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ELEVATED TEMPERATURE REVERSED-PHASE PAPER CHROMATOGRAPHY OF DERIVATIVES OF SELECTED DRUGS

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SUMMARY

Drug derivatives prepared by injection of a mixture of drug and reagent into a gas chromatograph can be collected and subjected to elevated temperature reversed-phase paper chromatography. This procedure provides additional parameters which have been found useful in identifying drugs in samples of blood, urine and liver.

A large number of drugs in current therapeutic use possess one or more functional groups. In many cases, these groups are either basic or acidic in their ionised form; in the unionised form, the drug may be lipid soluble and will be distributed between water and organic solvent according to the particular partition coefficient of the drug for that particular system. This interplay between the partition coefficient and the degree of ionisation of drug at a particular pH has been exploited by us in our system of elevated temperature reversed-phase paper partition chromatography for the separation of mixtures of drugs in biological material[1,2]. Briefly, the system consists of filter paper impregnated with tributyrin as the stationary phase, and water at about 90° as the mobile phase. It has been found that basic drugs are best chromatographed with the aqueous mobile phase at pH 4.6 and acidic and neutral drugs at pH 7.4. It is probable that adsorption forces between the cellulose of the filter paper and the drug itself cause deviation from ideal partition chromatography.

Fig. 1 shows the effect of increasing the temperature of such a chromatographic system on the resolution of a mixture of barbiturates. It will be noticed that the chromatographic 'run' is complete in about 15 min[3].

This procedure has now been used to chromatograph derivatives of drugs to assist in identification. We have found that the most convenient way of preparing many of these derivatives is by injection of a mixture of drug and reagent into a gas chromatograph. This technique has been described by us in detail very recently[4]. Primary amines, for example, can be made to react with aldehydes under these conditions. This can be a useful way of increasing the molecular weight of rather volatile drugs so that they can then be subjected to paper chromatography. For example, a few microlitres of a solution of amphetamine can be drawn up into a syringe followed by a few microlitres of a solution of *p*-dimethylaminobenzaldehyde *into the same syringe* and this mixture injected into a gas chromatograph fitted with a stream-

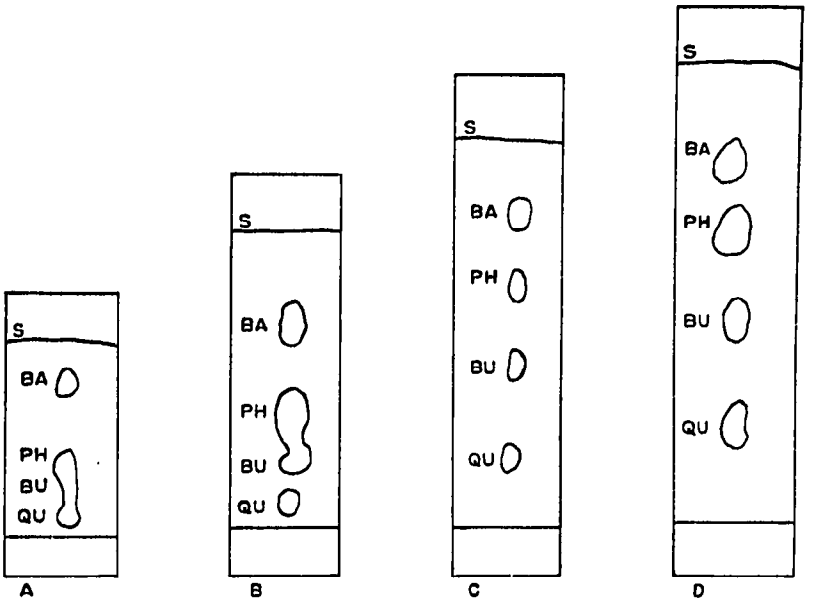

Fig. 1. Chromatographic resolution of a mixture of secobarbital (QU), butobarbital (BU), phenobarbital (PH) and barbital (BA) on Whatman No. 3 paper impregnated with tributyrin. The running time in each case was 15 min at (A) 20°, (B) 62°, (C) 86° and (D) 95°. The solvent in each case was $M/15$ phosphate buffer pH 7.4. S = solvent front. Note the rapid increase in the rate of separation as the temperature is increased.

splitter at its exit end. The Schiff base so formed can be collected and subjected to our elevated temperature paper chromatography to provide a further parameter for identification. Amphetamine itself is too volatile to be run in our paper system.

There are many obvious extensions of this technique. Vanillin, for example, can be used in place of *p*-dimethylaminobenzaldehyde when its phenolic hydroxyl group (which will also, of course, be present in the derivative) will allow other identification sprays to be used on the paper chromatogram.

Drugs containing phenolic or alcoholic hydroxyl groups can be readily acetylated by this technique. For example, if a solution of morphine and acetic anhydride is injected into a gas chromatograph, the resulting diacetylmorphine (heroin) can be collected, subjected to our paper chromatography and detected by means of iodoplatinate. Trimethylsilyl (TMS) derivatives of certain drugs can also be formed in this way but, whilst excellent results are obtained using gas–liquid chromatography, when attempts are made to collect the TMS derivatives in a melting point tube in the usual way, many of these derivatives hydrolyse spontaneously in the air, reforming the parent drug. Of course, even this property may be useful for identification purposes.

In some cases, for example with morphine, it has been found possible to collect the derivatives directly on to the tributyrin-impregnated paper used for the paper chromatographic run. In this way, transfer losses are avoided and also the applied spots are smaller in diameter than when applied by a capillary tube from a solution.

When applied to extracts of blood, urine or liver, it is preferable to make a division of the extract into five main fractions to include drugs which are:

(1) strong organic acids, *e.g.* salicylic acid,

(2) weak organic acids, *e.g.* barbiturates,
(3) neutral compounds, *e.g.* phenacetin,
(4) basic compounds, *e.g.* phenothiazines, and
(5) amphoteric compounds, *e.g.* morphine.

Appropriate volumes of these extracts are injected into the gas chromatograph together with the reagent in the same syringe. The collected material is then subjected to paper chromatography as described above. It is, of course, important to remember that the solvent used to dissolve residues of extracts must not react in the GLC apparatus with any drug which may be present. This may seem a little obvious at first, but it is surprising to find that some workers use acetone as solvent when the drug they are seeking may be a primary amine, *e.g.* amphetamine.

In conclusion, I would say that for *certain* drugs, identification can be rapidly made by preparing derivatives within a gas chromatograph and subjecting these derivatives to elevated temperature reversed-phase paper chromatography.

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