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AN ULTRA RAPID METHOD FOR THE EXTRACTION OF DRUGS FROM BIOLOGICAL FLUIDS

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SUMMARY

A rapid quantitative method is described for the extraction of amphetamine, methylamphetamine, pethidine and methadone. It has the advantages that the complete analysis is carried out in one vessel and, without an evaporation step, volatility problems are overcome. The method constitutes a ten-fold saving in extraction time over accepted procedures without concommitent losses in accuracy or sensitivity. The use of this method for the extraction of other drugs and its application to gas–liquid and thin-layer chromatography is discussed.

INTRODUCTION

Most methods in use today for the analysis of drugs in biological fluids involve three basic steps *viz.* solvent extraction, concentration, and finally detection or measurement. Considerable time and research effort has been spent over the past ten years in the development of more refined methods for the measurement of drugs in biological material. New gas–liquid chromatographic (GLC), techniques, with improved specificity and sensitivity, have been frequently reported. Few workers, however, have concentrated on simplifying the initial extraction procedures, which invariably take longer than the chromatographic step.

Toxicological drug extraction procedures are numerous, and include steam distillation and ion exchange, but the most usual is direct solvent extraction of samples. Such procedures may require pH adjustments on the aqueous phase followed by one or more extractions with excess of organic solvent for periods ranging from 5 min to 1 h. Thereafter, further purification steps on the organic phase may be necessary before this can be concentrated under reduced pressure or in a stream of nitrogen to a suitable volume for analysis. Each stage is time consuming and drug losses due to adsorption on to glassware, incomplete transfer of solvents and evaporation of volatile compounds may easily lower the recovery and therefore the sensitivity.

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By contrast, methods which eliminate prior extraction of drugs have been reported for barbiturates and amphetamines in urine[1,2]. These involve the direct injection of urine samples on to gas chromatographic columns but are subject to interference from endogenous compounds and lack sensitivity.

The determination of barbiturates in finger-prick blood samples has been carried out using a method which minimises the extraction procedure[3]. 100 μl of blood were mixed with an equal volume of chloroform on a vortex mixer, and after centrifugation, an aliquot of the organic phase was withdrawn and injected on to a GLC apparatus. Although this was said to be an improved modification of a previous method[4], no internal standardisation was used, and consequently even with multiple analysis the accuracy of the procedure was suspect. More recently, a third method, based on the above, but incorporating an internal standard, has been developed for the measurement of barbiturates and related compounds in small samples of blood[5].

We present here a general method for the rapid extraction of lipid-soluble drugs from body fluids and a description of its application to the detection and measurement in urine of four common drugs of addiction. This involves the addition of a very small amount of chloroform containing a suitable internal standard to a larger volume of aqueous phase. After mixing and centrifugation, a few microlitres of solvent are withdrawn and injected into a GLC apparatus or spotted on to a thin-layer plate. Volatility problems of solvent and drug are overcome, since the solvent is covered by aqueous phase throughout and no evaporation step is involved.

EXPERIMENTAL

Apparatus

Standard laboratory apparatus is used throughout. The complete extraction procedure is performed in glass-stoppered centrifuge tubes of 10-ml capacity, selected to have fine-tapered bases. The stoppers are water lubricated.

Reagents

All reagents used were obtained from Hopkin and Williams, except triptycene (which was obtained from RALPH N. EMANUEL), amphetamine sulphate (from Smith Kline and French), methylamphetamine hydrochloride (from May and Baker), pethidine hydrochloride (from McFarlane Smith), and methadone hydrochloride (from Burroughs Welcome).

Method

Two millilitres of urine were pipetted into a centrifuge tube and made alkaline with 0.2 ml 5 *N* NaOH. Fifty microlitres of internal standard made up in chloroform were added and mixed on a vortex mixer for about 30 sec. After centrifugation at 2,000 r.p.m. for 1 min, 1–4 μl of organic phase were carefully withdrawn into a microsyringe through the aqueous phase and injected on to a GLC apparatus.

Some difficulty was experienced with samples which formed an intermediate cloudy layer at the phase boundary, but with further centrifugation for 2 min this problem was overcome.

Chromatography

Amphetamine and methylamphetamine. A Pye 104 gas chromatograph equipped with a dual-flame ionisation detector was used and the signal recorded on a Honeywell recorder. The column was 80–100 mesh AW DMCS-treated Chromosorb G, coated with 10% KOH and 10% Apiezon L, packed into a 5 m × 4 mm I.D. silanised glass tube. It was operated at 160° with an injection port temperature of 210°. Gas flow rates were: hydrogen, 30 ml/min; nitrogen, 30 ml/min; air, 400 ml/min. Retention times under these conditions were: amphetamine, 4.7 min; methylamphetamine, 6.5 min; N,N-diethylaniline, 8.1 min.

