J Forensic Sci, Apr. 1977, Vol. 22, No. 2

L. P. Hackett,[1] *B.S. and L. J. Dusci,*[1] *B.S.*

The Use of Buffered Celite Columns in Drug Extraction Techniques and Their Proposed Application in Forensic Toxicology

Although forensic toxicology as a science has been with us for many years, problems are still encountered with quantitative drug extraction techniques from tissues. These techniques are important for, after death, tissue drug levels must be quantitated and interpretations made that may have important legal aspects.

Many published methods have drawbacks such as final reductions to a small volume to minimize the loss of volatile compounds or the need for distillation of tissues; some methods are specifically for a single drug and have not been investigated for a large range of compounds [*1-5*]. These methods require extreme care to ensure accurate small volumes, take several hours for extractions, or require much tissue manipulation. It is because of these problems that investigations are being carried out to find an efficient, clean extraction procedure that will give good recoveries for most drugs that are readily available to the general public.

The principle of the extraction technique used is based on a simple solvent extraction of the drugs by elution through an acidic or basic buffered celite column. Quantitation is achieved by the addition of a standard cholesterol solution at the final stage and analysis by temperature-programmed gas-liquid chromatography (GLC). The results are compared with standard solutions of the drugs under investigation containing the standard cholesterol solution.

Materials and Methods

A Hewlett-Packard Series 5700 A gas chromatograph equipped with a flame ionization detector was used. The column was a 4-ft (1.2-m) by 0.25-in. (6.35-mm) outside diameter glass coiled tube, packed with 3% OV-17 (Supelco) on Gas Chrom Q 80-100 mesh (Supelco). The instrument settings were as follows: injection port temperature, 300°C; nitrogen carrier gas flow rate, 60 ml/min; hydrogen flow rate, 60 ml/min; and air flow rate, 240 ml/min. As we were using cholesterol as an internal standard all the work was carried out using a temperature program which was started at 150°C, held for 2 min, and then increased at 8°C/min to 290°C. This temperature was then held isothermally for 8 min.

The reagents used were 1*M* HCl, 1.8*M* H_2SO_4, 0.45*M* NaOH, 5*M* NaOH, sodium dihydrogen orthophosphate, disodium tetraborate (borax), celite 545 AW (Supelco), sodium carbonate, diethyl ether, dichloromethane, hexane, and acetonitrile. All reagents

Received for publication 9 Aug. 1976; revised manuscript received 13 Oct. 1976; accepted for publication 14 Oct. 1976.

[1]Chemists, Toxicology Section, State Health Laboratories, Perth, Western Australia.

used were of analytical grade and manufactured by British drug houses unless otherwise stated.

All the drugs investigated were made up to an accurate concentration of 5 to 10 mg in 10 ml of ethanol. Standard cholesterol solution was prepared at a concentration of 0.2 g/litre in dichloromethane.

Celite Preparations

Ten grams of disodium tetraborate was dissolved (with heating) in 50 ml water. The solution was added to 100 g celite 545 in a beaker and mixed well with a glass rod. The mixture was allowed to air dry for several days before use. This is referred to as borate/celite in the text. Ten grams of sodium dihydrogen orthophosphate was dissolved (with heating) in 50 ml water; this solution was added to 100 g celite 545 in a beaker and mixed well with a glass rod. The mixture was allowed to air dry for several days before use and is referred to as phosphate/celite in the text.

Procedures

Five extraction procedures have been investigated: Procedures 1 and 2 for basic drugs, Procedures 3 and 4 for acidic drugs, and Procedure 5 for neutral drugs. In a 100-ml beaker, 6 g borate/celite for basic or neutral drugs, or 6 g phosphate/celite for acidic drugs, is added to 4 ml water containing 40 μl (20 to 40 μg) of the standard solution under examination. The contents are mixed well with a glass rod and transferred to a cotton-wool-plugged glass chromatography column, 30 cm long by 2 cm inside diameter. The column is tapped vertically on the bench to ensure good, even packing.

