TECHNICAL NOTE

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The Preparation of Lipid-Free Extracts of Drugs from Blood for Gas Chromatographic Analysis

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ABSTRACT: A method is presented to separate co-extracted biological materials, in particular lipids, from drugs in chloroform extracts of blood. The chloroform is evaporated and the residue is dissolved in a small volume of petroleum ether and partitioned with eight times this volume of 5% aqueous hydrochloric acid. The acidic, basic, and neutral drugs are extracted into the aqueous phase, leaving unwanted materials in the petroleum ether. The recoveries of drugs representative of a variety of types are presented. Reduction of the levels of lipids by factors on the order of 1000-fold is reported.

KEY WORDS: toxicology, lipids, chromatographic analysis

Extraction of biological materials with organic solvents is a common means of obtaining solutions suitable for instrumental analysis. Gas chromatographic (GC) analysis of chloroform extracts for drugs eluting in the barbiturate region usually suffers from interference attributed to fatty acid esters [*1*]. Gas chromatographic separation of substances of interest from co-extracted materials has been recommended to improve mass spectra [*2*].

In our laboratory the use of GC and gas chromatography/mass spectrometry (GC/MS) with organic extracts of biological fluids has been hampered by co-extracted biological materials, especially in the acidic and neutral fractions and particularly in extracts from postmortem specimens in various states of decomposition. Removal of these interfering materials appears to be the best solution to the problem since it reduces the number of GC peaks to be examined, in many cases improves the mass spectra obtained by GC/MS, and provides an extract suitable for a general screen in a single program. A simple method for this removal has been developed in our laboratory. The method is effective and permits satisfactory recovery of all of the numerous drugs that we have tested.

Materials and Methods

The extractions and separations are carried out in 15- or 50-ml graduated Corex® centrifuge tubes according to the volumes involved. All glassware is washed thoroughly

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with soapy water and rinsed with tap water and then with acetone. A new Pasteur pipet is used for each transfer and then discarded. Plastic stoppers are used for closure of the centrifuge tubes during shaking. These stoppers may be reused, but the stoppers used during the final extractions are cleaned by soaking in chloroform for 5 min.

All solvents are Fisher certified American Chemical Society grade and the chloroform is redistilled before use.

Gas chromatography is carried out on 0.9-m (3-ft) glass columns, packed with 2% OV-17 on 80-100 mesh Chromosorb WHP (Analab), programmed from 80 to 260°C at the rate of 20°C/min and held at the final temperature for 5 min. The inlet temperature is 280 to 300°C (the drugs extracted as soluble hydrochlorides may require high inlet temperatures).

Cleanup Procedure

Place 10 ml of blood and 30 ml of chloroform in each of two 50-ml centrifuge tubes. Close the tubes with plastic stoppers and shake vigorously for 1.5 min, then centrifuge until three well-defined layers are obtained: aqueous, a semisolid plug, and chloroform. Remove the top layer in each tube with a Pasteur pipet and discard. Hold the plug aside with a narrrow spatula, decant the chloroform from both tubes into a fluted filter, and collect the filtrate in a 125-ml Erlenmeyer flask. Evaporate the chloroform to approximately 5 ml and transfer to a 15-ml centrifuge tube. With a Pasteur pipet add a drop of *n*-decane to the tube, place the tube in a beaker of water, heat, and continue evaporation until bubbling diminishes. The *n*-decane is added to diminish the loss of volatile drugs and will be substantially removed along with the lipids. Blow very briefly with a stream of air or nitrogen to remove most of the chloroform. Add 0.5 ml of petroleum ether and mix; then add 4 ml of 5% hydrochloric acid to the tube; stopper; and shake vigorously for 1 min. Wash down the sides of the tube and dilute the materials in solution in the petroleum ether with about 3 ml of petroleum ether. Centrifuge, remove the top layer (which contains the lipids) with a Pasteur pipet, and discard. Wash the sides of the tube and the top of the aqueous layer with about 3 ml of petroleum ether two more times. Scrupulously clean technique is required from this point on. Transfer the aqueous extract to a clean 15-ml centrifuge tube; add 6 ml of chloroform; stopper; shake vigorously for 1 min; and centrifuge. Transfer the aqueous layer to one 15-ml centrifuge tube and the chloroform to another. This chloroform will contain most of the drugs, including the basic ones that form chloroform-soluble salts with hydrochloric acid. Adjust the pH of the aqueous layer to approximately 8.5 with concentrated ammonia by using pH paper. Extract with an equal volume of chloroform, centrifuge, remove the chloroform layer, and combine with the chloroform extract recovered at acid pH. Place the centrifuge tube in a beaker of water, heat, and evaporatc to 0.1 ml.

Determination of Drug Recovery and Effectiveness of Lipid Removal

A number of drugs were tested for recovery from chloroform extracts of blood by adding them to the chloroform before being shaken with blood, according to the cleanup procedure. The quantities added in most instances corresponded to 1 μg/ml blood.

