Screening for Drug Abuse: Use of NaCl to Increase Drug Recovery from Papers Coated with Ion-Exchange Resin

George J. Alexander

Use of papers loaded with ion-exchange resins to adsorb drugs from urine specimens resulted in large losses during the procedure. The first step, removal of drugs from urine specimens, was 25–85% efficient. The second step, elution of drugs from paper for further processing, was approximately 40–70% complete. The efficiency of the first step was decreased and the efficiency of the second step was increased by addition of NaCl, except in the case of barbiturates. Presence of salt during elution increased the yield of dihydrophine by 20%, of methadone by 16%, of amphetamine by 34%, and of chlorpromazine by 40%, but did not enhance the yield of pentobarbital. Overall recovery rates were: 51% for the opiates, 57% for methadone, 72% for a phenothiazine tranquilizer, but only 35% for amphetamine and 15% for a barbiturate.

Additional Keyphrases: urinalysis • drug assay • toxicology

Uptake of drugs by and their subsequent elution from papers loaded with ion-exchange resins has played a key role in a technique commonly used for routine screening of urine specimens from persons suspected of drug abuse (1). Use of such papers offered obvious advantages in terms of shipping and storing specimens but came under justifiable criticism because of large losses of drugs during the process (2). Because the procedure is so convenient, I have re-investigated the losses at various steps in the procedure and sought to improve the efficiency of drug recoveries.

Materials and Methods

Recoveries of drugs during all stages of the procedure have been followed, either by means of radioisotope-labeled compounds or by fluorometric analysis.

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Drugs. [7,8-3H]Dihydromorphine, [1-3H]methadone hydrobromide and [2-14C]pentobarbital were purchased from New England Nuclear, Boston, Mass. 02118. [3H]-l-Amphetamine sulfate was purchased from the Radiochemical Centre, Amersham, England. Homogeneity of these compounds was tested on thin-layer chromatographic plates with ethyl acetate/methanol/ammonia (17/2/1 by vol) as the developing system (3), and 95% of the radioactivity was present at the correct position (Rf 0.32 ± .04 for dihydrophine, 0.63 ± .11 for amphetamine, 0.92 ± .07 for methadone, and 0.65 ± .05 for pentobarbital). The labeled compounds were diluted with appropriate nonradioactive material and aliquots of 20 to 150 nCi (10–40 μg) were individually added directly to scintillation vials for radioassay, and to control urine specimens for extraction on paper loaded with ion-exchange resin. All tests were performed in quadruplicate. Because no radiolabeled phenothiazine tranquilizer was available to us, pure crystalline chlorpromazine (kindly provided by Smith, Kline and French, Philadelphia, Pa. 19101) was used. During extraction, the amounts present were assayed fluorometrically (4).

Extraction. Urine specimens (20 ml) were diluted with an equal volume of tap water and drugs adsorbed from them onto paper squares loaded with ion-exchange resin (6 × 6 cm, SA-2; Reeve-Angel Co., Clifton, N. J. 07014), as recommended by Dole et al. (1). The papers were washed briefly with tap water and dried. The treated (“spent”) urines were retained for assay of residual radioactivity. The adsorbed drugs were then eluted from the papers with chloroform/isopropanol (3/1 by vol) at the appropriate pH (2.2 for barbiturate, 9.3 for morphine, methadone, and phenothiazine, and 11.0 for amphetamine). Portions of all ion-exchange squares, urine, buffers, and solvent layers were transferred to scintillation vials, when necessary evaporated to dryness or to a small volume, treated with solubilizer (Scintisol GP; Isolab, Elkhart, Ind. 46514) and ethanol, and counted after addition of the counting cocktail [2,5-diphenyloxazole and 1,4-bis-2-(5-phenyloxazoly)-benzene phosphors in toluene] in a Tricarb Scintillation counter.

Neurotoxicology Research Unit, New York State Department of Mental Hygiene, 1500 Waters Place, Bronx, N. Y. 10461.
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**Effect of NaCl.** At both steps in the regular procedure in which the ion-exchange paper was used for drug isolation, the effect of salinity was investigated. Urines containing radiolabeled methadone were diluted with either an equal volume of tap water or of saturated (at room temperature) NaCl solution. Sodium chloride was added to the buffers that were used, along with equal volumes of organic solvent, to extract drugs from the paper squares. Salt concentrations in the buffers varied from 0 to 100 g/liter, to test for an increased salting out effect.

