgium. 4-{Ethyl-[2-(4-methoxyphenyl)-1-methylethyl]amino}-1-butanol was prepared as described elsewhere[9]. Acetic acid 100% (UCB, Belgium), ammonia 25% (UCB, Belgium) and all other products and solvents used were of analytical grade and were used as received.

Equipment

A Zeiss KM 3 densitometer with micro-optics (Zeiss, Oberkochen, F.R.G.), equipped for densitometric measurements at 263 nm in the reflectance mode, was used. The spots were applied to the plate as solutions in methanol by means of a Nanomat HPTLC spotting device (Camag, Muttenz, Switzerland) equipped with a 200-nl fixed-volume nanopipette (Pt–Ir). Plates were developed in a HPTLC twin-trough chamber (Camag), and peak areas were calculated with a Spectra-Physics minigrator (Spectra-Physics, Darmstadt, F.R.G.).

Chromatography

Plates. Different types of commercially available reversed-phase precoated HPTLC plate were used: HPTLC RP-2, HPTLC RP-8, and HPTLC RP-18 F_{254s} pre-coated plates (Merck) and Nano-SIL C_{18}-50, Nano-SIL C_{18}-75 and Nano-SIL C_{18}-100 UV_{254} precoated plates (Macherey-Nagel).

Mobile phases. The mobile phases were prepared by mixing the stated volume percentages. If pH adjustments are made, an aqueous solution containing the desired ammonia concentration is adjusted to pH with glacial acetic acid and mixed with the organic modifier component of the mobile phase.

Development procedure. After predevelopment of the plates for 2 h with methanol, the plates were spotted and dried in air for 15 min before being placed in a twin-trough chamber and equilibrated at room temperature (21 ± 2°C) for 10 min with 5 ml of the eluent (first compartment of the chamber). Prior to chromatography, 5 ml of the mobile phase is pipetted into the second compartment of the development chamber.

The quantitative determination was carried out with a mobile phase consisted of methanol–water–ammonia (80:18:2) on RP-8 F_{254s} precoated plates (Merck) over a development distance of 5 cm.

Procedure for the quantitative determination of mebeverine hydrochloride

Internal standard solution. Dissolve 250 mg of papaverine hydrochloride in 100 ml of methanol.

Standard solution. Weigh accurately *ca.* 25 mg of mebeverine hydrochloride reference standard into a 25-ml volumetric flask; add 10.0 ml of the internal standard solution and dilute to 25.0 ml with methanol. Apply 200 nl of this solution to the plate.

Sample preparation. Weigh a quantity of ground and homogenized tablet powder, corresponding to 25 mg of mebeverine hydrochloride, into a 25-ml volumetric flask. Add 10.0 ml of the internal standard solution and 10 ml of methanol, and dilute the suspension to 25.0 ml with methanol, after ultrasonic dispersion for 5 min. Mix and centrifuge the suspension for 5 min at 480 *g*. Apply 200 nl of the supernatant to the plate.

RESULTS AND DISCUSSION

Investigation of the mobile phase properties

Ammonia concentration. An eluent system based on the mobile phase previously proposed for the liquid column chromatographic determination of mebeverine hydrochloride[9], *i.e.* methanol–water–hexylamine (75:25:0.5), adjusted to pH 5 with phosphoric acid, was tested first, but without any positive result. Replacing hexylamine by ammonia was necessary to obtain a mobile phase that was chromatographically useful. Addition of ammonia to the eluent is necessary to move the basic compounds I, III and V from the starting point (Fig. 1). Since papaverine hydrochloride (pK_a 6.4)[11] is already partially deprotonated in the eluent containing no ammonia (methanol–water, 80:20), less interaction occurs with the residual silanol groups at the surface of the HPTLC RP-8 F_{254s} precoated plate, and a reasonable R_F value (R_F 0.22) is obtained for this compound. The relative position of the plateau obtained for each basic compound with increasing amount of ammonia in the eluent is dependent on the lipophylic characteristics of the solute: the higher the plateau is situated the more hydrophilic the (uncharged) basic compound is. The concentration of ammonia necessary to reach the plateau is dependent on the pK_a value for each particular solute; the higher the pK_a value the more ammonia is necessary to yield a R_F value independent of the ammonia concentration in the eluent.

