Journal of Chromatography, 83 (1973) 405–420

CHROM. 6870

EVALUATION OF LIQUID–LIQUID SYSTEMS FOR THE CHROMATOGRAPHIC SEPARATION OF THE PSYCHOTROPIC DRUG THIORIDAZINE AND ITS METABOLITES

R. G. MUUSZE
St. Joris Hospital, Delft (The Netherlands)
and
J. F. K. HUBER
Laboratory of Analytical Chemistry, University of Amsterdam, Amsterdam (The Netherlands)

SUMMARY

A number of liquid–liquid systems were evaluated for their usefulness in the chromatographic separation of the neurolepticum thioridazine and its metabolites.

The distribution coefficients of these compounds determined by a static method are presented. The influence of the pH is discussed. Corresponding distribution coefficients and retention data are correlated. A linear regression is found with some deviations depending on the type of compound and solid support.

The choice of the optimal phase system is discussed and chromatograms are presented.

INTRODUCTION

The compound thioridazine (Melleril®, Sandoz) is used as a tranquillizer in psychiatry. Its metabolism occurs mainly by sulphoxidation and N-dealkylation[1]. Some of the metabolites also have psychotropic activity. Table I shows the structure of thioridazine and some of its metabolites and indicates the psychotropic activity of these phenothiazine compounds.

For the study of the pharmacokinetic behaviour and the control of psychiatric patients, a rapid and sensitive method of analysis for thioridazine and its metabolites in blood is needed. Such a method is not available at present[2–4] and had to be developed. The method of choice for the separation of thioridazine and its metabolites is liquid chromatography. Very low-level detection is feasible if the compounds are converted into fluorigenic species, which can be determined by fluorimetry. As a first approach, thin-layer chromatography was used to analyze a test mixture of the compounds in Table I. The results shown in Fig. 1 demonstrate that the separation of the six compounds was not yet satisfactory. Another drawback is the laborious working method involved. It was therefore decided to improve the method of analysis by using high-pressure liquid chromatography. As we expected to find a possible corre-

TABLE I

STRUCTURE AND PSYCHOTROPIC ACTIVITY OF THIORIDAZINE AND ITS METABOLITES FORMED BY SULPHOXIDATION AND N-DEMETHYLATION

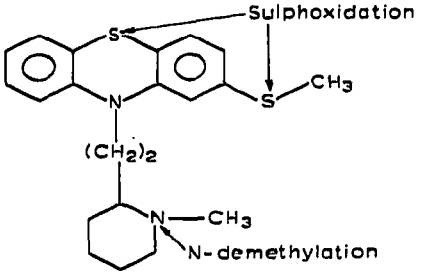
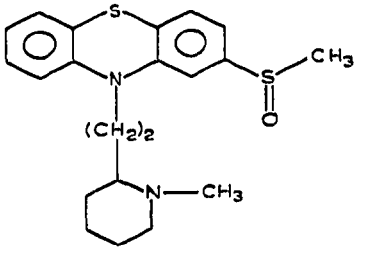
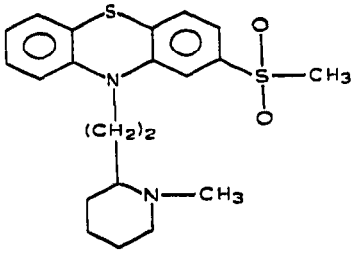
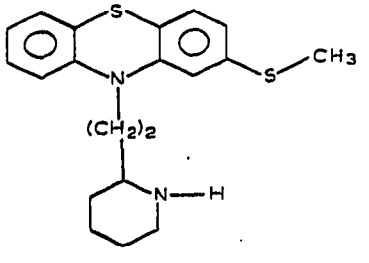
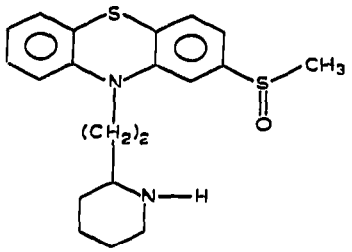
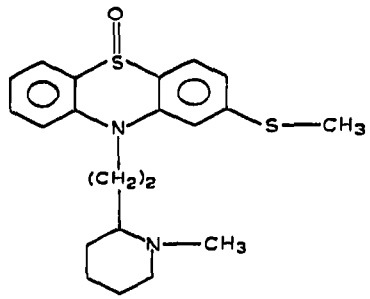
Structure	*Name*	*Psychotropic activity*
	Thioridazine (T)	Major tranquillizer
	Thioridazine-2-sulphoxide (T2SO)	Minor tranquillizer
	Thioridazine-2-sulphone ($T2SO_2$)	Major tranquillizer
	Northioridazine (NT)	Antidepressant

TABLE I *(continued)*

Structure	*Name*	*Psychotropic activity*
	Northioridazine-2-sulphoxide (NT2SO)	Inactive
	Thioridazine-5-sulphoxide (T5SO)	Inactive

lation between the psychotropic activity and the distribution coefficient, liquid–liquid chromatography was chosen.

