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IDENTIFICATION AND DETERMINATION OF N-SUBSTITUTED PIPERAZINES BY THIN-LAYER AND GAS CHROMATOGRAPHY

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SUMMARY

The results of the separation, identification and determination of some N-substituted piperazines by thin-layer (TLC) and gas chromatography (GC) are presented. The separation and identification of these compounds were performed by TLC using the elution system methanol–ammonia (100:1.5) and spraying with Dragendorff reagent. For quantitative determinations a GC method was applied, using a glass column, packed with 5% SE-30 on silanized Gas-Chrom Q.

INTRODUCTION

Piperazines have been determined as their salts or as complex derivatives by using ammonium molybdate, ammonium phosphomolybdate, vanadium pentoxide, chromium trioxide, silicotungstic acid, oxalic acid, acetic acid and or carbon disulphide as reagents[1–3]. The quantitative determination of piperazine derivatives by non-aqueous titration with perchloric acid before and after acetylation has been recommended[4–6].

Difficulties arose in the determination of a complex mixture of piperazine derivatives that cannot be analysed by classical methods. Although some reports refer to the determination of piperazine in the presence of N-hydroxyethylpiperazine and N,N′-dihydroxyethylpiperazine[7] and of piperazine in the presence of N-methyl- and N,N′-dimethylpiperazine by precipitation with carbon disulphide in chloroform, followed by titration with perchloric acid before and after acetylation[8,9], such methods can be aplied only to the determination of three components at most. Therefore, in order to analyse a mixture of N-substituted piperazines, either thin-layer (TLC) or gas chromatographic (GC) methods must be used for their separation.

Dao Huy Giao *et al.*[10] used TLC to separate *cis*- and *trans*-dimethyl-2,5-diphenyl-1,4-piperazine, dimethyl-2,6-diphenyl-1,4-piperazine, *cis*-dimethyl-2,5-di-*p*-tolyl-1,4-piperazine and dimethyl-2,6-di-*p*-tolyl-1,4-piperazine, using benzene as solvent. Tornquist[11] described a GC assay for piperazine and aminoethylpiperazine on a column packed with 10% Carbowax 20M and 3% potassium hydroxide on Chromosorb W HMDS. Ferapontov and Karpeyskaya[12] and Balandin *et al.*[13] used a column packed with 1% PEG 2600 and 0.5% potassium hydroxide on sodium chloride to separate piperazine, N-hydroxyethylpiperazine and N-aminoethylpiperazine.

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Fürst and Mannetstätter[14] separated piperazine, N-methylpiperazine and N,N'-dimethylpiperazine on a column packed with PEG 20M and 1% sodium hydroxide.

The following compounds were studied in this work: piperazine (I), N-hydroxyethoxyethylpiperazine (II), N,N'-bis(hydroxyethoxyethyl)piperazine (III), N-*p*-chlorobenzhydryloxyethylpiperazine (IV), N-phenylbenzyl-N'-hydroxyethoxypiperazine (V), N-*p*-chlorophenylbenzyl-N'-hydroxyethoxypiperazine (Hydroxyzin) (VI) and N,N'-bis(*p*-chlorobenzhydryloxyethoxyethyl)piperazine (VII). (Table I).

The literature on the TLC identification of such compounds deals only with the analysis of VI (Hydroxyzin), which is used as a drug. Published data on the eluent systems, the adsorbents and the R_F values of Hydroxyzin, determined in various biological media, have been given in detail[15]. Cardini *et al.*[16], Caddy *et al.*[17], Hartvig and Handl[18] and Fauda *et al.*[19] have determined Hydroxyzin in various biological media by GC, either after derivatization to the corresponding acetyl derivative (by acetylation) or after oxidation to benzhydrol.

EXPERIMENTAL

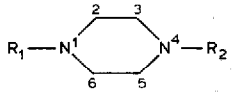
Standard compounds

Compounds II–VII were prepared in the laboratory and their purities were checked by elemental analysis.

