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Note

Dual-bed thin-layer chromatography for the separation and detection of drugs of abuse in urine

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The convenient applicability of urine analysis to the problems of detection and control of drug abuse in the areas of clinical, forensic and industrial medicine has prompted the recent appearance of numerous articles in this area of investigation[1–9]. Because of its relative simplicity and the high degree of resolution obtainable, thin-layer chromatography (TLC), used by itself or in combination with other methods, has become the most widely-used general technique for such analyses.

For most current procedures, prior extraction of the urine (previously adjusted for pH), or, as we have recently reported[1], of a lyophilized residue of the urine is necessary in order to obtain samples suitable for TLC. The successful application of column chromatography for the separation of opiates, barbiturates and amphetamines from urine has also been described[6,7]. The eluted materials must, of course, be subjected to subsequent analysis by TLC, ultraviolet (UV) spectroscopy or gas chromatography. We now wish to report the development of a novel system of one-dimensional TLC involving the use of both a weak anion exchanger and silica gel, each embedded on one half of a single glass plate. Material to be chromatographed is applied to the anion exchanger on one half of the plate, and this technique allows for direct application of untreated urine, as well as that of a previously partially purified sample.

EXPERIMENTAL

Materials

Dual-bed TLC plates, 20 × 20 cm, embedded with Cellex PAB weak anion exchanger** and silica gel, each 20 × 10 cm. Plates of both 0.4 mm (400 μ) and 1.0 mm (1000 μ) thickness are used, and the Cellex PAB half of each plate is scored into 1-cm and 2.5-cm vertical sections for the 0.4-mm and 1.0-mm plates, respectively. All chemicals and solvents employed are of reagent-grade quality and are used without further purification.

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** PAB = *p*-aminobenzyl = $-CH_2-C_6H_4-NH_2$.

Methods for preparation of sample

The following three procedures have been found to give comparable and highly satisfactory results:

(1) To aliquots of 2–20 ml of untreated urine are added nine times the volume of absolute ethanol. The resulting solutions are shaken briefly, filtered and then reduced azeotropically to a volume of about 1 ml by flash evaporation or by evaporation on a steam-bath. The solution is then chromatographed as described below.

(2) Aliquots (acidified) of the urine samples may be subjected to lyophilization followed by liquid–solid extraction to obtain solutions which upon evaporation yield suitable residues for subsequent chromatography. This procedure has been previously described in detail[1]. It should be noted that prior acidification of the urine is not required for the present purposes.

(3) Aliquots of 2 to 10 ml of each urine sample (no pH adjustment) may be applied directly to the Cellex PAB half of the plate. This procedure eliminates the preparation steps described above although the time required for complete application of each sample is somewhat longer owing to the necessity of evaporating an aqueous phase from the surface of the plate. Nevertheless, the authors have found this procedure to be best suited to the unique nature of the chromatographic plate employed.

Chromatography

All samples to be chromatographed are applied to the Cellex PAB portion of the plate in a uniform brush-like manner utilizing the full height of each scored vertical section except for 0.25-in. margins at both top and bottom. When applying the solution, the capillary or tube should be placed at the center of each scored column to avoid the possibility of flooding of adjacent columns or of the intervening space. Drying of the plate between multiple applications of a given sample or after final application of sample is achieved by means of a stream of hot air. The choice as to the use of either the 400-μ or 1000-μ plates will depend on the volume of sample to be applied. Thus the reduced volumes obtained from Procedure 1 described above are used to solubilize any residual solids in the beaker (if necessary, small additional aliquots of ethanol may be added for this purpose), and then applied to separate scored sections of a 400-μ plate (original aliquot 5 ml or less) or 1000-μ plate (original aliquot >5 ml). The residues obtained from the lyophilization, Procedure 2, are treated as previously described[1] and also applied to the 400-μ plates. Any arbitrary quantity of sample can, of course, be lyophilized as described, and the authors recommend that if quantities of urine greater than 5 ml are so treated the final chromatographic operation be performed on 1000-μ plates. The direct application of untreated urine can be performed on either the 400-μ plates for quantities of 2–3 ml or on the 1000-μ plates for larger quantities up to 10 ml.

