Journal of Chromatography, 247 (1982) 39–45
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,974

COMPARISON OF AMINE MODIFIERS USED TO REDUCE PEAK TAILING OF 2-PHENYLETHYLAMINE DRUGS IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

R. GILL*, S. P. ALEXANDER and A. C. MOFFAT
Home Office Central Research Establishment, Aldermaston, Reading, Berks. RG7 4PN (Great Britain)
(Received April 19th, 1982)

SUMMARY

Chromatographic retention and peak shapes for a group of five 2-phenylethylamine drugs in reversed-phase high-performance liquid chromatography have been examined with a series of eluents containing various amines as part of the buffer system. An ODS-silica column was used with aqueous methanolic eluents containing orthophosphoric acid and sodium hydroxide to which the appropriate amines were added. Eleven eluents containing different amines have been examined along with two control eluents containing only inorganic buffer components. Large improvements in peak shape are demonstrated by the addition of some amines. The importance of selecting amines of suitable hydrophobic character and molecular geometry is discussed.

INTRODUCTION

It has long been recognised that drugs with structures containing basic nitrogen atoms can show tailing peaks in reversed-phase high-performance liquid chromatography (HPLC) when using bonded hydrocarbonaceous packing materials[1]. These problems are believed to arise from interactions between the drugs and the adsorption sites on the silica matrix of the packing material[2–7].

Peak shapes can sometimes be improved by using acidic eluents containing hydrophobic anions (*e.g.* alkylsulphonic acids) but this is not always successful. The addition of an amine or a quaternary ammonium compound to an eluent has proved to be an effective method of reducing peak tailing and controlling retention[2–10]. Apparently the amines mask the silanol groups on the packing material, limiting the adsorption of basic drugs. A wide range of masking agents has been used including amines of low molecular weight (*e.g.* triethylamine) which have high solubility in aqueous eluents and can be used as part of the buffer system. Alternatively, low concentrations (typically 1 m*M*) of long chain hydrophobic masking agents (*e.g.* N,N-dimethyloctylamine or N,N,N-trimethylnonylammonium ions) can be added to eluents in addition to the buffer components. The molecular geometry of the masking agent is most important and this point has been discussed by Bij *et al.*[6]. Nevertheless,

0021-9673/82/0000–0000/$02.75

few results have been published which compare the chromatographic behaviour of a given group of compounds in the presence of a range of different masking agents although such information is required to aid the choice of appropriate additives. Recent work by Sokolowski and Wahlund[5] has examined the behaviour of several tricyclic antidepressant with eight masking agents. The present work examines changes in retention and peak shape for a group of five 2-phenylethylamine drugs with eluents containing a range of amine derivatives as part of the buffer.

EXPERIMENTAL

Apparatus

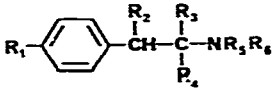
Chromatography was performed with a Waters M6000 pump, a Rheodyne 7120 injection valve (fitted with a 20-μl loop) and a Perkin-Elmer LC-75 variable-wavelength detector operated at 250 nm. The column was stainless steel (16 cm × 5 mm I.D.; Shandon Southern Products, Runcorn, Great Britain) and packed with ODS-Hypersil (5 μm) by a slurry procedure using isopropanol for dispersing the packing material and hexane as the pressurising solvent.

Materials

Methanol, sodium hydroxide, potassium hydroxide and orthophosphoric acid were AnalaR grade (BDH, Poole, Great Britain). Norpseudoephedrine and tyramine were obtained as the hydrochlorides from Aldrich (Gillingham, Great Britain). *d*-Amphetamine sulphate and dimethylamphetamine hydrochloride were obtained from Smith, Kline and French Labs. (Welwyn Garden City, Great Britain). Phentermine was obtained from Riker Labs. (Loughborough, Great Britain). The structures of the five drugs are given in Table I.

TABLE I

STRUCTURES OF 2-PHENYLETHYLAMINES



Compound	*Substituents*					
	R_1	R_2	R_3	R_4	R_5	R_6
Amphetamine	H	H	Me	H	H	H
Dimethylamphetamine	H	H	Me	H	Me	Me
Norpseudoephedrine	H	OH	Me	H	H	H
Phentermine	H	H	Me	Me	H	H
Tyramine	OH	H	H	H	H	H

N-Methyl-D-glucamine was obtained from Sigma (Poole, Great Britain). All other amines were from Fluka (Fluorochem, Glossop, Great Britain). Dibutylamine, diethylamine, hexylamine, triethanolamine, triethylamine and tris(hydroxymethyl)-aminomethane were of puriss grade. N,N-Dimethylethanolamine, N,N-dimethyl-

ethylamine, N,N-dimethylhexylamine and N-methylhexylamine were of purum grade. All amines were used without further purification.

