CHROMATOGRAPHY OF SOME BARBITURIC ACID DERIVATIVES ON MODIFIED CELLULOSE ION-EXCHANGE PAPER

HAROLD V. STREET
Department of Forensic Medicine,
University of Edinburgh (Great Britain)

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Numerous workers, *e.g.* ALGERI AND WALKER[1], WRIGHT[2], and CURRY[3] have used chromatography on "ordinary" filter paper in attemps to separate the various derivatives of barbituric acid. Conventional chromatography of this type takes at least six or seven hours to effect an unequivocal resolution of the barbiturates and, even after this time, not all the derivatives can be resolved.

The work of KNIGHT[4,5], who used chromatography on modified cellulose ion-exchange papers for the separation of mixtures of metallic cations and of amino acids, indicated that it might be possible to separate some of the barbiturates by this method, with considerable increase in speed of resolution. Experiments along these lines were carried out and, initially, proved to be disappointing in that resolution could not be satisfactorily obtained with aqueous "solvents". However, by using certain organic solvents saturated with buffer, it was found that separation of some barbiturate derivatives could be achieved with the cellulosic ion-exchange papers in a shorter time than with conventional chromatography on "ordinary" filter paper.

To avoid confusion, it is to be noted that the work described in this article refers only to chromatography on modified cellulose ion-exchange papers and *not* to ion-exchange papers prepared by impregnating filter paper with ion-exchange resins as described by LEDERER[6], TUCKERMANN[7], TUCKERMANN, OSTERYOUNG AND NACHOD[8], and LEWANDOWSKI AND JARCZEWSKI[9].

EXPERIMENTAL

Type of paper

Best results were obtained with the Whatman (DE20) anion-exchange paper. In this paper the cellulose has been modified by the introduction of diethylaminoethyl groups.

Solvents

The best solvent tried was *tert.*-amyl alcohol–0.1 *M* EDTA*, 1:1 (upper phase)—Sol-

* EDTA refers to the disodium salt of ethylenediaminetetra-acetic acid.

vent (A). A solvent which gave partial resolution was *n*-butanol–0.1 *M* EDTA, 1:1 (upper phase)—Solvent (B).

The following solvents were tried but were found to be unsatisfactory:

(i) Amyl alcohol–concentrated ammonia solution, 9:1 (upper phase).
(ii) *n*-Butanol–6 *N* ammonia solution, 1:1 (upper phase).
(iii) *n*-Butanol–0.2 *M* sodium carbonate solution, 1:1 (upper phase).
(iv) *n*-Butanol–0.2 *M* sodium bicarbonate solution, 1:1 (upper phase).
(v) *n*-Butanol containing 10% ethanol–0.1 *M* EDTA, 1:1 (upper phase).
(vi) As (v) but ethanol added to upper phase *after* separation instead of before.
(vii) *n*-Butanol–chloroform–0.1 *M* EDTA, 9:1:10 (upper phase).
(viii) Butan-2-ol–0.1 *M* EDTA, 1:1 (upper phase).

Barbiturate solutions

The barbiturates used were Phenobarbitone, Barbitone, Butobarbitone, Pentobarbitone, Amylobarbitone and Quinalbarbitone.

These were made up as 2% (w/v) solutions in ethanol. 2–5 μl of these solutions were "spotted" on the paper.

Chromatography

For preliminary trials, the ascending method was used. A sheet of DE20 paper was cut to the size and shape shown in Fig. 1. After the barbiturates had been spotted on

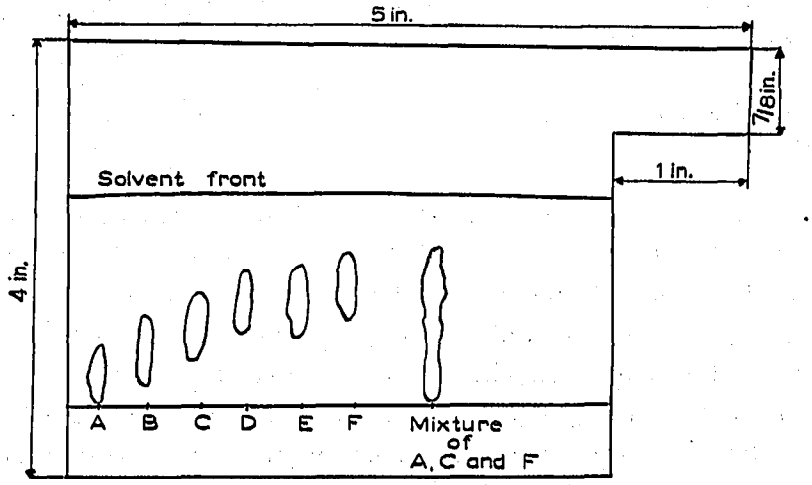

Fig. 1. Ascending chromatography of Phenobarbitone (A), Barbitone (B), Butobarbitone (C), Pentobarbitone (D), Amylobarbitone (E) and Quinalbarbitone (F) on DE20 ion-exchange paper. Time 45 min; solvent = *tert.*-amyl alcohol saturated with 0.1 *M* EDTA (upper phase).