Development of the plates is achieved with the system ethyl acetate–methanol –ammonium hydroxide (85:10:1) in the case of 400-μ plates or (85:10:10) in the case of 1000-μ plates to any convenient height of at least 5 cm. The plates are dried thoroughly and then visualized as previously described[1,2,4].

RESULTS AND DISCUSSION

As can be seen from Fig. 1, the use of the dual-bed TLC plates achieves in

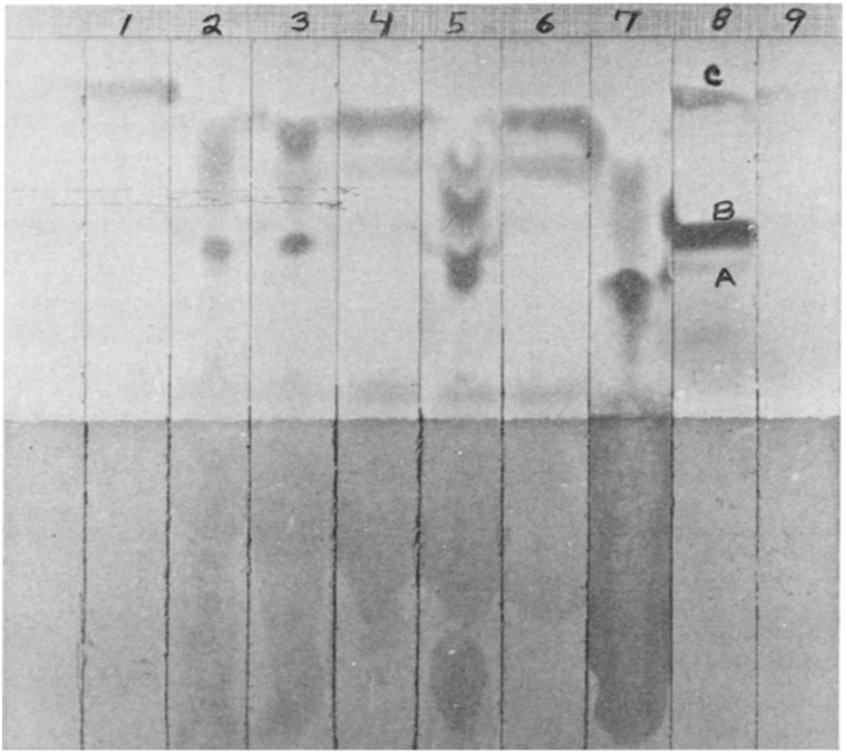

Fig. 1. Chromatography of eight untreated urine samples on dual-bed TLC plates (1.0 mm). 1–7, 9 = Urine samples; 8 = standard: morphine (A), quinine (B), methadone (C).

one chromatographic step a preliminary ion-exchange purification of the urine followed by an efficient resolution on silica gel of any drugs of abuse present in the sample. The passage of the urine through the uncharged ($-R-NH_2$) anion-exchange gel results in removal of undesired material of the strong acid type, including much pigmented matter, which would otherwise interfere with the clarity of the subsequent chromatographic resolution. Unconjugated basic drugs such as morphine, methadone, amphetamine, propoxyphene, meperidine, chlorpromazine, etc., as well as the common barbiturate sedatives pass unabsorbed through the ion exchanger and are resolved and detected on the silica gel as previously described[1,2,4]. The feasibility of directly chromatographing aliquots of untreated urine eliminates the need for prior purification of sample while retaining accuracy and reliability in the screening process. The use of the azeotrophic preparation of sample requires more manipulation prior to the chromatographic step, but allows for a much faster application of sample.

As has been emphasized in all our previous work, independent confirmation of the presence of drugs whose presence is indicated by TLC is essential for clinical or medico-legal purposes. In the present case, such confirmation can be easily achieved for many drugs by elution of material from the silica gel followed by the application of one or more of the techniques of microcrystallography, gas chromatography, UV spectroscopy and fluorometry[1,10,11]. The procedures for extraction of the basic drugs from the silica gel have been described in the references cited and it should

be noted that the prior use of multiple chromophoric spray reagents does not interfere with this technique.

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