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CHROMATOGRAPHIC CHARACTERIZATION OF PHENOTHIAZINE DRUGS BY A REVERSED-PHASE THIN-LAYER TECHNIQUE

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SUMMARY

A reversed-phase thin-layer chromatographic technique was used for the characterization of 26 phenothiazine drugs. With two chromatographic systems having the same stationary phase and phase volume ratio, but mobile phases of different pH*, all but two of the compounds could be identified. R_F values in the different systems were standardized by applying a reference compound to the plates next to each compound under investigation; the corrected R_F values were calculated from the differences in the R_M values of the compounds and the reference compound, and the theoretical R_M value of the reference. It was shown that R_F values for different chromatographic systems with the same stationary phase could be predicted with reasonable accuracy. The pH* of the mobile phase, for which a maximum difference in R_F values was obtained for pairs of compounds, could also be calculated and corresponded well with the observed values.

INTRODUCTION

Numerous thin-layer chromatographic (TLC) procedures for the characterization of phenothiazines have been described¹⁻⁹. Most workers used adsorption chromatography on silica gel for these drugs and a few^{1,8} used cellulose-coated plates. During reversed-phase thin-layer chromatographic (RP-TLC) experiments for the determination of the relative partition coefficients of some phenothiazines¹⁰, it became apparent that this technique could be useful for the separation and identification of these drugs and possibly of other groups of drugs. Reversed-phase techniques for the characterization of phenothiazines involving paper chromatography¹¹⁻¹³ and highperformance liquid chromatography have been described^{14,15}. Some of the workers^{12,14} pointed out the importance of the pH and composition of the mobile phase for the chromatographic behaviour of the drugs. In the work described here, the extent to which the R_F values of phenothiazines can be predicted when a RP-TLC method is used, in which disturbing adsorption phenomena have been proved to be absent, was investigated.

EXPERIMENTAL

Materials

Hydrochlorides of promazine, chlorpromazine, triflupromazine and prometh-

azine were obtained from various commercial sources and recrystallized from isopropanol. All other phenothiazines in were gifts from manufacturers and were used as supplied. Oleyl alcohol (Schuchardt, Munich, G.F.R.) containing 92-96% of *cis*-9octadecen-1-ol was distilled (135-140°; 0.05 mm Hg) and passed through a column of aluminium oxide (Merck, Darmstadt, G.F.R.). The density at 25° was 0.845 g/ml. Dioxan (Merck, "reinst") was freed from acid by passing it through a column of basic aluminium oxide (Merck). Distilled water was used throughout. Kieselguhr G (Merck) was used as supplied. All other materials were of reagent grade.

Thin-layer chromatography

The method used was as described previously¹⁰. Kieselguhr G (24 g) was shaken for 90 sec with a mixture of 1.25% (v/v) oleyl alcohol, 7 ml acetone and dioxan to 60 ml. Glass plates (20×20 cm) were coated with a 0.25-mm layer using standard equipment. The volatile components of the solvent were allowed to evaporate at room temperature for at least 16 h. Then 0.3% solutions of the phenothiazines or their salts in methanol were made (if impossible, saturated solutions were prepared) and 1 μ l of the solutions was spotted on to the plates, in varying order, on a line 2 cm from the lower edge of the plate, at 1.5-cm intervals. A migration of 10 or 15 cm was obtained by cutting the layer at 12 or 17 cm, respectively, from the lower edge. Each plate was placed in a chromatographic chamber that had been equilibrated for several hours with the mobile phase, the temperature being maintained at 25° throughout. The mobile phases were methanol-water mixtures. After development, the plates were dried at room temperature for 15 min and then sprayed with V⁵⁺ reagent¹ (650 mg of ammonium vanadate + 80 ml of concentrated sulphuric acid, water to 1000 ml) or with Dragendorff's reagent.

Measurement of dissociation constants

The concentration-dependent (acid) dissociation constants, ${}_{s}K_{a}c^{*}$, of a number of phenothiazines in the 50% (w/w) methanol-water mixture were measured by the titration method described by Benet and Goyan¹⁶. This method was applied earlier¹⁷ for six phenothiazines with satisfactory results. The pH^{*}^{**} meter (Metrohm Präzisions E510 pH meter) was standardised against methanol-water mixtures as described by Bates¹⁸ and Bates *et al.*¹⁹, using a Metrohm (EA121) combination glass electrode. A 50-g amount of methanol-water mixture containing 0.1 *M* potassium chloride and 10^{-3} *M* drug was titrated at 25.0 \pm 0.1° with the exclusion of light against 0.1–0.2 *N* sodium hydroxide solution or, hydrochloric acid, in at least 15 portions. The titrant, having the same methanol concentration as the test solution, was added from a 0.5ml Metrohm (E457) microburette calibrated to 0.0001 ml. Nitrogen was bubbled through the magnetically stirred solution throughout the titration. The pH^{*} was read 1 min after each addition. Free bases were titrated against 0.1 or 0.2 *N* hydrochloric acid; hydrochlorides were titrated against 0.1 or 0.2 *N* sodium hydroxide solution. In

The subscript indicates that a methanol-water mixture is involved. ${}_{s}K_{a}^{c}$ is thus the dissociation constant in a methanol-water mixture; the superscript c indicates that the "constant" depends on the concentration (ionic strength) in the solution.

^{**} pH meter readouts of measurements in methanol-water mixtures, after standardising the meter against a methanol-water buffer solution of the same methanol content, are denoted by the symbol pH*.

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all other instances (maleates, etc.) the free base of the drug was prepared by extracting an alkaline suspension of the drug with dichloromethane (DCM); after washing the DCM layer with water it was filtered and evaporated under reduced pressure. The residual free base was dissolved in methanol and to an aliquot of the methanolic solution an equal weight of water was added and the mixture titrated against 0.1 or 0.2 N hydrochloric acid. The $p({}_{s}K_{a}^{c})$ value of dixyrazine was also determined in 30% (w/w) methanol.

THEORETICAL

It was shown in a previous paper¹⁰ that under the conditions of the thin-layer experiments as described above, adsorption of phenothiazines on the support (Kieselguhr G) does not occur to any measurable extent; that is, the chromatographic process is based entirely on partitioning of the compounds between the stationary phase (oleyl alcohol) and the mobile phase (methanol-water mixtures). For a certain methanol-water mixture as the mobile phase, the R_M of a basic compound can then be expressed by¹⁰

$$R_M = \log_s P + \log_s f + \log r \tag{1}$$

where ${}_{s}P$ = partition coefficient [= the concentration in the stationary phase (in mole/l, divided by the concentration in the mobile phase (in mole/l)], ${}_{s}f = {}_{s}K_{a}{}^{c}/({}_{s}K_{a}{}^{c} + [\mathrm{H}^{+}]_{s})$, the fraction of the drug present as the free base ([H⁺]_s = molal concentration of protonated solvent), and r is the phase volume ratio, which is a constant for a given chromatographic system.

Substituting $R_M = \log (1/R_F - 1)$ in eqn. 1 yields, after rearrangement

$$\frac{R_F}{1-R_F} = \frac{1}{{}_sP \cdot r} + \frac{1}{{}_sP \cdot r \cdot {}_sK_a^c} \cdot [\mathrm{H}^+]_s \tag{2}$$

Graphs of $R_F/(1 - R_F)$ against $[H^+]_s$ should result in straight lines with slopes equal to $1/_s P \cdot r \cdot {}_s K_a^c$ and intercepts of $1/_s P \cdot r$. R_F can also be written as a function of $[H^+]_s$ by rearrangement of eqn. 2:

$$R_F = \frac{a_0 + a_1 \cdot [\mathrm{H}^+]_s}{1 + a_0 + a_1 \cdot [\mathrm{H}^+]_s}$$
(3)

where $a_0 = 1/{}_s P \cdot r$ and $a_1 = 1/{}_s P \cdot r \cdot {}_s K_a^c$.

For two compounds A and B, the difference in their R_F values, ΔR_F , can be expressed by

$$\Delta R_F = R_{F_{A}} - R_{F_{B}} = \frac{a_{0_{A}} + a_{1_{A}} [H^+]_{s}}{1 + a_{0_{A}} + a_{1_{A}} \cdot [H^+]_{s}} - \frac{a_{0_{B}} + a_{1_{B}} \cdot [H^+]_{s}}{1 + a_{0_{B}} + a_{1_{B}} \cdot [H^+]_{s}}$$

A maximum (or minimum) value of ΔR_F , $\Delta R_{F_{max}}$, is reached for $d(\Delta R_F)/d[H^+]_s = 0$. [H⁺]_s can be resolved from the resulting equation to give

$$[H^+]_s = \frac{-Y \pm \sqrt{Y^2 - 4XZ}}{2X}$$
(4)

(5)

where $X = (a_{1_A} \cdot a_{1_B}^2 - a_{1_B} \cdot a_{1_A}^2)$; $Y = 2 a_{1_A} \cdot a_{1_B} (a_{0_B} - a_{0_A})$; and $Z = 2 (a_{1_A} \cdot a_{0_B} - a_{0_A})$; $a_{0_A} \cdot a_{1_B} + a_{1_A} \cdot a_{0_B}^2 - a_{1_B} \cdot a_{0_A}^2 + a_{1_A} - a_{1_B}$.

The R_M value of the free base is a linear function of the methanol concentration, C(%, v/v), in the mobile phase²⁰⁻²² and can be represented by

$$R_M = R_{M_W} + bC$$

where b = constant and $R_{M_W} = \log P + \log r (P = \text{partition coefficient in the cleyl alcohol-water system); <math>R_{M_W}$ can be considered as the R_M value with water as the mobile phase.

RESULTS

The phenothiazines were chromatographed with a series of methanol-water

TABLE I

 $R_{\rm F} \times 100$ VALUES OF PHENOTHIAZINES FOR THREE METHANOL CONCENTRATIONS AND VARIOUS pH* VALUES OF THE MOBILE PHASE

Compound	pH*	of the r	nobile j	phase*	using 3	0% (w)	w) me	thanol	pH*	of the	mobile	phase**
	7.09	7.29	7.50	7 69	8.01	8.09	8.68	10.63	7.10	7.32	7.51	7.76
Thiopropazate	6	5	4	4	4	3			22	16	12	9
Thiethylperazine	9	7	5	4	3	3			26	20	15 -	11
Trifluoperazine	10	7	5	4	3	3			32	23	16	12
Prochlorperazine	14	9	8	5	4	3			39	27	20	15
Butaperazine	17	13	10	7	5	5			47 ·	34	26	21
Fluphenazine	23	18	15	11	9	8		5	57	44	32	29
Triflupromazine	26	19	14	8	5	4	1		60	47	31	22
Thioridazine	32	28	17	11	5	4	1		64	53	37	27
Perphenazine	33	22	21	14	11	11		8	62	48	39	34
Chlorpromazine	36	32	22	14	7	5	- 1		68	56	40	29
Perazine	40	33	26	19	13	12		б	70	58	45	38
Dixyrazine	43	- 34	31	22	17	15		10	72	61	50	44
Diethazine	49	37	31	21	11	8	4		-75	69	49	39
Profenamine	55	41	34	24	13	9	3		79	73	55	43
Pecazine	55	44	37	23	13	.10	4		79	68	55	45
Levomepromazine	56	44	36	24	14	10	5		80	70	55	43
Promethazine	57	45	37	25	15	12	6	3	78	69	52	41
Alimemazine	· 57	45	37	25	14	11	5		78	71	54	43
Thioproperazine	70	63	58	45	36	30		25	87	78	73	68
Methopromazine	71	64	58	43	26	21	11	3	87	80	72	62
Promazine	71	64	57	43	26	21	10	3	86	80	71	62
Acetophenazine	77	76	70	60	52	48		44	88	82	80	78
Aminopromazine	78	73	71	61	.40	35	19	5	89	86	81	76
Propericiazine	84	83	75	59	44	36	30	20	92	87	82	77
Mesoridazine	90	87	87	81	70	63	49	29	92	91	90	88
Oxomemazine	95	94	94	88	83	81	74	60	95	94	92	92

* 0.5 M solutions were used at $pH^* = 7.09$, $pH^* = 7.29$ and $pH^* = 7.50$; 0.2 M solutions at $pH^* = 7.69$; at all other pH^* values 0.1 M solutions were used.

