THE SEPARATION AND IDENTIFICATION OF SOME SYMPATHOMIMETIC AMINES BY PAPER PARTITION CHROMATOGRAPHY

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In pharmaceutical analysis micro methods are sometimes required for the identification of sympathomimetic amines. In a previous publication[1] one of the authors has reported the optical crystallographic properties of some commonly used salts of these amines. The observation of the optical data of pure crystalline substances is a good micro-identification method, especially when combined with micro melting point determinations.

The purpose of this paper is to report some results obtained by the authors in chromatographic experiments with some sympathomimetic amines. The technique elaborated is believed to be useful in pharmaceutical analysis, since identification of a substance by paper partition chromatography does not require its isolation in the pure crystalline state. The micro-identification of amphetamine and desoxy-ephedrine should be of particular interest, because these substances are sometimes wrongly used to produce a stimulating effect. The technique of paper partition chromatography may probably be used also in biochemical analysis for the detection of small quantities of sympathomimetic amines. Richter[2] has shown that amphetamine and ephedrine are not oxidised to any appreciable extent in the human body. The free amines could be extracted unchanged from the urine by organic solvents. The paper chromatographic separation of adrenaline and noradrenaline has been described in several publications,[3,4,5,6] and ephedrine has been included in a chromatographic study of alkaloids.[7] James[3] has also developed chromatograms of some synthetic sympathomimetic amines, which like adrenaline are catechol derivatives (corbasil, epinine). With the exception of adrenaline and ephedrine the sympathomimetic amines listed in Table I, have not as yet been subjected to a general chromatographic study, as far as the chemical literature is known to the authors.

We have developed chromatograms of 9 sympathomimetic amines with different organic solvents, water being the stationary phase. 4 of the solvent systems which have been examined in the present work, gave a satisfactory separation of the amines. The composition of these solvent systems and the observed R_F values are shown in Table I. The R_F values given in Table I are mean values from series of chromatograms where 25 μg. of the amines was employed. The temperature was maintained between 18° and 20° C. during the experiments. It is seen from Table I that the solvent systems have no resolving capacity on racemates (substances 2, 3, 4, 7) and that the separation of the two diastereoisomeric

TABLE I

Substance number	Pharmacopœial or commercial name	R_F values at 19° ± 1° C. I: *n*-Butanol-water-acetic acid 40:50:10	II: *n*-Butanol-toluene-water-acetic acid 100:100:50:50	III: Ethyl acetate-water-acetic acid 30:30:10	IV: Chloroform-water-acetic acid 100:50:40
1	Adrenaline (*l*)	0·37	0·04	0·27	0·00
2	Oxedrine (*d,l*) (Sympathol)	0·53	0·11	0·37	0·01
3	Supriphen (*d, l*)	0·60	0·15	0·42	0·02
4	Isodrine (*d,l*) (Veritol)	0·69	0·25	0·51	0·14
5	Pseudoephedrine (*d*)	0·73	0·35	0·57	0·52
6	Ephedrine (*l*)	0·75	0·37	0·58	0·55
7	Amphetamine (*d,l*)	0·78	0·47	0·68	0·69
8	Desoxyephedrine (Pervitine)	0·81	0·52	0·71	0·84
9	Tuamine	0·84	0·58	0·75	0·80

substances, *l*-ephedrine and *d*-pseudoephedrine, is not sufficient to resolve a mixture of them.

With the exception of tuamine, all the sympathomimetic amines listed in Table I, have the phenylethylamine structure. On chromatograms run with the 4 different solvent systems, the R_F values of the phenylethylamine derivatives will always arrange themselves in the same order from No. 1 to No. 8. The movement of a sympathomimetic phenylethylamine derivative seems to depend upon the number of hydroxyl groups in the molecule, and also upon the character of a hydroxyl group present—if phenolic or alcoholic. The effect of the hydroxyl groups on the observed R_F values is shown in Table II. Substance 4 is supposed to move more slowly than substance 6, since the presence of a phenolic hydroxyl group will give the amine an amphoteric character, and thus probably change the partition coefficient in favour of the water phase.

TABLE II

Substance number	General formula R_1–C$_6$H$_3$–CH(R$_2$)–CH(NH–CH$_3$)–CH$_3$: R_1	R_2	Relative movement at 19° ± 1° C. The R_F value of the slowest moving substance is taken as unity: Solvent system I	Solvent system II	Solvent system III	Solvent system IV
3	OH	OH	1·0	1·0	1·0	1·0
4	OH	H	1·15	1·66	1·21	7·0
6	H	OH	1·25	2·46	1·38	27·5
8	H	H	1·35	3·46	1·69	42·0

The observations shown in Table II are, of course, too limited to permit the establishment of exact rules for the relative movement of phenylethylamine derivatives on chromatograms. But it should be mentioned that James[3] has observed a considerably higher R_F value for epinine (adrenaline without the alcoholic hydroxyl group) than for adrenaline on chromatograms developed with a phenol-water system, water being the stationary phase.

For the detection of the spots we have employed 3 different spraying

reagents. (i) All the amines included in this study have sufficient strength as bases to give blue spots on spraying with bromocresol green dissolved in ethanol, when the chromatograms have been run with a solvent system containing acetic acid. (ii) By heating of the chromatograms after spraying with ninhydrin all the amines except amphetamine and tuamine may be localised as violet spots on a white background. (iii) If the chromatograms are sprayed with diazotised *p*-nitraniline and then passed through an ethanolic solution of sodium hydroxide, all the amines with phenolic character will appear as spots with characteristic colours, and, further, amphetamine and tuamine will appear as red spots.

