GAS CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME SYMPATHOMIMETIC AMINES IN USE IN ANOREXIGENIC DRUGS

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INTRODUCTION

Some papers have already been published on the gas chromatographic analysis of sympathomimetic amines[1,2]; their separation is not easy to perform. This is mainly attributable to the high basicity and polarity of these compounds, as a result of which adsorption on the supports used may occur. The adsorption phenomenon manifests itself as a pronounced tailing of the peaks, the result being that the retention times become dependent on the quantity of amine injected and the separating power of the column declines considerably[3]. This makes it difficult to identify sympathomimetic amines in drugs, especially in a mixture.

It is therefore of great importance that inert supports, such as Teflon, be used, or that the support be treated with, *e.g.*, KOH in methanol or with hexamethyldisilazane, etc. in order to inactivate the adsorptive sites at the surface. Further improvement may be achieved by applying a high percentage of stationary phase to the support.

A second problem is decomposition of the amines during separation. This process is promoted by the high injector and detector temperatures. Because of this, a gas chromatograph with glass columns and on-column injection has to be used. It also appears from data from the literature and our own research that some stationary phases promote this phenomenon[5]; however, it is possible to stabilize the amines by converting them into other compounds, such as acetates[6–8].

EXPERIMENTAL

An F & M gas chromatograph, Model 400, with a flame ionization detector was used. The column consisted of glass; length about 105 cm, and diameter 25 mm. The injector-heater zone of the column was filled with 60–80 mesh glass beads and closed with a plug of glass wool. Nitrogen was used as carrier gas.

A preliminary investigation showed that the best results were obtained if a silanized support was used.

The following column packings were tested on a Diatoport S* (60–80 mesh) support: (1) 18.8 % Apiezon N; (2) 20.6 % Versamid 900; (3) 12.3 % triethanolamine;

* Diatomaceous earth specially treated and silanized with dimethyldichlorosilazane, supplied by F & M Scientific Corporation.

TABLE I

SYMPATHOMIMETIC AMINES INVESTIGATED

No.	*Name**	*Chemical name*
1	Amphetamine	α-Methylphenylethylamine
2	Methamphetamine	N,α-Dimethylphenylethylamine
3	Isopropylhexedrine	N,β-Dimethylcyclohexane-ethylamine
4	Phenylpropanolamine	α-Methyl-β-hydroxy-phenylethylamine
5	Diethylpropion	α-Diethylaminopropiophenone
6	Methylphenidate	α-Phenyl-2-piperidineacetic acid methyl ester
7	Phenmetrazine	3-Methyl-2-phenylmorpholine
8	Phendimetrazine	3,4-Dimethyl-2-phenylmorpholine
9	Chlorphentermine	p-Chloro-α,α-dimethylphenylethylamine
10	Phentermine	α,α-Dimethylphenylethylamine

* WHO name where possible.

(4) 19.7 % Hyprose; and (5) 22.5 % Carbowax 20M. Carbowax 20M was not further used because of decomposition of the amines No. 3, 4, 9 and 10 in Table I.

1–2 μl of a 2.5 % solution of the amine bases in chloroform was injected on to the column by means of a 10 μl Hamilton syringe. This was followed by a mixture of butanol-1, octanol-1 and nonanol-1, a mixture of *n*-alkanes with an even carbon number, C_6 to C_{16} inclusive, as reference, and finally a mixture of all amine bases in chloroform.

Prior to identification by the gas chromatograph, the sympathomimetic amines were extracted from a basic solution of the drug containing them with ether or chloroform. If the amine was present in the form of an HCl salt, an extract could be made direct by boiling the drug with ethanol in the case of solid drugs, as the retention times of the free bases and the HCl salts proved to be the same.

So as to be less dependent on minor changes in the carrier gas velocity the retention times found, after correction for the column dead time, were divided by the retention time of nonanol-1.

TABLE II

COLUMNS USED AND ASYMMETRY FACTOR

Column temperature = approx. 140°, see Table III.