** 0.5 M solutions were used at $pH^* = 7.10$; 0.2 M solutions at $pH^* = 7.32$, $pH^* = 7.51$, $pH^* = 7.76$ and $pH^* = 8.01$; at all other pH^* values 0.1 M solutions were used.

*** 0.5 M solutions were used at $pH^* = 6.84$, $pH^* = 7.12$; 0.2 M solutions at $pH^* = 7.48$, $pH^* = 7.61$; at all other pH^* values 0.1 M solutions were used.

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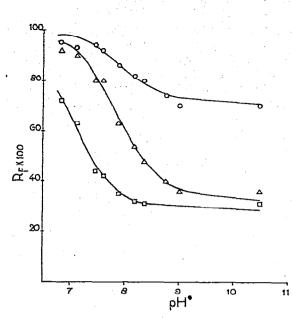
mixtures as the mobile phase. The methanol concentrations were 30, 40 and 50% (w/w). At each methanol concentration, a number of ammonia-ammonium chloride buffer solutions with different pH* were used as the mobile phase. At "low" pH* values, 0.5 or 0.2 M ammonia-ammonium chloride solutions were used, while at higher pH* values 0.1 M solutions were used. pH* values of about 10.6 were reached by adding 6 N ammonia (in the same methanol-water mixture) to a 0.1 M potassium chloride solution.

At least four different chromatograms were obtained of the phenothiazines with each mobile phase. The mean hR_F (= $R_F \cdot 100$) values are shown in Table I. In Fig. 1 the hR_F values of three compounds are plotted against the pH^{*} of the mobile phase [50% (w/w) methanol].

The concentration of ammonium chloride in the buffer solutions has, at lower pH^* values, a marked influence on R_F . Running chromatograms with mobile phases

using	40% (w/w) I	methanol	pH*	of the	mobile	phase*	** using	g 50%	(w/w) i	methan	ol	
8.01	8.23	8.64	10.60	6.84	7.12	7.48	7.61	7.90	8.20	8.38	8.78	9.02	10.50
9	9		9	47	40	30	29	27	25	25			25
9	7		6	56	48	30	27	22	20	19			18
10	9		6	63	54	35	34	25	22	22			20
12	10		8	68	58	36	34	27	24	24			22
16	14		11	72	63	44	42	35	32	31			31
24	22		19	74	66	55	54	48	47	47			46
15	10	5	4	79	73	49	49	30	22	18	16	14	13
17	9	5 5	1	80	75	55	53	33	22	19	11	9	8
28	27		22	80	74	57	56	50	47	47			47
19	12	7	4	. 81	76	55	54	34	25	21	17	14	14
31	26		21	83	79	61	5 9	50	47	47			45
37	33		28	84	79	66	64	57	54	54			53
27	18	10	5	87	84	64	63	41	31	27	19	17	16
31	19	9	3	88	85	68	67	45	33	27	18	13	12
33 -	21	13	6	89	86	69	66	47	37	32	25	21	21
32	22	13	7	89	87	68	68	47	37	33	26	23	22
32	23	16	11 -	89	85	65	65	47	40	36	32	31	29
33	22	13	7	90	86	68	68	47	37	32	25	23	20
61	57		52	- 91	87	83	82	76	74	74			75
51	38	25	12	92	90	80	80	63	54	48	40	36	36
50	37	24	10	92	91	80	79	62	52	45	37	33	33
73	72	••• •	68	93	92	87	85	82	82	82			83
68	57	39	13	92	90	88	86	76	68	63	51	44 [·]	43
69	61	54	47	95	93	88	86	79	74	74			71
85	78	68	48	95	93	94	92	86	82	80	74	70	70
91	88		82	96	96	96	94	92	91	90			91

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Fig. 1. Effect of pH* of the mobile phase [50% (w/w) methanol] on the hR_F values of mesoridazine (\bigcirc), methopromazine (\triangle) and butarerazine (\square). Each point represents the mean value of at least four hR_F measurements. The curves have been drawn to fit eqn. 3 using the values of a_0 and a_1 from Table 'II.

consisting of 0.1 *M* ammonia-ammonium chloride buffers in 50% (w/w) methanol with pH^{*} values lower than 7.6 resulted in R_F values lower than the theoretical values¹⁰. Increasing the buffer concentration to 0.2 *M* or, at the lowest pH^{*} values, to 0.5 *M* gave more reproducible R_F values, which corresponded well with the theoretical values (eqn. 3). The reproducibility of the R_F , R_M and ΔR_M values was investigated using for mobile phases 30% (w/w) methanol buffer solutions of low pH^{*} (7.09) and of high pH^{*} (10.6), and a 50% (w/w) methanol-buffer solution of high pH^{*} (10.5). The results are shown in Table II.

For a number of phenothiazines, the dissociation constants in methanol-water mixtures were calculated * (eqn. 2) from the values of the intercepts and the slopes of the graphs of $R_{\rm f}/(1-R_{\rm F})$ against $[{\rm H^+}]_s$. The results for 50% methanol are shown in Table III, together with the $p(_sK_a^c)$ values that were found by titration. Plots of $R_{\rm F}/(1-R_{\rm F})$ against $[{\rm H^+}]_s$ for three compounds with 30% (w/w) methanol are shown in Fig. 2.

The time needed for a migration of 10 cm was about 45 min for all of the mobile phases. Detection limits were estimated to be $0.5-1 \mu g$. About 100 μg chlor-promazine, after application to the plate, was chromatographed with 50% (w/w) methanol (pH* = 10.5). After development, the chlorpromazine zone was collected

^{*} Only R_F values between 0.1 and 0.85 and pH* values between $p({}_{s}K_{c}^{c}) \pm 1.5$ were included in the calculations. [H+], was calculated from pH*, using values for the activity coefficients of [H+] that were calculated with the extended Debye-Hückel equation²³ with the necessary constants from refs. 24-26.

TABLE II

VALUES OF $hR_{\rm F}$, $R_{\rm M}$ AND $\Delta R_{\rm M}$ AND THEIR STANDARD DEVIATIONS (s) FOR SOME PHENOTHIAZINES

The compounds given in italics were applied next to each other on the plates.

Compound	n*	hR _F	\$	R_M	s	ΔR_{M}^{δ}	s
Prochlorperazine	8	13	2.1	0.81	0.072	0.91	0.059
Butaperazine	8	17	2.9	0.69	0.085	0.78	0.029
Perphenazine	14	33	5.4	0.31	0.107	0.44	0.036
Perazine	8	40	5.3	0.19	0.095	0.29	0.048
Alimemazine	14	57 🕻	6.8	-0.13	0.122	0.00	0.000
Promethazine	14	57	6.6	-0.12	0.118	0.01	0.019
Promazine	14	71	4.3	-0.40	0.093	-0.27	0.039
Mesoridazine	8	90	1.9	-0.98	0.102	-0.87	0.046

30% (w/w) methanol, pH* = 10.63^{***}

Compound	n*	hR _F	S	R _M	S	$\Delta R_M^{\$\$}$	s
Dixyrazine	12	10	0.8	0.94	0.034	0.84	0.012
Propericiazine	12	20	0.8	0.60	0.022	0.50	0.017
Mesoridazine	12	29	0.9	0.40	0.029	0.30	0.021
Acetophenazine	12	44	1.7	0.10	0.029	0.00	0.000

50% (w/w) methanol, $pH^* = 10.50^{***}$

Compound	n*	hR _F	S	R _M	s	$\Delta R_M^{\$\$\$}$	5
Triflupromazine	8	13	0.9	0.84	0.037	0.90	0.025
Butaperazine	12	31	1.3	0.35 ⁺	0.025	0.40	0.021
Perazine	12	45	1.9	0.08	0.032	0.13	0.015
Dixyrazine	12	53 😚	2.0	-0.05	0.035	0.00	0.000
Propericiazine	12 ,	71	1.9	-0.39	0.039	0.34	0.011
Mesoridazine	12	70	1.8	-0.43	0.039	-0.38	0.022
Acetophenazine	12	83	1.3	-0.68	0.038	-0.63	0.011
Oxomemazine	8	91	1.0	-1.02	0.058	-0.98	0.029

n = number of determinations.

** 0.5 M buffer solution.

*** 0.1 M buffer solution.

 ${}^{\dagger} \Delta R_M = R_M$ of the compound minus R_M of alimemazine.

 ${}^{\frac{1}{2}\frac{1}{2}}\Delta R_M = R_M$ of the compound minus R_M of acetophenazine.

^{\$\$\$} $\Delta R_M = R_M$ of the compound minus R_M of dixyrazine.

and eluted with 0.1 N hydrochloric acid. The suspension was centrifuged and the resulting clear solution was made alkaline and shaken with DCM. The DCM layer was washed with water and extracted with 30 ml of 0.1 N hydrochloric acid; the ultraviolet absorbance spectrum of the aqueous layer had the ultraviolet absorbance characteristics of chlorpromazine.

DISCUSSION

Differences between the R_F values of the phenothiazines in reserved-phase chromatography are caused by differences in partition coefficients or in $p({}_{s}K_{a}{}^{c})$ values, or both. It can be seen (Table I) that the R_F values of all 26 phenothiazines are

TABLE III

SLOPES AND INTERCEPTS OF GRAPHS OF $R_F/1 - R_F$ AGAINST [H⁺], AND THE $p({}_{*}K_a^{\circ})$ VALUES IN 50% (w/w) METHANOL FROM CHROMATOGRAPHIC AND TITRIMETRIC DATA

 a_3 and a_1 = intercept and slope, respectively, of the plots of $R_F/(1 - R_F)$ versus $[H^+]_s$; s = standard deviation; n = number of measurements; four chromatograms were obtained for each compound with all mobile phases.

Compound	a ₀	S	<i>a</i> 1	S	n	$p(_{s}K_{a}^{c})$	
·	-		a galanta arti			Chromatography	Titration
Mesoridazine	2.44	0.099	2.18 · 10 ⁸	1.1.107	24	8.0	8.22
Pecazine	0.17	0.059	4.61 107	1.2.106	36	8.4	8.30
Propericiazine	2.31	0.067	6.60-107	6.4 · 106	16	7.5	7.46
Thioridazine	0.11	0.038	2.19.107	6.8 · 10 ⁵	-28	8.3	8.62
Butaperazine	0.40	0.037	8.80·10 ⁶	3.2 · 10⁵	24	7.3	7.23
Dixyrazine	1.09	0.094	1.77·10 ⁶	7.9 · 10⁵	- 24	7.2	7.15
Perphenazine	0.79	0.108	1.35-107	9.5·10 ⁵	24	7.2	7.01
Prochlorperazine	0.23	0.051	7.95·10 ⁶	4.3 · 10⁵	24	7.5	7.23
Chlorpromazine	0.14	0.029	2.33.107	5.6·10 ⁵	-32	8.2	8.24*
Promazine	0.41	0.079	7.54 · 107	3.1 - 106	28	8.3	8.37*
Triflupromazine	0.11	0.032	1.95.107	6.2 · 10 ⁵	32	8.2	8.13*
Methopromazine	0.48	0.692	7.61 · 107	3.6.10	28	8.2	8.20*
Levomepremazine	0.25	0.049	4.00.107	1.9.10	28	8.2	8.29
Promethazine	0.39	0.042	3.24 - 107	1.6·10 ⁶	28	7.9	7.94
Diethazine	0.16	0.037	3.44 - 107	1.4.106	28	8.3	8.31
Profenamine	0.12	0.043	4.23.107	1.7.106	28	8.6	8.69

* Results from ref. 17.

