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Note

Batch procedure for extraction of drugs from urine samples*

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The extraction procedures that precede any analytical method for the detection of drugs in urine are based on the use of organic solvents under specific pH conditions. The use of cation-exchange paper has been proposed[1] for the extraction of drugs from urine, with subsequent elution from the paper by three consecutive buffer–solvent systems to recover acidic, neutral and basic drugs. The widely accepted technique for extraction of drugs[2] is a single extraction with chloroform at pH 9.5. In this procedure, mechanical agitation of the urine and solvent for 5 min results in the extraction of sufficient amounts of basic, neutral and acidic drugs for the purpose of screening large numbers of samples.

Although other methods[3,4] are available for the detection of drugs, the extraction step remains inconvenient and time consuming. To overcome these problems, a variety of means of improving the extraction procedure have been investigated.

Promising results for extraction of drugs have been obtained[5] by dipping an air-bubbling probe into a tube containing the urine and solvent phases. The mixing motion produced a sufficient relative velocity to transfer the drugs to the solvent phase in a relatively short time.

In batch extraction, a solute is extracted from an aqueous solution by shaking with a second, immiscible phase until partition equilibrium has been attained. The rate of achievement of equilibrium depends on the rate of transfer of the extractable species from one phase to the other. The solute molecules must first move, by diffusion, from the aqueous solution through a relatively thin, stationary layer or film of the solvent on each side of the boundary. Provided that there is interfacial turbulence, the rate of passage through the phase boundary is rapid[6].

There is a practical limit to the degree of agitation that can be advantageously employed in equilibrating an extraction mixture. Although shaking is desirable for reducing the thickness of the stationary films on either side of the phase boundary, it is the velocity of one phase relative to that of the other which determines the film thickness. Too violent agitation serves no purpose other than imparting a high translational motion to the entire mixture without producing an appreciable increase in the relative motion of the two phases[7].

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Simple repeated inversion of a separating funnel containing the two phases imparts sufficient relative velocity to the phases for equilibrium to be obtained with relatively few inversions, even with high-molecular-weight solutes[8]. The purpose of this work was to evaluate the applicability of the stream extraction technique as a simple and rapid alternative to extraction by mechanical agitation.

EXPERIMENTAL

Control urine specimens spiked with the most frequently encountered drugs were prepared (Table I). Concentrations were at the approximate detection limits for the compounds in question[2]. Ten-milliliter aliquots were buffered to pH 9.5 with saturated ammonium chloride solution and then extracted with chloroform–isopropanol (96:4) by either mechanical agitation or pressurized stream method.

In the first method, each 10-ml urine sample was added to 50 ml of the extracting solvent in a 125-ml stoppered glass bottle. The bottles were agitated in a shaking machine (7.5-cm stroke, 180 cycles/min) for 5 min.

In the second method, urine aliquots were extracted by utilizing a stream of pressurized solvent forced directly on to each urine specimen contained in an open test-tube (15 × 3 cm). The extracting solvent was compressed using a modified automatic dispenser (any single-cam pipetting machine). A schematic diagram of the extraction apparatus is given in Fig. 1. The dispenser nozzle of the pipetter was restricted to a diameter of 0.5 mm and set for a filling volume of 10 ml of solvent in a continuous cycle at a speed of 30 strokes/min. This modification successfully allowed the delivery of one 10-ml aliquot of solvent into each sample tube with sufficient velocity to affect appreciable interfacial turbulence of the two phases. The various compounds present distributed themselves between the aqueous and organic layers according to their relative solubilities during this transient stage.

After the phases had separated again, the top aqueous phase was aspirated off and concentrates from both extraction procedures were developed on pre-coated thin-layer chromatographic (TLC) plates (silica gel, Brinkmann, Westbury, N.Y.,

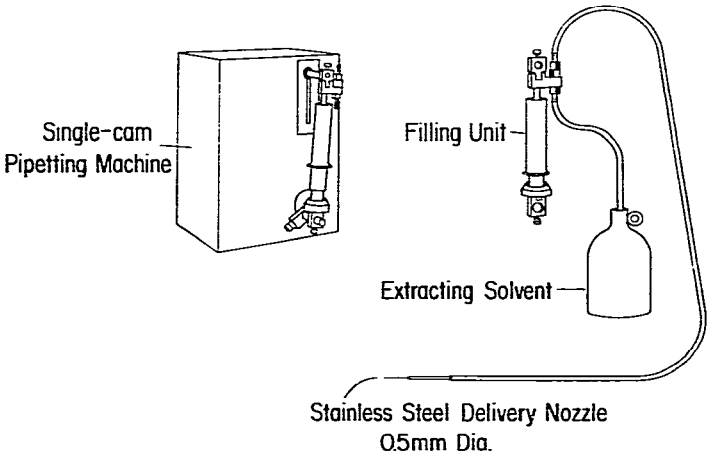

Fig. 1. Modified filling unit of the extraction apparatus.

U.S.A.) together with reference standards. The developing solvent was ethyl acetate–methanol–ammonia solution (17:2:1). The drugs were then identified using spray reagents that gave specific colors with individual drugs. The specificity of TLC analysis for drug detection has been verified[2,9].

The general methods for reagent preparation and the TLC techniques have been described in detail by Davidow *et al.*[2].

RESULTS AND DISCUSSION

The results from 100 urine samples extracted by the two methods are given in Table I. From the characteristic colors of the chromatographic spots and the R_F values for each drug it can be seen that both methods allowed the qualitative recovery of all of the drugs tested in amounts near the detection limits.

TABLE I

THIN-LAYER CHROMATOGRAPHIC DATA ON VARIOUS DRUGS EXTRACTED BY TWO DIFFERENT METHODS

Compound	*Amount (mg per 10 ml of urine)*	*Mean $R_F \times 100$**			*Spray reagents*	*Color reaction*
		A	*B*	*C*		
Amphetamine	0.050	79	76	78	Oven heating at 75° for 10 min, ninhydrin	Pink
Pentabarbital	0.050	76	73	75	Diphenylcarbazone, mercury(II) sulfate	Purple
Phenobarbital	0.050	45	42	44	Diphenylcarbazone, mercury(II) sulfate	Pink
Glutethimide	0.050	98	95	97	Diphenylcarbazone, mercury(II) sulfate	Pink
Chloropromazine	0.030	96	93	94	Oven heating at 75° for 2 min	Pink
Quinine	0.005	66	62	64	Ultraviolet light	Blue
Morphine	0.010	34	32	32	Iodoplatinate, Dragendorff	Dark blue
Codeine	0.010	56	55	57	Iodoplatinate, Dragendorff	Violet
Methadone	0.050	99	99	99	Iodoplatinate, Dragendorff	Orange-red

* A: Reference standards. B: Control samples extracted with a shaking machine. C: Control samples extracted with a pressurized stream.

A comparison of the R_F values obtained with the two methods with those for the reference standards suggests that the pressurized stream extraction gives a better resolution of the chromatographic spots. Moreover, it was observed that the individual spots representing the drugs extracted by the pressurized stream were more distinct, with little or none of the smearing that occurs with the mechanical agitation procedure. Both of these observations may be due to the fact that the mechanical agitation technique results in the increased extraction of polar and non-polar contaminants from the urine.

In addition to its simplicity, the stream extraction technique offers significant reductions in the total analysis cost and time. Only one fifth of the solvent is needed for extraction and, because of the rapidity of the technique, extractions can be achieved

at a rate of 30 per minute. The time needed for solvent evaporation was reduced by a factor of five and emulsion formation associated with violent agitation was eliminated.

The stream extraction using an open test-tube system as described here could be adapted to automation.

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