THEORETICAL

Distribution coefficient involving chemical equilibrium

When in solution, a solute can undergo dissociation and association reactions. The chromatographic migration velocity of a compound is determined by its total distribution coefficient between the stationary and mobile phases, irrespective of whether it is present in the phases as more than one chemical species[5]. Thioridazine and its metabolites are basic compounds so that the formation of cations can occur in a polar liquid. Therefore, at least two processes are involved in the distribution of these compounds between two liquid phases:

(1) distribution of the neutral basic compound, B, between the polar liquid phase, p, and the non-polar liquid phase, np:

$$B_p \rightleftharpoons B_{np}$$

(2) formation of a cation from the neutral basic compound, B, in the polar liquid phase:

$$B_p + (H^+)_p \rightleftharpoons (BH^+)_p$$

The total distribution coefficient, $K_{B(tot)}$, which considers all the species in which B is contained, is given by the following expression:

$$K_{B(tot)} = \frac{[B]_p + [BH^+]_p}{[B]_{np}} \quad (1)$$

where the symbol $[X]_k$ represents the concentration of the species X (*i.e.*, B and BH^+) in the phase k (*i.e.*, p and np).

The concentrations are also correlated by the partial distribution coefficient, K_B, of the single species B and by the formation constant, K_f, of the cation BH^+.

$$K_B = \frac{[B]_p}{[B]_{np}} \quad (2)$$

$$K_f = \frac{[BH^+]_p}{[B]_p\,[H^+]_p} \quad (3)$$

Combination of the expressions for the partial distribution coefficient, the formation constant and the total distribution coefficient gives the following equation:

$$K_{B(tot)} = K_B\,(1 + K_f\,[H^+]_p) \quad (4)$$

This equation approaches two limiting expressions:

$$K_{B(tot)} \simeq K_B$$

at $K_f\,[H^+]_p \ll 1$ and

$$K_{B(tot)} \simeq K_B K_f\,[H^+]_p$$

at $K_f\,[H^+]_p \gg 1$.

In the second case, the logarithmic form of the equation is a linear function of the pH in the polar liquid phase:

$$\log K_{B(tot)} = \log (K_B K_f) - (pH)_p \quad (5)$$

at $K_f\,[H^+] \gg 1$, where $(pH)_p = -\log [H^+]_p$

Correlation of retention times and distribution coefficients

From the theory of the chromatographic process, it follows that the retention time is a linear function of the distribution coefficients involved[6]. If the sample distributes between only two phases, the equation for the retention time is

$$t_{Ri} = t_{R0} + t_{R0}\,qK_i \quad (6)$$

where

t_{Ri} = retention time of component i
t_{R0} = retention time of the mobile phase
q = volume ratio of the stationary and mobile phases
K_i = distribution coefficient of component i between the stationary and mobile phases

Choice of phase system for chromatography

The choice of the phase system for a chromatographic separation can be based on the equation for the resolution, R_{ji}, of two components, j and i:

$$R_{ji} = (r_{ji} - 1)\frac{\kappa_i}{1 + \kappa_i} \cdot \left(\frac{L}{H_i}\right)^{\frac{1}{2}} \quad (7)$$

where

r_{ji} = selectivity factor = ratio of distribution coefficients of the components j and i in the stationary and mobile phases
κ_i = capacity ratio of component i
H_i = theoretical plate height for component i
L = column length

As a first approach, the nature of the phase system should be chosen with respect to the factor $(r_{ji}-1)\,\kappa_i/(1+\kappa_i)$. This factor should be made as large as possible with the restriction that extremely high capacity ratios should be avoided, because otherwise the separation time becomes too long. Another effect which has to be considered in principle is the dependence of the theoretical plate height on the nature of the phase system. This dependence, however, is usually a second order effect.

EXPERIMENTAL

Apparatus and procedures

The liquid chromatograph was assembled from custom-made and commercial parts. The eluent delivery system consisted of a thermostatted reservoir, a high-pressure membrane pump (Orlita DMP1515) and a flow-through Bourdon-tube manometer acting as damping device for flow pulsations. Sampling was carried out by means of a sampling valve (Siemens) with a loop of 27 μl volume. The columns were constructed from borosilicate glass tubes of 250 mm length, 2.8 mm I.D. and 12.0 mm O.D. Low dead-volume column connectors with smooth flow paths were used[7]. The solid supports for the stationary liquid were silica or diatomite, specific surface areas 15 and 2 m^2/g, respectively, average particle diameter 15 μm. They were prepared from commercial products (Spherosil, Rhône-Progil, and Kieselgur, Merck) by grinding and classifying in air classifier (Alpine 100 MZR). The detector was a single wavelength UV photometer (LDC) equipped with a micro-cell, volume 8 μl, operating at 254 nm. The detector signal was recorded on a potentiometric recorder (Servogor RE511, Goertz).

The static determination of liquid–liquid distribution coefficients was carried out in a thermostatted vessel according to a previously described method[8] involving the measurement of UV absorption.

