SEPARATION OF BARBITURATES BY REVERSED PHASE PAPER CHROMATOGRAPHY

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The scientific literature of the past decade contains numerous articles which describe attempts to separate barbituric acid derivatives by means of paper chromatography. These separations, with one exception, make use of conventional chromatography on either untreated or buffered filter paper with various combinations of organic solvents and aqueous solutions. Examples of separations of this type are to be found in the papers by WRIGHT[1], ALGERI AND WALKER[2], JACKSON[3], and CURRY[4,5].

Very recently, STREET[6] has described the separation of a number of 5,5-substituted barbituric acid derivatives by chromatography on modified cellulose ion-exchange paper sheets. Whilst this work was being undertaken, I was carrying out investigations[7] concerned with the separation of barbiturates from normal blood constituents. It was during the course of these experiments that it occurred to me that because the R_F values of the barbiturates on either modified or unmodified cellulose are related to their duration of pharmacological action, then it might be possible to exploit the differences of mobility in the organic phase by using reversed phase chromatography.

The literature does not appear to contain any references to publications dealing with the separation of barbiturates by reversed phase paper chromatography. The following account is a description of the behaviour of a number of barbiturates when subjected to this type of chromatography.

EXPERIMENTAL

Preparation of papers for reversed phase chromatography

Best results were obtained by dipping a sheet of Whatman No. 1 paper in a freshly prepared 10 or 20 % (v/v) solution of olive oil B.P. (in acetone), blotting to remove excess solution and allowing to dry in the air. The dried paper was then cut into a square (4 in.) with a tongue at one corner. For chromatography, the paper was folded into a cylinder and one top corner was fastened to the tongue by a paper-clip. For horizontal circular chromatography, Whatman No. 1 slotted papers were dipped in olive oil solution just prior to being used. 5 % olive oil solutions gave rise to ill-defined edges to the spots, resulting in poor resolution.

Whatman siliconed paper and Whatman No. 1 paper impregnated with liquid paraffin (10 % solution in diethyl ether) were also tried but the results were not satisfactory. The siliconed paper could not be wetted with aqueous methanol solutions containing less than 95% (v/v) methanol; paper treated with liquid paraffin gave only very slight differences in R_F values of the barbiturates.

Experimental procedure

A few microlitres of 2% (w/v) ethanolic solutions of various barbiturates were spotted on the treated paper. Solvent was placed in a jar of dimensions a little greater than the paper cylinder, and fitted with a ground glass stopper. Ascending chromatography was then carried out until the solvent front had risen about 3 in. The paper was then removed and examined, whilst wet, in the light from a lamp emitting mainly 254 mμ radiation. The 5,5-substituted barbituric acid derivatives show up in this light as dark purple absorbing areas.

A Kawerau apparatus* was used for horizontal circular chromatography.

RESULTS AND DISCUSSION

The figures illustrating the results are almost self-explanatory. They show the positions to which the barbiturates have moved in the various solvents in the times stated. Initially, aqueous methanol solutions were used as solvent. It was found that there was very little difference in the distribution pattern of the barbiturates for solvents of 0.1, 0.5, 1, 2, 5, 10 and 20 % aqueous methanol. Fig. 1 shows the pattern obtained with 1 % methanol. 50 % methanol moved all the barbiturates to approximately the same position with an R_F value of about 0.75.

A logical extension of the fact that 0.1 % methanol produced some separation of the barbiturates was to omit the methanol altogether. Fig. 2 shows the results of such a "run" in which de-ionised water was used as the solvent. It is seen that this pattern is practically the same as when methanol is present.

The next step was to use aqueous solvents but to vary their pH value. According to BIGGS[8] and BÜCHI AND PERLIA[9], the pK_1 values of the commoner barbiturates lie between 7.6 and 8.6. It was considered that some differences in speed of migration might be found to depend on whether the solvent pH was greater or less than the pK_1 value of the particular barbiturate. However, it was soon noticed that not only the pK value but also other factors were exerting some influence. Fig. 3 shows the pattern obtained with M/15 phosphate buffer at pH 7.4. Almost identical patterns to Fig. 3 were obtained with 0.2 M phosphate buffer at pH 7.7, 0.2 M phosphate buffer at pH 8.04, 0.1 M borate buffer at pH 8.3 and 0.1 M borate buffer at pH 8.6. Above pH 8.6, with 5 % sodium carbonate solution and also with 0.01 N ammonia solution, the barbiturates all moved at the same speed with an R_F value of about 0.8.

The "running" times for most of these experiments were between 20 and 40 min.

* Obtainable from Shandon Scientific Co., Ltd., London.

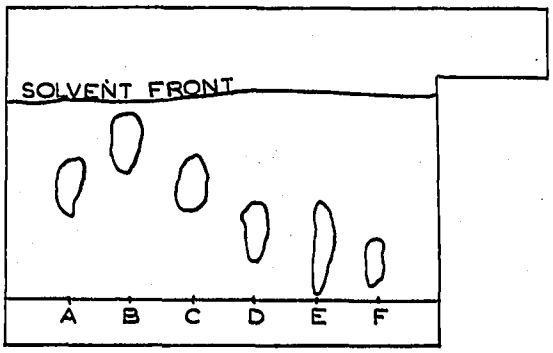

Fig. 1. Ascending chromatography of barbiturates on 20 % olive oil impregnated paper. Solvent = 1 % aqueous methanol. Time of "run" = 20 min. A = Phenobarbitone; B = Barbitone; C = Butobarbitone; D = Pentobarbitone; E = Amylobarbitone; F = Quinalbarbitone.