Name	*Column No.*			
	1	*2*	*3*	*4*
Amphetamine	1.5	1.4	2.3	1.3
Methamphetamine	1.5	1.3	1.1	1.8
Propylhexedrine	1.6	1.4	1.2	2.8
Phenylpropanolamine	1.4	—	—	—
Diethylpropion	1.1	1.0	0.9	1.1
Methylphenidate	—	—	—	—
Phenmetrazine	1.1	1.2	—	1.2
Phendimetrazine	1.3	1.1	0.9	1.1
Chlorphentermine	1.1	1.4	1.0	1.2
Phentermine	1.3	1.2	1.1	1.3
Nonanol-1	1.3	1.1	1.1	1.2
Hexadecane	1.1	1.1	1.1	—

TABLE III

RELATIVE RETENTION VALUES WITH RESPECT TO NONANOL-1 OF THE SYMPATHOMIMETIC AMINES INVESTIGATED

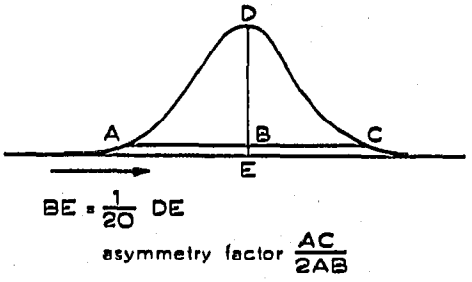
Name	*Column*									
	18.8% Apiezon N			*20.6% Versamid 900*			*12.3% triethanolamine*		*19.7% Hyprose*	
Amphetamine	0.99	1.00	1.07	0.48	0.62	0.58	1.60	1.68	1.28	1.15
Methamphetamine	1.42	1.47	1.48	0.48	0.64	0.81	0.99	1.04	0.97	0.87
Isopropylhexedrine	1.46	1.41	1.39	0.42	0.49	0.63	0.29	0.37	0.54	0.39
Phenylpropanolamine	3.18	2.77	2.55	—	—	—	—	—	—	—
Diethylpropion	10.14	7.15	5.57	3.02	3.08	2.98	1.95	2.06	2.80	2.53
Phenmetrazine	8.18	6.03	5.07	3.46	3.69	3.56	—	14.90	7.65	9.51
Phendimetrazine	9.11	6.71	5.57	2.73	3.03	2.98	4.53	4.59	4.07	3.87
Chlorphentermine	5.07	4.21	3.59	2.08	2.15	2.18	5.90	5.36	3.28	3.71
Phentermine	1.27	1.29	1.34	0.56	0.67	0.83	1.42	1.46	1.13	1.04
Ethanol	—	—	—	—	—	—	0.03	0.04	—	—
Butanol-1	—	—	—	0.04	0.04	0.13	0.08	0.11	0.12	0.05
Octanol-1	0.49	0.56	0.66	0.52	0.59	0.68	0.61	0.66	0.66	0.58
Octane	—	—	—	0.06	0.09	—	—	—	—	—
n-Decane	0.42	0.47	0.54	0.09	0.16	0.20	—	—	—	—
n-Dodecane	1.73	1.47	1.39	0.23	0.30	0.37	0.02	—	—	0.03
n-Tetradecane	8.26	4.64	3.48	0.85	0.76	0.79	0.05	0.10	0.15	0.10
n-Hexadecane	—	14.17	8.75	3.02	2.10	1.69	0.12	0.24	0.35	0.31
Retention time of nonanol (min)	7.30	1.70	0.56	18.3	3.70	1.20	15.90	5.61	2.02	14.40
Gas velocity at outlet (ml/min)	80	80	90	60	50	60	100	90	70	70
Column temperature (°C)	101	138	180	99	138.5	180	80	100	142	100
Injector temperature (°C)	236	265	257	242	247	247	203	205	247	220
Detector temperature (°C)	235	257	265	238	246	255	216	210	258	223
Pressure at inlet (atm.)	1.55	1.7	1.8	1.5	1.6	1.6	1.8	1.6	1.8	1.8
Weight of column packing (g)	2.5021			2.6416			2.0503		2.2440	

RESULTS

Peak tailing

The columns chosen do not entirely suppress the tailing. Nevertheless, it is possible to achieve a reasonably small asymmetry factor even with amphetamine (Table II).