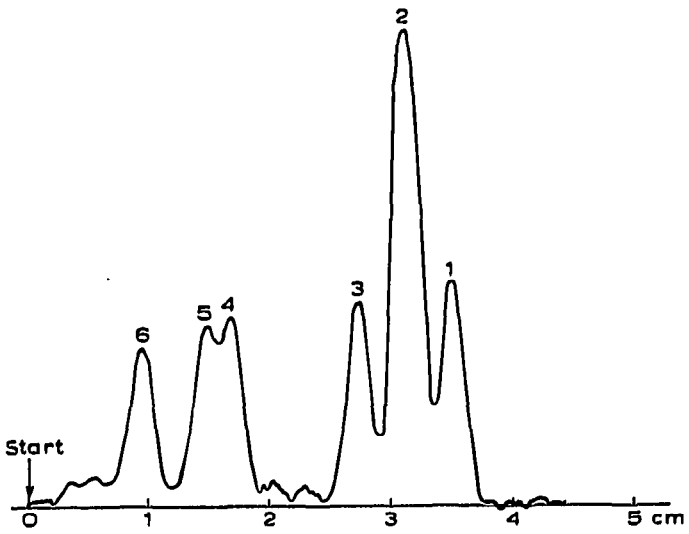


Fig. 1. Thin-layer chromatogram of a test mixture of thioridazine and its metabolites (see Table I) Chromatographic system: silica gel, layer thickness 0.1 mm; acetone–methyl acetate–aqueous ammonia (50:50:4). Detection: activation with ozone, reflection fluorescence measurement (Vitatron TLD100). 1 = T; 2 = $T2SO_2$; 3 = NT; 4 = T5SO; 5 = T2SO; 6 = NT2SO.

Separation characteristics	*T*	*$T2SO_2$*	*NT*	*T5SO*	*T2SO*	*NT2SO*
Capacity ratio	2.5	2.9	3.6	7.8	8.9	14.5
Selectivity factor	1.16	1.2	2.2	1.14	1.6	
Resolution	3.5	4.3	10.8	2.4	5.5	

TABLE II

COMPOSITION OF AQUEOUS BUFFER SOLUTIONS FOR DIFFERENT pH VALUES

pH	*Concentration (g/l)*		
	Citric acid monohydrate	*NaOH*	*HCl*
3.0	8.470	3.224	2.176
4.0	11.768	4.480	1.604
5.0	20.256	7.840	—
6.0	12.526	6.320	—
	KH_2PO_4	*$Na_2HPO_4 \cdot 2H_2O$*	
7.0	3.538	7.266	—
	Boric acid	*NaOH*	*HCl*
8.0	6.928	2.234	1.610
9.0	3.092	0.852	3.728
10.0	3.092	1.756	3.728
	$Na_2HPO_4 \cdot 2H_2O$	*NaOH*	
11.0	4.450	0.136	—
12.0	4.450	0.892	—

Chemicals

Liquid–liquid systems were prepared from spectroscopic-grade solvents. The compositions of the buffers (Titrisole, Merck) which were used to adjust the pH in the aqueous phase are presented in Table II. Samples of thioridazine and some of its metabolites were obtained from Sandoz.

RESULTS AND DISCUSSION

Static liquid–liquid distribution coefficients

As there was no information available on the selectivity of liquid–liquid systems with respect to phenothiazines, a suitable system for the chromatographic separation of these compounds had to be found by trial and error. The distribution coefficients of thioridazine and its metabolites in a number of arbitrarily chosen binary, ternary and quarternary liquid–liquid systems at 20° and different pH values were determined by static measurements. The results are given in Table III. Ternary and quaternary systems are characterized by the composition of the total phase system by which the equilibrium composition of the two coexisting liquid phases is defined. It must be remembered, however, that the same phase pair can be obtained from systems with different total composition, the only difference being the volume ratio of the two phases. An exact definition of the phase equilibrium would require the determination of the composition of the single phases. As the object of this work was the screening of liquid–liquid systems for chromatographic separations, it was renounced to investigate the phase equilibria. Most of the measurements were made in duplicate. The precision of the measurement was moderate, on average 10% in the basic medium and 16% in the acidic medium. The average precisions of the corresponding data given in Table III are therefore about 8 and 13%, respectively.

The lipophilic nature of a compound can be characterized by its distribution coefficient in a liquid–liquid system for the non-polar phase with respect to the polar phase. This value is the reciprocal of the data in Table III, from which it can be concluded that the sequence of the compounds according to the non-polar–polar liquid–liquid distribution coefficient corresponds with the sequence according to the psychotropic activity indicated in Table I.

In order to prove the interdependence of the distribution coefficient and the pH according to eqn. 5, a logarithmic plot of the corresponding data in Table III is presented in Fig. 2. In agreement with the theoretical prediction, the logarithm of the distribution coefficient decreases proportionally with the pH in the aqueous phase and tends towards a constant value at high pH. The slope of the lines in the linear range is the same for different compounds and phase systems. Its value is about 0.7, however, instead of the value of 1 predicted by eqn. 5. This deviation is not surprising, because in an exact treatment activities had to be used in the definition of the formation constant instead of concentrations. The pH can be adjusted by the addition of a buffer, base or acid to the phase system. Additions of volatile compounds are preferable, as such compounds can be removed together with the other volatile components of the phase system by evaporation if necessary, *e.g.*, for mass spectrometric studies. At increasing concentration, the additive will not only change the pH but will also influence the partial distribution coefficient of the neutral species of the sample. In order to test this effect, the content of 2-aminopropane was varied without

TABLE III

DISTRIBUTION COEFFICIENTS OF THIORIDAZINE AND ITS METABOLITES AT 20° IN LIQUID–LIQUID SYSTEMS

The distribution coefficient is given for the polar with respect to the non-polar phase. $\bar{K}_t$ = mean value of the distribution coefficient; $s_{\bar{K}}$ = standard deviation of the mean value.

