CHROM. 5709

LIQUID–SOLID EXTRACTION OF LYOPHILIZED BIOLOGICAL MATERIAL FOR FORENSIC ANALYSIS

I. APPLICATION TO URINE SAMPLES FOR DETECTION OF DRUGS OF ABUSE

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SUMMARY

A new method is described for the detection of drugs of abuse in urine, based on the techniques of lyophilization and liquid–solid extraction. Urine samples are acidified with acetic acid and then freeze-dried. The residues are then extracted and the extracts subjected to thin-layer chromatography. The method achieves much greater recovery of all classes of compounds than standard extraction procedures.

INTRODUCTION

The analysis of urine samples for both drugs of abuse and compounds of pharmaceutical interest continues to be of prime importance in forensic and medical fields. Recently, we reported on an improved thin-layer chromatographic (TLC) method for large-scale urine screening[1]. We now wish to report the development of a new method for the quantitative liquid–solid extraction of acidic, basic and neutral drugs from lyophilized urine samples.

This technique eliminates the partition of a given drug between two liquid phases, thus significantly increasing the recovery of the material. Furthermore, water soluble drugs that currently escape detection, or are recovered in negligible quantities by standard liquid–solid extraction procedures, may now be quantitatively recovered and assayed accordingly.

Future papers in this series will deal specifically with this and other aspects of the use of lyophilization, liquid–solid extraction as a general technique in forensic chemistry. The present paper describes its application to the detection of drugs of abuse in urine analysis.

EXPERIMENTAL

Apparatus

A Virtis freeze drier, No. 10, with a condenser capacity of 40 pounds. Chromatography was done on Brinkmann silica gel plates, 250 μ, 20 × 20 cm, and on Eastman

Chromatogram sheet 6060, silica gel 100 mμ. The lyophilization is performed in 25-ml erlenmeyer flasks.

Reagents

All chemicals and solvents are of reagent grade and used without further purification. The preparation of the chromatographic spray reagents has been described elsewhere[1,2].

Lyophilization procedure

Place 5.0 ml of a sample in a 25-ml erlenmeyer flask. Add 2.0 ml of glacial acetic acid. Briefly shake the flask and then place it in the freeze drier. The contents are frozen to −50°. The chamber is then evacuated to a residual pressure of 1×10^{-4} atm. The sample is then heated to 80° for a minimum of 1 h. Following lyophilization, the sample is removed from the freeze drier and allowed to cool to room temperature and then extracted.

Extraction procedure I

In the course of development, we have established two different methods of extraction. Both the acidic/neutral (A/N) and basic (B) drugs may be extracted together and simultaneously chromatographed in a single solvent system. Add 3 ml of methanol to the lyophilized sample and thoroughly mix. The flasks are allowed to stand for 1 h. Add 9 ml of acetone, shake briefly and filter the resulting suspension into a 20-ml beaker. The precipitate is washed once with 2 ml of methanol (1:3), acetone solution and then discarded. The filtrate is labeled I, then evaporated to dryness on a hot plate or steam bath. The resulting residue is suitable for chromatography[2].

Extraction procedure II

If separation of the A/N from the basic drugs is desired, add 10 ml of ether to the dried residue of extraction procedure I. The sample is allowed to stand for 30 min. The supernatant is carefully filtered into a 20-ml beaker labeled A/N, and evaporated to dryness on a steam bath or hot plate. This solvent wash contains the A/N drugs. Although the acetate salts of morphine and the other basic compounds are not appreciably soluble in ether, small amounts of certain acetates (codeine, quinine, methadone, etc.) will occasionally be carried over into the A/N fraction. It should be emphasized that this does not in any way interfere with the detection and analysis of either class of compound since both may be simultaneously chromatographed and independently visualized as indicated.

Chromatography

For the first extraction procedure, add enough methanol to the beaker to redissolve the residue. An appropriate aliquot of the methanolic solution is then spotted on the thin-layer plate (Brinkmann). The plate is then developed to a height of 12 cm in the system ethyl acetate–methanol–ammonium hydroxide (85:10:3).

Where the second extraction procedure has been used, the residues of both the A/N and the basic fractions should be redissolved in methanol. The basic plate is spotted and developed as indicated above, while the A/N plate may be developed using either 250 μ SG plates or the Eastman Chromatogram sheet 6060.

With the SG 250 μ plate, development should be to a height of 10 cm in the solvent system hexane–ethanol (85:15). With the Eastman plates, the development is to a height of 10 cm in the system hexane–ethanol (92:8). Because of the presence of some basic drugs in the A/N fraction, a series of basic drug standards should be run with the acid/neutral standards.