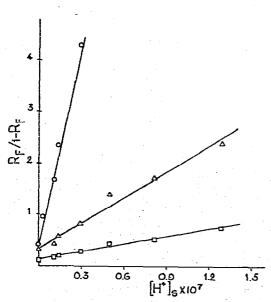


Fig. 2. $R_F/(1 - R_F)$ as a linear function of $[H^+]_s$ of the mobile phase [30% (w/w) methanol] for mesoridazine (\bigcirc), thioproperazine (\triangle) and dixyrazine (\square).

highly dependent on the pH* and methanol concentration of the mobile phase. There is no mobile phase with which all of the phenothiazines have different R_F values. However, the combination of 30% (w/w) methanol (pH* = 7.09) and 50% (w/w) methanol ($pH^* = 10.5$) makes identification within this group possible for almost all compounds. Three drugs (pecazine, levomepromazine and alimemazine) were difficult to separate for all compositions and pH* of the mobile phase. Obviously, these compounds have about the same partition coefficients and $p(_{s}K_{a}^{c})$ values. Of these, levomepromazine is characterized by the blue colour after spraying with V^{5+} reagent; the other two give an orange spot. Therefore, only alimemazine and pecazine are difficult to distinguish from one another by these two systems. Their separation could possibly be achieved by using another stationary phase of different polarity, for instance an n-alkane. Methopromazine and promazine were also very close together on almost all of the chromatograms, but, like the levomepromazine-alimemazine pair, methopromazine gives a blue spot and promazine an orange spot with V^{5+} reagent. This analogy is not surprising: methopromazine is promazine plus a CH_3O group at the C, position and levomepromazine is alimemazine plus a CH_3O group at the C_2 position. For both compounds the difference in R_F values is greatest with 50% (w/w) methanol ($pH^* = 10.5$). From the values in Table II, it is clear that the reproducibility of R_F values is better for mobile phases with higher pH^{*} values and that in all instances the reproducibility is best for extreme R_F values. The standard deviations of the R_M values also are lower for mobile phases with higher pH* values, but for a given chromatographic system there is no significant difference in the standard deviations of R_M values of very different magnitude. Variation in the R_M values of a compound obtained from different chromatograms must therefore be the result of plate to plate differences in the chromatographic system that cause a change in R_M that is equal in magnitude for all compounds. At high pH* values, variability in R_M may be caused by a variation from plate to plate in the phase volume ratio. At lower pH* values, a second source of error is probably the dissociation equilibrium of the drug in the mobile phase. The chromatographic conditions for compounds on one plate, however, seem to be much less prone to variation, because the standard deviations of the ΔR_M values^{*} are much smaller than the standard deviations of the R_M values. The error in the ΔR_M value of two compounds can be decreased even more by applying these compounds next to each other on the plates, as can be seen from Table II. The best characterization of the chromatographic behaviour of compounds in **RP-TLC** therefore appears to be obtained when a plate reference compound is used; this compound should be applied next to each of the compounds under test and the $\Delta R_{\rm M}$ values, between the compounds under investigation and this reference compound, determined. Addition of ΔR_M to the (standard) R_M value of the reference compound gives the corrected R_M values, from which the corrected R_F values (R_F corr.) can be calculated. Promethazine for 50% (w/w) methanol, methopromazine for 40% (w/w) methanol and dixyrazine for 30% (w/w) methanol were chosen as reference compounds. The R_F values of these compounds, at different pH* values of the mobile phase, do not become extremely high or low at extremes of pH*; also, their chromatographically determined $p(sK_a^{c})$ values are in excellent agreement with those ob-

 $[\]Delta R_M$ values are defined here as the differences in R_M values of pairs of compounds on the same chromatogram.

tained by titration (Table IV). The theoretical (standard) R_F , $R_F/(1 - R_F)$ and R_M values (calculated by using eqns. 2 and 3) at different pH* values for each of these compounds are presented in Table IV.

From the $R_F/(1 - R_F)$ values of the drugs, calculated from the corrected R_F values obtained with two mobile phases of different pH*s, the values of the slope (a_0) and intercept (a_1) of the graphs of $R_F/(1 - R_F)$ against $[H^+]_s$ can be calculated, as well as R_F values for all mobile phases that have the same methanol concentration but different pH* values. Examples are given in Table V. The calculated hR_F values, ob-

TABLE IV

CALCULATED VALUES OF hR_F , $R_F/(1 - R_F)$ AND R_M OF THE REFERENCE COMPOUNDS FOR THREE METHANOL CONCENTRATIONS

I =ionic strength (molality scale).

pH [≠]	I	hR _F	$R_{\rm F}/I - R_{\rm F}$	R_{M}
6.84	0.5	89.4	8.453	-0.927
7.12	0.5	82.2	4.629	-0.666
7.48	0.2	67.3	2.060	0.314
7.61	0.2	61.9	1.628	-0.212
7.90	0.1	49.2	0.968	0.014
8.20	0.1	40.4	0.677	0.169
8.38	0.1	36.6	0.578	0.238
8.78	0.1	31.6	0.462	0.336
9.02	0.1	30.0	0.429	0.368
10.5	0.1	27.9	0.387	0.413

40% (w/w) methanol, methopromazine**

₽H*	I	hR _F	$R_F/I - R_F$	R_M
7.10	0.5	87.6	7.040	-0.848
7.32	0.2	79.8	3.952	0.597
7.51	0.2	72.4	2.618	-0.418
7.76	0.2	60.8	1.554	-0.191
8.01	0.2	48.9	0.958	0.018
8.23	0.1	38.3	0.620	0.208
8.64	0.1	26.3	0.357	0.448
10.60	0.1	16.0	0.191	0.719

30% (w/w) methanol, dixyrazine***

·.	R _M	$R_F/I - R_F$	hR _F	I	<i>₽Н</i> * ∶
	0.115	0.767	43.4	0.5	7.09
	0.272	0.534	34.8	0.5	7.29
	0.418	0.382	27.6	0.5	7.50
	0.551	0.281	22.0	0.2	7.69
	0.686	0.206	17.1	0.1	8.01
	0.720	0.191	16.0	0.1	8.09
	0.822	0.151	13.1	0.1	8.68
	0.863	0.137	12.1	0.1	10.63
	0.005				

* $R_F/(1 - R_F) = 0.385 + 3.242 \cdot 10^7$; $p(_sK_a^c) = 7.93$ $[p(_sK_a^c)$ by titration = 7.94]. ** $R_F/(1 - R_F) = 0.189 + 5.266 \cdot 10^7$; $p(_sK_a^c) = 8.45$ $[p(_sK_a^c)$ by titration = 8.44]¹⁷. *** $R_F/(1 - R_F) = 0.137 + 4.885 \cdot 10^6$; $p(_sK_a^c) = 7.55$ $[p(_sK_a^c)$ by titration = 7.59].

tained with a third mobile phase, correspond reasonably well with the experimentally determined and corrected hR_F values, and the $p({}_{s}K_{a}^{c})$ values, calculated from a_0 and a_1 , are in good agreement with those in Table III. For a certain mobile phase, the pH^{*} at which ΔR_F for two compounds will be maximal ($\Delta R_{F_{max}}$) can be calculated from eqn. 4. The necessary values of a_0 and a_1 of both compounds can be determined by obtaining two chromatograms of the compounds (and the reference) with mobile phases of different pH^{*}. For instance, using the corrected $hR_{\rm F}$ values (Table V) of pecazine and prochlorperazine with 50% (w/w) methanol (pH* = 10.48 and 7.38), a_0 and a_1 for both compounds were calculated; for pecazine $a_0 = 0.206$ and $a_1 = 4.22 \cdot 10^7$, and for prochlorperazine $a_0 = 0.256$ and $a_1 = 7.36 \cdot 10^6$. Inserting these values in eqn. 10 yields $[H^+]_s = 6.90 \cdot 10^{-8}$. For a 0.5 M ammonia-ammonium chloride buffer solution in 50% (w/w) methanol, the activity coefficient of H⁺ is 0.58; the pH^{*} of the mobile phase at which ΔR_F will have a maximum value is therefore 7.40 and the calculated ΔR_F at pH* 7.40 is 0.38. The observed ΔR_F value at pH* = 7.38 (Table V) is 0.37. At pH* = 7.12 ΔR_F is 0.28, and at pH* = 7.48 ΔR_F is 0.33 (Table I). The calculated values of $\Delta R_{F_{max}}$ and the pH* at which $\Delta R_{F_{max}}$ is reached correspond with the observed values.

 $\Delta R_{\rm Fmax}$ for two compounds can thus be calculated from the corrected $R_{\rm F}$ values on two different chromatograms. However, when several compounds are to be separated on the same chromatogram, the use of eqn. 4 for all of the pairs of compounds would be cumbersome. It is then much more convenient to calculate the $R_{\rm F}$ values of each compound at different pH* values, again by inserting in eqn. 2 the $R_{\rm F}$ values of the compound on two different chromatograms. The most suitable pH* for the separation of the compounds can then be determined from the well known $R_{\rm F}$ versus pH graphs.

Another question is whether or not the R_F value of a compound can be estimated at other methanol concentrations in the mobile phase, after having measured R_F with a mobile phase of a given methanol concentration. R_M values of very lipophilic compounds decrease at a higher rate with increasing methanol concentration compared with R_M values of less lipophilic compounds.

It has been found experimentally²⁷ for a series of phenothiazines and benzodiazepines that the slope, b, of the lines $R_M = R_{M_W} + bC$ (eqn. 5)^{*} is a linear function of the R_M value at a certain methanol concentration:

$$b = a + \beta R_M \tag{6}$$

where α and β are constants whose values depend on the methanol concentration of the mobile phase. At zero methanol concentration the equation becomes

$$b = a + B R_{M_w} \tag{7}$$

(the R_{M_w} values were obtained by extrapolation of the lines $R_M = R_{M_w} + bC$ to zero methanol concentration).

Values of a and β at different methanol concentrations are shown in Table VI. From the corrected hR_F values of a certain compound, obtained for instance with two

* R_M and R_{M_W} in eqn. 5 have been calculated from the R_F values of the non-protonated drugs.

TABLE V

p(K, f) AND IRF VALUES OF SOME PHENOTHIAZINES, CALCULATED FROM THE CORRECTED IRF VALUES (IRF cont.) WITH TWO : MOBILE PHASES CONTAINING 50% (w/w) METHANOL

The compounds were applied on the plates in the same order as they are presented here.