Pethidine and methadone. A Perkin-Elmer F11 gas chromatograph with a dual-flame ionisation detector unit was used with a Hitachi recorder. The column used was 2.5% E-301 coated on HMDS-treated Chromosorb G, 80–100 mesh, and packed into 2 m × 1/8-in. O.D. stainless-steel tubing. The oven temperature was 170° for pethidine and 200° for methadone with an injection port temperature of 300°. Gas flow rates were: hydrogen, 30 ml/min; nitrogen, 30 ml/min; air, input pressure 25 p.s.i. Retention times under these conditions were: pethidine, 5.2 min; diisopropyl phthalate, 2.4 min; methadone, 5.2 min; triptycene, 8.0 min.

Measurement

Calibration curves were plotted prior to analysis by injecting drug standards made up in the same internal standard solution used for extraction. The ratio of (peak height of drug)/(peak height of internal standard) was calculated and plotted against drug concentration (Figs. 1 and 2).

Amphetamine and methylamphetamine. Concentrations of 20, 40, 100, 160 and 200 μg/ml of drug were made up in a solution of 100 μg/ml of N,N-diethylaniline in chloroform. These standards represented 1, 2, 5, 8 and 10 μg, respectively, of the drug in the final 50 μl, assuming 100% recovery.

Pethidine. The internal standard solution was a solution of diisopropyl phthalate, 20 μl in 200 ml of chloroform. 100 mg of pethidine hydrochloride were dissolved in 50 ml of internal standard solution, and the free base was liberated by titration with a few drops of 5 *N* sodium hydroxide. This solution was diluted with internal standard solution to give 2, 1.6, 1.2, 0.8, 0.4, 0.2, and 0.04 mg/ml. These standards corresponded to 100, 80, 60, 40, 20, 10 and 2 μg of pethidine in the final 50 μl, assuming 100% recovery.

Methadone. The procedure described for pethidine was carried out using methadone hydrochloride to produce standards of the same concentrations as above. The internal standard solution was 100 mg of triptycene in 250 ml of chloroform.

Recoveries were calculated for all drugs by adding known amounts to control urine. After extraction and gas chromatography the results were compared with those derived after injection of the standards. Five determinations were carried out at each concentration.

RESULTS

Figs. 3 and 4 show typical chromatograms of extracts of urine containing methadone and amphetamine, respectively, using this method. The solvent peak is small and there are no interfering peaks from endogenous urinary constituents.