Procedure 1—Diethyl ether is passed through the borate/celite column at a rate of approximately 4 ml/min and 30 ml is collected. The ether is extracted with 6 ml 1.8*M* H_2SO_4 and the acid is transferred to a 10-ml pointed centrifuge tube. This is now placed in a 60 °C water bath with an air blow until the volume is reduced to approximately 5 ml. This step ensures that any dissolved ether present is expelled. The solution is made alkaline by the addition of 4.5 ml 5*M* NaOH and cooled. A total of 200 μl of cholesterol standard in dichloromethane is added and then the tube is shaken vigorously by hand and centrifuged at 1000 rpm for 2 min. A 5-μl sample is analyzed directly from the bottom organic layer and compared with a mixture of 1 μl of the standard and 5 μl of the cholesterol solution.

Procedure 2—Diethyl ether is passed through the borate/celite column at a rate of approximately 4 ml/min and 30 ml is collected. The ether is extracted with 6 ml 1.8*M* H_2SO_4. The acid is made alkaline with excess solid sodium carbonate and re-extracted with 30 ml diethyl ether. The ether is filtered into a tube, where 200 μl of standard cholesterol solution is added, and then taken to dryness. The residue is dissolved in 200 μl ethanol, and 5 μl is injected onto the GLC for analysis.

Procedure 3—In a 100-ml beaker, 6 g phosphate/celite is added to 4 ml water containing 40 μl of the standard solution under examination. The contents are mixed well and the column prepared as for Procedures 1 and 2. The column is washed with 30 ml hexane and the compounds are then eluted with dichloromethane at a rate of approximately 4 ml/min until 30 ml is collected. The dichloromethane is extracted with 10 ml 0.45*M* NaOH, the soda is made acidic with 5 ml 1*M* HCl and re-extracted with 30 ml dichloromethane. The organic layer is filtered, 200 μl standard cholesterol is added, and the mixture is taken to dryness. The residue is dissolved in 200 μl ethanol for examination by GLC.

Procedure 4—Diethyl ether is passed through the borate/celite column at a rate of approximately 4 ml/min and 30 ml is collected. The ether is extracted with 6 ml 1.8*M* H_2SO_4 and the acid is rejected. The ether is washed with 10 ml water, filtered, and taken

to dryness; the residue is redissolved in 10 ml acetonitrile saturated with hexane and 10 ml of hexane; the mixture is shaken; and the hexane is rejected. The acetonitrile is taken to dryness, redissolved in 20 ml dichloromethane and 10 ml 0.45*M* NaOH, and shaken well. The dichloromethane is rejected, 5 ml 1.0*M* HCl is added to the alkali, and the mixture is extracted with 25 ml dichloromethane. This is filtered, 200 μl standard cholesterol is added, and the mixture is taken to dryness. The residue is dissolved in 200 μl ethanol for analysis by GLC.

Procedure 5—Procedure 5 is the same as Procedure 4 except that the dichloromethane, after extraction with NaOH, is filtered and taken to dryness for the analysis of neutral drugs by GLC.

Examination of Tables

The results obtained from the behavior of each drug under the extraction procedures described are shown in Tables 1-3. Table 1 is a list for basic drugs, Table 2 for acidic drugs, and Table 3 for neutral drugs.

For all tables a triple positive sign (+ + +) indicated greater than 75% recovery, a double positive sign (+ +) indicated 50 to 75% recovery, a single positive sign (+) indicated 10 to 50% recovery, and a negative sign (−) indicated less than 10% recovery. Indications are given where breakdown or volatility is suspected.

Results

Basic Drugs

The use of a direct extraction step incorporating a small volume of organic solvent gave very good quantitative results for most of the basic drugs tested, including the volatile ones. Difficulty in quantitation by GLC may occur if there is a large excess of the basic compound over the internal standard used, but this could be compensated by adding a larger volume of the cholesterol solution and rerunning the sample on the GLC. Several drugs were found to be heat or acid labile, or both, in Procedure 1, but the heating to remove dissolved ether must be included to obtain good quantitative results. Low results obtained for oxazepam and nitrazepam could be due to the high pH used in the direct extraction procedure, and possibly the use of carbonate solution to give a pH of approximately 10 would enhance their recoveries without detriment to the other bases.

Procedure 2 is the method of preference as the sample may be taken to dryness and reconstituted to a known volume for quantitation isothermally after identification has been achieved on the temperature program. Unfortunately, the use of this procedure results in a significant loss of volatile drugs.