| $pH^* = 10.48^*$ $pH^* = 8.03^*$ $pH^* = 7.38^{\bullet \bullet}$ $p(I_* = 7.37^{\bullet \bullet})$ $pH^* = 7.37^{\bullet \bullet}$ IIR_p <t< th=""><th>$pH^* = 10.48^*$ $pH^* = 8.03^*$ $pH^* = 7.38^*$ $p(1^* = 7.38^*)$ $p(1^* = 7.37^*)$ iR_F iR_F</th></t<> <th>$pH^* = 10.48^*$ $pH^* = 8.03^*$ $pH^* = 7.38^*$ $p(_4K_{c}^c)$ $pH^* = pH^* = 7.37^*$ lR_r lR_r lR_r lR_r lR_r $lR_r = pH^* = 7.37^*$ lR_r lR_r lR_r lR_r $lR_r = 7.38^*$ $pH^* = 7.37^*$ 66.5 67.3 87 85.4 93 93.8 8.03 $lR_r = 7.37^*$ 66.5 67.3 87 85.4 93 93.8 8.03 lR_r RR_r RR_r</th> <th>$pH^* = 10.48^\circ$$pH^* = 8.03^\circ$$pH^* = 7.38^\circ$$p(I_1K_{corr})$$pH^* = 7.38^\circ$$pH^* = 7.37^\circ$$\bar{I}R_{F}$<th>$pH^* = 10.48^{\circ}$ $pH^* = 8.03^{\circ}$ $pH^* = 7.38^{\circ}$ $pH^* = 7.37^{\circ}$ iR_P iR_P</th><th>$pH^* = 10.48^{\circ}$ $pH^* = 8.03^{\circ}$ $pH^* = 7.39^{\circ}$ $p(I_n^* I_n)$ $pH^* = 7.37^{\circ}$ $I_R P IR_F$ $I_R P$</th><th></th><th>$\begin{array}{c c c c c c c c c c c c c c c c c c c$</th><th>$pH^* = 10.49^*$ $pH^* = 8.03^*$ $pH^* = 7.38^*$ $p(R^*)_{1}$ $pH^* = 7.37^*$ R_0 R_R R_R^* R_R^*</th><th></th><th>*Hd</th><th></th><th></th><th></th><th></th><th></th><th></th><th>30% (w/1</th><th>30% (w/w) methanol</th><th></th><th>-</th><th></th><th></th></th> | $pH^* = 10.48^*$ $pH^* = 8.03^*$ $pH^* = 7.38^*$ $p(1^* = 7.38^*)$ $p(1^* = 7.37^*)$ iR_F | $pH^* = 10.48^*$ $pH^* = 8.03^*$ $pH^* = 7.38^*$ $p(_4K_{c}^c)$ $pH^* = pH^* = 7.37^*$ lR_r lR_r lR_r lR_r lR_r $lR_r = pH^* = 7.37^*$ lR_r lR_r lR_r lR_r $lR_r = 7.38^*$ $pH^* = 7.37^*$ 66.5 67.3 87 85.4 93 93.8 8.03 $lR_r = 7.37^*$ 66.5 67.3 87 85.4 93 93.8 8.03 lR_r RR_r | $pH^* = 10.48^\circ$ $pH^* = 8.03^\circ$ $pH^* = 7.38^\circ$ $p(I_1K_{corr})$ $pH^* = 7.38^\circ$ $pH^* = 7.37^\circ$ $\bar{I}R_{F}$ <th>$pH^* = 10.48^{\circ}$ $pH^* = 8.03^{\circ}$ $pH^* = 7.38^{\circ}$ $pH^* = 7.37^{\circ}$ iR_P iR_P</th> <th>$pH^* = 10.48^{\circ}$ $pH^* = 8.03^{\circ}$ $pH^* = 7.39^{\circ}$ $p(I_n^* I_n)$ $pH^* = 7.37^{\circ}$ $I_R P IR_F$ $I_R P$</th> <th></th> <th>$\begin{array}{c c c c c c c c c c c c c c c c c c c$</th> <th>$pH^* = 10.49^*$ $pH^* = 8.03^*$ $pH^* = 7.38^*$ $p(R^*)_{1}$ $pH^* = 7.37^*$ R_0 R_R R_R^* R_R^*</th> <th></th> <th>*Hd</th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th>30% (w/1</th> <th>30% (w/w) methanol</th> <th></th> <th>-</th> <th></th> <th></th> | $pH^* = 10.48^{\circ}$ $pH^* = 8.03^{\circ}$ $pH^* = 7.38^{\circ}$ $pH^* = 7.37^{\circ}$ iR_P | $pH^* = 10.48^{\circ}$ $pH^* = 8.03^{\circ}$ $pH^* = 7.39^{\circ}$ $p(I_n^* I_n)$ $pH^* = 7.37^{\circ}$ $I_R P IR_F$ $I_R P$ | | $ \begin{array}{c c c c c c c c c c c c c c c c c c c $ | $pH^* = 10.49^*$ $pH^* = 8.03^*$ $pH^* = 7.38^*$ $p(R^*)_{1}$ $pH^* = 7.37^*$ R_0 R_R R_R^* | | *Hd | | | | | | | 30% (w/1 | 30% (w/w) methanol | | - | | |
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---|---------------------|------|-----------|------|---------|-----------------|------------|--------------------------------------|---------------------------------|--------------------|------|----------|------------|---|
| $IR_{\rm V}$ | iR_r | <i>hRv</i> <th< th=""><th>$hR_{\mu}$$hR_{r$</th><th>IRF IRF IRF<th>IRe IRe IRE<th>IRe IRe IRe<th>hR_{e} hR_{e} hR_{e}</th><th>R_{F} R_{F} <</th><th></th><th></th><th>= 10.48*</th><th>*Hd</th><th>= 8,03*</th><th>*Hd</th><th>= 7.38**</th><th>$p(_{\mathbf{x}}K_{\mathbf{u}}^{c})$</th><th>pH* =</th><th>$pH^* =$</th><th>*Hd</th><th>= 7.37**</th><th></th><th></th></th></th></th></th<> | hR_{μ} hR_{r} hR_{r | IRF IRF <th>IRe IRe IRE<th>IRe IRe IRe<th>hR_{e} hR_{e} hR_{e}</th><th>R_{F} R_{F} <</th><th></th><th></th><th>= 10.48*</th><th>*Hd</th><th>= 8,03*</th><th>*Hd</th><th>= 7.38**</th><th>$p(_{\mathbf{x}}K_{\mathbf{u}}^{c})$</th><th>pH* =</th><th>$pH^* =$</th><th>*Hd</th><th>= 7.37**</th><th></th><th></th></th></th> | IRe IRE <th>IRe IRe IRe<th>hR_{e} hR_{e} hR_{e}</th><th>R_{F} R_{F} <</th><th></th><th></th><th>= 10.48*</th><th>*Hd</th><th>= 8,03*</th><th>*Hd</th><th>= 7.38**</th><th>$p(_{\mathbf{x}}K_{\mathbf{u}}^{c})$</th><th>pH* =</th><th>$pH^* =$</th><th>*Hd</th><th>= 7.37**</th><th></th><th></th></th> | IRe IRe <th>hR_{e} hR_{e} hR_{e}</th> <th>R_{F} R_{F} <</th> <th></th> <th></th> <th>= 10.48*</th> <th>*Hd</th> <th>= 8,03*</th> <th>*Hd</th> <th>= 7.38**</th> <th>$p(_{\mathbf{x}}K_{\mathbf{u}}^{c})$</th> <th>pH* =</th> <th>$pH^* =$</th> <th>*Hd</th> <th>= 7.37**</th> <th></th> <th></th> | hR_{e} | R_{F} < | | | = 10.48* | *Hd | = 8,03* | *Hd | = 7.38** | $p(_{\mathbf{x}}K_{\mathbf{u}}^{c})$ | pH* = | $pH^* =$ | *Hd | = 7.37** | | |
|
 | 66.5 67.3 87 85.4 93 93.8 8.1^{11} 96^{14} 86 85.4 27.5 48.5 70.5 70.5 70.5 70.5 8.1^{11} 76^{14} 86 85.4 17 17.2 49 45.6 74 76.4 8.4^{11} 78^{14} 37.5 36.3 6.5 51.2 55.5 53.3 58 63.8 7.3^{14} 78^{14} 37.5 36.3 26.5 47 66.5 70.8 7.3^{14} 78^{14} 78^{14} 37.5 36.3 49.5 51.2 55.5 53.6 65.5 70.8 7.3^{14} 57^{143} 31.9 49.5 51.2 55.5 53.6 65.5 70.8 7.3^{14} 57^{143} 31.9 44 43.8 47.1 56 63.0 7.2^{144} 49^{110} 20 19.5 28 46.5 70.8 7.3^{14} 57^{143} 57^{143} 31.9 20.5 20.4 24.5 37.7 44.0 7.5^{144} 70.8 7.9^{14} 8 8.1 45 42.6 73 76.3 8.6^{14} 78^{14} 40.8 8 8.1 73 8.6^{14} 78^{14} 28.1 41 40.8 8 8.1 8.6^{14} 78^{14} 8.5 8.3 8.6^{14} 71^{16} 8 8.1 69.5 54.5 58.8 8.2^{14} 63^{14} |
 | 66.5 67.3 87 85.4 93 93.8 8.1^{11} 96^{14} 86 85.4 $27,5$ 48.5 70.5 70.5 70.5 8.1^{11} 76^{14} 86 85.4 17 17.2 49 45.6 74 76.4 8.4^{11} 78^{14} 37.5 36.3 6.5 6.9 35 33.3 58 63.8 8.6^{11} 70^{14} 16 16.2 26.5 47 6.8 7.2^{18} 8.6^{11} 70^{14} 16 16.2 26.5 51.2 55.5 53.6 65.5 70.8 7.3^{14} 57^{143} 33.3 49.5 51.2 55.5 53.6 63.0 7.2^{114} 37.9 31.9 44 43.8 47.1 56 63.0 7.2^{114} 49^{110} 20 19.5 20.4 24.5 53.5 57.6 7.2^{114} 76.1 49^{110} 20 19.5 21.6 20.4 24.5 73 76.3 8.6^{11} 78^{14} 40.8 8.3 8 8.1 47.5 69.5 73 76.3 8.6^{11} 78^{14} 40.8 8.3 8 8.1 11.12 31.5 29.5 58.8 8.2^{14} 63^{14} 17 16.9 11 11.2 31.5 29.5 54.5 58.8 8.2^{14} 63^{14} 17 17 16.9 11 11.2 31.5 <
 | ridazine 665 67.3 87 85.4 93 93.8 8.1 ¹⁴ 96 ¹⁴ 86 ¹⁶ 86 85.4 90
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zine 17 17.2 49 45.6 74 76.4 8.4 ¹⁴ 78 ¹⁴ 37.5 36.3 35
ridazine 6.5 6.9 35 33.3 58 63.8 8.6 ¹⁴ 70 ¹⁴ 16 16.2 9
ethazine 26.5 47.1 56 63.0 7.2 ¹¹⁴ 49 ¹¹⁰ 20 19.5 23
benazine 49.5 51.2 55.5 53.6 63.0 7.2 ¹¹⁴ 49 ¹¹⁰ 20 19.5 23
tethazine 28 48.5 47.1 56 63.0 7.2 ¹¹⁴ 49 ¹¹⁰ 20 19.5 23
benazine 28 48.5 47.1 56 63.0 7.5 ¹¹⁴ 49 ¹¹⁰ 20 19.5 23
inchrazine 28 8.1 45.5 70.8 7.3 ¹¹⁴ 26 ¹¹⁴ 8.5 8.3 31.9 35
benazine 28 48.5 47.1 56 63.0 7.5 ¹¹⁴ 49 ¹¹⁰ 20 19.5 23
inchrazine 28 48.5 47.1 56 63.0 7.5 ¹¹⁴ 26 ¹¹⁴ 8.5 8.3 19
benazine 28 48.5 47.1 56 63.0 7.5 ¹¹⁴ 49 ¹¹⁰ 20 19.5 23
inchrazine 20.5 20.4 24.5 23.5 34.5 58.8 8.2 ¹⁴ 78 ¹⁴ 26 ¹¹⁴ 8.5 8.3 10
inchrazine 27.5 47.5 69.5 88.8 8.2 ¹⁴ 63 ¹⁴ 17 16.9 15
inchrazine 11 11.2 31.5 29.5 54.5 58.8 8.2 ¹⁶ 63 ¹⁰ (w/w) methanol chromatograms. The <i>hR</i> , value of
<i>i</i> Diversine 6.1
 | soridarine 66.5 67.3 87 85.4 93 93.8 8.1 ¹¹ 96 ¹¹ 96 ¹¹ 86 85.4 90
methrazine 27.5 6.3 48.5 70.5 70.5 70.5 70.6 74 76.4 8.4 ¹¹ 78 ¹¹ 37.5 36.3 35
oridazine 6.5 6.9 35 33.3 58 63.8 8.6 ¹¹ 70 ¹¹ 16 16.2 9
methrazine 26.5 4.9 45.6 74 76.4 8.4 ¹¹ 78 ¹¹ 37.5 36.3 35
oridazine 26.5 6.9 35 33.3 58 63.8 8.6 ¹¹ 70 ¹¹ 16 16.2 9
premazine 49.5 51.2 55.5 53.6 63.0 7.2 ¹¹¹ 49 ¹¹¹ 20 19.5 23
phemazine 20.4 24.5 23.5 37.6 65.5 70.8 7.3 ¹¹ 57 ¹¹¹ 33 31.9 35
phemazine 20.4 24.5 73 76.3 8.6 ¹¹ 78 ¹¹ 20 ¹¹ 20 19.5 23
methrazine 8 8.1 45 42.6 73 76.3 8.6 ¹¹ 78 ¹¹ 26 ¹¹¹ 20 19.5 23
contentrazine 20.5 20.4 24.5 63.0 7.2 ¹¹¹ 49 ¹¹¹ 20 19.5 23
methrazine 20.5 20.4 24.5 63.0 7.2 ¹¹¹ 49 ¹¹¹ 20 19.5 23
Methrazine 27.5 47.5 69.5 8.8 8.2 ¹¹ 63 ¹¹ 17 16.9 15
10 michrazine 11 11.2 31.5 29.5 54.5 58.8 8.2 ¹¹ 63 ¹¹ 17 16.9 15
10 michrazine 40.5
11 11.2 31.5 29.5 54.5 58.8 8.2 ¹¹ 63 ¹¹ 17 16.9 15
10 michrazine sus used as the reference compound in the same way as pripmethrazine for the 50% (w/w) methranol chromatograms. The <i>hR_i</i> value of
11 <i>HR</i> values of the four measured <i>hR</i> , values on the plate.
1 <i>HR</i> values for 50% (w/w) methranol (pH [*] = 7.3 ¹) were estimated from two corrected <i>h</i> .6, values for 50% (w/w) methranol | esoridazine 66.5 67.3 87 85.4 93 93.8 8.1 ¹¹ 96 ¹¹ 96 ¹¹ 86 85.4 90
omethazine 27.5 4.8.5 70.5 70.5 70.5 70.8 8.4 ¹¹ 78 ¹¹ 37.5 36.3 35.3 35
atzine 17 17.2 4.9 45.6 74 76.4 8.4 ¹¹ 78 ¹¹ 37.5 36.3 35
oriethazine 6.5 6.9 35 33.3 5.8 6.3.8 8.6 ¹¹ 70 ¹¹ 16 16.2 9
oriethazine 4.9.5 51.2 55.5 53.6 6.5.5 70.8 7.3 ¹¹ 57 ¹¹¹ 31.9 33 31.9 35
phonazine 4.9.5 51.2 55.5 53.6 6.3.0 7.2 ¹¹¹ 4.9 ¹¹¹ 20 19.5 23
oriethazine 28 46.5 47.1 5.6 6.3.0 7.2 ¹¹¹ 4.9 ¹¹¹ 20 19.5 23
oriethazine 28 46.5 47.1 5.6 6.3.0 7.2 ¹¹¹ 4.9 ¹¹¹ 20 19.5 23
oriethazine 28 46.5 70.8 7.3 ¹¹ 4.4.0 7.5 ¹¹¹ 4.9 ¹¹¹ 20 19.5 23
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oriethazine 20.5 20.4 24.5 23.5 37. 44.0 7.5 ¹¹¹ 4.9 ¹¹¹ 20 ¹¹ 10.8 27
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oriethazine 21.1 11.2 31.5 29.5 54.5 58.8 8.2 ¹¹ 63 ¹¹ 17 ¹ 16.9 15
· Tonic strength = 0.1.
· Tonic strength = 0.1.
· Tonic strength = 0.5.
· Tonic strength = 0.5
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IIR _{F cate} , | | hR. | hRP corr | liRF cuic. | |
| $ \begin{array}{cccccccccccccccccccccccccccccccccccc$