Components of the solvent systems	*pH*	*Solutes*											
		T		*NT*		*T2SO₂*		*T2SO*		*NT2SO*		*T5SO*	
		$\bar{K}_t$	$s_{\bar{K}}$	$\bar{K}_t$	$s_{\bar{K}}$	$\bar{K}_t$	$s_{\bar{K}}$	$\bar{K}_t$	$s_{\bar{K}}$	$\bar{K}_t$	$s_{\bar{K}}$	$\bar{K}_t$	$s_{\bar{K}}$
Water–octanol-1	7.0	<0.01		<0.01		0.07		0.12		1.2		0.10	
Water–diethyl ether	3.0	9.0		14.6		—		—		—		—	
	4.0	1.90		6.8		—		—		—		—	
	5.0	0.06		1.00		—		—		49		—	
	6.0	—		—		1.50		—		3.7		—	
	7.0	—		—		0.15		1.90		1.30		11.8	
	8.0	—		—		—		0.24		—		2.1	
Water–heptane	5.0	1.03		—		—		—		—		—	
	6.0	0.17		1.60		—		—		—		—	
	7.0	—		0.33		4.0		—		—		—	
	8.0	—		—		0.63		—		—		—	
	9.0	—		—		—		1.76		14.9		19.1	
	10.0	—		—		—		0.44		3.0		3.5	
	11.0	—		—		—		—		1.30		1.50	
	12.0	—		—		—		—		0.47		—	
	14.0	—		—		—		—		—		0.20	
Components of the solvent systems (vol.-%)													
Water–cyclopentane–2-aminopropane	32.68 65.36 1.96	<0.01		<0.01		<0.01		0.02	0.004	0.06	0.007	0.44	0.04
Water–cyclohexane–2-aminopropane	32.68 65.36 1.96	<0.01		<0.01		<0.01		0.02		0.10		0.60	
Water–2,2,4-trimethylpentane–2-aminopropane	32.68 65.36 1.96	<0.01		0.01		0.20	0.06	0.32	0.02	0.69	0.12	5.7	1.1
Water–ethanol–2,2,4-trimethylpentane–2-aminopropane	4.42 36.96 56.95 1.67	1.10	0.06	1.70	0.07	5.0	0.14	5.8	0.6	10.1	1.0	10.5	0.8
Water–ethanol–2,2,4-trimethylpentane–2-aminopropane	2.63 17.15 77.87 2.35	1.50	0.06	2.7	0.06	13.0	1.7	16.0	0.2	20	1.2	22	2.5

TABLE III *(continued)*

Components of the solvent systems (vol.-%)		*Solutes*											
		T		*NT*		*T2SO₂*		*T2SO*		*NT2SO*		*T5SO*	
		R_f	$s_{\bar{R}}$	R_f	$s_{\bar{R}}$	R_f	$s_{\bar{R}}$	R_f	$s_{\bar{R}}$	R_f	$s_{\bar{R}}$	R_f	$s_{\bar{R}}$
Water–	3.02	1.00	0.04	1.94	0.01	21	1.3	33	1.1	45	1.5	47	1.3
ethanol–	12.73												
2,2,4-trimethyl-pentane–	81.80												
2-aminopropane–	2.45												
Water–	11.71	0.24	0.02	0.51	0.02	15	1.4	32	1.1	66	4.6	77	3.9
ethanol–	16.81												
2,2,4-trimethyl-pentane–	69.39												
2-aminopropane–	2.09												
Water–	11.90	0.22		0.43		—		—		—		—	
ethanol–	17.08												
2,2,4-trimethyl-pentane–	70.53												
2-aminopropane–	0.49												
Water–	11.39	0.66		0.74		—		—		—		—	
ethanol–	16.36												
2,2,4-trimethyl-pentane–	67.53												
2-aminopropane–	4.72												
Water–	11.71	0.24		0.76		7.7		25		76		64	
ethanol–	16.81												
2,2,4-trimethyl-pentane–	69.39												
1-dimethyl-amino-2-propyne	2.09												
Acetonitrile–	32.68	1.40	0.10	3.1	0.16	27	0.8	12.0	0.07	23	0.7	31	2.0
2,2,4-trimethyl-pentane–	65.36												
2-aminopropane	1.96												
Acetonitrile–	31.75	1.60		3.2		—		—		—		—	
2,2,4-trimethyl-pentane–	63.49												
2-aminopropane	4.76												
Acetonitrile–	33.22	1.60		3.5		—		—		—		—	
2,2,4-trimethyl-pentane–	66.45												
2-aminopropane	0.33												
Acetronitrile–	29.41	1.80		4.2		29		17.0		28		38	
ethanol–	3.27												
2,2,4-trimethyl-pentane–	65.36												
2-aminopropane	1.96												
Acetonitrile–	16.34	1.70		2.3		7.7		5.6		5.8		7.0	
ethanol–	16.34												
2,2,4-trimethyl-pentane–	65.36												
2-aminopentane	1.96												

(Continued on p. 414)

