Journal of Chromatography, 503 (1990) 205–215
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM 22 132

High-performance liquid chromatography of levomepromazine (methotrimeprazine) and its main metabolites

T. LOENNECHEN and S. G. DAHL*
Institute of Medical Biology, University of Tromsø, N-9001 Tromsø (Norway)
(First received June 14th, 1989; revised manuscript received October 16th, 1989)

SUMMARY

The phenothiazine drug levomepromazine (methotrimeprazine) has five metabolites which previously have been identified in plasma from psychiatric patients. These are formed by sulphoxidation, N-demethylation, O-demethylation and aromatic hydroxylation in two different positions. A high-performance liquid chromatographic system is described for the analysis of levomepromazine and its main metabolites on a Supelcosil C_{18}-DB column, based on ion-pair formation with sodium docecyl sulphate. The effects of variations in pH, buffer concentration, counter-ion concentration, temperature and concentration and composition of the organic solvent were examined. The six components may be analysed in 27.4 min at room temperature using 25 m*M* sodium dodecyl sulphate in 500 m*M* ammonium acetate buffer (pH 5.0)–5% v/v tetrahydrofuran in acetonitrile (50:50, v/v) as the mobile phase.

INTRODUCTION

The phenothiazine drug levomepromazine (methotrimeprazine) was introduced in 1959 as an agent with both neuroleptic[1] and analgesic[2] effects. Since then the drug has mainly been used in psychiatry, as a sedative adjuvant to other psychotropic drug treatment. Ten different levomepromazine metabolites have been identified in urine from psychiatric patients by nuclear magnetic resonance spectroscopy[3] and combined gas chromatography–mass spectrometry[4–6]. Five of these metabolites, all of which are formed by a single biotransformation step of the parent drug (Fig. 1), were found in the highest concentrations in urine. These metabolites, levomepromazine sulphoxide (LMSO), N-desmethyllevomepromazine (N-DLM), 3-hydroxylevomepromazine (3-OH-LM), 7-hydroxylevomepromazine (7-OH-LM) and O-desmethyllevomepromazine (O-DLM), have also been identified in plasma from patients treated with levomepromazine[6,7].