To show the amounts and the points at which the lipids are eluted, several specimens of blood from autopsies were extracted with chloroform and temperature-programmed gas chromatograms of the concentrated extracts were obtained both before and after cleanup.

Results and Discussion

The recoveries of drugs, shown in Table 1, are based on limited data and are offered primarily as evidence for the adequacy of extraction into aqueous acid, not only of basic drugs, but also of the acidic and neutral ones. The values obtained are lower than they would have been if the drugs had been added to the chloroform after filtration. However, we wished to determine recoveries more closely approximating those that would result from processing blood specimens that contain drugs when drawn from individuals. The recoveries are sufficiently reproducible so that calibration will permit the calculation of actual concentrations of free drugs in the blood. The oven temperatures at which the drugs were eluted are included for comparison with similar elution temperatures of materials that are removed by the cleanup (Table 2). The GC patterns of the potentially interfering lipids in different blood specimens show a high degree of similarity except in quantity.

Only the two components that eluted at 208 and 222°C would usually provide serious interference with large numbers of drugs at the level of 10 μg/ml or more. The substance eluted after 2.3 min at the final temperature of 260°C (probably cholesterol) would follow most drugs. Nevertheless, others (see Specimen 1, Table 2) would occasionally provide serious interference at a drug level of 10 μg/ml of blood, frequently impair quantitative accuracy and the quality of mass spectra of drugs not separated from the lipids by the GC, and prevent adequate determination of drugs at levels on the order of 1 μg or less per millilitre of blood. The presence of drugs at these lower levels may be important [3].

If the drug in question chromatographs well, the cleanup procedure will permit the detection and quantitation by GC of most drugs at levels of 0.01 μg/ml in 20 ml of blood (after cleanup only two lipids remained at similar levels). Injection of an extract equivalent to 2 ml or more of blood is desirable when a high degree of sensitivity is needed.

The analyst should be alert to the possibility that the use of large sample volumes in cases where drug levels exceed 10 μg/ml could result in precipitation and unsatisfactory distribution between the aqueous acid and petroleum ether. Furthermore, drugs may precipitate when the extract is concentrated for instrumental analysis. On some occasions

TABLE 1—*Recovery of drugs by acid-extraction method.*

Drug	Recovery, %	Programmed Elution Temperature, °C
Ethosuximide	78	130
Methyprylon	79	177
Amobarbital	62	195
Secobarbital	62	202
Glutethimide	75	210
Meprobamate	77	212
Methadone	64	230
Propoxyphene	72	233
Imipramine	75	240
Pentazocine	58	243
Codeine	71	260
Diazepam	77	260 + 0.3 min
Diphenylhydantoin	67	260 + 0.4 min
Chlordiazepoxide	60	260 + 0.7 min
Trifluoperazine	41	260 + 0.7 min
Flurazepam	74	260 + 1.4 min
Hydroxyzine	74	260 + 2.1 min

TABLE 2—*Levels of lipids in blood,*[a] *μg/ml (estimated).*

Programmed Elution Temperatures of Various Lipids, °C	Blood Specimen			
	1	2	3	4
182	10	0.6	0.8	3
208	100	17	10	40
222	160	21	10	50
243	40	0.4	0.5	...
256	...	0.2	1.2	...
260 + 0.3 min	...	1.1	0.9	0.9
260 + 0.7 min	...	0.6	1.0	...
260 + 2.3 min	60	48	25	6.5

[a]Extracted by chloroform. Note: following cleanup of Specimen 4 by the acid-extraction process, none of the major lipids exceeded an estimated 0.03 μg/ml of blood; minor ones apparently were reduced correspondingly.

we have observed turbidity in the solution in 0.5 ml of petroleum ether. In such instances the turbidity can be removed by increasing solvent volumes in the same relative amounts throughout. Alternatively, an aliquot may be processed if the larger volumes are objectionable. However, removal of the turbidity may be unnecessary if both layers are clear after the extraction with aqueous acid and there is no apparent precipitate.

Caffeine is not removed by the cleanup process and may be present at substantial levels. Nicotine and its metabolite cotinine are not removed. Neither nicotine nor cotinine is present in blood in large amounts though their concentrations in urine may be much greater.

The cleanup procedure is applicable to chloroform extracts of urine, blood, and tissue homogenates. The size of specimen taken for analysis may vary with needed sensitivity, availability, and the quantity of substances, such as fats, in relation to the volume of petroleum ether used. Large sample sizes tend to minimize the degree of interference from substances included as experimental artifacts; thus, 20 ml of blood has been found by us to be a good choice for the size of specimen. Much smaller specimens may yield adequate information.

Summary

The initial chloroform extract is evaporated, taken up in petroleum ether, and partitioned with a volume of dilute hydrochloric acid somewhat larger than that of the petroleum ether. The interfering lipids are left in the petroleum ether while drugs (including acidic and neutral ones) are extracted to the aqueous phase. Good recoveries and good reproducibility are obtained.

References

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