TABLE I

THE N-SUBSTITUTED PIPERAZINES ANALYSED

The following compounds were studied: piperazine (I), N-hydroxyethoxyethylpiperazine (II), N,N-bis-(hydroxyethyl)piperazine (III), N-*p*-chlorobenzhydryloxyethylpiperazine (IV), N-phenylbenzyl-N'-hydroxyethosxypiperazine (V), N-*p*-chlorophenylbenzyl-N-hydroxyethoxypiperazine (Hydroxyzin) (VI) and N,N'-bis(*p*-chlorobenzhydryloxyethoxyethyl)piperazine (VII).



Compound	R_1	R_2
I	H	H
II	$CH_2CH_2OCH_2CH_2OH$	H
III	$CH_2CH_2OCH_2CH_2OH$	$CH_2CH_2OCH_2CH_2OH$
IV	$CH_2CH_2OCH_2CH_2OCH(C_6H_4Cl)(C_6H_5)$	H
V	$CH_2CH_2OCH_2CH_2OH$	$CH(C_6H_5)_2$
VI	$CH_2CH_2OCH_2CH_2OH$	$CH(C_6H_4Cl)(C_6H_5)$
VII	$CH_2CH_2OCH_2CH_2OCH(C_6H_4Cl)(C_6H_5)$	$CH_2CH_2OCH_2CH_2OCH(C_6H_4Cl)(C_6H_5)$

Thin-layer chromatographic plates

We used 10 × 18 cm plates, coated with a 0.35-mm layer of silica gel G either untreated or impregnated with 0.1 *M* sodium hydroxide, 0.1 *M* potassium hydroxide or 0.1 *M* sodium hydrogen carbonate solution.

Mobile phases

Acetone, methanol, methanol–12 *M* ammonia (90:1.5), chloroform–methanol (90:10). chloroform–acetone–12 *M* ammonia (80:20:1) and benzene–ethanol–12 *M* ammonia (95:15:5) were used.

Spray reagent

Dragendorff reagent was employed.

Test solutions

Solutions containing 0.1000 g of substance in 10 ml of ethanol were prepared.

Procedure

Samples of 100 μg of compounds I–VII were spotted on the starting line. The plate was then placed in the chromatographic tank containing the eluent and developed up to nine-tenths of its length. The plate was dried at room temperature and sprayed with Dragendorff reagent.

GC apparatus

GC analyses were performed on a Carlo Erba Fractovap 2400 V gas chromatograph equipped with a flame-ionization detector, connected to a Spectra-Physics Minigrator computer/integrator, with glass columns (2 m × 4 mm I.D.) packed with 5% SE-30 on silanized Gas-Chrom Q (80–100 mesh).

Operating conditions

The column temperature was programmed from 120 to 250°C at 10°C/min and held isothermally at 250°C for 20 min. The injector and detector temperatures were 300°C. The nitrogen carrier gas was at a flow-rate of 80 ml/min. Samples solutions containing 20 g of compound in 100 ml of toluene were used and the sample volume was 1 μl.

RESULTS AND DISCUSSION

For the TLC separation of the basic compounds I–VII two methods were tested: (1) a basic adsorbent (silica gel G, impregnated with 0.1 *M* sodium hydroxide, 0.1 *M* potassium hydroxide or 0.1 *M* sodium hydrogen carbonate solution) and a neutral eluent system, and (2) an untreated silica gel and a basic eluent system (containing ammonia).

The R_F values for impregnated silica gel G plates, developed with eluents of various polarities, such as acetene, chloroform–methanol and methanol are listed in Table II. Table III lists the R_F values of the compounds on untreated silica gel G in various eluent systems.