TABLE III *(continued)*

Components of the solvent systems (vol.-%)	*pH*	*Solutes*											
		T		*NT*		*T2SO₂*		*T2SO*		*NT2SO*		*T5SO*	
		$\bar{R}_t$	$s_{\bar{R}}$	$\bar{R}_t$	$s_{\bar{R}}$	$\bar{R}_t$	$s_{\bar{R}}$	$\bar{R}_t$	$s_{\bar{R}}$	$\bar{R}_t$	$s_{\bar{R}}$	$\bar{R}_t$	$s_{\bar{R}}$
Water–27.78 methyl ethyl ketone–69.44 acetic acid 2.78	2.5	0.4	0.14	0.5	0.16	0.6	0.05	1.8	0.07	1.4	0.18	1.9	0.14
Water–27.78 ethyl acetate–69.44 acetic acid 2.78	2.5	2.0	0.3	1.80	0.2	23	1.1	66	2.1	45	2.2	141	7.0
Water–27.78 diethyl ether–69.44 acetic acid 2.78	2.5	25	5	16.0	3.6	400	70	2500	360	1500	210	3000	360
Water–27.78 diisopropyl ether–69.44 acetic acid 2.78	2.5	36	2.4	22	1.8	360	30	680	49	410	71	790	11
Water–28.17 diisopropyl ether–70.42 acetic acid 1.41		46		45		400		—		—		—	
Water–28.47 diisopropyl ether–71.17 acetic acid 0.36		25		150		1290		—		—		—	

changing the mutual volume ratio of the other compounds in a ternary and quaternary system. In accordance with eqn. 5 and Fig. 2, the distribution coefficient should decrease with increasing concentration of 2-aminopropane (the pH increases), but a slight opposite trend was found, as can be seen in Table III, but the effect is not particularly significant. This is different to the case when the content of acetic acid was varied, as can be seen in Table III. A large decrease in the distribution coefficient with increasing acetic acid concentration was found. A possible explanation of this behaviour could be the formation of ion pairs in the polar phase, which can distribute like a neutral species between the polar and non-polar phases, which results in a decrease of the polar–non-polar distribution coefficient. It is likely that the lower precision of the distribution coefficient measurement in acidic medium is caused by such effects.

In analogy with previous results with other compounds[8], it may be expected that the distribution coefficients of thioridazine and its metabolites run over a maximum if increasing amounts of an additional compound that is miscible with the other

components of the phase system are added. As can be seen from Table III, this expectation is confirmed if ethanol is added to the water–2,2,4-trimethylpentane–2-aminopropane and the acetonitrile–2,2,4-trimethylpentane–2-aminopropane systems.

Correlation of retention data and distribution coefficients

In order to prove whether or not a pure liquid–liquid distribution was involved in the chromatographic process, chromatographic retention times and static distribution coefficients of thioridazine and its metabolites were correlated. Different phase systems and solid supports were used. Fig. 3 shows the results. It can be seen that only the very inert diatomite support gave a perfect linear regression according to eqn. 6. With the silica support, significant deviations were observed. This effect was probably caused by the acidic surface of this support.

Chromatographic separation

According to eqn. 7, the following condition should be fulfilled for all of the components that are eluted successively from the chromatographic column:

$$\frac{R_{jl}^{\min.}}{\sqrt{N_l}} \leqslant (r_{jl} - 1) \cdot \frac{\kappa_l}{1+\kappa_l}$$

where $R_{jl}^{\min.}$ is the required resolution and, $N_l = L/H_l$ is the theoretical plate number of component i. Assuming a theoretical plate number of 1600 and a minimum resolution of 4, the term $(r_{jl}-1)\kappa_l/(1+\kappa_l)$ should be at least 0.1. At a high capacity ratio, this value is achieved with a selectivity factor of 1.1, while at a low capacity ratio the necessary selectivity factor is higher, *e.g.*, 1.3 at a capacity ratio of 0.5. The liquid volume that can be held stationary by a solid support is limited. The maximum stationary liquid load depends on the wettability of the solid support by the stationary and mobile phases, the geometry of the pore system and the flow-rate of the mobile phase. On average, the maximum stable phase ratio, q, is found to be of the order of 0.1. For this reason, the distribution coefficient should be greater than 5 in order to obtain a capacity ratio greater than 0.5. A first choice of potential phase systems for the chromatographic separation of thioridazine and its metabolities can be made from Table III. It should be noted that the distribution coefficients in Table III are defined arbitrarily as concentration ratios in the polar and non-polar phases. On the other hand, the chromatographic distribution coefficient is defined as the concentration ratio in the stationary and mobile phases. Depending on the nature of the solid support, the polar or the non-polar phase can be held stationary, so that both the normal and the reversed value of a distribution coefficient have to be considered from the chromatographic point of view. Both are equivalent, because the phase system can be reversed. Consequently, the systems in which most of the distribution coefficients are greater than 5 or less than 0.2 were chosen from Table III as a first approach for further investigation. These systems are presented in Table IV. In addition to the distribution coefficients, the selectivity factors, $r_{(n+1)n}$, are given for sequential values of the distribution coefficient in a given phase system. None of the phase systems was found to be an ideal choice and therefore chromatographic separation experiments were carried out with several systems. Typical chromatograms are shown in Fig. 4.