Eluents

Sodium hydroxide (28 g) was dissolved in distilled water (4930 ml) and orthophosphoric acid (70 ml) added slowly. On cooling, methanol (500 ml) was added, giving a solution with a measured pH of 2.4. Aliquots of this stock solution were taken and the appropriate weight of each amine added to give a concentration of 0.05 *M*. Two control eluents were also prepared in which the amine was replaced by sodium hydroxide or potassium hydroxide. The measured pH values of the eluents are shown in Table II.

Chromatographic procedure

The five drugs were dissolved in methanol (1–5 mg/ml) for injection on to the column (5–10 μl). The quantity of each drug injected was maintained constant for all eluents while the detector sensitivity was adjusted accordingly. Measurements were taken with a flow-rate of 2.5 ml/min.

With each change of eluent the column was first washed with 50% aqueous methanol (50 ml), then methanol (100 ml). After flushing with the new eluent (30 ml) it was recycled for *ca.* 1 h at a flow-rate of 2.5 ml/min before any injections were made. Some eluents were run several times throughout the experiments and measurements repeated to ensure that no irreversible changes occurred to the column.

Chromatographic retention data were expressed as capacity ratios, $k' = (t_R - t_0)/t_0$ where t_R and t_0 are the retention times of the drug and a non-retained compound, respectively. The shape of each peak was assessed by measurement of the asymmetry factors A_s[11], calculated by dropping a perpendicular from the peak maximum and measuring the distance from this line to the leading edge (a) and the trailing edge (b) at the 10% peak-height level. $A_s = b/a$: therefore, a symmetrical peak has $A_s = 1$ while a tailing peak has $A_s > 1$.

RESULTS AND DISCUSSION

A series of eleven eluents which differed only by the nature of the aliphatic amine in the buffer has been examined. These eluents were prepared from phosphoric acid and sodium hydroxide in 9.1% methanol to which the appropriate amines were added (0.05 *M*). In addition two control eluents were examined in which the amine was replaced by an inorganic base (sodium hydroxide or potassium hydroxide). Amphetamine, dimethylamphetamine, norpseudoephedrine, phentermine and tyramine were selected as test compounds as they were previously seen to have a wide spread of retention properties with such chromatographic systems[10]. All eluents were acidic (pH $\leqslant$ 3.10, Table II) and the drugs would therefore be protonated and pass through the column in association with phosphate ions.

The k' values for the five drugs with the thirteen eluents are given in Table II along with peak-asymmetry data. The amine additives are listed in approximate ascending order of hydrophobic character. In addition to the asymmetry factors (A_s) for each drug–eluent combination an average A_s for the five drugs with each eluent is presented.

TABLE II

HPLC RETENTION AND PEAK-SHAPE DATA FOR FIVE DRUGS WITH VARIOUS BASES ADDED TO THE ELUENT

Eluents: Orthophosphoric acid (0.19 *M*) + sodium hydroxide (0.13 *M*) + base (0.05 *M*) in 9.1 % methanol.

*Base**	*Eluent pH*	*Tyramine***	*Norpseudoephedrine***	*Amphetamine***	*Dimethylamphetamine***	*Phentermine***	*Average* A_s
Potassium hydroxide	2.95	1.41 (2.72)	7.11 (4.39)	13.57 (6.21)	14.47 (7.57)	29.71 (4.30)	5.04
Sodium hydroxide	2.95	1.23 (2.21)	6.69 (2.93)	12.71 (5.64)	19.93 (6.49)	31.88 (3.88)	4.23
N-Methylglucamine	2.65	1.48 (3.28)	7.63 (2.94)	13.31 (4.77)	16.15 (4.49)	31.00 (4.31)	3.96
Triethanolamine	2.90	1.28 (2.03)	6.43 (3.24)	12.91 (3.90)	18.48 (6.93)	29.91 (4.68)	4.16
N,N-Dimethylethanolamine	3.00	1.22 (1.70)	6.37 (2.85)	11.95 (3.39)	15.95 (3.70)	27.95 (3.52)	3.03
N,N-Dimethylethylamine	2.75	1.21 (1.80)	6.17 (2.37)	12.88 (2.30)	15.34 (3.68)	27.37 (3.10)	2.65
Tris(hydroxymethyl)-aminomethane	2.95	1.18 (2.22)	6.18 (3.10)	11.12 (7.15)	12.21 (5.90)	23.67 (5.93)	4.86
Diethylamine	3.10	1.05 (1.77)	5.52 (2.25)	10.07 (3.22)	13.12 (4.17)	23.33 (3.91)	3.06
Triethylamine	2.95	1.95 (2.00)	4.63 (2.49)	8.48 (3.95)	10.38 (3.84)	19.50 (3.73)	3.20
Dibutylamine	3.00	0.19 (1.87)	1.18 (1.30)	2.19 (1.53)	2.34 (2.48)	4.21 (1.70)	1.78
Hexylamine	2.95	0.19 (2.34)	1.31 (1.01)	2.14 (1.34)	2.40 (2.01)	4.58 (2.02)	1.74
N-Methylhexylamine	3.00	0.19 (1.80)	1.11 (0.97)	1.81 (2.00)	1.93 (1.34)	3.72 (1.85)	1.59
N,N-Dimethylhexylamine	3.00	0.19 (1.81)	1.12 (1.52)	1.91 (1.46)	1.97 (1.45)	3.91 (2.02)	1.65