As can be seen compounds I–VII did not migrate on either impregnated or

TABLE II

R_F VALUES OF THE COMPOUNDS STUDIED IN VARIOUS ELUENT SYSTEMS AND ON IMPREGNATED SILICA GEL G

Solvent	*Silica gel G impregnated with*	*Compound*						
		I	*II*	*III*	*IV*	*V*	*VI*	*VII*
Acetone	0.1 *M* NaOH	0.00	0.05	0.00	0.25	0.375	0.375	0.42
	0.1 *M* KOH	0.00	0.05	0.00	0.27	0.410	0.410	0.42
	0.1 *M* $NaHCO_3$	0.00	0.05	0.00	0.25	0.370	0.370	0.42
Chloroform–methanol	0.1 *M* NaOH	0.00	0.09	0.09	0.56	0.61	0.61	0.70
	0.1 *M* KOH	0.00	0.11	0.11	0.54	0.58	0.58	065
	0.1 *M* $NaHCO_3$	0.00	0.14	0.14	0.60	0.74	0.74	0.80
Methanol	0.1 *M* NaOH	0.05	0.09	0.28	0.40	0.55	0.55	0.60
	0.1 *M* KOH	0.05	0.10	0.35	0.45	0.64	0.64	0.68
	0.1 *M* $NaHCO_3$	0.12	0.17	0.42	0.48	0.69	0.69	0.70

untreated silica gel G when the eluent system did not contain hydroxyl groups (*e.g.*, acetone, chloroform or benzene). The best results were obtained with methanol, containing a small amount of ammonia, as the eluents, or with adsorbents impregnated with small amounts of sodium hydroxide, potassium hydroxide or sodium hydrogen carbonate.

Using the TLC procedure described under Experimental we were able to separate compounds I–VII. The location and detection of the spots were performed with Dragendorff reagent, which with piperazine derivatives givess brown spots of various shades.

For the quantitative determination of the N-substituted piperazines and for the separation of compounds V and VI GC was tested. Because of their low vapour pressure, we used a stationary phase that is stable at high temperatures: columns packed with 3% of OV-225 on Gas-Chrom Q, 5% SE-30 on Chromosorb W HP, 5% SE-30 on Gas-Chrom and 10% XE-60 on Chromosorb W AW. The only column able to separate the N-substituted piperazines was that packed with 5% SE-30 on

TABLE III

R_F VALUES OF THE COMPOUNDS STUDIED AND VARIOUS ELUENT SYSTEMS AND ON UNTREATED SILICA GEL G

Solvent	*Compound*						
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*
$CHCl_3$–$(CH_3)_2O$–12 *M* NH_3 (80:20:1)	0.00	0.00	0.00	0.20	0.25	0.25	0.30
C_6H_6–C_2H_5OH–12 *M* NH_3 (95:15:5)	0.00	0.07	0.00	0.40	0.51	0.51	0.58
CH_3OH–12 *M* NH_3 (90:1.5)	0.10	0.22	0.68	0.75	0.80	0.80	0.88

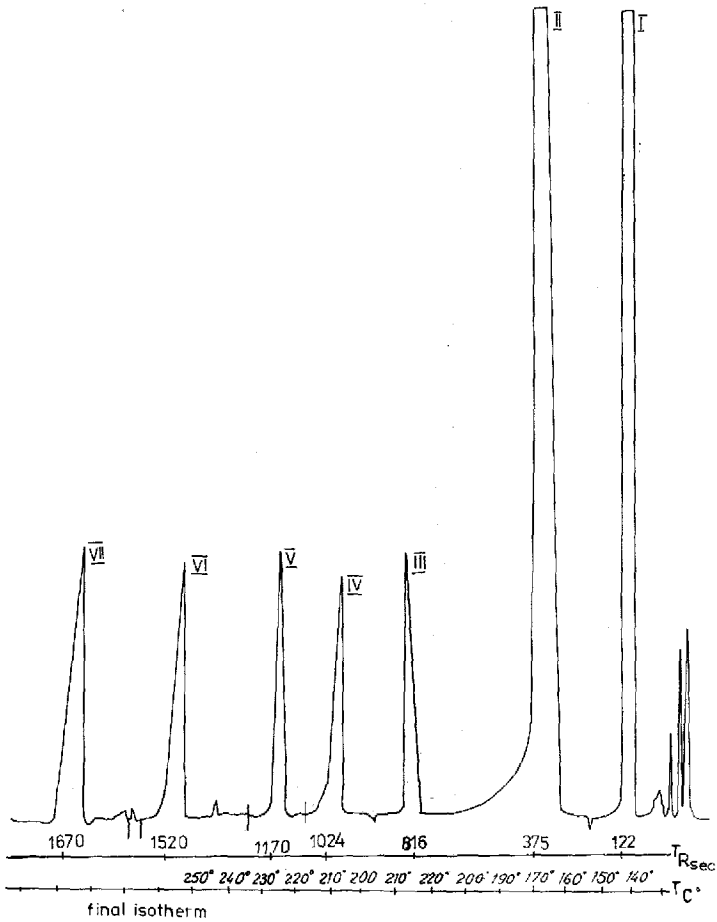


Fig. 1. Separation of N-substituted piperazines by GC on a column of 5% SE-30 on Gas-Chrom Q with a linear temperature gradient. For compound identification, see Table I.