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23 and 6.5 6.3.0 7.2¹¹¹ 78¹¹¹ 26¹¹¹ 20 19.5 23 and 6.5 6.3.0 7.2¹¹¹ 78¹¹¹ 26¹¹¹ 20 19.5 23 and 6.5 6.3.0 7.2¹¹¹¹ 26¹¹¹ 20 10.8 27 and 7.5¹¹¹ 26¹¹¹ 12 2.5 5.4.5 58.8 8.2¹¹¹ 6.3¹¹¹ 78¹¹ 26¹¹¹ 20 20.8 27 and 7.5¹¹¹ 11.2 31.5 29.5 54.5 58.8 8.2¹¹¹ 6.3¹¹¹ 78¹¹¹ 26¹¹¹ 16.9 15 and 6.5 for 6.6¹¹ 78¹¹¹ 26¹¹¹ 11.2 31.5 29.5 54.5 58.8 8.2¹¹¹ 6.3¹¹¹ 17 16.9 15 and 6.5¹¹ for strength = 0.1. 11.1.2 31.5 29.5 54.5 58.8 8.2¹¹¹ 6.5¹¹¹ 6.5¹¹¹ 17 16.9 15 and 6.5¹¹¹ for strength = 0.1. 11.1.2 31.5 29.5 54.5 58.8 8.2¹¹¹ 6.5¹¹¹ 78¹¹¹ 16.9 15 and 6.5¹¹¹ for strength = 0.5. 110 in strength = 0.5. 100 in the sune way as proprinthazine for the 50% (w/w) methanol (pHⁿ = 7.3¹¹) were stimated from two corrected h.6, walters for 50% (w/w) methanol. 110 in the sune and a 0.5 0% (w/w) methanol. 100 in the sune way as proprinted with h.6, walters at pHⁿ = 10.48 and 8.03. 100 in the sune the 200 in the sune stimated from two corrected h.6, walters for 50% (w/w) methanol. 110 in the sune tin</td> <td>omethazine $27,5$ 48.5 70.5 70.5 70.5 70.5 70.5 $74.76,4$ 8.4^{11} 78^{11} 37.5 36.3 35.5 35.5 35.5 35.5 35.5 35.5 35.5 35.5 35.6 53.5 35.6 53.5 35.6 55.5 70.6 8.6^{11} 70^{11} 16.5 16.2 9 35.5 35.6 55.5 70.8 57.11 37.5 35.5 35.6 65.5 70.8 7.2111 49.11 20.5 29.5 35.6 63.0 7.2111 49.11 20.5 35.5 35.6 63.0 7.2111 49.11 20.5 27.5 27.5 27.5 20.5 20.5 27.5 28.211 63.11 17.7 10.2 10.2 117</td> <td>Promethazine 27,5 48,5 70,5 70,8 74, 76,4 8,41, 78,1 37,5 36,3 35,3 35,3 55,5 50,6 5,6 5,6 5,6 5,5 70,8 7,3 1,6 7,0 1,1 6,1 6,2 9 7,0 7,0 1,0 1,6 1,6 2,5 9 7,0 7,0 1,0 1,6 1,6 2,5 9 7,0 8 7,3 1,1 2,2 1,2 2,2 2</td> <td>Mesoridazine</td> <td>66.5</td> <td></td> <td>87</td> <td>[</td> <td>93</td> <td>93.8</td> <td>8.1 00</td> <td>9611</td> <td></td> <td>86</td> <td>85.4</td> <td>00</td> <td></td> | omethazine 27.5 48.5 70.5 and 8.4^{11} 78 ¹¹ 78 ¹¹ 37.5 36.3 35 35 and 3.5^{2} action 17 17.2 49 45.6 74 76.4 8.4 ¹¹ 78 ¹¹ 37.5 36.3 35 35 35.5 5.5 5.6 3.3 3.3 5.8 6.3.8 8.6 ¹¹ 70 ¹¹ 16 16.2 9 5 51.2 55.5 51.2 55.5 53.6 6.3.0 7.2 ¹¹¹ 49 ¹¹¹ 20 19.5 23 6.3 and 7.2 ¹¹¹ 49 ¹¹¹ 20 19.5 23 and 6.5 6.3.0 7.2 ¹¹¹ 49 ¹¹¹ 20 19.5 23 and 6.5 6.3.0 7.2 ¹¹¹ 49 ¹¹¹ 20 19.5 23 and 6.5 6.3.0 7.2 ¹¹¹ 49 ¹¹¹ 20 19.5 23 and 6.5 6.3.0 7.2 ¹¹¹ 49 ¹¹¹ 20 19.5 23 and 6.5 6.3.0 7.2 ¹¹¹ 49 ¹¹¹ 20 19.5 23 and 6.5 6.3.0 7.2 ¹¹¹ 70 ¹¹¹ 40 ¹¹¹ 20 19.5 23 and 6.5 6.3.0 7.2 ¹¹¹ 70 ¹¹¹ 26 ¹¹¹ 20 19.5 23 and 6.5 6.3.0 7.2 ¹¹¹ 78 ¹¹¹ 26 ¹¹¹ 20 19.5 23 and 6.5 6.3.0 7.2 ¹¹¹ 78 ¹¹¹ 26 ¹¹¹ 20 19.5 23 and 6.5 6.3.0 7.2 ¹¹¹ 78 ¹¹¹ 26 ¹¹¹ 20 19.5 23 and 6.5 6.3.0 7.2 ¹¹¹¹ 26 ¹¹¹ 20 10.8 27 and 7.5 ¹¹¹ 26 ¹¹¹ 12 2.5 5.4.5 58.8 8.2 ¹¹¹ 6.3 ¹¹¹ 78 ¹¹ 26 ¹¹¹ 20 20.8 27 and 7.5 ¹¹¹ 11.2 31.5 29.5 54.5 58.8 8.2 ¹¹¹ 6.3 ¹¹¹ 78 ¹¹¹ 26 ¹¹¹ 16.9 15 and 6.5 for 6.6 ¹¹ 78 ¹¹¹ 26 ¹¹¹ 11.2 31.5 29.5 54.5 58.8 8.2 ¹¹¹ 6.3 ¹¹¹ 17 16.9 15 and 6.5 ¹¹ for strength = 0.1. 11.1.2 31.5 29.5 54.5 58.8 8.2 ¹¹¹ 6.5 ¹¹¹ 6.5 ¹¹¹ 17 16.9 15 and 6.5 ¹¹¹ for strength = 0.1. 11.1.2 31.5 29.5 54.5 58.8 8.2 ¹¹¹ 6.5 ¹¹¹ 78 ¹¹¹ 16.9 15 and 6.5 ¹¹¹ for strength = 0.5. 110 in strength = 0.5. 100 in the sune way as proprinthazine for the 50% (w/w) methanol (pH ⁿ = 7.3 ¹¹) were stimated from two corrected h.6, walters for 50% (w/w) methanol. 110 in the sune and a 0.5 0% (w/w) methanol. 100 in the sune way as proprinted with h.6, walters at pH ⁿ = 10.48 and 8.03. 100 in the sune the 200 in the sune stimated from two corrected h.6, walters for 50% (w/w) methanol. 110 in the sune tin | omethazine $27,5$ 48.5 70.5 70.5 70.5 70.5 70.5 $74.76,4$ 8.4^{11} 78^{11} 37.5 36.3 35.5 35.5 35.5 35.5 35.5 35.5 35.5 35.5 35.6 53.5 35.6 53.5 35.6 55.5 70.6 8.6^{11} 70^{11} 16.5 16.2 9 35.5 35.6 55.5 70.8 57.11 37.5 35.5 35.6 65.5 70.8 7.2111 49.11 20.5 29.5 35.6 63.0 7.2111 49.11 20.5 35.5 35.6 63.0 7.2111 49.11 20.5 27.5 27.5 27.5 20.5 20.5 27.5 27.5 27.5 27.5 27.5 27.5 27.5 27.5 27.5 27.5 27.5 27.5 27.5 27.5 28.211 63.11 17.7 10.2 10.2 117
 | Promethazine 27,5 48,5 70,5 70,8 74, 76,4 8,41, 78,1 37,5 36,3 35,3 35,3 55,5 50,6 5,6 5,6 5,6 5,5 70,8 7,3 1,6 7,0 1,1 6,1 6,2 9 7,0 7,0 1,0 1,6 1,6 2,5 9 7,0 7,0 1,0 1,6 1,6 2,5 9 7,0 8 7,3 1,1 2,2 1,2 2,2 2 | Mesoridazine | 66.5 | | 87 | [| 93 | 93.8 | 8.1 00 | 9611 | | 86 | 85.4 | 00 | |
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 | 1717.24945.67476.4 8.4^{11} 78^{16} 37.5 36.3 6.56.93533.358 63.8 8.6^{11} 70^{11} 16 162.2 26.5 4768 63.8 8.6^{11} 70^{11} 16 162.2 26.5 4768 63.8 7.3^{11} 57^{111} 33 31.9 49.5 51.2 55.5 53.6 65.5 70.8 7.3^{11} 57^{111} 33 31.9 49.5 43.8 48.5 47.1 56 63.0 7.2^{111} 49^{111} 20 19.5 28 46.5 67 7.2^{111} 76.3 8.6^{11} 78^{11} 20 19.5 28 8.1 45.5 73.7 76.3 8.6^{11} 78^{11} 20 19.5 28 8.1 45.5 73.7 76.3 8.6^{11} 78^{11} 40.8 8 8.1 47.5 69.5 8.8^{11} 63^{11} 17 16.9 27.5 47.5 54.5 58.8 8.2^{11} 63^{11} 17 16.9 11 11.2 31.5 29.5 54.5 58.8 8.2^{11} 63^{11} 17 16.9 17 16.9 17 16.9 17 16.9 17 16.9
 | zinc 17 17.2 49 45.6 74 76.4 8.4 ¹⁰ 78 ¹¹ 37.5 36.3 35
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* Ionic strength = 0.1
* Ionic strength = 0.1.
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 | azine 17 17.2 49 45.6 74 76.4 8.4 ¹¹ 78 ¹¹ 37.5 36.3 35
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 | caratice 17 17.2 49 45.6 74 76.4 8.4.1 78.1 37.5 36.3 35
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orientiazine 11 11.2 31.5 29.5 54.5 58.8 8.2.11 6.3.11 17 16.9 15
orientiazine 2.7.5 0.5.0 0.0.0000 in the same way as primethazine for the 50% (w/w) methanol chromatograms. The <i>h.R.</i> value of <i>Nrazine</i> is an east the form as used as the reference compound in the same way as primethazine for the 50% (w/w) methanol $(pH^* = 7.37)$ were estimated from two corrected <i>h.R.</i> values for 50% (w/w) methanol.
10.6.10.6.10.8 0.0.1.
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<i>the values on the plate.</i>
<i>the values for 90%</i> (w/w) methanol (pH[*] = 7.37) were estimated from two corrected <i>hR_t</i> values for 50% (w/w) methanol.
<i>the values on the plate.</i>
<i>the values for 90%</i> (w/w) methanol (pH[*] = 7.37) were estimated from two corrected <i>hR_t</i> values for 50% (w/w) methanol.
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<i>the ca</i></td> <td>Thioriductine$6.5$$6.9$$3.5$$3.3.3$$5.8$$6.4.1$$70^{11}$$16$$16.2$$9$Promethazine$26.5$$3.1.3$$5.8$$6.3.0$$7.3^{11}$$5.1^{11}$$3.1.9$$3.5^{11}$$3.5^{$</td> <td>Pecazine</td> <td>11</td> <td>17.2</td> <td>49</td> <td></td> <td>74</td> <td>76.4</td> <td>8.448</td> <td>18 81</td> <td></td> <td>37.5</td> <td>36.3</td> <td>35</td> <td></td> | Indicatine $6.5 \ 6.9 \ 35 \ 31.3 \ 5.8 \ 6.8 \ 8.6^{11} \ 70^{11}$ If $16 \ 16.2 \ 9$
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- Informazine vas used as the reference compound in the same way as primethazine for the 50% (w/w) methanol chromatograms. The <i>hR</i>^p value of the strength = 0.1.
- Information and the reference compound in the same way as primethazine for the 50% (w/w) methanol. (hR^p values of 10, with methanol (hH^{p+} = 7.3¹¹) were estimated from two corrected <i>hR</i>^p values for 50% (w/w) methanol.
- <i>I hR</i>^p values at pH^{p+} = 10.48 and 8.03.
- <i>I hR</i>^p values at pH^{p+} = 10.48 and 7.38.</td> <td>romethazine</td> <td>26.5</td> <td></td> <td>47</td> <td></td> <td>68</td> <td>•</td> <td></td> <td></td> <td>•</td> <td></td> <td></td> <td></td> <td></td>
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 | hyperazine49.551.255.553.665.570.87.311571113331.935erphenazine4443.848.547.15663.07.2111491112019.523omethazine2846.5677.2111491112019.52323conchorperazine2846.5677.211491112019.52323conchorperazine288.14547.67376.38.64478442727conchazine20.520.424.558.88.214634478442727conchazine2111.121.554.558.88.21463441716.915conchazine1111.231.529.554.558.88.21463441716.915conchazine2111.121.554.558.88.21463441716.915conchazine2111.121.524.558.88.24463441716.915concestrend in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>luR</i> , values11.12.379.6concestrend with <i>R</i> values on the plate.1 <i>I</i> .Rvalues for 50% (w/w) methanol.1.41.4effectuated with <i>R</i> values at pH* = 10.48 and 7.38.8.031.71.71.61.7 | Jixyrazine 49.5 51.2 55.5 53.6 65.5 70.8 7.3 ¹¹ 57 ¹¹¹ 31 31.9 35
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- Informazine vas used as the reference compound in the same way as primethazine for the 50% (w/w) methanol chromatograms. The <i>hR</i> ^p value of the strength = 0.1.
- Information and the reference compound in the same way as primethazine for the 50% (w/w) methanol. (hR ^p values of 10, with methanol (hH ^{p+} = 7.3 ¹¹) were estimated from two corrected <i>hR</i> ^p values for 50% (w/w) methanol.
- <i>I hR</i> ^p values at pH ^{p+} = 10.48 and 8.03.
- <i>I hR</i> ^p values at pH ^{p+} = 10.48 and 7.38. | romethazine | 26.5 | | 47 | | 68 | • | | | • | | | | |
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 | $\begin{array}{cccccccccccccccccccccccccccccccccccc$ | 44 43.8 48.5 47.1 56 63.0 7.2 ¹⁰¹ 49 ¹⁰¹ 20 19.5 28 46.5 67 67 7.5 ¹⁰¹ 49 ¹⁰¹ 20 19.5 28 46.5 67 67 7.5 ¹⁰¹ 8.5 8.3 20.5 20.4 24.5 23.5 37 44.0 7.5 ¹⁰¹ 26 ¹⁰¹ 8.5 8.3 8 8.1 45 42.6 73 76.3 8.6 ¹⁰¹ 78 ¹⁰ 41 40.8 27.5 47.5 69.5 54.5 58.8 8.2 ¹⁰ 63 ¹⁰¹ 17 16.9
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* Ionic strength = 0.1.
• Tonic strength = 0.5.
• Dixyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>h.R.</i> value of a strength -0.5 .
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* Ionic strength = 0.1.
* Ionic strength = 0.5.
** Ionic strength = 0.5.
** Divyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>inR</i> , value of yrazine is one of the four measured <i>inR</i> , values on the plate.
* <i>inR</i> , values for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected <i>inR</i> , values for 50% (w/w) methanol | ochlorperazine 20.5 20.4 24.5 37. 44.0 7.5^{111} 26^{111} 8.5 8.3 10 ofenamine 8 8.1 45 42.6 73 76.3 8.6^{11} 78^{11} 41 40.8 27 ofenamine 8 8.1 45 42.6 73 76.3 8.6^{11} 78^{11} 41 40.8 27 ornethazine 11 11.2 31.5 29.5 54.5 58.8 8.2^{11} 63^{11} 17 16.9 15 iflupromazine 11 11.2 31.5 29.5 54.5 58.8 8.2^{11} 63^{11} 17 16.9 15 * Ionic strength = 0.1. 11.2 31.5 29.5 54.5 58.8 8.2^{11} 63^{11} 17 16.9 15 * Ionic strength = 0.5. 16 15 ** Ionic strength = 0.5. * Ionic strengt is one of the four measured her
 | ochlorperazine 20.5 20.4 24.5 23.5 37 44.0 7.5 ¹¹¹ 26 ¹¹¹ 8.5 8.3 10
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\cdot Ionic strength = 0.1.
\cdot Ionic strength = 0.1.
\cdot Ionic strength = 0.5.
\cdot Tonic strength = 0.5.
\cdot Ionic strength = 0.5.
\cdot Tonic strength = 0.5.