An asymmetry factor of less than 1 indicates overloading of the column (see Fig. 1).



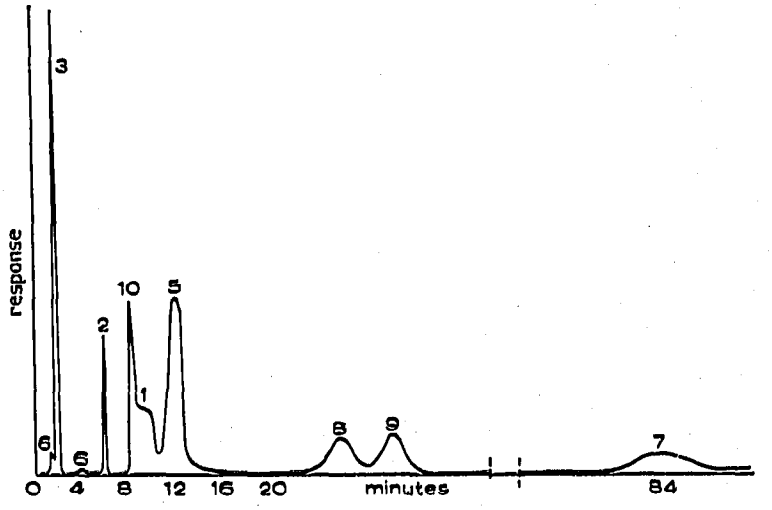


Fig. 1. Peak shape defined by the asymmetry factor.

Fig. 2. Gas chromatogram, on a triethanolamine column of a mixture of 10 sympathomimetic amines as described in Table III. Column temperature: 100°. Numbers above peaks, see Table I.

Relative retention (Table III and Figs. 2 and 3)

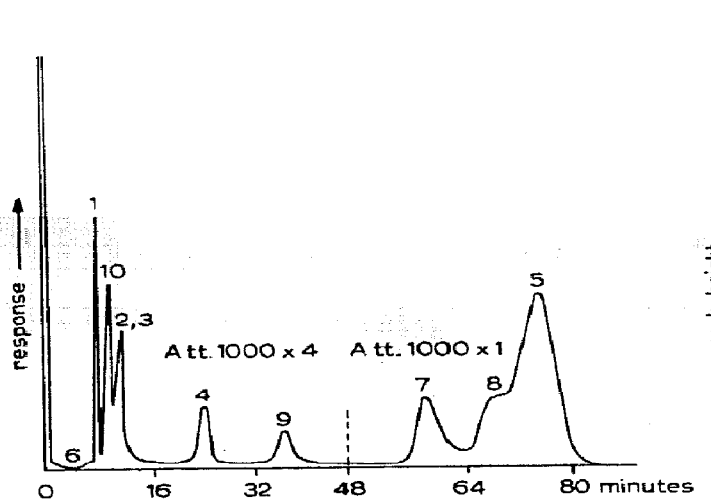
Methylphenidate did not give a clear response on any of the columns; total decomposition occurred in almost all cases. This phenomenon was also noticeable with phenylpropanolamine, though it was not as pronounced.

In the case of the Apiezon column, the calculated Kovats indices[9] were found

TABLE IV

THE KOVATS INDEX AND EFFECT OF COLUMN TEMPERATURE ON THE APIEZON COLUMN

Name	*Column temperature*		
	180°	*138°*	*100°*
Amphetamine	1145	1136	1120
Methamphetamine	1214	1200	1172
Propylhexedrine	1200	1192	1176
Phenylpropanolamine	1333	1369	1278
Diethylpropion	1502	1501	1426
Phenmetrazine	1482	1485	1399
Phendimetrazine	1502	1495	1417
Chlorphentermine	1406	1381	1338
Phentermine	1192	1176	1156
Nonanol-1	1530	1332	1122