Their biological activities and plasma concentrations indicate that some levo-

0021-9673/90/$03.50

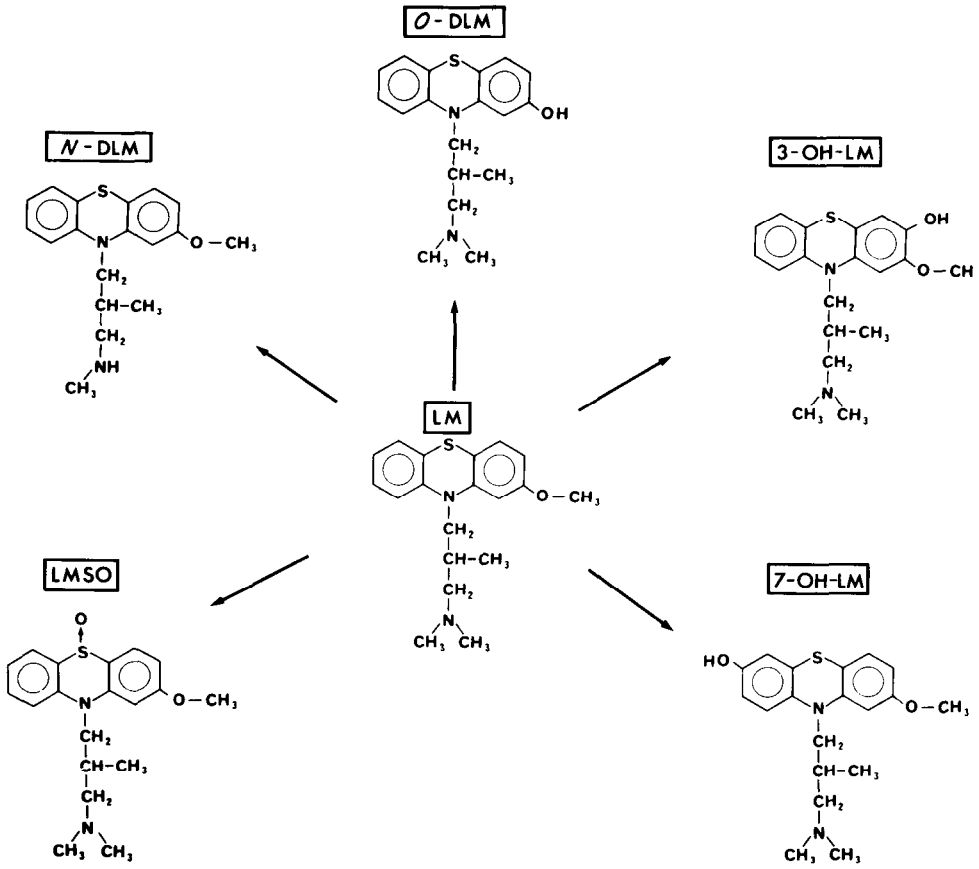

Fig. 1. Structures of levomepromazine (LM), levomepromazine sulphoxide (LMSO), N-desmethyllevomepromazine (N-DLM), 3-hydroxylevomepromazine (3-OH-LM), 7-hydroxylevomepromazine (7-OH-LM) and O-desmethyllevomepromazine (O-DLM).

mepromazine metabolites may contribute to the effects of the drug in man[8]. Phenothiazine neuroleptics show considerable between-patient variation in plasma drug levels and in metabolite-to-parent-drug plasma concentration ratios[9]. It may be expected that the contributions from metabolites to the effects of levomepromazine are subject to inter-individual variations. In order to examine this, it would be useful to have a chromatographic system that separates the six components in less than 30 min.

Normal-phase high-performance liquid chromatography (HPLC) has been reported to yield unsatisfactory results in the analysis of chlorpromazine, chlorpromazine sulphoxide and 7-hydroxychlorpromazine[10]. Similar results were obtained in our laboratory in attempts to analyse levomepromazine and its metabolites by normal-phase HPLC.

This paper describes an isocratic HPLC method for the analysis of LM and its five main metabolites (Fig. 1), based on reversed-phase ion-pair chromatography. The effects of variations in temperature and pH of the mobile phase and of the concentrations of the ion-pair forming agent, buffer and organic modifier on the retention and separation of the six compounds were examined.

EXPERIMENTAL

Chemicals

Levomepromazine hydrochloride, N-desmethyllevomepromazine maleate, levomepromazine sulphoxide and methoxypromazine (MPZ) maleate were generously donated by Rhône-Poulenc Industries (Paris, France). O-Desmethyllevomepromazine, 3-hydroxylevomepromazine and 7-hydroxylevomepromazine were synthesized from levomepromazine by a non-enzymatic method[3]. Stock solutions of levomepromazine and its metabolites were prepared at a concentration of 2 m*M* in methanol and stored at −20°C for up to 30 days.

Phenothiazine derivatives are prone to photodecomposition[10], and exposure to light was therefore avoided throughout the experimental procedures. In order to minimize adsorptive losses, all glassware was silanized as described previously[7], after initial cleaning with chromic acid–sulphuric acid. Solutions (0.01 m*M*) of levomepromazine and metabolites, which were used in the HPLC assay, were made by diluting stock solutions with mobile phase without tetrahydrofuran.

Analytical-reagent grade sodium dodecyl sulphate, which was used as a lipophilic counter ion, was obtained from Koch-Light Labs. (Colnbrook, U.K.). Ammonium acetate and acetic acid were obtained from Merck (Darmstadt, F.R.G.) and HPLC-grade tetrahydrofuran (THF), acetonitrile and methanol from Rathburn Chemicals (Walkerburn, U.K.).