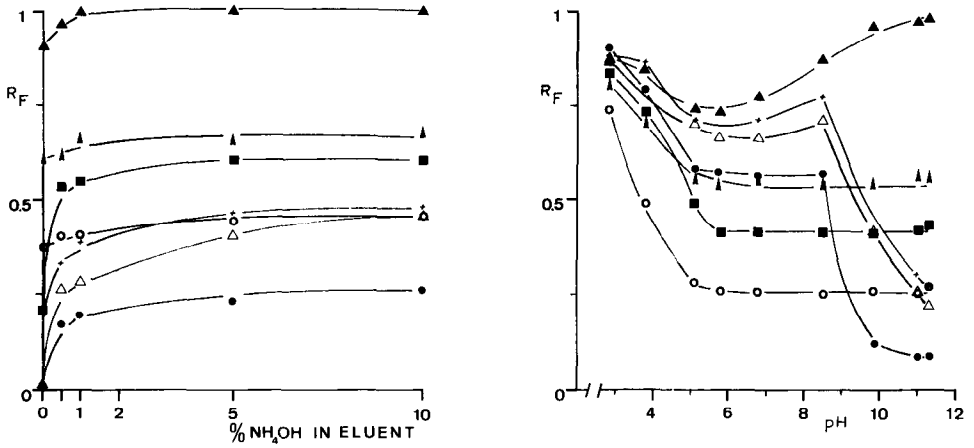

Fig. 1. R_F values as a function of the ammonia concentration in the eluent. Eluent, methanol–water–ammonia (80:20:0→10); HPTLC RP-8 F_{254s} precoated plate; detection, reflectance at 263 nm. Key: 1 = N-[ethyl-(4-methoxyphenyl)isopropyl]amine (V) (△); 2 = 4-{ethyl-[2-(4-methoxyphenyl)-1-methyl-ethyl]-amino}1-butanol (III) (+); 3 = mebeverine hydrochloride (I) (●); 4 = veratric acid (II) (▲); 5 = papaverine hydrochloride (VI) (■); 6 = methylveratrate (VII) (◭); 7 = 4-iodobutylveratrate (IV) (○).

Fig. 2. Effect of the pH of the eluent on retention. Eluent, methanol–water–ammonia (75:23:2), adjusted to pH with glacial acetic acid. Plate and detection, see Fig. 1; key as in Fig. 1.

As expected, the neutral compounds IV and VII are virtually unaffected by an increasing ammonia concentration in the eluent, whereas the acidic compound (II) of the mixture shows a decreased retention as the charged form of the acid readily interacts with the polar mobile phase. The relationship of the R_F value to the percentage of ammonia in the eluent on reversed-phase HPTLC is surprisingly similar to that obtained with HPTLC on bare silica[12]. This is a strong evidence that retention

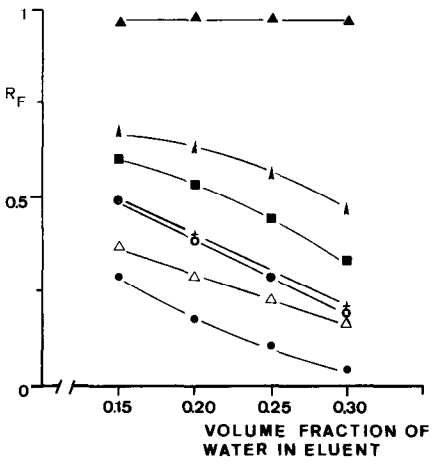

Fig. 3. R_F values as a function of the water content of the eluent. Eluent, methanol–water with 2% (v/v) NH_4OH. Plate and detection, see Fig. 1; key as in Fig. 1.

on the RP-8 layer is partially governed by residual silanol groups present at the surface of the alkyl modified layer.

pH value. The pH of the eluent, adjusted by replacing an amount of water by an equivalent amount of glacial acetic acid, seems to have a dramatic effect on the retention and elution sequence (Fig. 2). In the low pH range (pH less than 4) retention is small, resulting in an insufficient separation. At intermediate pH values the best overall resolution is obtained but a baseline separation of I and the internal standard (VI) from all other impurities is obtained only at pH greater than 10. Thus, the quantitative determination is performed with an eluent consisting of methanol–water–ammonia (80:18:2) without pH adjustment. The apparent pH of this mobile phase is 11.30.