Visualization of the thin-layer plates

(1) All plates should be thoroughly dried before visualization. The following series of chromophoric reagents is applied to the plate used following extraction procedure I. This system is essentially as previously described[2].

(a) Ninhydrin solution followed by 2-min exposure to long-wave (360 mμ) UV light, amphetamine and methamphetamine with both be visualized. (b) Diphenylhydrazone solution. (c) Mercuric sulfate solution. Barbiturates and glutethemide will appear as darkish spots. (d) The plates should be heated for 1–2 min under a stream of hot air or placed in an oven heated to 75° for 2 min. Phenothiazine drugs and their sulfoxide metabolites will appear as reddish or violet spots. (e) 1% methanolic sulfuric acid followed by examination of the plate under UV light (360 mμ) will reveal the presence of quinine. (f) Iodoplatinate solution. The plate should now be inspected for the presence of basic drugs. (g) Dragendorff reagent. The final inspection of the plate should be made 5–10 min after application of the Dragendorff solution.

(2) The following procedure should be used with samples extracted by the second method.

TABLE I

R_F VALUES OF A SERIES OF BASIC AND ACIDIC NEUTRAL COMPOUNDS

Solvents: System 1 = ethyl acetate–methanol–ammonium hydroxide (85:10:3), (modified Davidow). 12-cm development on Brinkmann SG plates. System 2 = hexane–ethanol (92:8). 12-cm development on Eastman Chromatogram sheet 6060.

Compound	*System 1*	*Microdot*	*System 2*
Codeine	0.53	(0.52)	0.13
Methadone	0.96	(0.94)	0.31
Morphine	0.28	(0.32)	0.14
Meperidine (Demerol)	0.90	(0.84)	
Methapyrilene	0.92	(0.86)	
Nicotine	0.88	(0.78)	0.33
Propoxyphene (Darvon)	0.97	(0.96)	0.87
Quinine	0.74	(0.52)	0.11
Amphetamine	0.73	(0.64)	0.14
Methamphetamine	0.65	(0.58)	
Amitriptyline (Elavil)	0.92	(0.86)	0.47
Imipramine (Tofranil)	0.91	(0.82)	
Methaqualone (Quaalude)	0.95	(0.92)	
Chlorpromazine (Thorazine)	0.94	(0.82)	0.31
Prochlorperazine (Composine)	0.85	(0.64)	
Diazepam (Valium)	0.94		0.66
Amobarbital	0.02		0.47
Pentobarbital	0.87		0.43
Phenobarbital	0.53		0.32
Secobarbital	0.88		0.48
Glutethimide (Doriden)	0.94		0.53

For the basic plate: (a) Ninhydrin solution with UV exposure. (b) 1 % methanolic sulfuric acid. (c) Heat for 1–2 min under a hot air stream or place in an oven set at 75° for 2 min. (d) Examine under UV light (360 mμ). (e) Iodoplatinate reagent. (f) Dragendorff reagent.

For the acid/neutral plate: (a) Diphenylcarbazone solution. (b) Mercuric sulfate solution. (c) UV examination for the detection of quinine. (d) Dragendorff reagent for the detection of basic drugs carried over in the A/N extraction. (e) Vanillin solution with gentle heat for the detection of carbamates.

Table I gives the R_F values of a series of basic and acidic/neutral compounds in the two solvent systems described. The colors obtained from the visualization of the plates with the chromophoric reagents have been described elsewhere[1,2].

RESULTS AND DISCUSSION

The results obtained with the techniques described in this paper illustrate both the ease of applicability and the significant advantages of the lyophilization and liquid–solid extraction procedures. The lyophilization step provides a convenient method by which any volume of urine may be conveniently concentrated and stored. For the purpose of large-scale urine screening, upwards of five-hundred urine samples may be easily lyophilized at the same time. The direct extraction of the lyophilized residue affords a highly efficient extraction of all classes of drugs, eliminating the partition of material between two liquid phases. The determining factor now becomes the rate of solubility of a particular drug in the solvent employed. This is why the methanol suspension is allowed to stand for 1 h.

The addition of acetone prior to filtration precipitates many inorganic salts, thus yielding a much cleaner extract. Subsequent analysis of the extracts is greatly facilitated by performing chromatography simultaneously on both the acid/neutral and basic drugs in a single solvent system.

The authors wish to re-emphasize that the results obtained from TLC are not to be considered definitive for the presence of any compound, and that additional independent analysis should be carried out. Many basic compounds are readily confirmed by the technique of microcrystallography following their elution from the thin-layer plate. Spectrophotometric analysis may also be performed on material eluted from thin-layer plates.

Although the present paper deals primarily with the use of lyophilization and liquid–solid extraction for the detection of abuse in urine, future papers to be published in this series will describe the applicability of this technique to forensic analysis in general.

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