Equipment

HPLC was carried out with a Rheodyne (Cotati, CA, U.S.A.) Model 7125 injector fitted with a 20-μl loop, two pumps connected with a gradient master (Consta Metric; Laboratory Data Control, Riviera Beach, FL, U.S.A.) and an absorbance detector (Spectroflow Model 773 UV–VIS; Kratos, Ramsey, N.T., U.S.A.) operated at 254 nm and 0.001 a.u.f.s. Quantification was performed with an SP 4270 integrator (Spectra-Physics, San Jose, CA, U.S.A.).

The reversed-phase columns (analytical column, 250 nm × 4.6 mm I.D.; precolumn, 20 mm × 4.6 mm I.D.) were Supelcosil C_{18}-DB, 5 μm particle size (Supelco, Bellefonte, OA, U.S.A.), and Supelguard LC_{18}-DB, 5 μm particle size (Supelco). The analytical column had been alkylated by the manufacturer in order to optimize the separation of basic compounds.

Analysis

Two solutions, delivered to the chromatographic system by separate pumps, were prepared. The aqueous solution was prepared by adding 100 m*M* sodium dodecyl sulphate to a *M* ammonium acetate solution, followed by addition of concentrated acetic acid to the desired pH (37–38 ml in order to obtain pH 5.0) and distilled water to a total volume of 2 l. The organic solution consisted of acetonitrile or a mixture of THF and acetonitrile. The total flow-rate was kept constant at 1.5 ml/min and the effect of the concentration of organic modifier in the mobile phase was studied.

The dead volume of the system was measured as the first distortion of the baseline after injection of mobile phase. The capacity factor for each solute under the given experimental conditions was calculated by the equation $k' = (t_R - t_o)/t_o$, where

t_R and t_0 are the retention times for the solute and the solvent front, respectively.

Separation factors for two compounds are expressed as the ratio between the capacity factors of the last (k_2') and first (k_1') eluting peak, $\alpha = k_2'/k_1'$.

RESULTS AND DISCUSSION

Problems with separation from other components may arise in the analysis of levomepromazine and metabolites in biological extracts. We therefore examined how the capacity factors (k') and separation factors (α) of LM and its metabolites could be adjusted by changing the composition of the mobile phase.

Methanol alone as the mobile phase, at a flow-rate of 1 ml/min, produced retention times ranging from 43.67 to 11.65 min for LM and its metabolites and broad, tailing peaks. Acetonitrile alone as the mobile phase resulted in the elution of all components together with the solvent front within 1.45 min. Further experiments with acetonitrile and 50 mM ammonium acetate as the mobile phase demonstrated