the paper, the sheet was folded into a cylinder and fastened with a paper-clip. For longer runs, a long flat strip of the paper was used.

When the ascending method produced results which suggested that resolution might be possible with a longer run, the procedure was repeated by using horizontal circular chromatography in the apparatus designed by KAWERAU[10].

The barbiturates could be detected on either the wet or the dried paper by inspection in 254 mμ ultra violet light.

RESULTS

Fig. 1 shows the results of a 45 min run on DE20 paper (ascending) using solvent (A). Because these results seemed promising, the experiment was repeated with a longer sheet for a longer period. Figs. 2 and 3 show the results of a 2 h and 4 h run respectively in solvent (A).

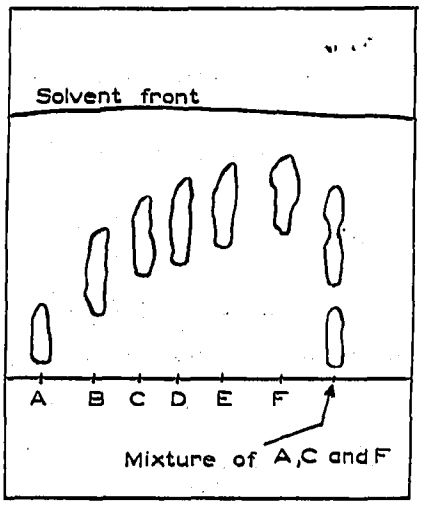

Fig. 2. Details as for Fig. 1 except that time = 2 h.

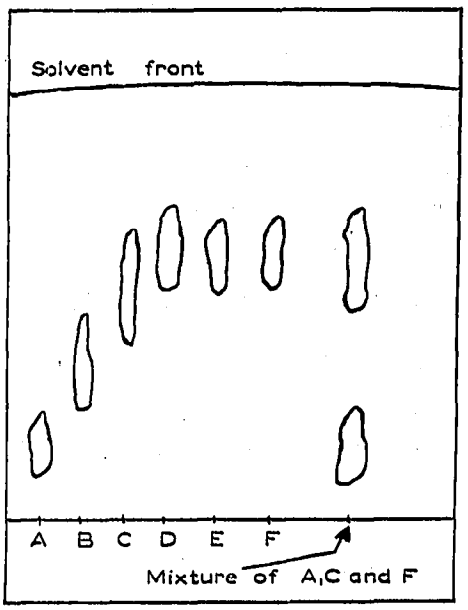

Fig. 3. Details as for Fig. 1 except that time = 4 h.

Preliminary inspection indicated that a still longer run might resolve the Phenobarbitone, Barbitone, Butobarbitone and Pentobarbitone. However, when a longer run was carried out, the spots became too diffuse and resolution was poor. Horizontal circular chromatography (using DE20 paper) was, therefore, tried. This proved successful and the results shown in Fig. 4 indicate that a mixture of Phenobarbitone, Barbitone, Butobarbitone and Quinalbarbitone can be clearly resolved in a 4 h run.

Using solvent (B), *i.e.* with *n*-butanol in place of *tert.*-amyl alcohol, the results were similar to, but not so good as those using solvent (A). Fig. 5 shows the results of a 2 h run (ascending) using solvent (B).

DISCUSSION

It has not been found possible to separate a mixture of Pentobarbitone, Amylobarbitone and Quinalbarbitone by the technique described. However, by spraying the paper with an acidified solution of potassium permanganate, the Quinalbarbitone can be differentiated from the other barbiturates. Quinalbarbitone reduces the per-

manganate and appears as a yellow spot on a pink background. Pentobarbitone and Amylobarbitone can be differentiated by the method of CURRY[11].