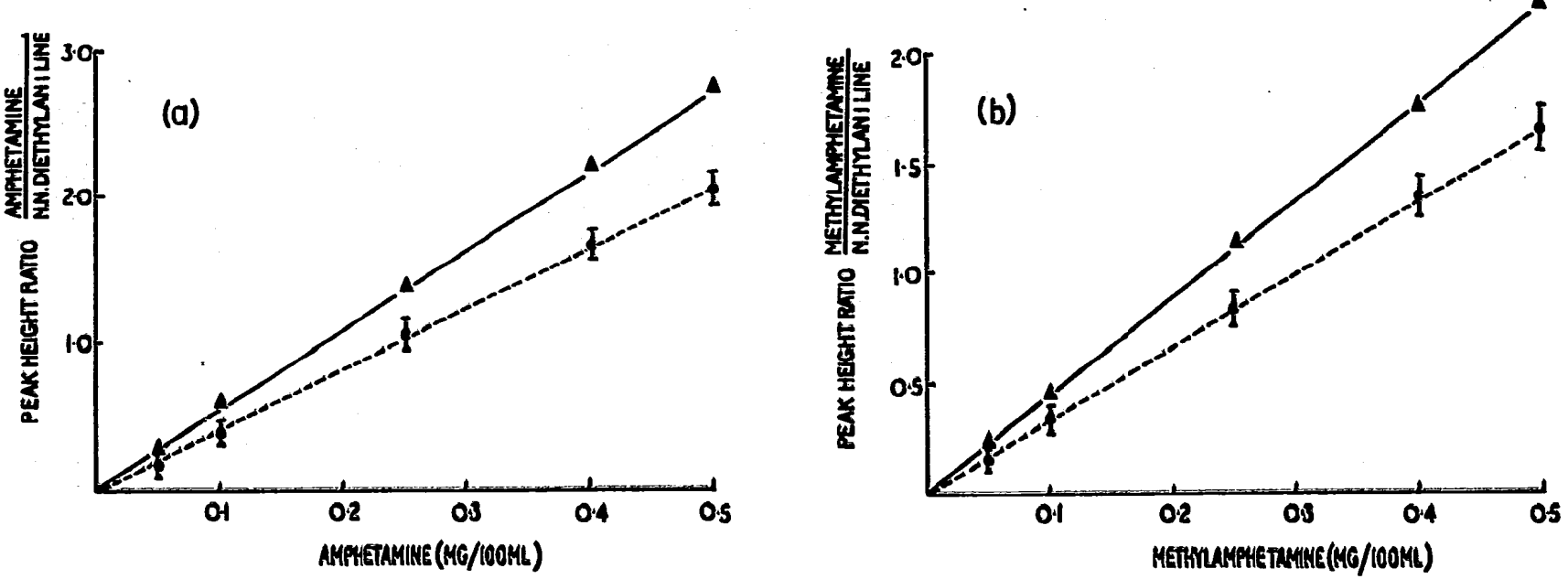


Fig. 1. Calibration graphs relating peak heights of (a) amphetamine and N, N-diethylaniline and (b) methylamphetamine and N, N-diethylaniline. ▲——▲, standards; ●- - -●, after extraction.

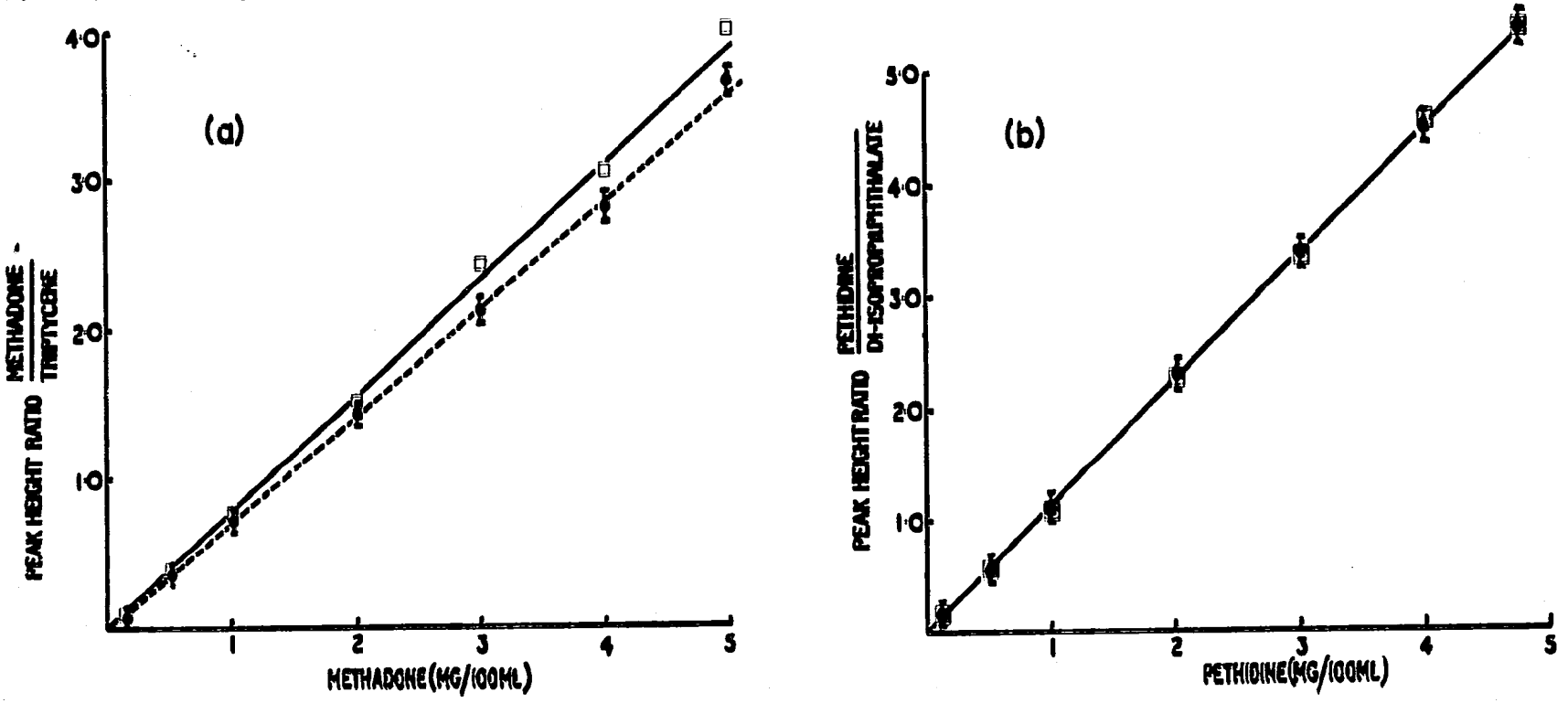
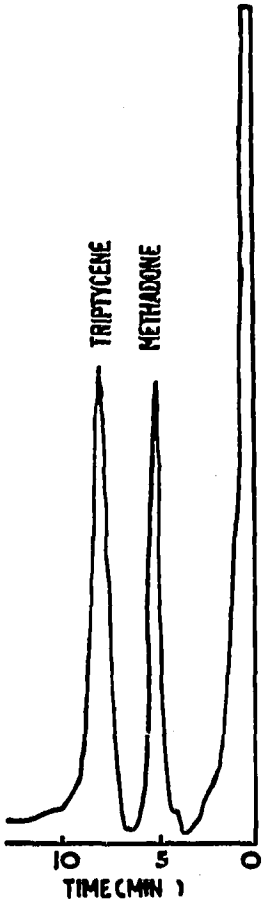