This is a practical example of the possibility of carrying out separations at high pH values (pH greater than 8), an advantage of reversed-phase HPTLC over reversed-phase high-performance liquid chromatography (HPLC), where separations must be carried out at pH values less than 8 in order not to dissolve the stationary phase material[13].

The retention of the basic compounds as a function of the pH of the eluent is based on the ionization of the surface silanol groups and on the ionic character of the solutes[14]. An initial decrease in R_F value is observed with rising pH as the silanols became negatively charged, resulting in strong interactions with the positively charged basic compounds. At intermediate pH values retention becomes constant, both the basic samples and the silanol groups being fully charged. The strong decrease in R_F value observed for compounds I, III, V and VI at pH *ca.* 9 is due to the deprotonation of the molecules causing enhanced interactions with the lipophilic octyl stationary phase, and consequently the retention increase dramatically.

Water content. The dependence of the R_F values on the water content of the mobile phase is shown in Fig. 3. As the polarity of the eluent increases a monotone decrease in R_F values is observed for all samples. A plot of the R_M values, calculated according the formula[15]

$$R_M = \log\frac{(1 - R_F)}{R_F}$$

instead of R_F values, *versus* the water content of the eluent gives straight lines for all compounds but veratric acid ($r > 0.9950$). In accordance with the well-known reversed-phase theory[16], we might assume that only one mechanism dominates the

TABLE I

α VALUES AS A FUNCTION OF THE TYPE OF ORGANIC MODIFIER IN THE ELUENT

α values are expressed as the ratio of the R_F value of the compound with higher R_F value to the R_F value of the compound with lower R_F value. For key to numbering in parentheses, see Fig. 1.

Modifier	$R_F(1):R_F(3)$	$R_F(2):R_F(1)$	$R_F(6):R_F(5)$	$R_F(7):R_F(2)$
Methanol (80%)	1.61	1.41	1.19	1.05
Acetonitrile (77.5%)	1.32	1.55	1.10	1.29
Acetone (70%)	1.21	1.37	1.10	1.12

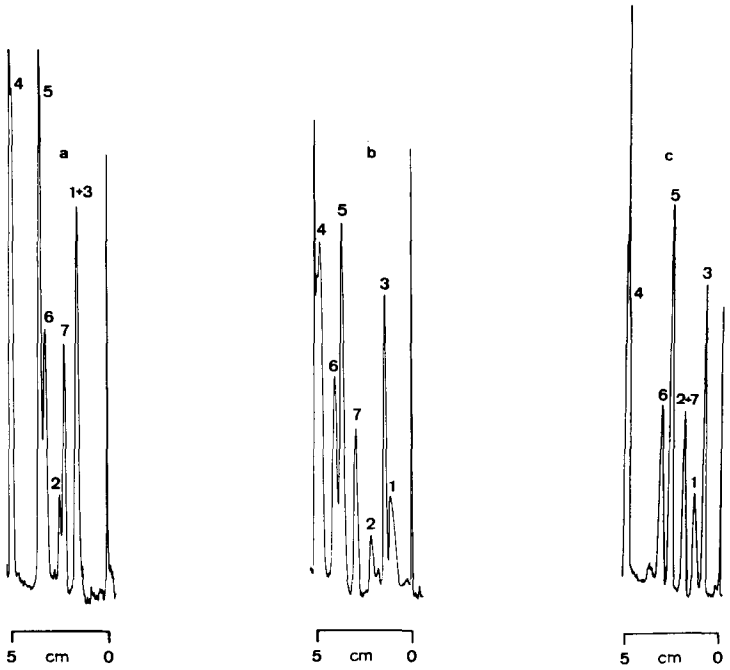

Fig. 4. Separation of a mixture of mebeverine hydrochloride, some potential contaminants and papaverine hydrochloride as a function of the type of organic modifier in the eluent: (a) acetone–water–ammonia (70.5:27.5:2); (b) acetonitrile–water–ammonia (77.5:20.5:2); (c) methanol–water–ammonia (80:18:2). Plate and detection, see Fig. 1; key as in Fig. 1.