* The bases are given in approximately ascending order of hydrophobic character.

** Data presented as $k'(A_s)$.

The control eluents, having no amine additives, showed very poor peak shapes with average A_s values greater than 4. The eluent containing sodium ions showed slightly shorter retention times and better peak shapes than that containing potassium ions. Both control eluents show a general increase in A_s as the retention of the drugs increases, *i.e.* the hydrophobic drugs show more tailing. Asmus and Freed[12] have shown that very hydrophilic 2-phenylethylamines such as catecholamines give good peak shapes on ODS-silica with eluents containing only inorganic buffers. Table II shows that phentermine gives a better peak shape than dimethylamphetamine despite its greater retention. It can be seen that this occurs not only with the control eluents but also with most of the other eluents. This probably indicates that the two α-methyl groups on phentermine hinder the interaction of the amino group with the adsorption sites on the packing material.

The retention data shown in Table II are plotted in Fig. 1 and it can be seen that the five drugs are eluted in the same order with all eluents. Elution times decrease as the added amines become more hydrophobic while those eluents containing polar amines bearing several hydroxyl groups (*e.g.* N-methylglucamine and triethanolamine) show only small changes from the control eluents. Retention can thus be controlled by the selection of an appropriate amine additive.

The average A_s values in Table II indicate that large improvements in peak shape can be obtained by adding amines to the eluent. As with chromatographic retention the A_s values generally decrease as the hydrophobic character of the amine additive increases. Several amines of high polarity containing hydroxyl substituents

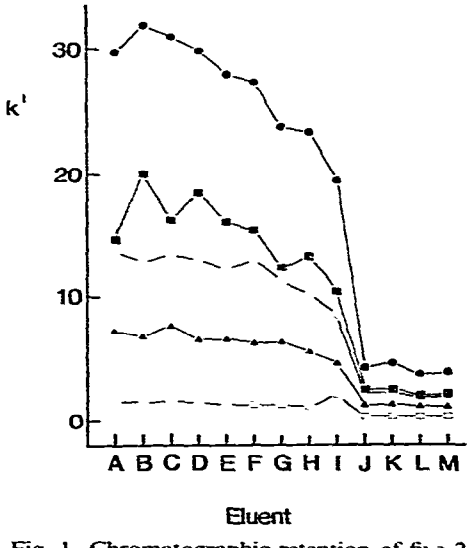


Fig. 1. Chromatographic retention of five 2-phenylethylamine drugs with 13 eluents containing added bases. Eluents: orthophosphoric acid (0.19 *M*) + sodium hydroxide (0.13 *M*) in 9.1% methanol with added base (0.05 *M*). A = Potassium hydroxide; B = sodium hydroxide; C = N-methylglucamine; D = triethanolamine; E = N,N-dimethylethanolamine; F = N,N-dimethylethylamine; G = tris(hydroxymethyl)aminomethane; H = diethylamine; I = triethylamine; J = dibutylamine; K = hexylamine; L = N-methylhexylamine; M = N,N-dimethylhexylamine. They are in approximate order of increasing hydrophobic character. □ = Tyramine; ▲ = norpseudoephedrine; ○ = amphetamine; ■ = dimethylamphetamine; ● = phentermine.

were included in the study and pairs of compounds can be selected which represent the substitution of an amine additive with hydroxyl groups, *e.g.* N-methylglucamine represents a pentahydroxy derivative of N-methylhexylamine and triethanolamine represents a trihydroxy derivative of triethylamine. In all cases the introduction of hydroxyl groups into the structure of an amine additive led to greater peak tailing.

It has been suggested that amine additives of the type $R–N(CH_3)_2$ are particularly good for reducing peak tailing[5]. Two examples of this type are included in the present work where R = ethyl and R = hexyl. The average A_s values (2.65 and 1.65, respectively) demonstrate that an increase in chain length of the amine additive gives a significant improvement in peak shape. Such a change represents an increase in hydrophobic nature without significant alteration of the molecular geometry around the amino group.

