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Note

Specialized thin-layer chromatographic system for some common drug identification problems

P. A. F. PRANITIS and A. STOLMAN
Connecticut State Department of Health, P.O. Box 1689, Hartford, Conn. 06101 (U.S.A.)
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Because of the widespread abuse of barbiturates and narcotics, it is incumbent on the toxicologist to properly identify these drugs in blood and urine samples and in autopsy material. The thin-layer chromatographic (TLC) solvent system described here adequately separates those barbiturates which are commonly encountered in combination. It also separates morphine from quinine metabolites which result from heroin and quinine combinations.

EXPERIMENTAL

Solvent system

The composition of the solvent system was ethyl ether–petroleum ether (b.p. 30–60°)–ethanol–conc. ammonium hydroxide (70:22:3:5). The solvents were mixed for 15 sec in a separatory funnel and the entire solution was then transferred to a Brinkmann rectangular glass developing tank (4 in. × 12 in. × 9 in.). The system in the tank was allowed to equilibrate for 3 min at room temperature (22°) before the plate was inserted and developed. A freshly prepared solvent mixture will produce the best separations.

Preparation of plates

Plates were prepared by spreading a layer of silica gel G on glass plates with a thickness of 0.25 mm. The dried plates were activated at 110° for 120 min before use. The plate spotted with the drugs was developed for a distance of 10–15 cm.

Spray reagents

A mercurous nitrate spray for the detection of barbiturates was prepared as follows: 1 g mercurous nitrate was dissolved in 49 ml of water and 1 ml of concentrated nitric acid. The acid solution was then diluted to 100 ml with water.

A potassium iodoplatinate spray for the detection of morphine was prepared as follows: 4.5 g of potassium iodide was dissolved in 45 ml of water and then mixed with 5 ml of 5% platinic chloride. The mixture was then diluted to 150 ml with water.

TABLE I

R_F VALUES OF BARBITURATES

Barbiturate	R_F
Methohexital	0.91
Secobarbital	0.54
Amobarbital	0.40
Butabarbital	0.34
Phenobarbital	0.09

RESULTS AND DISCUSSION

Separation of barbiturates

A commonly encountered combination of barbiturates is found in Tuinal (Eli Lilly & Co., Indianapolis, Ind., U.S.A.) (amobarbital and secobarbital). Most TLC systems[1-4] do not separate amobarbital and secobarbital adequately and rapid identification of the presence of both drugs would be beneficial to the clinician. The TLC system presented in this paper separates amobarbital and secobarbital so that there is no ambiguity about the presence of two different barbiturates. R_F values of these and other barbiturates are shown in Table I with this solvent system.

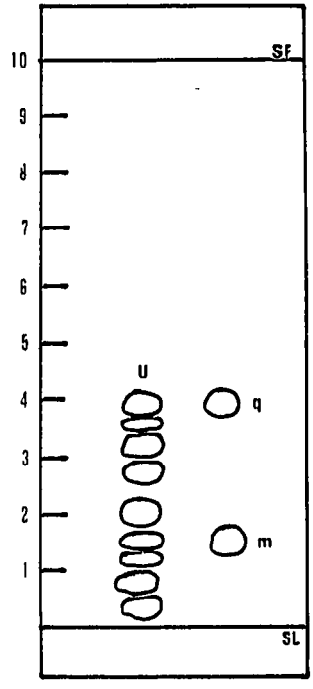

Fig. 1. Thin-layer chromatogram of probationer's urine showing morphine, quinine, and quinine metabolites. Solvent system: ethyl acetate–methanol–concentrated ammonium hydroxide (85:10:5). u = Probationer's urine; *q* = quinine standard; m = morphine standard; SL = starting line; SF = solvent front.

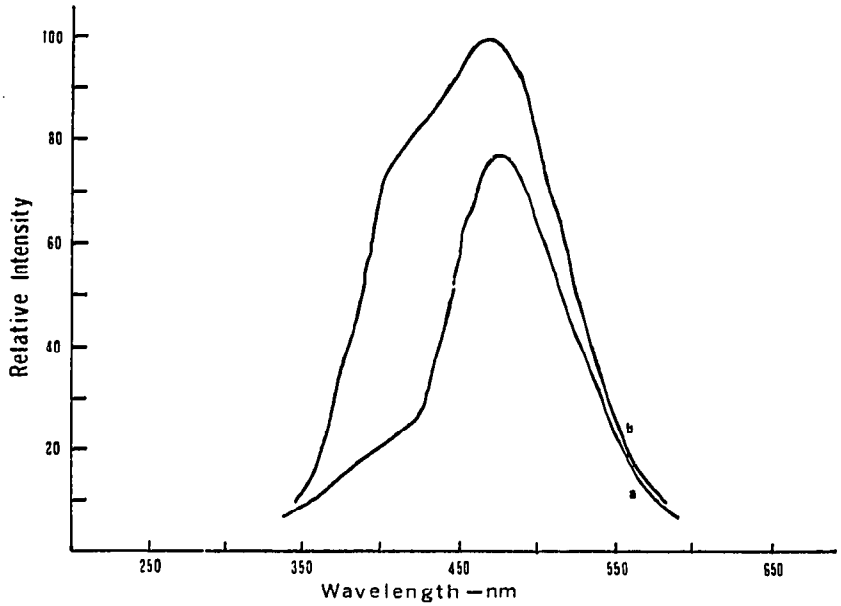

Fig. 2. SPF scan of the morphine spot eluted from the plate shown in Fig. 1. The maximum fluorescence is displaced by 40 nm due to the interference of a fluorescent quinine metabolite. The excitation wavelength is 255 nm. a = Morphine spot; b = reagent added to produce fluorescent pseudomorphine.