\cdot Calculated with <i>hRv</i> values on the plate.
\cdot Interval to a stress of the four measured <i>hRv</i> values for the 50% (w/w) methanol chromatograms. The <i>hRv</i> value of xyrazine is one of the four measured <i>hRv</i> values on the plate.
\cdot Acculated with <i>hRv</i> values at pH* = 10.48 and 8.03.
\cdot Calculated with <i>hRv</i> values at pH* = 10.48 and 7.38. | Prochlorperuzine 20.5 20.4 24.5 23.5 37 44.0 7.5 ¹¹¹ 26 ¹¹¹ 8.5 8.3 10
Profenamine 8 8.1 45 42.6 73 76.3 8.6 ¹¹ 78 ¹¹¹ 26 ¹¹¹ 41 40.8 27
Promethazine 27.5 47.5 69.5 73 76.3 8.6 ¹¹¹ 78 ¹¹¹ 17 16.9 15
Promethazine 11 11.2 31.5 29.5 54.5 58.8 8.2 ¹¹¹ 63 ¹¹¹ 17 16.9 15
10 in cistength = 0.1.
10 in cistength = 0.5.
10 in cistength = 0.5.
11 for the 50% (w/w) methanol chromatograms. The <i>hR</i> _r value of the four measured <i>hR</i> _r values for the 50% (w/w) methanol chromatograms. The <i>hR</i> _r value of the four measured <i>hR</i> _r values on the plate.
1 <i>hR</i> _r values for 30% (w/w) methanol (pH [*] = 7.37) were estimated from two corrected <i>hR</i> _r values for 50% (w/w) methanol.
1 <i>hR</i> _r values at pH [*] = 10.48 and 8.03.
11 Calculated with <i>hR</i> _r values at pH [*] = 10.48 and 7.38. | romethazine | 28 | | 46.5 | | 67 | 1j- | | | | | | | |
| 8 8.1 45 42.6 73 76.3 8.6 ¹¹ 78 ¹⁰ 41 40.8 27.5 47.5 69.5 69.5 54.5 58.8 8.2 ¹⁰ 63 ¹⁴ 17 16.9
 | 8 8.1 45 42.6 73 76.3 8.6 ¹¹ 78 ¹⁰ 41 40.8 27.5 47.5 69.5 69.5 54.5 58.8 8.2 ¹⁰ 63 ¹⁶ 17 16.9 fremeth = 0.1 11.2 31.5 29.5 54.5 58.8 8.2 ¹⁰ 63 ¹⁶ 17 16.9 | 8.1 45 42.6 73 76.3 8.6 ⁴⁴ 78 ⁴⁴ 41 40.8 47.5 69.5 69.5 8.2 ⁴⁴ 63 ⁴⁴ 17 16.9 11.2 31.5 29.5 58.8 8.2 ⁴⁴ 63 ⁴⁴ 17 16.9
 | 8 8.1 45 42.6 73 76.3 8.6 ¹¹ 78 ¹⁰ 41 40.8 27.5 47.5 69.5 69.5 54.5 58.8 8.2 ¹⁰ 63 ¹⁰ 17 16.9 ne 11 11.2 31.5 29.5 54.5 58.8 8.2 ¹⁰ 63 ¹⁰ 17 16.9 fremeth = 0.1. 0.5 54.5 58.8 8.2 ¹⁰ 63 ¹⁰ 17 16.9
 | $\begin{array}{rcrcrcccccccccccccccccccccccccccccccc$
 | Menamine 8 8.1 45 42.6 73 76.3 8.6 ¹¹ 78 ¹¹ 41 40.8 27
muchazine 27.5 47.5 69.5 58.8 8.2 ¹¹ 63 ¹¹ 17 16.9 15
fupromazine 11 11.2 31.5 29.5 54.5 58.8 8.2 ¹¹ 63 ¹¹ 17 16.9 15
* Ionic strength = 0.1.
* Tonic strength = 0.5.
** Tonic strength = 0.5.
** Dixyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>hR</i> , value of yrazine is one of the four measured <i>hR</i> , values on the plate.
* <i>hR</i> , values for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected <i>hR</i> , values for 50% (w/w) methanol | of enamine 8 8.1 45 42.6 73 76.3 8.6 ⁴⁴ 78 ⁴⁴ 41 40.8 27
ornethazine 27.5 47.5 69.5 58.8 8.2 ⁴⁴ 63 ⁴⁴ 78 ⁴⁴ 41 40.8 27
ornethazine 11 11.2 31.5 29.5 54.5 58.8 8.2 ⁴⁴ 63 ⁴⁴ 17 16.9 15
\cdot Ionic strength = 0.1.
\cdot Ionic strength = 0.5.
$\cdot \cdot$ Ionic strength = 0.5.
$\cdot \cdot$ Dixyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>hR</i> _r value of xyrazine is one of the four measured <i>hR</i> _r values on the plate.
$\cdot \cdot$ Ir <i>R</i> _r values for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected <i>hR</i> _r values for 50% (w/w) methanol.
 | ofenamine 8 8.1 45 42.6 73 76.3 8.6 ⁴⁴ 78 ⁴⁴ 41 40.8 27
correthazine 27.5 47.5 69.5 54.5 58.8 8.6 ⁴⁴ 78 ⁴⁴ 41 40.8 27
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10nic strength = 0.6.
11.1.2 31.5 29.5 54.5 58.8 8.2 ⁴⁴ 63 ⁴⁴ 17 16.9 15
10nic strength = 0.5.
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11.1.2 31.5 29.5 54.5 58.8 8.2 ⁴⁴ 63 ⁴⁴ 17 16.9 15
11.1.2 31.5 29.5 54.5 58.8 8.2 ⁴⁴ 63 ⁴⁴ 17 16.9 15
11.1.2 31.5 29.5 54.5 58.8 8.2 ⁴⁴ 63 ⁴⁴ 10.8 methanol chromatograms. The <i>htt</i> _r value of xyrazine is one of the four measured <i>ht</i> _r values on the plate.
10.1.8 methanol (pH* = 7.37) were estimated from two corrected <i>ht</i> _r values for 50% (w/w) methanol.
11.1.2 10.48 and 8.03.
11.1.2 10.48 and 7.38. | Profenamine 8 8.1 45 42.6 73 76.3 8.6 ¹⁰ 78 ¹¹ 41 40.8 27 Promethazine 27.5 47.5 69.5 54.5 58.8 8.2 ¹⁰ 63 ¹¹ 17 16.9 15 Promethazine 27.5 47.5 69.5 54.5 58.8 8.2 ¹⁰ 63 ¹¹ 17 16.9 15 Promotisterength 0.1 31.5 29.5 54.5 58.8 8.2 ¹⁰ 63 ¹¹ 17 16.9 15 Promis strength 0.1 11 12 31.5 29.5 54.5 58.8 8.2 ¹⁰ 63 ¹¹ 17 16.9 15 Promis strength 0.1 11 12 31.5 29.5 54.5 58.8 8.2 ¹⁰ 63 ¹¹ 17 16.9 15 Promis strength 0.1 11 12 31.5 29.5 54.5 58.6 (w/w) methanol chromatograms. The <i>hA</i> , whue of hAr, values for 50% (w/w) methanol. 16 17 16.9 15 Provide the four 10.68 10.48 10.4 | rochlorperazine | 20.5 | 20.4 | 24.5 | | 37. | 44.0 | 7.5 *** | | 26444 | 8.5 | 8,3 | 10 | |
| 27.5 47.5 69.5 ne 11 11.2 31.5 29.5 54.5 58.8 8.2 ¹¹ 63 ¹¹
 | 27.5 47.5 69.5 ne 11 11.2 31.5 29.5 54.5 58.8 8.2 ¹¹ 63 ¹¹ fremeth = 0.1 11 11.2 31.5 29.5 54.5 58.8 8.2 ¹¹ 63 ¹¹ | 11.2 47.5 69.5 58.8 8.2 ¹¹ 63 ¹¹ 17 16.9
 | 27.5 47.5 69.5 nc 11 11.2 31.5 29.5 54.5 58.8 8.211 63.11 trength = 0.1. 0.5 54.5 58.8 8.211 63.11 17 16.9
 | rethrazine 27.5 47.5 69.5 54.5 58.8 8.2^{11} 63^{11} 17 16.9 15 1000 strength = 0.1.
• Ionic strength = 0.1.
• Dixyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>lnR</i> value of a string is 200 of the four measured <i>LP</i> reduces the four or the four methanol chromatograms. The <i>lnR</i> value of the four measured <i>LP</i> reduces the four or the four strength = 0.5.
 | whetharine 27.5 47.5 69.5 (http://doi.org/10.11.2 27.5 47.5 58.8 8.21 631 17 16.9 15 10 fubromazine 11 11.2 31.5 29.5 54.5 58.8 8.21 631 17 16.9 15 \cdot Ionic strength = 0.1. \cdot Ionic strength = 0.5. \cdot Ionic strength = 0.5. \cdot Ionic strength = 0.5 \cdot \cdot Ionic strength = 0.5 \cdot \cdot Interference compound in the same way as premetharine for the 50% (w/w) methanol chromatograms. The <i>hR</i> , value of variance is one of the four measured <i>hR</i> values on the plate. \cdot <i>i.hR</i> , values for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected <i>hR</i> , values for 50% (w/w) methanol | ornethazine 27.5 47.5 69.5 54.5 58.8 8.2^{14} 63^{14} 17 16.9 15 10 10 11.2 31.5 29.5 54.5 58.8 8.2^{14} 63^{14} 17 16.9 15 15 10 10 if and strength = 0.1.
Tomic strength = 0.5.
The reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>lnR</i> value of tyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>lnR</i> value of tyrazine is one of the four measured <i>lnR</i> values on the plate.
** Calculated with <i>hR</i> values at pH* = 10.48 and 8.03.
 | correthazine 27.5 47.5 69.5 54.5 58.8 8.2^{11} 63^{11} 17 16.9 15 16.9 15 16.9 11 11.2 31.5 29.5 54.5 58.8 8.2^{11} 63^{11} 17 16.9 15 16.9 15 10 inic strength = 0.1.
• Ionic strength = 0.1.
• Dixyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>hR_r</i> value of xyrazine is one of the four measured <i>hR_r</i> values on the plate.
• <i>hR_r</i> values for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected <i>hR_r</i> values for 50% (w/w) methanol.
• Calculated with <i>hR_r</i> values at pH* = 10.48 and 8.03.
• Calculated with <i>hR_r</i> values at pH* = 10.48 and 7.38. | Promethazine 27.5 47.5 69.5
Frifupromazine 11 11.2 31.5 29.5 54.5 58.8 8.2^{11} 63^{14} 17 16.9 15
* Ionic strength = 0.1.
* Ionic strength = 0.5.
** Ionic strength = 0.5.
** Dixyrazine was used as the reference compound in the same way as prepriethazine for the 50% (w/w) methanol chromatograms. The <i>iR</i> _F value of lixyrazine was used as the reference compound in the same way as prepriethazine for the 50% (w/w) methanol chromatograms. The <i>iR</i> _F value of lixyrazine is one of the four measured <i>hR</i> _F values on the plate.
** Calculated with <i>hR</i> _F values at pH* = 10.48 and 8.03.
** Calculated with <i>hR</i> _F values at pH* = 10.48 and 7.38. | rofenamine | 8 | 8.1 | 45 | | 73 | 76.3 | 8.644 | 1818 | •• | 41 | 40.8 | 27 | • |
| 11 11.2 31.5 29.5 54.5 58.8 8.2 ¹¹ 63 ¹¹ 17
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ngth = 0.1.
nuth = 0.5</td> <td>apromazine1111.231.529.554.558.8$8.2^{14}$$63^{14}$$17$16.915* Ionic strength = 0.1.* Ionic strength = 0.5.* Dixyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The hR_{V} value of a strength and the four methanol chromatograms. The hR_{V} value of the four methanol chromatograms. The hR_{V} value of the four strength and th</td> <td>Rupromazine 11 11.2 31.5 29.5 54.5 58.8 8.2¹¹ 63¹¹ 17 16.9 15
* Ionic strength = 0.1,
* Ionic strength = 0.5,
** Ionic strength = 0.5,
** Dixyrazine was used as the reference compound in the same way as premethazine for the 50% (w/w) methanol chromatograms. The $hR_{\rm F}$ value of yrazine is one of the four measured $hR_{\rm F}$ values on the plate.
* $hR_{\rm F}$ values for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected $hR_{\rm F}$ values for 50% (w/w) methanol</td> <td>iflupromazine 11 11.2 31.5 29.5 54.5 58.8 8.2¹¹ 63¹¹ 17 16.9 15
\cdot Ionic strength = 0.1.
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$\cdot \cdot$ Dixyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>lnR</i>^r value of tyrazine is one of the four measured <i>lnR</i>_r values on the plate.
$\cdot \cdot \cdot R$ values for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected <i>lnR</i>_r values for 50% (w/w) methanol.</td> <td>iflupromazine 11 11.2 31.5 29.5 54.5 58.8 8.2¹¹ 63¹¹ 17 16.9 15
* Ionic strength = 0.1.
* Ionic strength = 0.1.
* Dixyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>hR_v</i> value of xyrazine is one of the four measured <i>hR_v</i> values on the plate.
* <i>hR_v</i> values for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected <i>hR_v</i> values for 50% (w/w) methanol.
** Calculated with <i>hR_v</i> values at pH* = 10.48 and 8.03.
*** Calculated with <i>hR_v</i> values at pH* = 10.48 and 7.38.</td> <td>Triflupromazine 11 11.2 31.5 29.5 54.5 58.8 8.2¹⁰ 63¹⁰ 17 16.9 15
Tonic strength = 0.1.
Tonic strength = 0.5.
The information of the four measured information of the same way as promethazine for the 50% (w/w) methanol chromatograms. The information of interval in one of the four measured information in the same way as promethazine for the 50% (w/w) methanol chromatograms. The information of information of the four measured information of the plate.