retention process under these experimental conditions. Indeed as already mentioned, the pH of the eluent is greater than 11; at this pH the basic compounds are fully deprotonated and are not longer able to interact with the deprotonated silanol groups, so that only solvent effects govern the retention.

Type of organic modifier. Besides the concentration, the type of organic modifier in the eluent is another important parameter in the chromatographic optimization process[17]. Indeed, one is less restricted in the choice of organic solvent as mobile

TABLE II

DEVELOPMENT TIME AS A FUNCTION OF TYPE AND CONCENTRATION OF THE ORGANIC MODIFIER IN THE ELUENT

Modifier	*Development time (min)**
Methanol (75%)	35
Methanol (80%)	26
Acetonitrile (80%)	11
Acetonitrile (77.5%)	13
Acetone (80%)	18
Acetone (70%)	24
N,N-Dimethylformamide (80%)	>180

* Development distance, 5 cm.

phase (component) in reversed-phase HPTLC than in reversed-phase HPLC. Also the speed of testing potential useful solvents for a given separation problem is a great advantage of this analytical technique.

Initially three solvent systems were selected, *i.e.* acetonitrile–water, acetone–water and N,N-dimethylformamide-water, each containing 2% ammonia. With N,N-dimethylformamide as the organic modifier, the separation took more than 3 h for a development distance of 5 cm and very broad peaks with poor resolution were observed, so this eluent system was not further investigated. It seems that N,N-dimethylformamide is more useful in TLC when added in small amounts to the eluent as a third phase component. The polarity of the eluent mixtures was calculated according to Snyder and Kirkland[18] in order to obtain the same elution strength in all solvent mixtures, because we were solely interested in possible selectivity changes. Thus acetone–water (70:30) and acetonitrile–water (77.5:22.5) will give about the same solvent strength as methanol–water (80:20).

The selectivity change as a function of the type of organic modifier is shown in Table I. It is obvious that methanol provides the best selectivity for as baseline separation of compounds I and VI from all impurities. The separation of compounds III and IV is easily achieved with acetonitrile as the organic modifier, and quite different elution orders can be obtained depending on the modifier used (Fig. 4).

The development time is strongly influenced by the concentration and type of organic modifier (Table II). Increasing the methanol concentration from 75% to 80% decreases the migration time by 25%. Developing the RP-8 layer with methanol took about twice as long as with acetonitrile; acetone shows a development time between that for methanol and that for acetonitrile. It is clear that acetonitrile should be used, if satisfactory separations are obtained, in order to decrease the analysis time.

