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Note

High-performance liquid chromatographic separation of 1-benzyl-4-(2′-pyridinecarbonyl)piperazine and its potential metabolites

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It has been shown[1,2] that of the N-substituted piperazides of pyridinecarboxylic acids 1-benzyl-4-(2′-pyridinecarbonyl)piperazine (1) possesses therapeutically utilizable antidepressant activity. The four potential metabolites of 1 are N-(2′-pyridinecarbonyl)-piperazine (2), N-benzylpiperazine (3), pyridine-2-carboxylic acid (4) and benzyl alcohol (5) (Fig. 1).

In a given case these five compounds may be simultaneously present in the living organism so their detection and determination in the presence of each other is important. As no reports have been published in this field, we have developed a high-performance liquid chromatographic method for the separation of the above five compounds. Although 4 (refs. 3,4) and 5 (refs. 5,6) have been investigated earlier by liquid chromatography, no simultaneous determination of the two derivatives has been accomplished.

EXPERIMENTAL

A Hewlett-Packard 1082A instrument working in the isocratic mode and equipped with an automatic sample injector and a UV detector (254 nm) was used.

The columns applied were as follows: 200 × 4.6 mm I.D. 10 μm Hewlett-Pack-

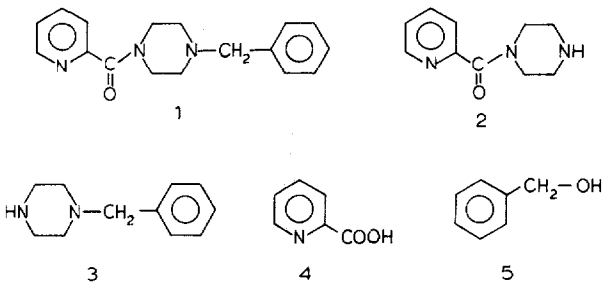

Fig. 1. Structures of compounds.

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TABLE I

COLUMNS AND ELUENTS USED FOR THE MEASUREMENTS

Column	
LiChrosorb RP-8	Methanol–water
	Isopropanol–water
	Acetonitrile–water
	Mixtures of Sörensen phosphate buffer and acetonitrile
	Mixtures of citrate buffer and methyl Cellosolve
LiChrosorb Si 100	Chloroform–methanol
	Cyclohexane–isopropanol
	Dichloroethane
LiChrosorb-DIOL	Chloroform–methanol

ard LiChrosorb RP-8, home-made 250 × 4.6 mm I.D. 5 μm LiChrosorb Si 100 and 250 × 4.6 mm I.D. 10 μm Chrompack LiChrosorb-DIOL.

The examinations were carried out with standards of analytical purity. For the eluents, solvents and reagents of the same purity were applied, and when necessary the solvents were purified by the usual procedures.

RESULTS AND DISCUSSION

The five compounds have different properties so their separation with one solvent system seemed to be very difficult. To find appropriate eluent systems a systematic investigation was performed using reversed-phase chromatographic and adsorption chromatographic methods involving three types of columns and eight eluents. The solvent systems applied are summarized in Table I.

The absorption chromatographic studies on the 5 μm LiChrosorb Si 100 silica gel column did not give satisfactory results as compound 4 appeared as an unacceptably broad peak and the separation of the other four compounds also proved to be extremely difficult. These findings are illustrated by the measurements carried out with cyclohexane–isopropanol mixtures (Table II).

Compound 2 could be eluted with long retention times (15 and 30 min) as broad uncharacteristic peaks in both eluent systems.

TABLE II

RETENTION TIMES (min) WITH CYCLOHEXANE–ISOPROPANOL MIXTURES

Solvent flow-rate = 1.0 cm^3/min.

Compound	*Cyclohexane–isopropanol*	
	7:3	*9:1*
1	7.15	16.6
5	3.21	3.91
3	3.21	4.54

TABLE III

RETENTION TIMES (min) CHLOROFORM–METHANOL MIXTURES

Solvent flow-rate = 2.0 cm^3/min.

Compound	*Chloroform–methanol*		
	7:3	*8:2*	*9:1*
1	1.46	1.48	1.96
5	1.41	1.41	1.43
2	3.54	4.80	9.22
3	6.86	10.45	15.30*

* Broad, useless peak.

Better results were obtained with chloroform–methanol mixtures, but only three of the five compounds could be successfully separated (Table III). It should be noted that the limit of detection of 1 was the lowest (25 ng/ml) in this solvent system.

The application of dichloromethane as the eluent or the LiChrosorb-DIOL column with chloroform–methanol mixtures gave poor results. On the other hand, a satisfactory separation of 1 and its four metabolites was achieved by reversed-phase chromatography on the LiChrosorb RP-8 column using the five eluents given in Table I. Some of these solvents proved to be appropriate for the separation of a mixture of the five compounds.

In general, with methanol–water mixtures it was found that the elution of 2 was much more difficult than that of the other four compounds. In an isocratic run the application of only two kinds of methanol–water mixtures resulted in satisfactory separation. With methanol–water (8:2) the retention time of 2 was 5.1 min (solvent flow-rate 3.0 cm^3/min) and for the other compounds it was *ca.* 2 min. Using methanol–water (1:1 or 4:6) compounds 1,3,4 and 5 could be readily separated (Table IV, Fig. 2).