Information of the four measured information (pH[*] = 7.37) were estimated from two corrected information for 50% (w/w) methanol.
Information with information of the four measured information in the same way as provided information in the same way as provided information in the same way as provided information of the four measured information in the same way as provided in the same way and so the same way as provided in the sam</td> <td>romethazine</td> <td>27.5</td> <td></td> <td>47.5</td> <td></td> <td>69.5</td> <td></td> <td></td> <td></td> <td>-</td> <td></td> <td></td> <td></td> <td></td> | 11.2 31.5 29.5 54.5 58.8 8.2 ¹¹ 63 ¹¹ 17
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* Ionic strength = 0.1,
* Ionic strength = 0.5,
** Ionic strength = 0.5,
** Dixyrazine was used as the reference compound in the same way as premethazine for the 50% (w/w) methanol chromatograms. The $hR_{\rm F}$ value of yrazine is one of the four measured $hR_{\rm F}$ values on the plate.
* $hR_{\rm F}$ values for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected $hR_{\rm F}$ values for 50% (w/w) methanol
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\cdot Ionic strength = 0.1.
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$\cdot \cdot$ Dixyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>lnR</i> ^r value of tyrazine is one of the four measured <i>lnR</i> _r values on the plate.
$\cdot \cdot \cdot R$ values for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected <i>lnR</i> _r values for 50% (w/w) methanol. | iflupromazine 11 11.2 31.5 29.5 54.5 58.8 8.2 ¹¹ 63 ¹¹ 17 16.9 15
* Ionic strength = 0.1.
* Ionic strength = 0.1.
* Dixyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>hR_v</i> value of xyrazine is one of the four measured <i>hR_v</i> values on the plate.
* <i>hR_v</i> values for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected <i>hR_v</i> values for 50% (w/w) methanol.
** Calculated with <i>hR_v</i> values at pH* = 10.48 and 8.03.
*** Calculated with <i>hR_v</i> values at pH* = 10.48 and 7.38.
 | Triflupromazine 11 11.2 31.5 29.5 54.5 58.8 8.2 ¹⁰ 63 ¹⁰ 17 16.9 15
Tonic strength = 0.1.
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Information of the four measured information (pH [*] = 7.37) were estimated from two corrected information for 50% (w/w) methanol.
Information with information of the four measured information in the same way as provided information in the same way as provided information in the same way as provided information of the four measured information in the same way as provided in the same way and so the same way as provided in the sam | romethazine | 27.5 | | 47.5 | | 69.5 | | | | - | | | | |
|
 | |
 |
 | * Ionic strength = 0.1.
• Ionic strength = 0.5.
• Dixyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>hth</i> value of a structure of the four measured <i>t</i> . Determine the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>hth</i> value of the four measured the four measured the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>hth</i> value of the four measured the four measure | * Ionic strength = 0.1.
• Ionic strength = 0.5.
• Dixyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The $hR_{\rm r}$ value of yrazine is one of the four measured $hR_{\rm r}$ values on the plate.
• $hR_{\rm r}$ values for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected $hR_{\rm r}$ values for 50% (w/w) methanol
 | * Ionic strength = 0.1.
• Ionic strength = 0.5.
• Dixyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>hRr</i> value of xyrazine is one of the four measured <i>hRr</i> values on the plate.
• <i>hRr</i> values for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected <i>hRr</i> values for 50% (w/w) methanol.
 | • Ionic strength = 0.1.
• Ionic strength = 0.5.
• Divyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>hR</i> _r value of xyrazine is one of the four measured <i>hR</i> _r values on the plate.
• <i>hR</i> _r values for 30% (w/w) methanol (pH [*] = 7.37) were estimated from two corrected <i>hR</i> _r values for 50% (w/w) methanol.
• Calculated with <i>hR</i> _r values at pH [*] = 10.48 and 8.03.
• Calculated with <i>hR</i> _r values at pH [*] = 10.48 and 7.38. | Tonic strength = 0.1.
Tonic strength = 0.5.
Tonic strength = 0.5.
Tonic strength = 0.5.
Tonic strength = 0.5.
Tonic strength = 0.5.
The <i>hR</i> _r values on the plate.
<i>i hR</i> _r values for 30% (w/w) methanol (pH [*] = 7.37) were estimated from two corrected <i>hR</i> _r values for 50% (w/w) methanol.
<i>i R</i> _r values for 30% (w/w) methanol (pH [*] = 7.37) were estimated from two corrected <i>hR</i> _r values for 50% (w/w) methanol.
i <i>R</i> _r values of the four measured <i>hR</i> _r and 8.03.
Calculated with <i>hR</i> _r values at pH [*] = 10.48 and 7.38. | Triflupromazine | 11 | 11.2 | 31.5 | | 54.5 | 58,8 | 8.246 | 63 11 | • | 17 | 16.9 | 15 | |
|
 | |
 | Juic succession – 0.1. Succession – 0.5
 | Tonic strength = 0.1.
• Tonic strength = 0.5.
• Dixytrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The hR_{radius} is one of the four measured hP_{radius} on the and on the same way as promethazine for the 50% (w/w) methanol chromatograms. The hR_{radius}
 | • Ionic strength = 0.5.
• Ionic strength = 0.5.
• Dixyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The h.R. yrazine is one of the four measured h.R. values for 50% (w/w) methanol (DH [*] = 7.37) were estimated from two corrected h.R. values for 50% (w/w) methanol (DH [*] = 7.37) were estimated from two corrected h.R. values for 50% (w/w) methanol | • Tonic strength = 0.1.
• Tonic strength = 0.5.
• Divyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The hR
syrazine is one of the four measured $hR_{\rm F}$ values on the plate.
• $hR_{\rm F}$ values for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected $hR_{\rm F}$ values for 50% (w/w) methanol.
• Calculated with $hR_{\rm F}$ values at pH* = 10.48 and 8.03.
 | • Ionic strength = 0.1.
• Tonic strength = 0.5.
• Divyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The $hR_{\rm XY}$ are of the four measured $hR_{\rm F}$ values on the plate.
• $hR_{\rm F}$ values for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected $hR_{\rm F}$ values for 50% (w/w) methanol.
• Calculated with $hR_{\rm F}$ values at pH* = 10.48 and 8.03.
• Calculated with $hR_{\rm F}$ values at pH* = 10.48 and 7.38. | • Tonic strength = 0.1.
• Tonic strength = 0.5.
• Divyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>hR</i> , divyrazine is one of the four measured <i>hR</i> _r values on the plate.
• <i>hR</i> _r values for 30% (w/w) methanol ($\rho H^* = 7.37$) were estimated from two corrected <i>hR</i> _r values for 50% (w/w) methanol.
• Calculated with <i>hR</i> _r values at $\rho H^* = 10.48$ and 8.03.
• Calculated with <i>hR</i> _r values at $\rho H^* = 10.48$ and 7.38. | Triflupromazine | 11 | 11.2 | 31.5 | . 1 | 54.5 | 58.8 | 8.216 | 63 11 | | 17 | 16.9 | 15 | |
| *** Divyrazine was used as the reference compound in the same way as promethazine for the 50% (w/w) methanol chromatograms. The <i>hR</i> _r value yrazine is one of the four measured <i>hR</i> _r values on the plate.
*** Pixyrazine is one of the four measured <i>hR</i> _r values on the plate.
*** A subject of the four measured <i>hR</i> _r values on the plate.
*** Calculated with <i>hR</i> _r values at pH* = 10.48 and 8.03.
*** Calculated with <i>hR</i> _r values at pH* = 10.48 and 7.38.
 | Narratine way used us the releatence compound in the same way as promethazine for the 30% (W/W) methanol chromatograms. The $hR_{\rm F}$ variation is one of the four measured $hR_{\rm F}$ values on the plate.
⁹ hR _F values for 30% (W/W) methanol (pH [*] = 7.37) were estimated from two corrected $hR_{\rm F}$ values for 50% (W/W) methanol.
¹⁰ Calculated with $hR_{\rm F}$ values at pH [*] = 10.48 and 8.03.
¹¹¹ Calculated with $hR_{\rm F}$ values at pH [*] = 10.48 and 7.38. | of the four measured $hR_{\rm F}$ values on the plate.
of the four measured $hR_{\rm F}$ values on the plate.
I with $hR_{\rm F}$ values at pH* = 10.48 and 8.03.
I with $hR_{\rm F}$ values at pH* = 10.48 and 7.38.
 | Revalues for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected $h_{\rm Re}$ values for 50% (w/w) methanol. The standard of the two corrected $h_{\rm Re}$ values for 50% (w/w) methanol. Alculated with $h_{\rm Re}$ values at pH* = 10.48 and 3.03.
 | ¹⁴ Calculated with <i>hRr</i> values at pH [*] = 10.48 and 8.03.
¹⁵ Calculated with <i>hRr</i> values at pH [*] = 10.48 and 7.38.
 | ¹¹⁹ Calculated with $hR_{\rm r}$ values at pH ⁹ = 10.48 and 7.38. |
 | | | | | | | | | • | : | | • | | | | |
| The hRr values for the formation of the formation of the solution of the solution of the formation of the f | invariance was used us the corrections compound in the same way as promentazine for the 30% (w/w) memanol chromatograms. The $hR_{\rm F}$ invariance for four measured $hR_{\rm F}$ values on the plate.
¹ hR _F values for 30% (w/w) methanol (pH [*] = 7.37) were estimated from two corrected $hR_{\rm F}$ values for 50% (w/w) methanol.
¹⁰ Calculated with $hR_{\rm F}$ values at pH [*] = 10.48 and 7.38.
 | of the four measured $hR_{\rm r}$ values on the plate.
of the four measured $hR_{\rm r}$ values on the plate.
is for 30% (w/w) methanol (pH* = 7.37) were estimated from two corrected $hR_{\rm r}$ values for 50% (w/w) methanol.
I with $hR_{\rm r}$ values at pH* = 10.48 and 7.38.
 | Revealed with $hR_{\rm F}$ values at pH [*] = 10.48 and 7.38.
Alculated with $hR_{\rm F}$ values at pH [*] = 10.48 and 7.38.
 | ¹⁶ Calculated with <i>hR_r</i> values at pH [*] = 10.48 and 8.03.
¹⁹ Calculated with <i>hR_r</i> values at pH [*] = 10.48 and 7.38. | ¹⁴¹ Calculated with hR_F values at pH ⁴ = 10,48 and 7.38.
 |
 | | | | | | | | | | | | | | | | |