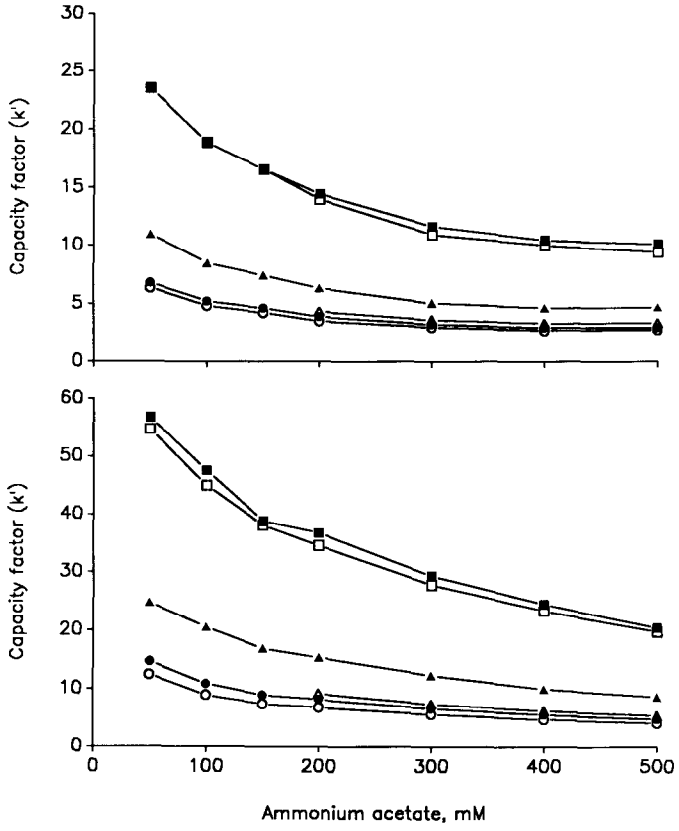

Fig. 2. Effect of buffer concentration on capacity factors (k') of levomepromazine and metabolites. Mobile phase: ammonium acetate buffer (pH 6.0) with 20 mM sodium dodecyl sulphate – acetonitrile, 45:55 (v/v) (upper curve) or 50:50 (v/v) (lower curve). (○) LMSO; (●) 7-OH-LM; (△) 3-OH-LM; (▲) O-DLM; (□) LM; (■) N-DLM.

TABLE I

EFFECT OF AMMONIUM ACETATE BUFFER CONCENTRATION ON SEPARATION FACTORS (α) OF LEVOMEPROMAZINE AND METABOLITES

Mobile phase: 20 m*M* sodium dodecyl sulphate in ammonium acetate buffer (pH 6.0) – acetonitrile (50:50, v/v). Flow-rate: 1.5 ml/min.

Compound	*Ammonium acetate concentration (mM)*						
	100	*150*	*200*	*250*	*300*	*400*	*500*
7-OH-LM/LMSO	1.18	1.21	1.20	1.20	1.18	1.17	1.16
3-OH-LM/7-OH-LM[a]	–	–	–	1.67	1.11	1.13	1.13
O-DLM/3-OH-LM[a]	–	–	–	2.25	1.66	1.18	1.59
LM/O-DLM	2.21	2.49	2.25	2.25	2.26	2.35	2.29
N-DLM/LM	1.04	1.06	1.02	1.07	1.06	1.05	1.03

[a] 3-OH-LM could not be detected at buffer concentrations below 200 m*M*.

that increasing the amount of buffer from 10 to 50% (v/v) increased the capacity factors of all compounds except LMSO, for which it was virtually unchanged. However, this system also yielded peaks that were far too wide for analytical purposes. This problem was overcome by increasing the salt concentration in the aqueous solvent.

Ammonium acetate buffer concentration

As shown in Fig. 2, increasing ammonium acetate concentration in the range 50–500 m*M* decreased the capacity factors of the compounds by 55–70%. This may have been caused by decreased formation of ion pairs with the dodecyl sulphate counter ion, owing to competition from acetate ions.

As indicated in Fig. 2, 3-OH-LM could not be detected at ammonium acetate concentrations below 200 m*M*. At lower buffer concentrations the 3-OH-LM peak was replaced by two other unidentified peaks in the chromatogram, indicating that the metabolite had decomposed.

Owing to the apparent instability of 3-OH-LM, ammonium acetate concentrations ranging from 400 to 500 m*M* were used in further experiments. As shown in Table I, the separation factors did not differ significantly with different ammonium acetate concentrations in the range 200–500 m*M*.

Concentration of ion-pair forming agent

Addition of sodium dodecyl sulphate to the mobile phase was intended to produce ionic associations between negatively charged dodecyl sulphate ions and protonated amino groups in the phenothiazine derivatives. This method would be expected to produce complexes with lower polarities than the analysed compounds alone. As expected, increased retention times of levomepromazine and metabolites were observed on addition of sodium dodecyl sulphate to the mobile phase.

As shown in Fig. 3, increasing the sodium dodecyl sulphate concentrations from 5 to 50 m*M* resulted in a slight increase in the capacity factors of 3-OH-LM, 7-OH-LM and O-DLM, a 2- to 3-fold increase in the capacity factors of O-DLM and

