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

A Comparison of the Borate-Celite Column Screening Technique with Other Extraction Methods in Forensic Toxicology

Extraction techniques are an important aspect of forensic toxicology, providing the starting point for drug identification and quantitation. They must be rapid and efficient, giving good recoveries of as wide a range of drugs as possible, and provide clean extracts. There is at present no method for extracting postmortem tissue that fulfills these requirements.

Several papers have been published on drug extractions in forensic toxicology [*1–17*] that describe techniques using protein precipitation, acid hydrolysis, ion exchange chromatography, column chromatography, and direct extraction. Many of these systems have not been adequately evaluated with samples containing the more modern and predominately basic drugs. The aim of this paper is to provide a comparison of some of these methods with tissue samples. Liver tissue from postmortem cases was used instead of either aqueous or tissue mixtures of drugs. This is the most reliable and accurate method for providing a comparison because both drug-binding effects and protein precipitation losses can be taken into account.

A column chromatography technique with buffered celite proposed by Hackett and Dusci [*14*] seemed to fulfill a number of the requirements listed above with aqueous standards. It was decided to use this method as a comparison with the more commonly used techniques. The methods used for the comparison of the acidic drugs included (1) borate/celite column, (2) direct extraction, (3) acid hydrolysis, and (4) tungstate precipitation. The methods used for the basic drugs included (1) borate/celite column, (2) direct extraction, (3) acid hydrolysis, and (4) ammonium sulfate precipitation.

Materials and Methods

Apparatus

A Hewlett-Packard series 5700A gas chromatograph equipped with a flame ionization detector was used. The column was a 4-ft (1.2-m) by ¼-in. (6.35-mm) outside diameter glass coiled tube, packed with 3% OV-17 on Gas Chrom Q 80-100 mesh (Supelco). The instrument settings were as follows: injection port temperature, 300 °C; detector temperature, 300 °C; nitrogen carrier gas flow rate, 60 ml/min; hydrogen flow rate, 60 ml/min; and air flow rate, 240 ml/min. In this work the oven temperature was set at temperatures varying from 150 to 290 °C. In screening for unknown drugs a temperature program was used, starting at 150 °C. This temperature was held constant for 2 min, then increased at 8 °C/min to 290 °C, which was held isothermally for 8 min.

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

Extraction Procedures

The extraction procedures used were slight modifications of the published methods [*4,10,12-14*].

Acidic and Neutral Drugs—In the borate/celite column procedure, 5 g macerated tissue, 10 g anhydrous sodium sulfate, and 6 g borate/celite were mixed and packed into a glass column. Ether (35 ml) was passed through the column and collected. This contained the acidic, neutral, and basic drugs. The ether was shaken twice with 10 ml 1.8*M* sulfuric acid to extract the basic drugs, then washed with saturated saline, filtered, and taken to dryness. The residue was dissolved in 10 ml hexane, and then 10 ml of acetonitrile saturated with hexane was added and the mixture shaken. The top hexane layer was discarded. The acetonitrile was taken to dryness, re-dissolved in 30 ml dichloromethane and 10 ml 0.45*M* sodium hydroxide, and shaken. The dichloromethane layer was filtered and taken to dryness for analysis of the neutral drugs. The sodium hydroxide solution was acidified with 5 ml 1.0*M* hydrochloric acid and the mixture extracted with dichloromethane. This was filtered and taken to dryness for analysis of the acidic drugs.

In the direct extraction procedure, 10 g macerated tissue, 10 ml water, and 5 ml 1.0*M* hydrochloric acid were mixed and shaken with 100 ml dichloromethane. The mixture was centrifuged and the aqueous layer discarded. The dichloromethane was filtered and taken to dryness. The residue was dissolved in 10 ml hexane, 10 ml of acetonitrile saturated with hexane was added, and the mixture was shaken. The extraction was continued as outlined for the borate/celite column procedure.

For the acid hydrolysis procedure, 10 g macerated tissue, 10 ml water, and 13.5 ml concentrated hydrochloric acid were mixed and placed on a boiling water bath for 15 min. The sample was cooled and then extracted as in the direct extraction procedure.

The tungstic acid precipitation technique was that proposed by Curry [*10*] with 10 g tissue. The extract was shaken with 100 ml dichloromethane and the aqueous layer discarded. The acidic drugs were extracted with 10 ml 0.45*M* sodium hydroxide, which was then acidified and re-extracted with dichloromethane. The original dichloromethane was filtered and taken to dryness for examination of the neutral drugs.

Basic Drugs—In the borate/celite column procedure, the sulfuric acid extract was made alkaline with solid sodium carbonate and extracted with 50 ml ether. The ether was filtered and taken to dryness for examination of the basic drugs.

For the direct extraction procedure, 10 g tissue and 15 ml 20% sodium carbonate were shaken with 100 ml ether. The mixture was centrifuged and the aqueous layer discarded. The ether was extracted twice 10 ml 1.8*M* sulfuric acid and the aqueous layer separated. The extraction was continued as in the borate/celite column procedure.

The acid hydrolysis procedure used was the same as for acidic drugs. The mixture was cooled and made just alkaline with potassium hydroxide pellets and then adjusted to pH 10 to 11 with solid sodium carbonate. The mixture was shaken with 100 ml ether and centrifuged. The ether layer was then extracted twice with 10 ml 1.8*M* sulfuric acid and continued as in the borate/celite column procedure.