Gas-Chrom Q. With a linear temperature increase from 120 to 250°C, compounds I–VII were separated in order of their boiling points.

Fig. 1 shows the chromatogram of a synthetic sample, prepared from authentic compounds, the purity of which was previously checked by GC. The identification was made by comparison of the retention times of the compounds in the mixture with those of the individual compounds determined separately. For precise quantitative determination, correction factors were used, their values being established by analysis of various standard mixtures. Compounds V and VI, which could not be separated by TLC, were clearly separated by GC. It should be emphasized that the Gas-Chrom Q support allowed a good separation of the piperazines, providing symmetrical peaks, without derivatization of the compounds or impregnation of the stationary phase with potassium hydroxide.

These methods have been used for the determination of the composition of

reaction mixtures in order to control a given reaction and to find the optimum reaction conditions.

REFERENCES

1 A. Castiglieni and M. Nivoli, *Fresenius Z. Anal. Chem.*, 117 (1939) 25; 119 (1940) 118; 133 (1951) 193; 135 (1952) 413; 138 (1953) 186; 142 (1954) 18; *Ann. Chim. Appl.*, 31 (1941) 129.
2 G. R. Bond, *Anal. Chem.*, 32 (1960) 1332.
3 W. K. Maynard, *J. Assoc. Off. Agric. Chem.*, 42 (1959) 610.
4 L. L. Ciaccia, S. R. Missan and G. McMullen, *Anal. Chem.*, 29 (1957) 1670.
5 P. F. Helgren, J. G. Theivagt and D. J. Cambell, *J. Am. Pharm. Assoc.*, 46 (1957) 639.
6 J. B. Milne, *J. Am. Pharm. Assoc.*, 48 (1959) 117.
7 E. Toldy, F. Cssillag, T. Bobak and J. Gyenes, *Magy. Chem. Foly.*, 67 (1961) 180.
8 T. Neto, H. Sawada, M. Tsuji and R. Seiyaru, *Chem. Abstr.*, 52 (1958) 8463; *Tanabe Seiyaku Kenky Mempe*, 2 (1957) 46.
9 R. Simionovici and L. Dimofte, paper presented at the *Conference Nationale de Pharmacie, Bucharest, November 1963.*
10 Dao Huy Giao, A. Verdier and A. Lattes, *J. Chromatogr.*, 41 (1969) 107.
11 J. Tornquist, *Acta Chem. Scand.*, 19 (1965) 777.
12 V. A. Ferapontov and E. J. Karpeyskaya, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1965) 2088.
13 A. A. Balandin, V. A. Ferapentov, E. J. Karpejeskaia and L. S. Gorshkeva, *Dokl. Akad. Nauk. SSSR*, (1966) 170.
14 W. Fürst and E. Mannetstätter, *Arch. Pharm. Ber. Dtsch. Pharm. Ges.*, 301 (1968) 569.
15 K. Florey (Editor), *Analytical Profiles of Drug Substances*, Vol. 7, Academic Press, New York, San Francisco, Londen, 1978, p. 330.
16 C. Cardini, V. Quercia and A. Cale, *J. Chromatogr.*, 37 (1968) 190.
18 B. Caddy, F. Fish and J. Tranter, *AnaLYST)London)*, 99 (1974) 555.
18 P. Hartvig and W. Handl, *Acta Pharm Suec.*, 12 (1975) 349.
19 H. G. Fauda, D. C. Hobbs and J. E. Stambaugh, *J. Pharm. Sci.*, 68 (1979) 1456.