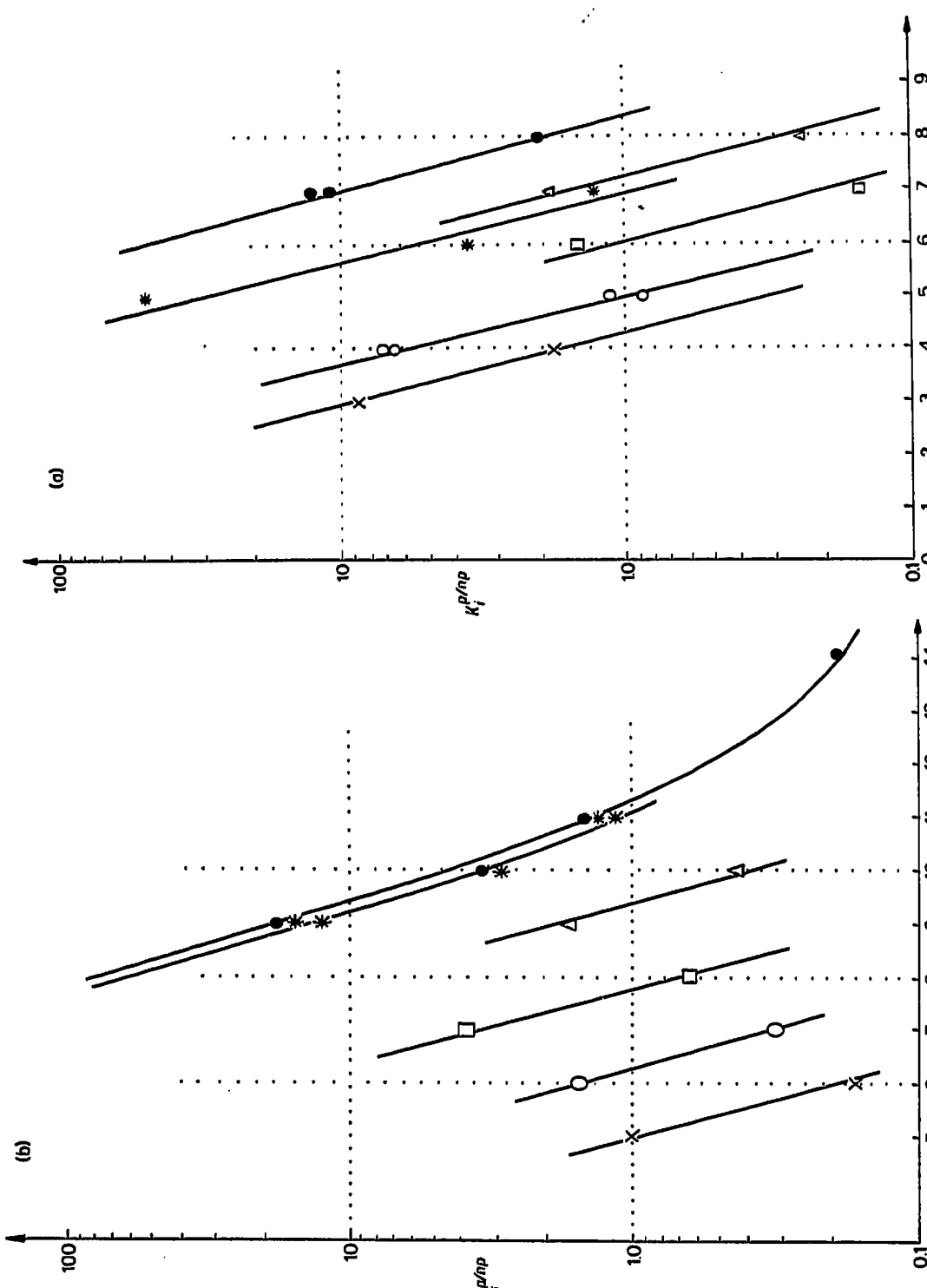


Fig. 2. Liquid–liquid distribution coefficient as a function of hydrogen ion concentration (logarithmic) (a) Water–diethyl ether system; (b) water–heptane system. pH in aqueous phase adjusted by means of buffers (Table II, pH 14 adjusted with 1 *N* NaOH). ×, T; ○, NT; □, $T2SO_2$; △, T2SO; ✱, NT2SO; ●, T5SO.