Since absolute R_F values are not given in this paper, it will be necessary for the definite identification of one of the substances listed in Table I, to include reference substances in the chromatograms of the unknown substance. For identification purposes replicate chromatograms should be run with at least 2 of the solvent systems. Further, it will be advisable to spray replicate chromatograms with the various spraying reagents described in this paper.

EXPERIMENTAL

Apparatus and technique. The same technique for descending chromatograms has been used as in a previous paper,[8] where more details are given.

Adrenaline was dissolved in 5 per cent. acetic acid to obtain a 0·5 per cent. solution. The other amines were applied as free bases in a suitable solvent (ethanol or ether) on the "starting line" drawn on a sheet of Whatman filter paper No. 1, 56 × 20 cm. Quantities of 25 μg. to 50 μg. were used in a volume of 5 to 10 μl.

With the exception of *d*-pseudoephedrine (B.D.H.) and adrenaline (Danish Pharmacopœia standard), the amines were all available as salts, which were identified according to one of the Scandianavian pharmacopœias or by crystallographic methods. Desoxyephedrine (pervitin) was only available as hydrochloride in tablets and in solution for injection from Temmler Werke, Berlin. The free amines were obtained from the commercial salts by an appropriate technique (precipitation, extraction).

The chromatograms were run with the organic phase of the following solvent mixtures. The proportion of the solvent systems are given by weight.

I. *n*-Butanol (b.pt. 116° to 117° C.)-water-acetic acid (95 per cent.), 40:50:10. This solvent system has been employed in previously published work on the chromatographic behaviour of adrenaline[5,6] and of ephedrine.[7]

II. *n*-Butanol (b.pt. 116° to 117° C.)-toluene (b.pt. 109° to 110° C.) -water-acetic acid (95 per cent.), 100:100:50:50. It is shown in Table I that the addition of toluene, a liquid with relatively low polarity, to the components of solvent system I, will cause a decrease in the observed R_F values and simultaneously assure a better separation of some of the amines. If the organic phase of the mixture toluene-water-acetic acid, 100:50:40, is used as the mobile phase, the movement of the amines on the chromatograms will be negligible.

III. Ethyl acetate (Norwegian Pharmacopœia standard)-water-acetic acid (95 per cent.), 30:30:10.

IV. Chloroform-water-acetic acid (95 per cent.), 100:50:40. The R_F values given in Table I for this solvent system, were obtained by the use of freshly prepared anhydrous and ethanol-free chloroform. Chromatograms were also run with commercial chloroform (B.P.), stabilised with ethanol. The ethanol content of the chloroform will cause a decrease in the observed R_F values of about 5 to 10 per cent. but it will not affect the satisfactory separation of the amines. The mobile phases are allowed to flow down the paper to a point about 45 cm. from the "starting line." This movement will require at, 18° to 20° C., about 18 hours for the solvent system I, about 6 hours for the solvent systems II and III, and 3 to 4 hours for the solvent system IV.

Spraying reagents.

1. Bromocresol green 0·5 g. is dissolved in 100 ml. of ethanol. When the chromatograms, carefully dried in the air at ordinary temperature, are sprayed with this solution, the amines (or more precisely the amine acetates) will appear as blue spots on a greenish yellow background. If the paper is afterwards exposed to acetic acid vapours, it will turn yellow, and thus a greater contrast between spots and background may be obtained. The spots are, however, easily distinguished without this operation.

2. The dried chromatogram is sprayed with a solution of 0·20 g. of ninhydrin in 5 ml. of concentrated acetic acid and 95 ml. of *n*-butanol. The sprayed paper sheet is then heated during 5 minutes at 105° to 110° C. when strongly coloured violet spots are given by substances 2, 3, 5 and 6 and weakly coloured spots are given by substances 1, 4 and 8. Tuamine and amphetamine do not give coloured spots, even when quantities of about 100 μg. have been applied.

3. The dried chromatogram is sprayed with a solution of diazotised *p*-nitraniline. 0·25 g. of *p*-nitraniline is dissolved by gentle heating in 25 ml. of N hydrochloric acid and the solution is diluted with ethanol to 50 ml. 0·10 g. of sodium nitrite is added to each 10 ml. of this solution before spraying (cooling under running water). The sprayed chromatogram is allowed to dry in the air for 3 to 5 minutes and then it is passed through a 0·5N solution of sodium hydroxide in ethanol. Excess of the sodium hydroxide solution should be removed by means of clean filter paper. The phenolic substances appear as differently coloured spots: adrenaline, dark blue; oxedrine, dark red; supriphen, dark red; isodrine, greenish grey. Further, amphetamine and tuamine appear with a characteristic pink colour. Substances 5, 6 and 8 give no coloured spots on the yellow background.

The colour reaction between amphetamine and diazotised *p*-nitroaniline has been employed as a colorimetric determination of amphetamine which was isolated from biological materials by steam distillation.[9] Tuamine will give the same red colour with this reagent (Samdahl, private comunication).

Summary

1. The separation of 9 sympathomimetic amines by paper partition chromatography with 4 different solvent systems is described and the observed R_F values are reported. The relation between the R_F values and the constitution of phenylethylamine derivatives is briefly discussed.

2. The spraying reagents described may be used to differentiate some of the amines and thus facilitate their identification.

3. The results are considered useful for micro-identification purposes.

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