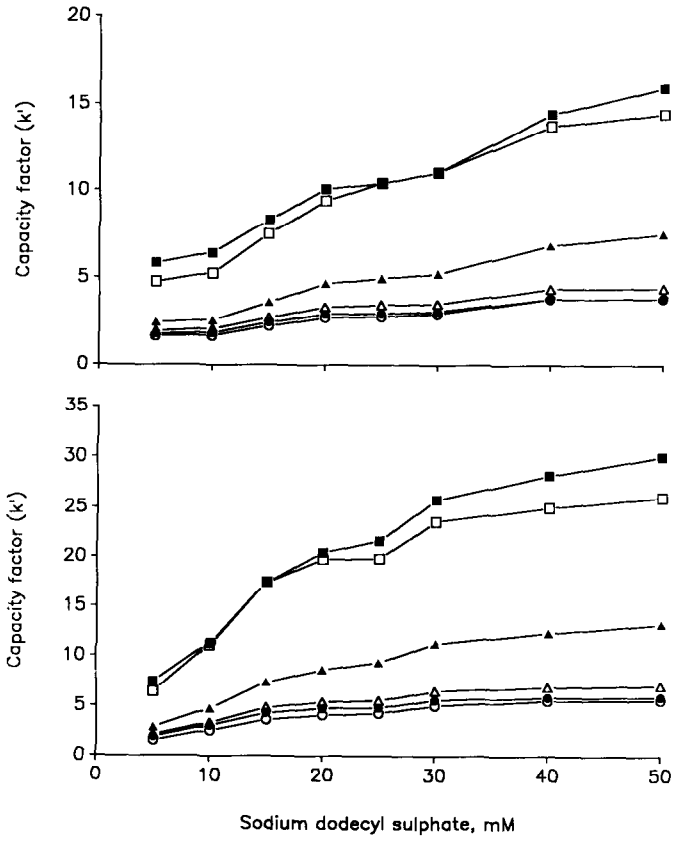

Fig. 3. Effect of sodium dodecyl sulphate concentration on capacity factors (k') of levomepromazine and metabolites. Mobile phase: 500 mM ammonium acetate buffer (pH 6.0) with sodium dodecyl sulphate – acetonitrile, 45:55 (v/v) (upper curve) or 50:50 (v/v) (lower curve). Symbols as in Fig. 2.

substantial increases in the capacity factors of levomepromazine and N-DLM. The difference between the effect of counter ion formation on the capacity factors of the compounds might be due to their different pK_a values (Table II). At pH 6.0, LM and

TABLE II

pK_a VALUES OF LEVOMEPROMAZINE AND METABOLITES

pK_a values were determined by titration of aqueous solutions of the compounds.

Compound	pK_{a_1}	pK_{a_2}
LM	8.8	–
N-DLM	8.1	–
LMSO	5.9	–
O-DLM	5.4	9.8
3-OH-LM	5.5	10.0
7-OH-LM	5.5	9.7

TABLE III

EFFECT OF SODIUM DODECYL SULPHATE CONCENTRATION ON SEPARATION FACTORS (α) OF LEVOMEPROMAZINE AND METABOLITES

Mobile phase: sodium dodecyl sulphate in 500 m*M* ammonium acetate buffer (pH 6.0) – acetonitrile (50:50, v/v). Flow-rate: 1.5 ml/min.

Compound	*Sodium dodecyl sulphate concentration (mM)*							
	5	*10*	*15*	*20*	*25*	*30*	*40*	*50*
7-OH-LM/LMSO	1.24	1.20	1.18	1.16	1.13	1.11	1.06	1.05
3-OH-LM/7-OH-LM	1.10	1.09	1.13	1.13	1.15	1.16	1.18	1.20
O-DLM/3-OH-LM	1.36	1.42	1.52	1.59	1.73	1.78	1.78	1.87
LM/O-DLM	2.23	2.33	2.36	2.29	2.12	2.09	2.03	1.97
NDLM/LM	1.15	1.02	1.00	1.03	1.10	1.09	1.13	1.15