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TABLE VI

s_a and s_b = standar values are included;	d deviations	of α and β	3, respectively	; $n = num$	ber of	compound	is whose R
Methanol (%, w/w)		Sa	β	Sβ	<i>n</i>	r	s
0	-0.0208	0.0006	-0.0089	0.0002	26	0.991	0.0015
30	-0.0306	0.0005	-0.0131	0.0005	26	0.983	0.0021
40	-0.0361	0.0005	-0.0152	0.0007	26	0.973	0.0027
50	-0.0435	0.0007	-0.0184	0.0011	26	0.960	0.0032

COFFEICIENTS AND STATISTICAL DATA OF THE CORRELATIONS &

50% (w/w) methanol buffers [57.98% (v/v)] of different pH* values, the hR_F values with a 30% (w/w) methanol buffer [36.20% (v/v)] can be estimated by calculating a_0 and a_1 for that compound at 50% (w/w) methanol. The (corrected) R_M value at high pH* can then be inserted in the equation (Table VI)

 $b = -0.0435 - 0.0184 R_{\rm M}$

Substitution of the values of b and R_M in eqn. 5 gives the value of R_{M_W} , and the R_M value of the free base with 30% (w/w) methanol can be calculated. From a_0 and a_1 at 50% (w/w) methanol, $p({}_{s}K_{a}{}^{c})$ in 50% (w/w) methanol can be calculated. The difference between $p(_{s}K_{a}^{c})$ in 50% (w/w) methanol and pK_{a}^{c} in water for phenothiazines has been found^{17,28} (Table III) to range roughly from 0.9 to 1.2, with a mean value of 1.1. Assuming, as a first approximation, a linear decrease in $p({}_{s}K_{a})$ with the methanol concentration^{*}, the $p(_{s}K_{a}^{c})$ value of the compound in 30% (w/w) methanol can be estimated by adding 0.4 to the $p(_{s}K_{a}^{c})$ value in 50% (w/w) methanol. From the R_{M} value at high pH* and $p(sK_a^{c})$ [both in 30% (w/w) methanol] the value of a_1 for 30% (w/w) methanol can be calculated, and with the values of a_0 and a_1 so obtained the R_F values of the drug for 30% (w/w) methanol at different pH* values can be calculated (eqn. 3). Examples are given in Table V. For most compounds, the estimated hR_F values are close to the observed hR_F values. In some instances, it may be helpful to change the phase:volume ratio, r, in order to obtain a better resolution for two compounds. Changing $\log r$ of the chromatographic system (by changing the oley) alcohol concentration in the impregnating mixture) results in an equal absolute change in the R_M values of both compounds. Changes in R_M will cause a maximal shift in R_F at R_M values around $R_M = 0$ ($R_F = 0.5$). For instance (Table I), in the system oleyl alcohol [1.25% (v/v) in the impregnating mixture]-30\% (w/w) methanol (pH^{*} = 7.29), the hR_F values of propericiazine and mesoridazine are 83 and 87, respectively. Increasing $\log r$ by 0.75, that is, making the oleyl alcohol concentration in the impregnating mixture about 7% (v/v)^{**}, results in R_M values of 0.06 for

^{*} It was shown¹⁷ that $p(K_a^c)$ does not change linearly with the methanol concentration; however, the error that is made by ignoring this non-linearity will in most instances be small compared with the error that is introduced by assuming a difference of 1.1 between $p(sK_a^c)$ in 50% (w/w) methanol and pK_a^c in water for every compound.

^{**} It is reasonable to assume that $r = k \cdot C_{ol}$ (ref. 10), where C_{ol} is the concentration of olcyl alcohol (%, v/v) in the impregnating mixture and k is a constant. A change in log C_{ot} therefore results in an equal change in log r.

propericiazine and -0.08 for mesoridazine. The hR_F values are then 46.5 and 54.5, respectively, and ΔR_F will have become twice as great. However, it was found that the detection is less sensitive at higher phase volume ratios. A considerable advantage of the reversed-phase method, as described above, is that two chromatograms of each compound under investigation and a reference compound are sufficient to predict with reasonable accuracy the R_F values for the compounds in chromatographic systems that have the same stationary phase (but not necessarily the same loading) and any mobile phase consisting of methanol-water mixtures of a certain pH*. It is also possible to predict for which pH* of a certain mobile phase the maximum difference in R_F is obtained. This same procedure should be applicable to any group of acidic or basic drugs for which adsorption on the support phase has been proved to be absent.

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