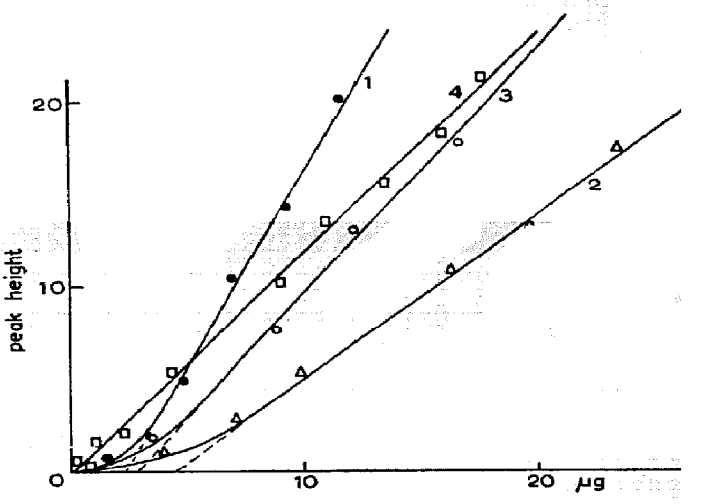


Fig. 3. Gas chromatogram on Apiezon N column, of a mixture of 10 sympathomimetic amines as described in Table III. Column temperature: 101°. Numbers above peaks, see Table I.

Fig. 4. Detector response *vs.* injected quantity of amphetamine. 1 = Apiezon column; 2 = Versamid column; 3 = Hyprose column; 4 = Hyprose column with amphetamine–acetone derivative.

to be the least dependent on temperature (Table IV). However, the separation of all the amines, except phenylpropanolamine, was better on the triethanolamine column. The retention times were not very dependent on the quantity of amine injected if this was not less than 1 μg (5 μg for Column 1) or not more than 100 μ.

Response

As the columns tested will be used in future for the quantitative analysis of sympathomimetic amines in anorexigenic drugs, it was necessary to determine in what region the relation between quantity injected and peak height is linear for amphetamine. Amphetamine was chosen for this investigation because the peak in the gas chromatogram of this substance tails more strongly than that of the other amines. The line representing the relation between peak height and quantity injected was found to be straight above 5 μg; below this quantity it curves away towards the origin of the system of coordinates. The minimum detectable quantity was still fairly small (Table V).

TABLE V

MINIMUM DETECTABLE QUANTITY OF AMPHETAMINE

Column No.	*1*	*2*	*3*	*4*	*4*
Minimum quantity (μg)	0.5	0.2	2.5	0.15	0.02*
Column temperature (°C)	138	140	100	142	140
Injector temperature (°C)	250	242	205	220	245
Detector temperature (°C)	255	245	210	237	253
Gas velocity at outlet (ml/min)	90	80	100	70	80

* A much better result was obtained by boiling amphetamine base with acetone. The linear relation between peak height and injected quantity was then maintained down to the minimum detectable quantity of 0.02 μg.

DISCUSSION

It is possible to identify which sympathomimetic amine has been used in an anorexigenic drug by analysing a chloroform or ethyl alcohol extract with a column consisting of 18.8 % Apiezon N on Diatoport S 60–80 mesh. Only the peaks for methamphetamine and isopropylhexedrine coincide. Although the latter two drugs are separated on a column of 12.3 % triethanolamine on Diatoport S, there is no response here, however, to phenylpropanolamine.

In quantitative analysis, it is advantageous to react the primary amines, especially those where considerable peak tailing occurs, with acetone. Further details of the quantitative analysis of sympathomimetic amines in drugs will be reported in the future.

SUMMARY

The identification and separation of some sympathomimetic amines which are used in anorexigenic drugs is described. These compounds can be successfully separated with Apiezon N on Diatoport S, with the exception of methamphetamine and isopropylhexedrine. The latter two can be separated with one of the other columns described.

It was found impossible to analyse methylphenidate. A detailed study of the minimum detectable quantity and the linearity of the peak height, plotted against quantity injected, was carried out on one of the amines, *viz.* amphetamine.

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