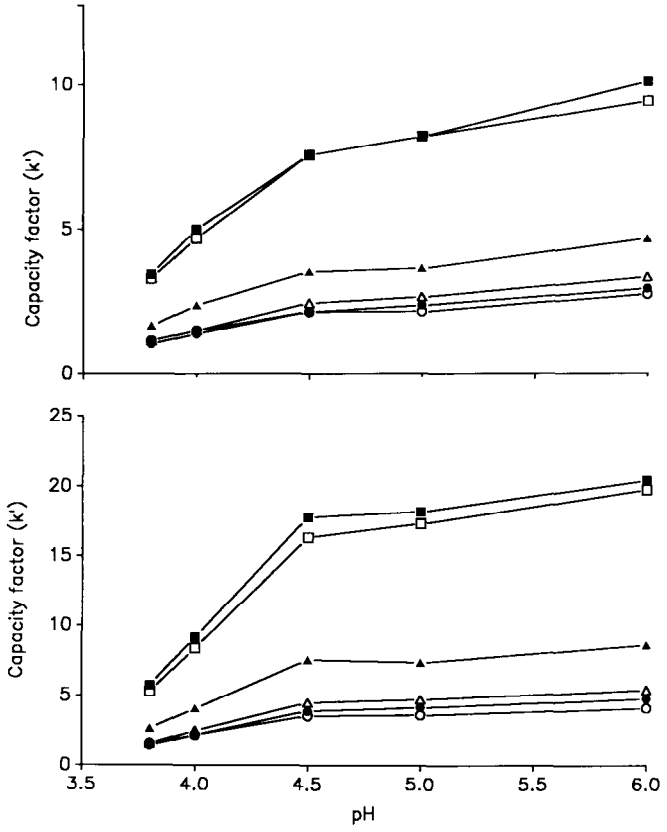

Fig. 4. Effect of pH on capacity factors (*k'*) of levomepromazine and metabolites. Mobile phase: 500 m*M* ammonium acetate buffer with 20 m*M* sodium dodecyl sulphate – acetonitrile 45:55 (v/v) (upper curve) or 50:50 (v/v) (lower curve). Symbols as in Fig. 2.

TABLE IV

EFFECT OF AQUEOUS PHASE pH ON SEPARATION FACTORS (α) OF LEVOMEPROMAZINE AND METABOLITES

Mobile phase: 20 m*M* sodium dodecyl sulphate in 500 m*M* ammonium acetate buffer – acetonitrile (50:50, v/v). Flow-rate: 1.5 ml/min.

Compound	*pH*				
	6.0	*5.0*	*4.5*	*4.0*	*3.8*
7-OH-LM/LMSO	1.16	1.16	1.10	1.0	0.91
3-OH-LM/7-OH-LM	1.13	1.14	1.16	1.14	1.10
O-DLM/3-OH-LM	1.59	1.56	1.68	1.64	1.69
LM/O-DLM	2.29	2.38	2.17	2.06	2.01
N-DLM/LM	1.03	1.05	1.09	1.09	1.09

TABLE V

EFFECT OF TEMPERATURE ON CAPACITY FACTORS (*k'*) OF LEVOMEPROMAZINE AND METABOLITES

Mobile phase: 25 m*M* sodium dodecyl sulphate in 500 m*M* ammonium acetate buffer (pH 5.0) – acetonitrile (50:50, v/v). Flow-rate: 1.5 ml/min.

Compound	*Temperature (°C)*	
	20	*50*
LMSO	3.82	3.40
7-OH-LM	4.31	3.70
3-OH-LM	5.00	4.30
O-DLM	8.65	6.87
LM	19.33	14.52
N-DLM	21.33	15.76

TABLE VI

EFFECT OF THF CONCENTRATION IN THE ORGANIC SOLVENT ON SEPARATION FACTORS (α) OF LEVOMEPROMAZINE AND METABOLITES

Mobile phase: 25 m*M* sodium dodecyl sulphate in 500 m*M* ammonium acetate buffer (pH 5.0) – acetonitrile with THF (50:50, v/v). Flow-rate: 1.5 ml/min.