Acidic Drugs

The common acidic drugs are well extracted by both Procedures 3 and 4, but the recovery of several of the less common acids is poor. The use of the acidic phosphate/celite column does not significantly improve the recovery of acids. Specific methods of analysis for some of the less common acids in biological fluids have been published [*6*, *7*] and may be applicable to tissue analysis with slight modifications using direct extractions.

Neutral Drugs

Good recoveries of most neutral drugs have been found. It may be noted that the

TABLE 1—*Recoveries of basic drugs by Procedure 1, which incorporates extraction into a small volume, and by Procedure 2, where the extract is taken to dryness.*

Drug	Procedure 1	Procedure 2
Alprenolol	+ + +	+ + +
Amitriptyline	+ + +	+ + +
Amphetamine	+ + +	‡
Atropine	+ + +	+ + +
Caffeine	+	+
Chlorcyclizine	+ + +	+ + +
Chlordiazepoxide	+ + +	+ + +
Chloroquine	+ + +	+ + +
Chlorphentermine	+ + +	‡
Chlorpromazine	+ + +	+ + +
Cocaine	+	+ + +
Codeine	+ + +	+ + +
Desipramine	+ + +	+ + +
Dextromoramide	+ + +	+ + +
Dextropropoxyphene	+ + +	+ + +
Diazepam	+ + +	+ + +
Dibenzepin	+ + +	+ + +
Diethylpropion	+ + +	‡
Diphenhydramine	+ + +	+ + +
Diphenylpyraline	*	+ + +
Doxepin	+ + +	+ + +
Ephedrine	+ + +	‡
Fenfluramine	+ + +	‡
Hyoscine	+ + +	+ + +
Imipramine	+ + +	+ + +
Lidocaine	+ + +	+ + +
Medazepam	+ + +	+ + +
Meperidine	+ + +	+ + +
Methadone	+ + +	+ + +
Methaqualone	+ + +	+ + +
Methamphetamine	+ + +	‡
Methylphenidate	*	‡
Morphine	–	–
Nicotine	+ + +	‡
Nikethimide	+ + +	‡
Nitrazepam	–	+ + +
Nortryptyline	+ + +	+ + +
Orphenadrine	*	+ + +
Oxazepam	–	+ +
Oxprenolol	+ + +	+ + +
Pentazocine	+ + +	+ + +
Pheniramine	+ + +	+ + +
Phenmetrazine	+ + +	‡
Phentermine	+ + +	‡
Promazine	+ + +	+ + +
Propericiazine	+ + +	+ + +
Protriptyline	+ + +	+ + +
Quinidine	+ + +	+ + +
Quinine	+ + +	+ + +
Strychnine	+ + +	+ + +
Thioridazine	+ +	+ +
Tranylcypromine	+ + +	‡
Trifluoperazine	+ + +	+ + +
Trimeprazine	+ + +	+ + +
Trimipramine	+ + +	+ + +

\+ = 10 to 50% recovery.
\+ + = 50 to 75% recovery.
\+ + + = 75 to 100% recovery.
– = less than 10% recovery.
* = variable recovery because of heat/acid lability.
‡ = variable recovery because of volatility.

TABLE 2—*Recovery of acidic drugs by Procedure 3, which consists of elution through a phosphate/celite column before extraction, and by Procedure 4, which consists of elution through a borate/celite column before extraction.*

Drug	Procedure 3	Procedure 4
Amobarbital	+ + +	+ + +
Butobarbital	+ + +	+ + +
Diphenylhydantoin	+ + +	+ + +
Ethosuximide	‡	‡
Mephobarbital	+ + +	+ + +
Pentobarbital	+ + +	+ + +
Phenobarbital	+ + +	+ + +
Phenylbutazone	−	+
Primidone	+	+
Salicylamide	+ +	−
Secobarbital	+ + +	+ + +
Sulthiame	+ +	+
Theophylline	+	−

− = less than 10% recovery.
+ = 10 to 50% recovery.
+ + = 50 to 75% recovery.
+ + + = 75 to 100% recovery.
‡ = variable recovery because of volatility.