Influence of the addition of sodium chloride on the separation. Although no

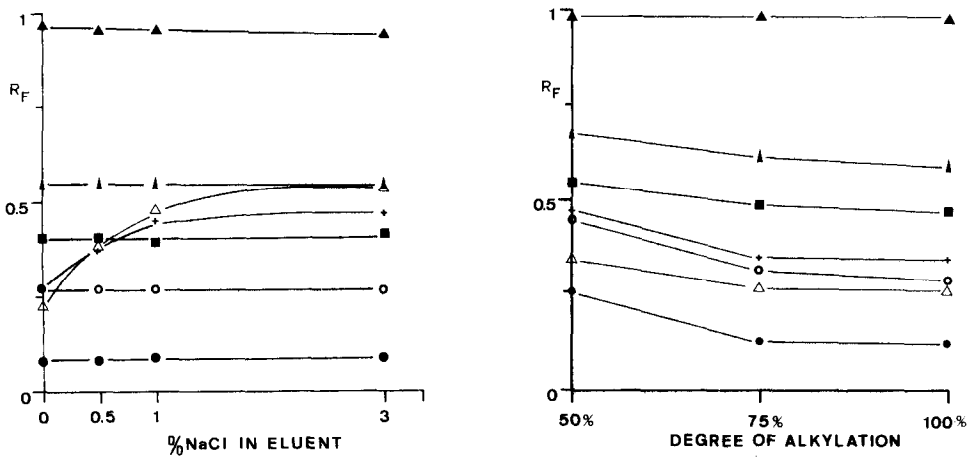

Fig. 5. R_F values as a function of the sodium chloride concentration in the eluent. Eluent, methanol–water–ammonia (75:23:2) with 0–3% sodiumchloride. Plate and detection, see Fig. 1; key as in Fig. 1.

Fig. 6. Effect of the degree of alkylation of the sorbent layer on retention. Eluent, methanol–water–ammonia (80:18:2); plates, Nano-SIL C_{18}-100, Nano SIL C_{18}-75 and Nano-SIL C_{18}-50 UV 254 precoated plates. Detection, see Fig. 1; key as in Fig. 1.

problems were encountered with the eluent systems used in this study in terms of non-wettability and/or swelling of the precoated layers, we studied the effect of sodium chloride, usually used to prevent such problems[19], on the selectivity and the efficiency of the separation. Fig. 5 shows that the addition of the salt results in a different elution order of the solutes because the R_F value of compounds III and V are increased. Moreover, a peak broadening effect occurs: addition of 3% sodium chloride results in an increase of 15% in the peak width calculated for both compounds I and VI. Only without the addition of salt satisfactory resolution is obtained.

The development time also depends on the salt concentration, and tends to increase with increasing sodium chloride concentration in the eluent.

Investigation of some sorbent layer characteristics

The degree of alkylation. The degree of alkylation of the layer materials might offer additional possibilities for changing the selectivities. Polar contributions to retention could be expected on partially alkylated layers, since the presence of free silanols results in lower R_F values for polar compounds[20]. The influence of the degree of alkylation of the sorbent layer was investigated on HPTLC SIL $C_{18\text{-}100}$, SIL $C_{18\text{-}75}$ and SIL $C_{18\text{-}50}$ UV_{254} precoated plates (Macherey-Nagel) and is shown in Fig. 6. The R_F values (and selectivities) are only slightly influenced by the degree of