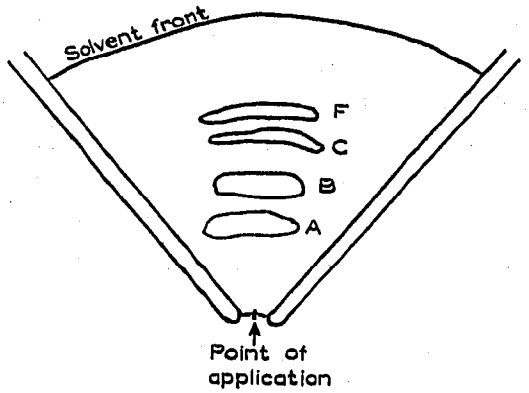

Fig. 4. Part of a circular chromatogram on DE 20 ion-exchange paper showing separation of a mixture of Phenobarbitone (A), Barbitone (B), Butobarbitone (C) and Quinalbarbitone (F). Solvent as in Fig. 1; time = 4 h.

It is interesting to note that CURRY[12] in his recent review article on Toxicological Analysis points out that Butobarbitone and Amylobarbitone are not separated unequivocally by *n*-butanol–ammonia–water (2:1:3) on "ordinary" paper. Since these

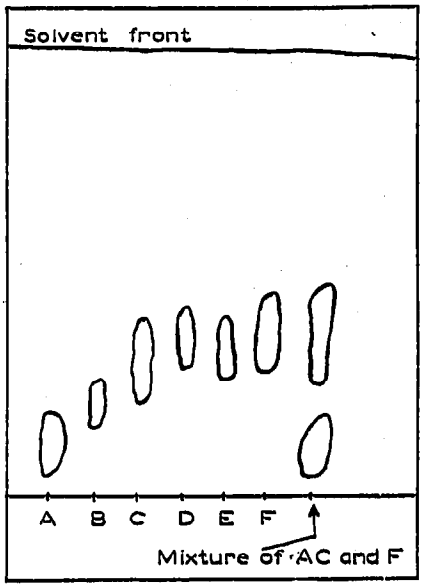

Fig. 5. Details as for Fig. 1 except that *tert.*-amyl alcohol was replaced by *n*-butanol and time = 2 h.

two barbiturates are separated clearly by the ion-exchange paper, it follows that a combination of chromatography on "ordinary" and on ion-exchange papers provides almost all the information necessary for identification of the 5,5-substituted barbiturates.

When solvent (A) or (B) is used with Whatman No. 1 filter paper, the barbiturates move with the solvent front. This shows that although the ion-exchange properties of the paper may have been suppressed to some extent by the use of organic solvents, there is still clearly a difference between the behaviour of the modified and unmodified cellulose.

It is interesting to note that the order of separation of these 5,5-substituted barbiturates is similar to that obtained by WRIGHT[2], *i.e.*, in order of the duration of their pharmacological action. There is one outstanding exception, however. It will be seen that in the procedure described here, Barbitone moves faster than Phenobarbitone, whereas in WRIGHT's[2] method the reverse is the case.

SUMMARY

Chromatography on a modified cellulose anion-exchange paper has been used to separate mixtures of certain 5,5-substituted barbiturates. Aqueous "solvents" were found to be unsatisfactory but organic solvents gave good resolution in a shorter time than with "ordinary" filter paper. Complete resolution of a mixture of Phenobarbitone, Barbitone, Butobarbitone and Quinalbarbitone can be achieved in 4 hours.

REFERENCES

1 E. J. ALGERI AND J. T. WALKER, *Am. J. Clin. Pathol.*, 22 (1952) 37.
2 J. T. WRIGHT, *J. Clin. Pathol.*, 7 (1954) 61.
3 A. S. CURRY, in C. P. STEWART AND A. STOLMAN, *Toxicology*, Vol. 2, Academic Press, New York, 1961.
4 C. S. KNIGHT, *Nature*, 183 (1959) 165.
5 C. S. KNIGHT, *Nature*, 184 (1959) 1486.
6 M. LEDERER, *Anal. Chim. Acta*, 12 (1955) 142.
7 M. M. TUCKERMANN, *Anal. Chem.*, 30 (1958) 231.
8 M. M. TUCKERMANN, R. A. OSTERYOUNG AND F. C. NACHOD, *Anal. Chim. Acta*, 19 (1958) 251.
9 A. LEWANDOWSKI AND A. JARCZEWSKI, *Talanta*, 4 (1960) 174.
10 E. KAWERAU, in I. SMITH, *Chromatographic and Electrophoretic Techniques*, Vol. I, 2nd Ed., Heinemann, London, 1960.
11 A. S. CURRY, *Nature*, 183 (1959) 1052.
12 A. S. CURRY, *J. Pharm. and Pharmacol.*, 12 (1960) 321.