Isopropanol–water mixtures were also investigated for the same purpose and the best results were obtained when the ratio was 3:7. The order of elution with these mixtures was the same as that of observed for the methanol–water systems (Table

TABLE IV

RETENTION TIMES (t_R) AND CAPACITY RATIOS (k') WITH METHANOL–WATER MIXTURES

Solvent flow-rate = 3.0 cm^3/min.

Compound	*Methanol–water*			
	1:1		*4:6*	
	t_R *(min)*	k'	t_R *(min)*	k'
4	1.06	0.45	1.76	1.05
5	1.71	1.34	3.43	2.98
3	2.19	2.00	4.91	4.71
1	4.35	4.95	9.50	10.05

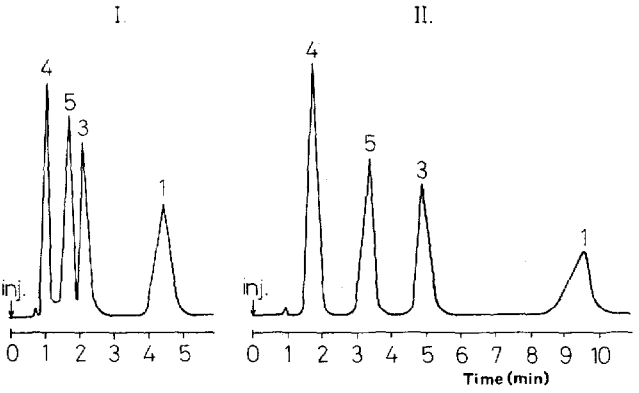

Fig. 2. Separation of 1-benzyl-4-(2'-pyridinecarbonyl)piperazine in methanol–water systems. Methanol–water ratios: I, 1:1; II, 4:6. Solvent flow-rates, 3.0 cm^3/min; column, 200 × 4.6 mm I.D. 10 μm LiChrosorb RP-8.

V). Compound 2 could be satisfactorily separated with mixtures of higher isopropanol content (isopropanol–water, 7:3).

The best results were achieved with buffer systems; each of the five compounds could be separated in an isocratic run with a single eluent. Suitable eluents are a mixture of pH 6.98 Sörensen phosphate buffer (600 ml of 11.87 g/l Na_2HPO_4 and 400 ml of 9.078 g/l KH_2PO_4) and 30% of acetonitrile, and a mixture of pH 6 citrate buffer (7.0 g of citric acid · xH_2O + 4.0 g of sodium hydroxide in 1 l of water) and 32% of methyl Cellosolve (2-methoxyethanol) (Table VI, Fig. 3). With a decrease in the amount of the organic solvent the degree of separation of 2 and 4 was increased with both buffers, but the retention time of the other compounds was also increased. Acetonitrile–water mixtures gave poorer results than those obtained with the previous systems.

It was established that the best method for the high-performance liquid chromatographic separation of 1-benzyl-4-(2'-pyridinecarbonyl)piperazine and its four metabolites in an isocratic run is reversed-phase chromatography on a C-8 stationary phase column with pH 6.98 phosphate buffer–acetonitrile (7:3) or with pH 6 citrate buffer–methyl Cellosolve (68:32).

The application of this method to the analysis of plasma and urine will be published separately.

TABLE V

RETENTION TIMES AND CAPACITY RATIOS WITH ISOPROPANOL–WATER (3:7)

Solvent flow-rate = 2.0 cm^3/min.

Compound	t_R *(min)*	k'
4	1.23	0.46
5	2.62	2.11
3	3.54	3.21
1	4.39	4.22

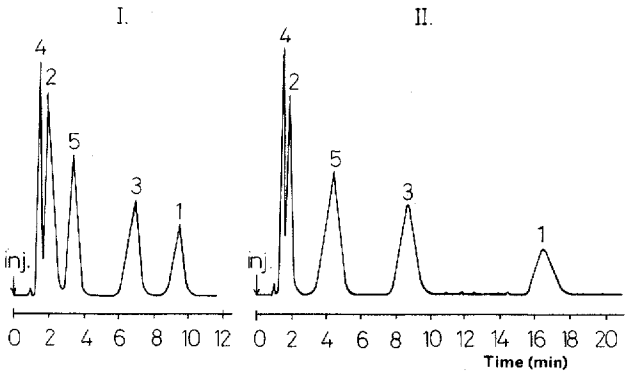

Fig. 3. Separation of 1-benzyl-4-(2'-pyridinecarbonyl)piperazine and its metabolites in buffer systems. Eluents: I, phosphate buffer (pH 6.98)–acetonitrile (7:3); II, citrate buffer (pH 6)–methyl Cellosolve (68:32). Solvent flow-rate, 2.0 cm^3/min; column, 200 × 4.6 mm I.D. 10 μm LiChrosorb RP-8.

TABLE VI

RETENTION TIMES AND CAPACITY RATIOS IN BUFFER SYSTEMS

Solvent flow-rate = 2.0 cm^3/min; t_R*: Solvent flow-rate = 3.0 cm^3/min.

Compound	*Phosphate buffer + 30% acetonitrile*		*Citrate buffer + 32% methyl Cellosolve*		
	t_R *(min)*	k'	t_R *(min)*	k'	t_R*
4	1.45	0.52	1.41	0.56	0.95
2	1.93	1.03	1.97	1.17	1.35
5	3.43	2.61	4.26	3.73	2.81
3	6.55	5.89	8.75	8.72	5.83
1	9.63	9.13	16.50	17.33	10.75

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