Fig. 2. Calibration graphs relating peak heights of (a) methadone and triptycene and (b) pethidine and diisopropyl phthalate. □——□, standards;



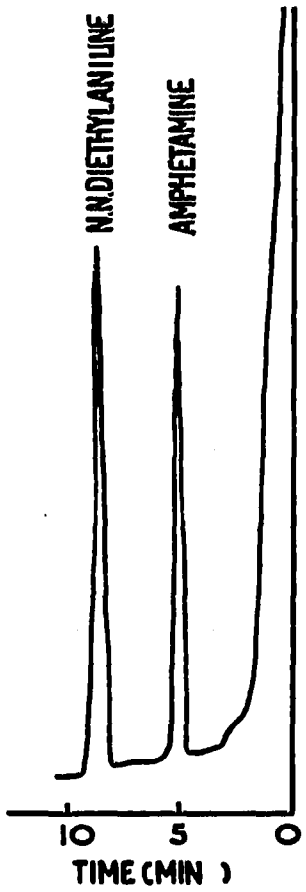


Fig. 3. Gas chromatogram of a chloroform extract of 2 ml urine containing 30 μg of methadone.

Fig. 4. Gas chromatogram of a chloroform extract of 2 ml urine containing 5 μg of amphetamine.

Recoveries are represented graphically (Figs. 1 and 2) in the form of standard and extraction calibration curves and also in Table I. Implicit in the calculation of the recoveries are the assumptions that there is no volume change in either phase after equilibration and no transfer of internal standard from organic to aqueous phase.

TABLE I

RECOVERY OF DRUGS FROM 2 ml OF URINE

Five determinations were made at each concentration.

	% *Recovery*	*Conc. range* (μg)
Amphetamine	78 ± 5	1–10
Methylamphetamine	80 ± 5	1–10
Methadone	83 ± 3	1–50
Pethidine	99 ± 1	1–50

DISCUSSION

The results obtained so far have indicated that this method is sensitive, accurate and reproducible for the analysis of amphetamine, methylamphetamine, pethidine

and methadone in urine. The most important advantage over previous methods is its speed and simplicity. For example, the complete analysis of a urine sample for amphetamine takes 12 min as compared to 30–40 minutes when using the method of BECKETT AND ROWLAND[6]. The reduced amount of glassware used minimises considerably the possibility of losing drugs by adsorption on to glass, and the elimination of an evaporation stage prevents the volatilisation of certain drugs, *e.g.* amphetamine[7], during such a step. Further, solvent impurities are not concentrated into a small volume prior to analysis and therefore solvents need not be redistilled before extraction. Endogenous urinary impurities, which may interfere with subsequent chromatography, are not extracted into the small volume of solvent used and consequently extracts are analytically clean.

The technique has been applied to a pharmacokinetic study of amphetamine excretion in human urine[8], a dissolution rate analysis of sustained relase fenfluramine (Ponderax) capsules[8], and to the screening of addict urines[9]. The feasibility of detecting drugs by thin-layer chromatography after this simple extraction has already been demonstrated in the case of pethidine. A poisoning by pethidine was investigated by this method and pethidine and norpethidine were found by thin-layer chromatography of a 25-μl aliquot of the chloroform extract of 2 ml of urine. These findings were subsequently confirmed by GLC analysis of the same extract[10].

It is envisaged that this type of procedure will be applicable to the analysis of many other drugs provided they are present in sufficient quantities within the biological sample under investigation and further that they have partition coefficients sufficiently in favour of the organic phase. Several variables have not yet been investigated, for example the pH of the aqueous phase and the nature of the extraction solvent. This latter need only be denser than the aqueous phase and must not form complexes with the drugs sought. Given that all these conditions will be obtainable for other drugs, the basic procedure should represent a considerable advance in the field of toxicological drug analysis.

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