The importance of the molecular geometry of the amine additives can best be assessed by comparing isomers. Triethylamine and hexylamine ($C_6H_{15}N$) give average A_s values of 3.20 and 1.74, respectively, clearly indicating that the tertiary amine interacts less strongly than the primary amine with the adsorption sites on the packing material. However, a decrease in the number of substituents on the nitrogen atom does not always lead to better peak shapes. N,N-Dimethylethylamine and diethylamine ($C_4H_{11}N$) give average A_s values of 2.65 and 3.06, respectively, the tertiary amine giving slightly better peak shapes than the secondary amine. Similar results are seen for N,N-dimethylhexylamine and dibutylamine ($C_8H_{19}N$), giving average A_s values of 1.65 and 1.78, respectively. It appears then that methyl groups on the nitrogen atom cause little steric hindrance to interaction with the adsorption sites. This point is further demonstrated by the hexylamine series [$R–NH_2$, $R–NHCH_3$, $R–N(CH_3)_2$, where $R = C_6H_{13}$]. In the eluents containing these amines, the average A_s values (1.74, 1.59 and 1.65, respectively) are all very similar.

Table II shows that those amines which lead to the greatest improvements in peak shape also cause the largest decrease in retention times. Acceptable peak shapes (average $A_s < 2$) are only obtained with eluents where the drugs all show $k' < 5$. It is clearly important that a particular amine additive continues to give good peak shapes when retention times are increased by changing other components in the eluent. Table III shows data for four drugs using a series of four eluents (A–D) each containing

TABLE III

HPLC RETENTION AND PEAK-SHAPE DATA FOR FOUR DRUGS USING ELUENTS CONTAINING N,N-DIMETHYLHEXYLAMINE

*Eluent**	*Tyramine***	*Amphetamine***	*Dimethylamphetamine***	*Phentermine***	*Average A_s*
A	0.19 (1.81)	1.91 (1.46)	1.97 (1.45)	3.91 (2.02)	1.69
B	0.35 (1.55)	2.81 (1.72)	3.39 (1.70)	6.53 (1.71)	1.67
C	0.63 (1.78)	4.23 (1.26)	5.98 (1.61)	10.14 (2.02)	1.67
D	0.90 (1.53)	7.30 (1.40)	11.22 (1.70)	17.93 (1.68)	1.58

* Eluent A = orthophosphoric acid (0.19 *M*) + sodium hydroxide (0.13 *M*) + amine (0.05 *M*) in 9.1% methanol; eluent B, C and D = orthophosphoric acid (0.2 *M*) + amine (*x M*) adjusted to pH 2.8 with sodium hydroxide where $x = 0.05$, 0.025 or 0.01 for B, C and D, respectively.

** Data presented as $k'(A_s)$.

N,N-dimethylhexylamine and showing a progressive increase in retention times. This increase is achieved first by the removal of methanol from the eluent and then by decreasing the concentration of amine from 0.05 to 0.01 *M*. Phentermine shows an increase in k' from 3.91 to 17.93. It can be seen that despite the increases in retention, the average A_s value remains below 1.7.

In conclusion, the present study has shown that the control of peak tailing for 2-phenylethylamine drugs in reversed-phase HPLC by the addition of an amine to the eluent is strongly influenced by the nature of the amine. The results provide some practical guidelines for the selection of suitable amine additives.

REFERENCES

1 P. J. Twitchett and A. C. Moffat, *J. Chromatogr.*, 111 (1975) 149.
2 I. M. Johansson, K.-G. Wahlund and G. Schill, *J. Chromatogr.*, 149 (1978) 281.
3 K.-G. Wahlund and A. Sokolowski, *J. Chromatogr.*, 151 (1978) 299.
4 A. T. Melin, M. Ljungcrantz and G. Schill, *J. Chromatogr.*, 185 (1979) 225.
5 A. Sokolowski and K.-G. Wahlund, *J. Chromatogr.*, 189 (1980) 299.
6 K. E. Bij, Cs. Horváth, W. R. Melander and A. Nahum, *J. Chromatogr.*, 203 (1981) 65.
7 S. O. Jansson, I. Andersson and B. A. Persson, *J. Chromatogr.*, 203 (1981) 93.
8 W. R. Melander, J. Stoveken and Cs. Horváth, *J. Chromatogr.*, 185 (1979) 111.
9 D. Westerlund and E. Erixson, *J. Chromatogr.*, 185 (1979) 593.
10 R. Gill, S. P. Alexander and A. C. Moffat, *J. Chromatogr.*, 218 (1981) 639.
11 R. G. Brownlee and J. W. Higgins, *Chromatographia*, 11 (1978) 567.
12 P. A. Asmus and C. R. Freed, *J. Chromatogr.*, 169 (1979) 303.