The ammonium sulfate procedure was a modification of the Nickolls method [*13*], in which 10 g tissue, 60 ml water, and 1.0 ml concentrated hydrochloric acid were mixed and placed on a boiling water bath and solid ammonium sulfate was added until the solution was saturated. The mixture was heated for 30 min, then cooled slightly and filtered. The filtrate was adjusted to pH 10 to 11 with solid sodium carbonate and extracted with 100 ml ether. The ether was separated and extracted twice with 10 ml 1.8*M* sulfuric acid and the extraction continued as in the borate/celite column procedure.

All samples were reconstituted in an aliquot of ethanol and injected on the gas chromatograph. The peak heights were compared directly with accurate reference standards.

Results and Discussion

Protein precipitation techniques have been used in forensic toxicology for a number of years as they provided a relatively efficient and clean extract with little or no solvent emulsions. These methods, however, were somewhat cumbersome and time-consuming, and the yields of most drugs were quite low. Direct extraction provided a rapid and highly efficient technique but occasionally suffered from solvent emulsion. For acid drugs, direct extraction of old and putrified tissue resulted in dirty extracts, unsuitable for analysis by gas chromatography. Although acid hydrolysis gave relatively clean extracts and good recoveries, it could not be used as a general screening method because some drugs are acid or heat labile and break down under these conditions.

The borate/celite method provided clean, emulsion-free extracts, gave excellent recoveries for acidic and basic drugs, and therefore was a suitable screening method for postmortem tissues. An explanation for these higher yields of drugs could be that the tissue was finely divided between the sodium sulfate and the buffered celite and offered a much larger surface area for the solvent to extract the drugs. This method worked equally well with other biological material such as blood, urine, and bile. A number of interesting points emerged from the results, as presented in Tables 1 and 2.

TABLE 1—*Results obtained for acidic and neutral drugs in liver with four different extraction techniques.*

Drug[a]	Case	Borate/Celite	Direct	Acid Hydrolysis	Tungstate
Phenobarbital	1	8.3	unobtainable	unobtainable	1.4
Amylobarbital	2	12.5	11.8	12.2	9.0
Dilantin®	3	0.8	0.9	0.1	0.3
Phenobarbital	3	44.0	44.5	41.0	27.0
Pentobarbital	4	8.7	7.8	9.8	6.4
Glutethimide	5	2.7	2.5	2.8	1.6
Barbital	6	13.2	11.9	7.9	6.1
Amylobarbital	7	24.8	21.0	23.0	18.4
Secobarbital	8	31.3	29.5	29.2	23.8

[a] All results expressed in mg/100 g (average of two determinations).

Acidic Drugs

The borate/celite method gave the best overall recoveries of the acid drugs presented. In one instance (Case 1) the liver sample was extremely putrified and results by the direct and acid hydrolysis methods using gas chromatography were unsatisfactory. Acid hydrolysis did not increase the yield of the barbiturates, the results being similar to the direct and borate/celite methods. The tungstate protein precipitation technique gave recoveries of approximately 75% of the direct extraction procedure, which was in agreement with data from other workers [*16*].

Basic Drugs

The borate/celite and the direct extraction method gave similar results for most of the basic drugs examined. However, amitriptyline was a notable exception. The yield of amitriptyline was not greater with acid hydrolysis than the borate/celite method, although, as we expected, acid hydrolysis gave higher recoveries than the direct extraction procedure.

TABLE 2—*Results obtained for basic drugs in liver with four different extraction techniques.*

Drug[a]	Case	Borate/Celite	Direct	Acid Hydrolysis	Ammonium Sulfate
Amitriptyline	1	3.9	1.0	2.4	0.1
Chlorpromazine	2	2.6	1.1	5.8	0.2
Propoxyphene	3	0.9	0.8	ND[b]	ND
Dibenzepin	4	3.2	1.9	3.6	0.3
Dibenzepin metabolite	4	2.9	2.9	3.9	0.2
Amitriptyline	5	9.1	5.3	7.0	0.9
Nortriptyline	5	0.3	0.2	1.4	ND
Methaqualone	6	10.8	11.3	9.8	0.3
Diphenhydramine	6	2.3	3.0	0.4	0.9
Propoxyphene	7	3.2	4.1	ND	0.5
Norpropoxyphene	7	2.0	2.0	0.4	0.3
Norpropoxyphene amide	7	1.9	1.9	1.5	0.7
Pentazocine	8	0.1	0.1	0.1	ND
Diazepam	9	0.2	0.1	ND	ND
Imipramine	10	1.0	1.1	1.5	ND
Desipramine	10	0.7	0.8	1.6	ND
Quinine	11	6.9	6.7	6.2	5.2
Quinine metabolite	11	0.6	0.7	0.6	1.1
Oxazepam	12	1.7	1.2	ND	0.2
Amitriptyline	13	0.8	0.4	1.3	0.1
Nortriptyline	13	0.5	0.2	1.1	ND
Strychnine	14	0.7	0.6	0.8	0.4

[a] All results expressed in mg/100 g (average of two determinations).
[b] ND = not detected.

A number of the drugs examined were found to be acid or heat labile and hence unable to be assayed as the parent drug, making the acid hydrolysis technique unsuitable as a screening method. These included oxazepam, diazepam, diphenhydramine, and propoxyphene, although norpropoxyphene and its amide were still observed.

Generally the ammonium sulfate procedures gave very poor results, except for quinine, its metabolite, and strychnine, where the results were compatible with the other techniques used. A number of the samples that had low drug levels were chosen for the comparison, to test the methods at the therapeutic range. The borate/celite procedure proved its effectiveness in this regard. It has proved to be a reliable, efficient, and rapid technique for the screening of unknown drugs in biological material.

Summary

The comparison of five different extraction techniques from postmortem tissues was reported. The borate/celite column chromatography technique generally gave the best yields and its use as a screening method in forensic toxicology was discussed.

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