TABLE 3—*Recoveries of neutral drugs by Procedure 5, which consists of a borate/celite system.*

Drug	Procedure 5
Caffeine	−
Carbamazepine	+ + +
Glutethimide	+ + +
Meprobamate	+ + +
Mephenytoin	+ + +
Methyprylon	+ +
Oxyphencyclimine	+
Phenacetin	+ + +
Primidone	−

− = less than 10% recovery.
+ = 10 to 50% recovery.
+ + = 50 to 75% recovery.
+ + + = 75 to 100% recovery.

normal hexane/alcohol/water clean-up procedure has been substituted by hexane/hexane-saturated acetonitrile. It was found that the use of hexane/alcohol/water gave low recoveries of many neutral drugs.

The choice of solvents is imperative, for if ether is used in the final stage, neutral drugs such as glutethimide and meprobamate are transferred into the sodium hydroxide phase and not separated from the acids. It may be noted that acetaminophen is not listed; however, it is recovered quantitatively from the borate/celite column after elution with ether and remains in the acetonitrile after washing with hexane. If it is to be quantitated, the acetonitrile can be taken to dryness, and the acetaminophen acetylated [*8*] and analyzed without separation of the acid fraction.

Application and Discussion

The aim of these experiments was to develop a rapid and efficient procedure for the estimation of drugs in tissues. A number of papers have been published for urine screening using single solvent extraction at pH 9.5. Either sodium borate [*9*] or ammonium hydroxide/chloride [*10*] was used to attain this pH. We have found borate to be suitable, giving good recoveries of most acidic, basic, and neutral drugs. A number of different solvents have been used, including chloroform, hexane, and ethyl acetate, but ether has proved to be the best and most consistent. Several of the procedures have been joined into a single extraction step using only 5 g of tissue. The proposed method is shown in Fig. 1. The addition of sodium sulfate enhances the solvent elution through the column, especially when fatty tissue or bile is used.

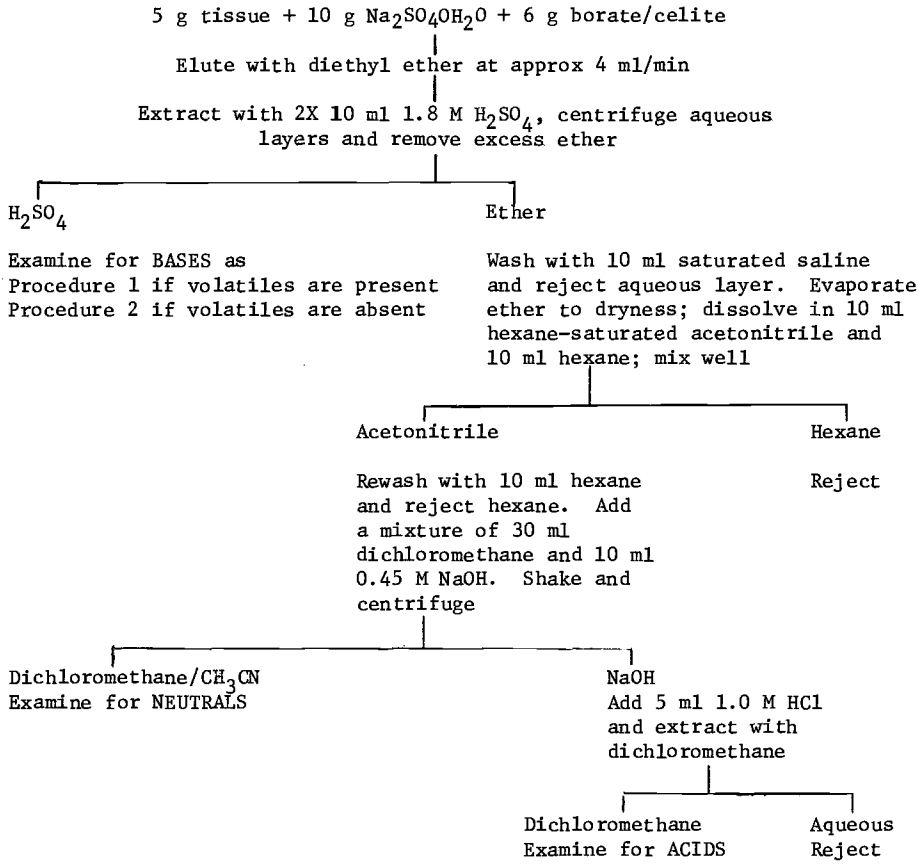

FIG. 1—*Proposed procedure for the analysis of acidic, basic, and neutral drugs in tissues.*

A series of recoveries has been carried out from liver samples with a variety of drugs added at a level of 1 mg/100 g. The drugs include amobarbital, pentobarbital, phenobarbital, diphenylhydantoin, glutethimide, diphenhydramine, methaqualone, amitriptyline, nortriptyline, oxazepam, diazepam, dibenzepin, quinine, strychnine, and chlorpromazine. In all cases the recoveries of the drugs were similar to those obtained using the aqueous reference standards (Tables 1-3).