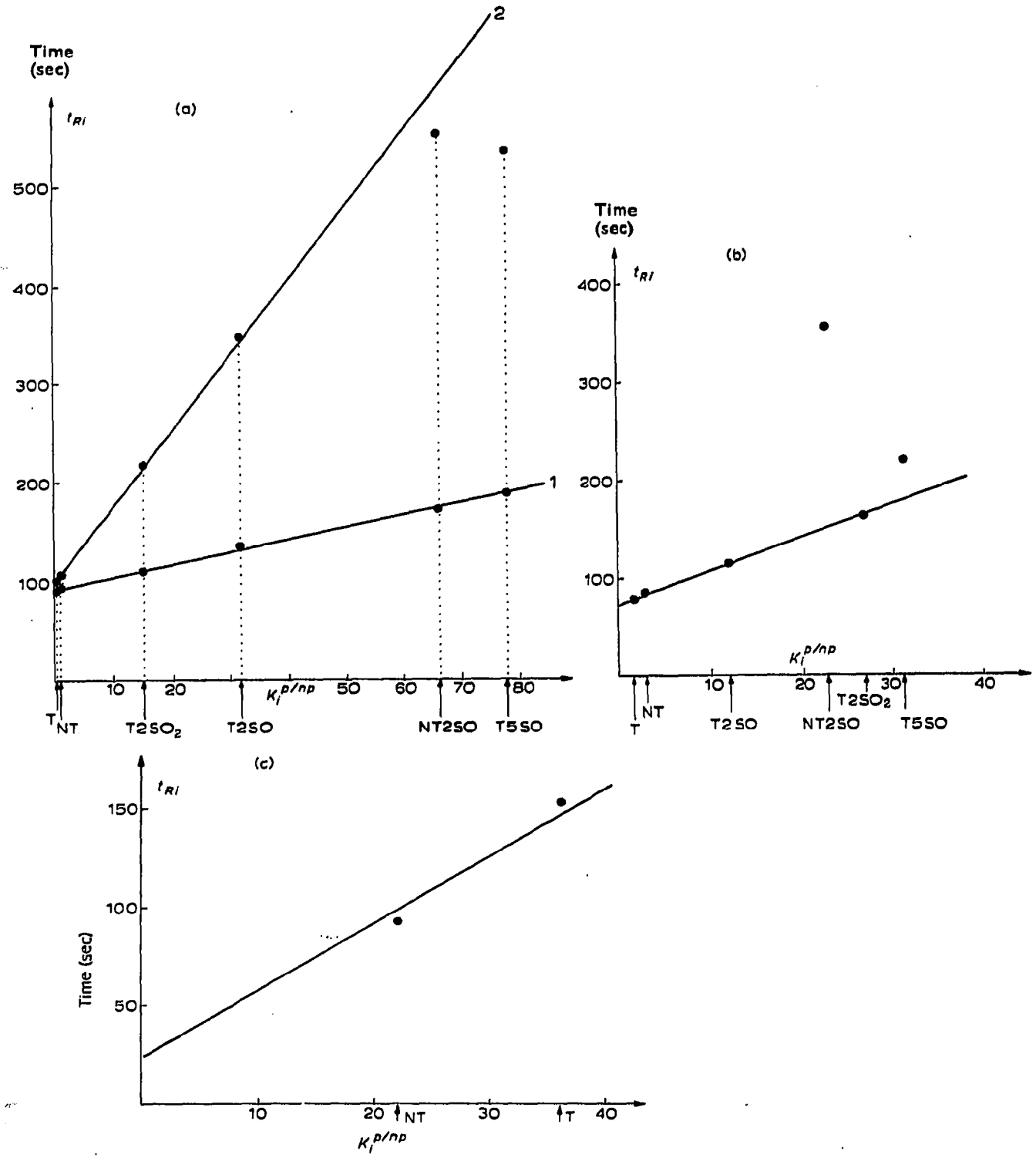


Fig. 3. Correlation of distribution coefficients and retention times. (a) Solvent system: water–ethanol–2,2,4-trimethylpentane–2-aminopropane (11.71:16.81:69.39:2.09). Solid support: 1, diatomite; 2, silica. (b) Solvent system: acetonitrile–2,2,4-trimethylpentane–2-aminopropane (32.68:65.36:1.96). Solid support: silica. (c) Solvent system: water–diisopropyl ether–acetic acid (27.78:69.44:2.78). Solid support: diatomite.