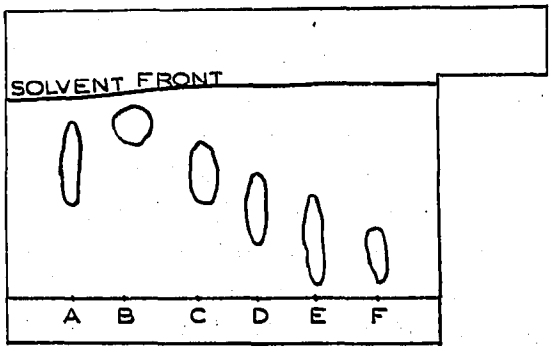

Fig. 2. Ascending chromatography of barbiturates on 20 % olive oil impregnated paper. Solvent = deionised water. A, B, C etc. same as in Fig. 1. Time of "run" = 20 min.

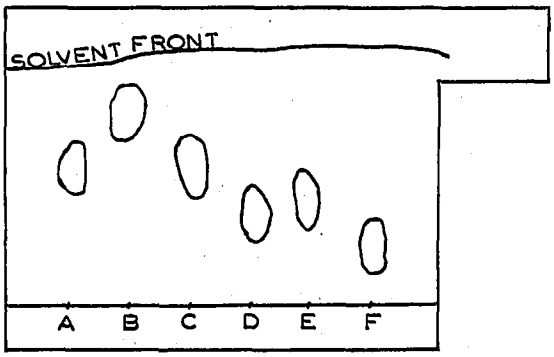

Fig. 3. Ascending chromatography of barbiturates on 20 % olive oil impregnated paper. Solvent = $M/15$ phosphate buffer, pH 7.4. Time of "run" = 40 min. A, B, C etc. same as in Fig. 1.

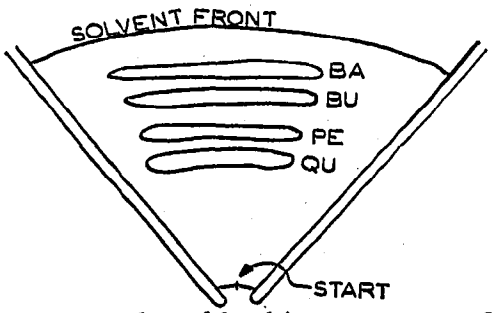

Fig. 4. Horizontal circular chromatography of barbiturates on 10 % olive oil impregnated paper. Solvent = $M/15$ phosphate buffer, pH 7.4. Time of "run" = 1 h 40 min. QU = Quinalbarbitone; PE = Pentobarbitone; BU = Butobarbitone; BA = Barbitone.

In the 40 min "runs", the barbiturates could be detected on the *wet* paper but, except for Barbitone and Phenobarbitone, they could not be seen after the paper had been dried. This was very disappointing because, when attempts were made to extend the running times of the most promising chromatograms, both by ascending and horizontal circular chromatography, the barbiturates could not be detected on the paper. Even exposure of the papers to ammonia vapour for several hours failed to reveal the compounds. Furthermore, the olive oil on the paper interfered with the usual reagents for detecting barbiturates.

All the initial experiments were carried out with 20 % olive oil solutions. By decreasing the concentration of olive oil to 10 %, it was found that the barbiturates could be detected on the paper even after drying and after longer runs. Fig. 4 shows the results of horizontal circular chromatography on Whatman No. 1 paper impregnated with olive oil (from a 10 % solution), of a mixture of Quinalbarbitone, Pentobarbitone, Butobarbitone and Barbitone. The time of the run was 1 h 40 min.

It is clear from Figs. 2 and 3 that in 20 to 40 min a partial resolution of slow-, medium- and quick-acting barbiturates can be achieved and, from Fig. 4, that the resolution can be greatly improved by a change to circular chromatography for 90 min. With this reversed phase technique, Phenobarbitone and Butobarbitone are not separable; neither are Pentobarbitone and Amylobarbitone. However, it is pertinent to compare the movement of all these barbiturates using reversed phase paper chromatography, with those obtained using ion-exchange paper chromatography[6] (*q..v*). It is also important to note that the time required for separation in each of these two techniques is far less than that required for *conventional* paper chromatography. A combination of conventional, ion-exchange and reversed phase paper chromatography should prove to be of considerable value in the analysis of mixtures of barbiturates.

SUMMARY

The behaviour is described of several barbiturates when subjected to chromatography on filter paper impregnated with olive oil. Results are given for various methanolic and aqueous inorganic solvents. The technique affords a fairly rapid separation of slow-, medium- and quick-acting barbiturates but the order of separation is different from those obtained with conventional and ion-exchange paper chromatography.

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