Compound	*THF concentration in acetonitrile (%)*								
	0	*2*	*3*	*4*	*5*	*8*	*12*	*20*	*25*
7-OH-LM/LMSO	1.13	1.17	1.18	1.20	1.21	1.27	1.35	1.46	1.56
3-OH-LM/7-OH-LM	1.16	1.14	1.15	1.15	1.14	1.13	1.12	1.11	1.09
O-DLM/3-OH-LM	1.73	1.65	1.67	1.66	1.64	1.63	1.60	1.51	1.46
LM/O-DLM	2.23	2.10	2.02	2.00	1.98	1.91	1.84	1.74	1.66
N-DLM/LM	1.10	1.12	1.12	1.12	1.13	1.13	1.14	1.13	1.13

N-DLM are almost completely protonated, whereas the other compounds are approximately 50% protonated and thus have fewer amino groups available for ion-pair formation.

With a mobile phase of sodium dodecyl sulphate in 500 m*M* ammonium acetate buffer (pH 6.0)–acetonitrile (45:55, v/v), the highest separation factors were obtained with sodium dodecyl sulphate concentrations in the range 5–20 m*M*. With a 50:50 (v/v) mixture of aqueous and organic solvents, the highest separation factors of levomepromazine and metabolites were obtained with sodium dodecyl sulphate concentrations from 25 to 30 m*M* (Table III).

pH in the aqueous phase

As shown in Fig. 4, the capacity factors of all the six compounds decreased with decreasing pH. This effect was most pronounced at pH values below 4.5. With a mobile phase of 20 m*M* sodium dodecyl sulphate in 500 m*M* ammonium acetate buffer–acetonitrile (45:55 v/v), the highest separation factors of levomepromazine and metabolites were obtained at pH 6.0. With a 50:50 (v/v) mixture of aqueous and organic solvents the highest separation factors were observed at pH 5.0–6.0 (Table IV).

As indicated in Table IV, LMSO eluted after 7-OH-LM at pH values below 4.0. The LMSO peak in the chromatogram also overlapped with the 3-OH-LM peak at pH 3.8.

Effect of temperature

Elevated temperatures are sometimes used in HPLC in order to improve the separation of small molecules or control *t* values. As shown in Table V, the capacity factors of levomepromazine and metabolites decreased on increasing the column and mobile phase temperature from 20 to 50°C. It also seems likely that levomepromazine and its metabolites would be even more vulnerable to oxidation or decomposition at

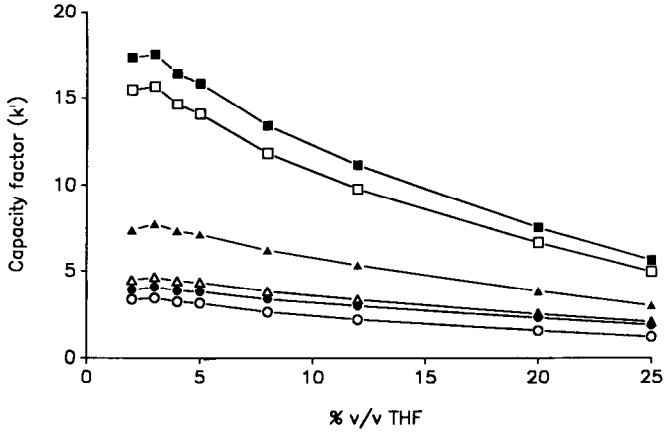

Fig. 5. Effect of THF concentration in the organic solvent on capacity factors (*k'*) of levomepromazine and metabolites. Mobile phase: 500 m*M* ammonium acetate buffer (pH 5.0) with 25 m*M* sodium dodecyl sulphate – acetonitrile with THF (50:50, v/v). Symbols as in Fig. 2.