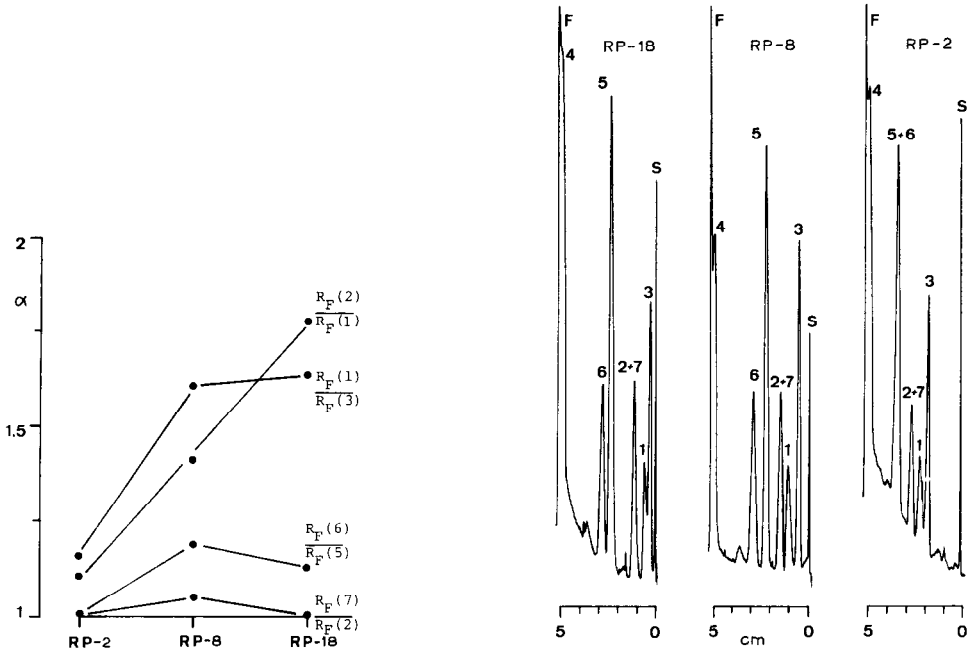

Fig. 7. Change in selectivity as a function of the hydrocarbon chain length (C_2,C_8,C_{18}). Eluent, methanol–water–ammonia (75:23:2); plates, HPTLC RP-2, RP-8 and RP-18 F_{254s} precoated plates. Detection, see Fig. 1; key as in Fig. 1.

Fig. 8. Separation of a mixture of mebeverine hydrochloride, some potential contaminants and papaverine hydrochloride as a function of the hydrocarbon chain length. Eluent, plate and detection, see Fig. 7; key as in Fig. 1. F = Front; S = starting point.

TABLE III

RESULTS OF THE QUANTITATIVE DETERMINATION OF MEBEVERINE HYDROCHLORIDE IN TABLETS

Plate number	*Sample*	*Percentage found of declared potency (100 mg per tablet)*
1	1	101.2
	2	97.9
2	3	98.2
	4	95.4
3	5	99.0
	6	99.7
4	7	97.8
	8	97.3
Average		98.3
Relative S.D.		1.69%

alkylation; in fact, the results are the opposite of what could be expected on a theoretical basis. Highest R_F values are obtained for all compounds on the SIL C_{18}-50 layer. Similar retention and selectivity were observed on SIL C_{18}-100 and SIL C_{18}-75. An explanation for these remarkable results might be the masking effect of the ammonia, present in the eluent, on the silanol groups.

The development time (for a 5-cm run) was slightly longer on SIL C_{18}-50 layer (26 min) than on the other two (23 min).

We may thus conclude that the degree of alkylation is of minor significance in the chromatographic optimization process.

The hydrocarbon chain length. The separations were performed on HPTLC RP-2, RP-8 and RP-18 F_{254s} precoated plates (Merck). The R_F values obtained on RP-2 plates were distinctly higher than those obtained on RP-8 and RP-18 plates (eluent methanol–water–ammonia) (75:23:2); the R_F values are lowest in all cases on RP-18 plates. The change in selectivity (α) is particularly important between RP-2 and RP-8/RP-18, and α did improve in all cases (Fig. 7). Selectivity changes are also observed between RP-8 and RP-18 layers, all be it less pronounced.

The influence of the hydrocarbon chain length on the separation is illustrated in Fig. 8. It can be seen that the RP-8 layer provides an excellent separation; therefore this type of layer was used in the quantitative determination.

Quantitative determination of mebeverine hydrochloride

The concentration of compound I in the tablets was calculated from a calibration graph constructed by plotting the peak-area ratio (drug to internal standard) *versus* the amount of drug in the reference solutions. The best-fit straight line was calculated, and a correlation coefficient $r > 0.9980$ was found over the concentration range tested (85–115% of the declared potency). Standard addition–recovery experiments performed on placebo mixtures showed a mean recovery of 100.1% [$n = 6$, relative standard deviation (R.S.D.) = 1.30].

The results of the quantitative determination, calculated on eight independent prepared samples and analysed on four different plates, are summarized in Table III.

The data show that the method gives accurate and precise results, comparable with these usually obtained in HPLC. Good agreement between the declared drug potency of the tablets (100 mg per tablet) and the assay results is obtained.

CONCLUSION

A flexible development system is described. For any given problem (determination of compound I in pharmaceuticals, purity control and stability control), the selectivity can be adjusted to avoid possible interferences. It is demonstrated that reversed-phase HPTLC is a useful analytical technique (especially attractive for routine analysis) and the results, in terms of separation control and reproducibility, are comparable with those obtained in HPLC.

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