Many proposed methods give good recoveries from aqueous solutions, or from

biological samples with drugs added to them, but the criteria and test of a procedure must ultimately be carried out in comparison with other methods on actual postmortem tissues from overdose cases. Detailed further studies of these comparisons are in preparation and will be reported subsequently. At present, the results obtained have been reproducible and in most cases much greater than when using conventional methods, including the direct extraction technique [*11*]. The advantages of the proposed technique are that little tissue manipulation or expertise is required; small sample weights and small volumes of solvents are acceptable; and rapid and good recoveries of a large number of drugs, including those that are volatile, are achieved. These assets make the method suitable for routine work in forensic toxicology.

Summary

The recoveries of acidic, basic, and neutral drugs through buffered celite columns are reported. The methods are rapid, simple, clean, and efficient, especially with compounds having a high volatility.

Acknowledgment

The authors wish to acknowledge the Commissioner of Public Health, Western Australia, for allowing this work to be published.

References

[*1*] Finkle, B. S., Cherry, E. J., and Taylor, D. M., "A G.L.C. Based System for the Detection of Poisons, Drugs and Human Metabolites Encountered in Forensic Toxicology," *Journal of Chromatographic Science,* Vol. 9, 1971, pp. 393–419.

[*2*] Tompsett, S. L., "Extraction Procedures in Clinical Toxicology," *Analyst,* Vol. 93, No. 1112, 1968, pp. 740–748.

[*3*] Feldstein, M. and Klendshoj, N. C., "The Analysis of General Unknowns in Toxicology," *Analyst,* Vol. 78, No. 922, 1953, pp. 43–47.

[*4*] Bogan, J., Rentoul, E., Smith, H., and Weir, W. P., "Homicidal Poisoning by Strychnine," *Journal of the Forensic Science Society,* Vol. 6, No. 4, 1966, pp. 166–169.

[*5*] Henwood, C. R., "Analysis of Amitriptyline and Its Analogues in Body Tissue," *Journal of the Forensic Science Society,* Vol. 15, No. 2, 1975, pp. 147–151.

[*6*] Hackett, L. P. and Dusci, L. J., "Gas Liquid Chromatographic Determination of Sulthiame in Human Plasma," *Clinica Chimica Acta,* Vol. 66, 1976, pp. 443–447.

[*7*] Dusci, L. J. and Hackett, L. P., "Gas Liquid Chromatographic Determination of Theophylline in Human Plasma," *Journal of Chromatography,* Vol. 104, 1975, pp. 147–150.

[*8*] Hedges, A., Kaye, C. M., Mackay, W. P., and Turner, P., "A Comparison of the Absorption of Effervescent Preparations of Paracetamol and Penicillin V (Phenoxymethylpenicillin) with Solid Dose Forms of These Drugs," *Journal of Clinical Pharmacology,* Vol. 14, No. 7, 1974, pp. 363–368.

[*9*] Goldbaum, L. R., Santinga, P., and Dominguez, A. M., "A Procedure for the Rapid Analysis of Large Numbers of Urine Samples for Drugs," *Clinical Toxicology,* Vol. 5, No. 3, 1972, pp. 369–379.

[*10*] Blass, K. G., Thibert, R. J., and Draisey, T. F., "A Simple, Rapid Thin-Layer Chromatographic Drug Screening Procedure," *Journal of Chromatography,* Vol. 95, 1974, pp. 75–79.

[*11*] Hackett, L. P., Dusci, L. J., and McDonald, I. A., "Extraction Procedures for Some Common Drugs in Clinical and Forensic Toxicology," *Journal of Forensic Sciences,* Vol. 21, No. 2, 1976, pp. 263–274.

Toxicology Section
State Health Laboratories
G.P.O. Box F312
Perth, Western Australia 6001