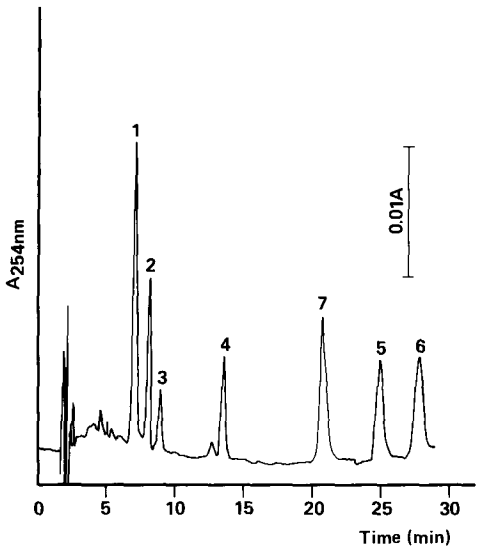

Fig. 6. Elution profile of a mixture containing 0.01 m*M* of levomepromazine and metabolites: 1 = LMSO (6.71 min); 2 = 7-OH-LM (7.80 min); 3 = 3-OH-LM (8.68 min); 4 = O-DLM (13.22 min); 5 = LM (24.53 min); 6 = N-DLM (27.43 min); 7 = methoxypromazine, internal standard (20.41 min). Mobile phase: 500 m*M* ammonium acetate with 25 m*M* sodium dodecyl sulphate (pH 5.0) – 5% (v/v) THF in acetonitrile (50:50, v/v).

elevated temperatures. For these reasons, all subsequent experiments were performed at 20°C.

Organic modifier

It has been shown that the addition of small amounts of tetrahydrofuran may improve the separation of non-polar components when acetonitrile is used as the organic modifier[11]. In order to improve the separation of LM and its metabolites, various concentrations of THF were added to the organic solvent.

THF concentrations of up to 25% (v/v) in acetonitrile did not increase the separation factors of 3-OH-LM–7-OH-LM and N-DLM–LM. The separation factor of 7-OH-LM–LMSO, on the other hand, increased from 1.1 to 1.6 (Table VI). However, addition of up to 25% (v/v) THF to acetonitrile resulted in a pronounced decrease in the capacity factors of LM and N-DLM, a smaller decrease in the capacity factor of O-DLM and a slight decrease in the capacity factors of the other three compounds (Fig. 5).

On addition of 5% (v/v) THF to acetonitrile, the capacity factor of the last-eluting compound, N-DLM, was reduced from 21.2 to 15.8, which reduced the retention time from 38 to 27.4 min. As a compromise between distinct separation and reasonably short retention times, a mobile phase composition of 500 m*M* ammonium acetate buffer (pH 5.0) with 25 m*M* sodium dodecyl sulphate –5% (v/v) THF in acetonitrile (50:50, v/v) was chosen. This system produced the chromatogram shown in Fig. 6.

Internal standard

In order to apply the method to quantitative analysis, a suitable internal standard would be required. Among several phenothiazine drugs and metabolites which were examined, methoxypromazine, which elutes between O-DLM and LM in this system (Fig. 6), was found to have the most suitable retention time.

REFERENCES

1 J. M. Huot and A. C. Kristof, *Can. Med. Assoc. J.*, 81 (1959) 546.
2 B. Paradis, *Anesthésie*, 16 (1959) 185.
3 S. G. Dahl, E. Kaufmann, B. Mompon and T. Purcell, *J. Pharm. Sci.*, 76 (1987) 541.
4 S. G. Dahl and M. Garle, *J. Pharm. Sci.*, 66 (1977) 190.
5 H. Johnsen and S. G. Dahl, *Drug Metab. Dispos.*, 10 (1982) 63.
6 S. G. Dahl, H. Johnsen and C. R. Lee, *Biomed. Mass Spectrom.*, 9 (1982) 534.
7 S. G. Dahl, T. Bratlid and O. Lingjærde, *Ther. Drug Monit.*, 4 (1982) 81.
8 S. G. Dahl, *Ther. Drug. Monit.*, 4 (1982) 33.
9 S. G. Dahl, *Clin. Pharmacokinet.*, 11 (1986) 36.
10 D. Stevenson and E. Reid, *Anal. Lett.*, 14 (1981) 741.
11 W. S. Hancock and J. T. Sparrow, *HPLC Analysis of Biological Compounds* (*Chromatographic Science Series*, Vol. 26), Marcel Dekker, New York, 1984.