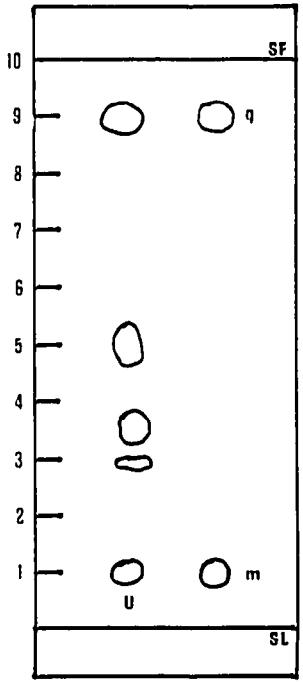

Fig. 3. Thin-layer chromatogram of the same probationer's urine showing the morphine separated from quinine and quinine metabolites. Solvent system: ethyl ether–petroleum ether (b.p. 30–60°)–ethanol–concentrated ammonium hydroxide (70:22:3:5). u = Probationer's urine; q = quinine standard; m = morphine standard; SL = starting line; SF = solvent front.

Separation of morphine from quinine metabolites

Heroin is frequently adulterated with quinine, and, in the body, morphine, quinine, and quinine metabolites are some of the excretory products. In checking samples of urine from probationers and parolees for narcotics, the TLC identification technique must ensure differentiation of morphine from quinine metabolites. In this laboratory, confirmation of the presence of morphine after the preliminary TLC screen is done by a modified spectrofluorometric (SPF) procedure described by Kupferberg *et al.*[5]. The suspected morphine spot is removed from the plate, extracted with saturated sodium borate, and the borate solution analyzed spectrofluorometrically for pseudomorphine[5]. Most of the common TLC solvent systems do not separate morphine from quinine metabolites (Fig. 1). When the suspected morphine spot is scraped from the plate for SPF confirmation, an ambiguous SPF scan (Fig. 2) is obtained due to the interference of a fluorescent quinine metabolite which was eluted from the silica gel along with the morphine. The TLC system described in this report separates quinine metabolites from morphine (Fig. 3) in the same urine sample. When the suspected morphine spot is than scraped from the plate for SPF analysis, the SPF scan (Fig. 4) represents maximum fluorescence of pseudomorphine at the corrected wavelength of 410 nm.

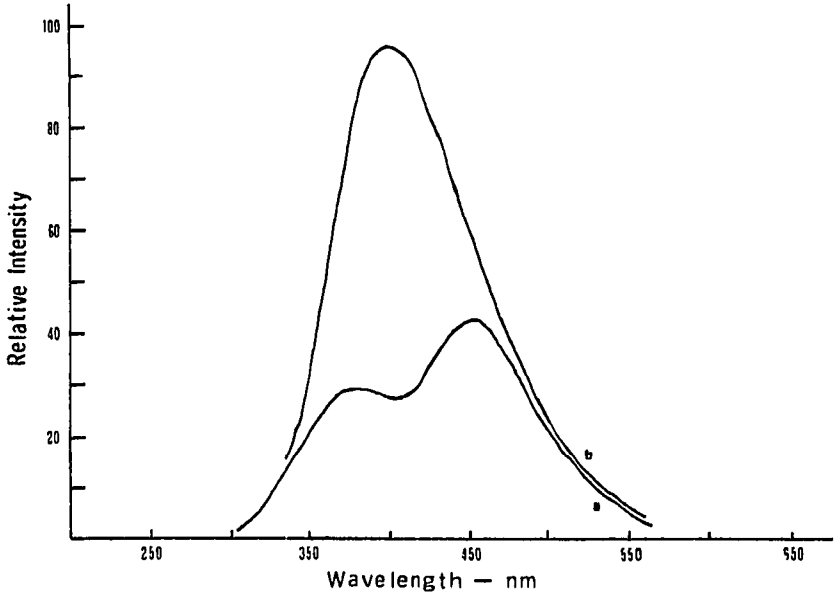

Fig. 4. SPF scan of the morphine spot eluted from the plate shown in Fig. 3. The maximum fluorescence occurs at the corrected wavelength of 410 nm. The excitation wavelength is 255 nm. a = Morphine spot, b = reagent added to produce the fluorescent pseudomorphine.

NOTE OF THE EDITOR

The separation of barbiturates as described here was tried in the Editor's laboratory using several ready thin layers (Kieselgel 60-F_{254} from Merck, Woelm, and

Carlo Erba). None of these layers gave the R_F values obtained by the authors, all the barbiturates moving only very slowly on these commercially prepared plates.

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