**Results**

The squares of ion-exchange paper adsorbed from urine 68.1% of dihydromorphine, 51.3% of amphetamine, and 84.1% of methadone. Figures were not available for phenothiazine, but apparently a similar proportion of that drug was adsorbed, as judged from data on overall recovery. Not surprisingly, only about 25.3% of barbiturate was adsorbed by the cation-exchange paper. I found almost 19% of the dihydromorphine, 12.5% of the phenothiazine, 7.6% of the methadone, 35.5% of the amphetamine and 65% of the barbiturate still present in the “spent” urines, and so could account for about 90% of the amounts of drugs originally present in the specimens. The step in which the drugs are eluted from the paper squares into solvent at appropriate pH, was 40–66% efficient, and I could account for 44.9% of the original opiates, 51.7% of the phenothiazine, 51.3% of methadone, 26.3% of the amphetamine, and 14.9% of the barbiturate in the eluent.

Addition of NaCl solution instead of water to the specimens before treatment with the resin-paper squares decreased the uptake of drug by the paper. An equal volume of saturated saline added to urine decreased the proportion of adsorbed methadone by 27%, and the amount of methadone left behind in the “spent” urine increased fivefold. Overall recovery of methadone from urine in the presence of this additional NaCl was 35.9%, down from the 51.3% recovered in the absence of salt.

Although added NaCl decreased the uptake of drugs from urine onto paper in step 1, it improved the elution of the alkaloid drugs from the paper into the solvent. For example, the proportion of dihydromorphine extracted in one of our experiments increased in the presence of 100 g of NaCl per liter from 62.9 to 75.6% of the amount in the square, equivalent to an increase from 42.8 to 51.5% of the total originally present in the urine specimen. The increase was related to the concentration of salt (20–100 g/liter). Recovery of methadone, amphetamine, and chlorpromazine—but not of barbiturate—also increased by as much as 40% in the presence of NaCl in the aqueous layer. A sharp decrease, for which no explanation is available, was observed in the case of chlorpromazine at the 100 g/liter salt concentration.

**Discussion**

Squares of ion-exchange paper offer obvious conveniences. The primary drawback is loss of sizable portions of the drugs present. We have confirmed that the losses are large, although perhaps not as extensive as originally feared (2). Perhaps improved manufacture of the SA-2 squares has played a role in improving drug yields.

Mulé, who reported overall recovery of only 22% of morphine and 2.4% of pentobarbital, used 50 ml of undiluted urine (2). Perhaps our use of 20 ml of urine and the dilution with tap water increased the recovery of the opiates and also of pentobarbital.

The use of ion-exchange resin paper provided convenience and a preliminary purification, but at a cost. The thin-layer chromatographic plates made after this step were easier to interpret than those for which the samples were obtained by direct solvent extraction (5). There were fewer spots, less streaking, and the spots were sharper and better defined. On the other hand, the loss during the procedure was serious, although the procedure has proved its usefulness and reliability when it is used with care (6). Use of newly developed, extremely sensitive detection methods such as radioimmunoassay and hemagglutination inhibition tests, in which nanograms and even picograms of material can be analyzed, can, it is hoped, compensate for the losses sustained during isolation.

Large amounts of NaCl in the urine at the time of uptake of drugs by the resin-loaded paper significantly decreased the uptake, presumably because Cl− competed with alkaloids for available sites on the paper, but the reverse was true for elution; the NaCl competitively displaced the drug from the resin. The proportion of drugs eluted from paper was related to salt concentration in the buffer during elution, except for the barbiturate. Obviously, elution of barbiturates is not controlled by ordinary ion-exchange processes. They probably are passively washed off the paper.

These observations prompt obvious recommendations for procedures to be followed in the use of ion-exchange papers. Urines should be diluted with distilled water, not tap water, and certainly not with isotonic saline. Papers should be washed with distilled water, and only sparingly. For thin-layer chromatographic analysis, low concentrations of drugs should be eluted from paper after adding 0.2–1.0 g of NaCl to the buffer. At the levels of recovery of most drugs few difficulties should arise, regardless of the detection procedure employed after extraction. However, in view of the low yield of barbiturates, direct solvent extraction of drugs from urine appears to be preferable whenever detection of low concentrations of barbiturates by thin-layer chromatography is critical.

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**References**