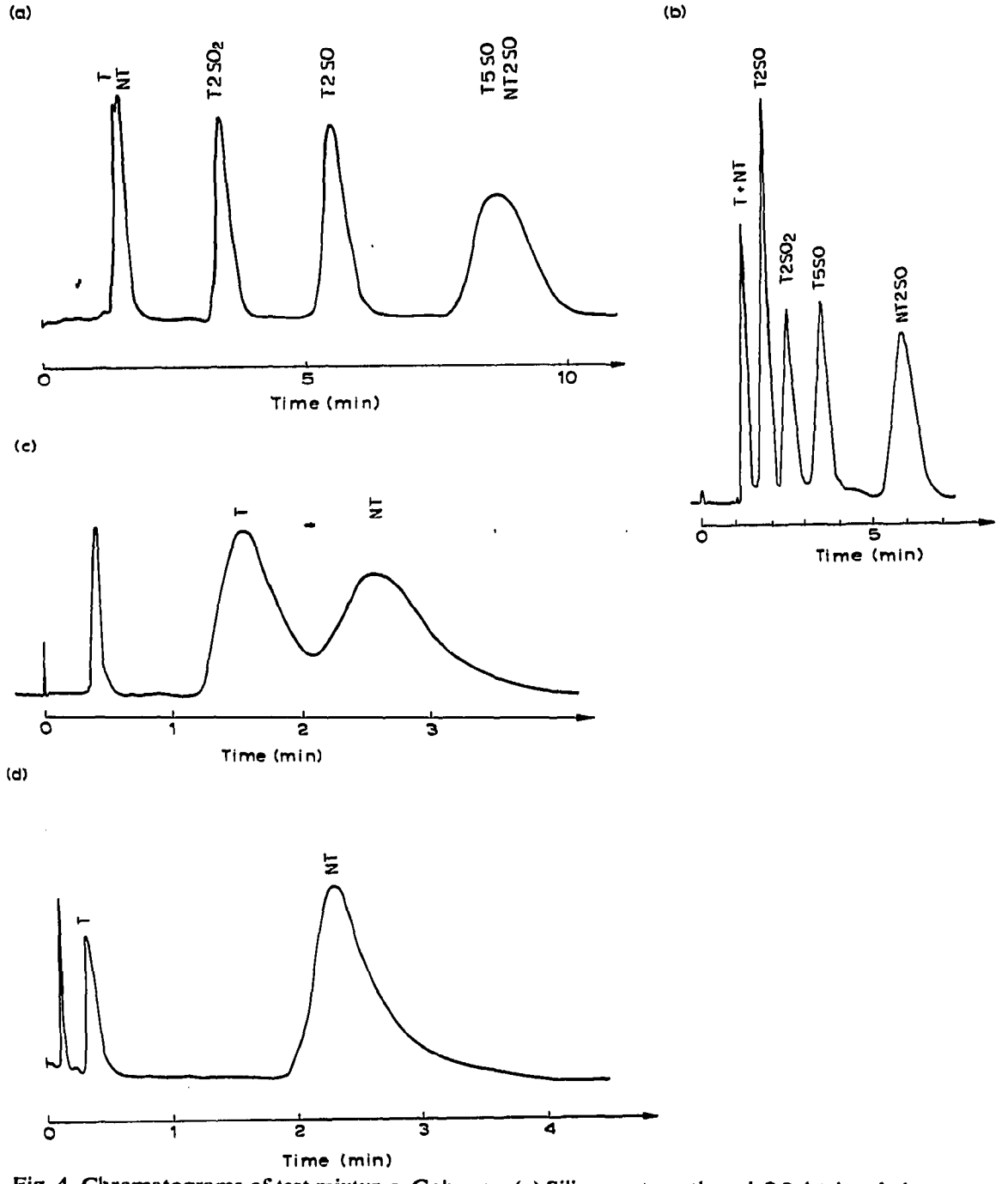


Fig. 4. Chromatograms of test mixtures. Columns: (a) Silica; water–ethanol–2,2,4-trimethylpentane–2-aminopropane (11.71:16.81:69.39:2.09); 1.25 ml/min; 58 bar; 250 × 2.7 mm. (b) Silica; acetonitrile–2,2,4-trimethylpropane–2-aminopropane (32.68:65.36:1.96); 250 × 2.7 mm. (c) Diatomite; water–diisopropyl ether–acetic acid (27.78:69.44:2.78); 3.2 ml/min; 250 × 2.7 mm. (d) Diatomite; water–diisopropyl ether–acetic acid (28.46:71.18:0.36); 125 × 2.7 mm.

TABLE IV

SEQUENTIAL SELECTIVITY FACTORS OF THIORIDAZINE AND ITS METABOLITES AT 20° FOR SELECTED PHASE SYSTEMS FROM TABLE III

Solvent compositions (vol.-%): A_1, water–ethanol–2,2,4-trimethylpentane–2-aminopropane (3.02:12.73:81.80:2.45); A_2, as A_1 (11.71:16.81:69.39:2.09); B_1, acetonitrile–ethanol–2,2,4-trimethylpentane–2-aminopropane (32.68:0.00:65.36:1.96); B_2, as B_1 (29.41:3.27:65.36:1.96); C_1, water–diisopropyl ether–acetic acid (27.78:69.44:2.78); C_2, as C_1 (28.46:71.18:0.36).

Phase system	*Selectivity factor*	*Solute (i)*					
		T	*NT*	*T2SO₂*	*T2SO*	*NT2SO*	*T5SO*
A_1	K_i	1.00	1.94	21	33	45	47
	n	1	2	3	4	5	6
	$r_{(n+1)n}$	1.9	11	1.6	1.4	1.05	
A_2	K_i	0.24	0.51	15	32	66	77
	n	1	2	3	4	5	6
	$r_{(n+1)n}$	2.1	29	2.1	2.1	1.17	
B_1	K_i	1.40	3.1	27	12	23	31
	n	1	2	5	3	4	6
	$r_{(n+1)n}$	2.2	3.9	1.17	1.9	1.15	
B_2	K_i	1.80	4.2	29	17	28	38
	n	1	2	5	3	4	6
	$r_{(n+1)n}$	2.3	4.0	1.04	1.6	1.3	
C_1	K_i	36	22	360	680	410	790
	n	2	1	3	5	4	6
	$r_{(n+1)n}$	10	1.6	1.14	1.16	1.7	
C_2	K_i	25	150	1290	—	—	—
	n	1	2	3	—	—	—
	$r_{(n+1)n}$	6.8	8.6	—	—	—	—

It can be seen that the chromatograms obtained with acidic phase systems are very unusual (Fig. 4c and d). The peaks are unusually broad and asymmetrical. These results were obtained on the same type of phase systems that have also shown an anomalous behaviour with respect to the pH-dependence of the distribution coefficient. It is likely that in both instances the same phenomenon is responsible for the irregularity, which would mean that the extreme peak width and asymmetry has to be attributed to the slow kinetics of the ion-pair formation.

The chromatograms obtained with the basic systems show that the separation of thioridazine and northioridazine forms the bottle-neck (Fig. 4a and b). Both compounds are psychotropic drugs and their separation is essential. Under isocratic conditions, better resolution of these compounds may be achieved in two ways, either with a higher theoretical plate number or with a higher selectivity factor, and work is being carried out on both aspects. Gradient elution and column switching are other possibilities for solving the problem, and work on the second method is also in progress.

ACKNOWLEDGEMENTS

The technical assistance of Mrs. C. E. M. van Ham-Vesters and Mrs. A. J. Visser-van der Weel is appreciated.

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