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Detection of Drugs Using XAD-2 Resin. III: A Routine Screening Procedure for Bile

A number of biological specimens may be routinely examined in medical examiner's cases. A useful specimen for toxicologic analysis is one that by its physiological nature concentrates drugs in easily detectable amounts, is readily available in sufficient quantity, does not require extensive preparation, and is applicable to simple screening techniques. Urine has been particularly amenable to XAD-2 resin screening [*1-11*]. However, in postmortem cases urine is often unavailable and bile becomes the sample of choice. Simple hydrolysis alters the consistency of bile so that it can be readily extracted by the XAD-2 resin methods [*12,13*]. In addition, the disposition and biotransformation of many drugs causes significant quantities of parent drugs and metabolites to be found in bile, often in concentrations higher than those in other biological specimens.

In a tissue distribution study of barbiturates, Sunshine et al [*14*] reported concentrations in bile to be lower than those in liver but higher than those in blood or urine. Dal Cortivo et al [*15*] showed that bile is an important route of elimination for imipramine and probably other drugs since the unhydrolyzed bile concentration of imipramine was found to be twice as high as the liver concentration and 14 times as high as the blood concentration. Doxepin concentrations in bile were comparable to those in liver but significantly higher than those in blood and urine [*16*]. In two meperidine fatalities, concentrations of the drug in the bile were approximately twice as high as those found in blood and liver [*17*]. Manning et al [*18*] reported a distribution study of methadone in which concentrations in bile were 2 to 12 times higher than those in blood, 3 to 14 times higher than those in brain, 1.2 to 5 times higher than those in kidney, and up to 4 times higher than those in liver.

The analysis of bile is particularly significant in the detection of morphine. Tissue distribution studies for this drug indicate that morphine is deposited in the bile in concentrations several times those in blood, urine, kidney, or liver. Coumbis and Kaul [*19*] reported bile concentrations of morphine to be eight times higher than liver concentrations and six times higher than kidney concentrations. Christopoulos and Kirch [*20*] concluded that bile and urine are specimens of choice for morphine detection, followed by liver and kidney. Morphine concentrations in bile were 7 to 14 times higher than those in liver and approximately twice those in urine. Richards et al [*21*] reported morphine levels in bile up to 2.5 times higher than those in urine and up to 150 times higher than those in blood.

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We have previously reported our general experiences with XAD-2 resin [*22*] and the resin's applicability to the analysis of liver [*23*]. This paper will present a routine screening procedure for the analysis of bile.

Materials and Methods

All chemicals were reagent grade. Drug Skreen® adsorbent cartridges packed with XAD-2 resin were from Brinkmann Instruments (Westbury, N.Y.). Thin-layer plates, silica gel GF, were from Analtech (Newark, Del.).

Preparation of Bile

Bile (10 ml) was mixed with concentrated sulfuric acid (1.2 ml) and distilled water was added to a total volume of 25 ml. The mixture was placed in a screw-capped jar (30 ml) and hydrolyzed by autoclaving at 103 to 131 kPa (15 to 19 psi) pressure for 20 min. After cooling, the mixture was filtered through Whatman No. 1 paper, and the pH was adjusted first to neutral with 40% sodium hydroxide and then to 8 to 8.5 by using solid sodium bicarbonate (Fig. 1).

Extraction Procedure

The buffered filtrate from the bile sample was poured onto an XAD-2 resin column that had been wet with 3 to 5 ml of distilled water. After the aqueous bile preparation had passed through the column by gravitational flow, suction was applied to remove any aqueous solution remaining on the resin. The resin in the column was then extracted with 25 ml of a mixture of 1,2-dichloroethane and ethyl acetate (2:3). The upper cotton plug of the column was removed, the organic mixture added to the column, and the eluate collected in a 30-ml evaporation tube. After the addition of one drop of sulfuric acid in methanol (1%), the eluate was evaporated to dryness under a stream of air or in a Brinkmann sample concentrator (Fig. 2).

Thin-Layer Chromatography

The residue was redissolved in several drops of chloroform/methanol (1:1) and transferred to a silica gel GF chromatographic plate. The plate was developed to 15 cm with the ethyl acetate/methanol/ammonium hydroxide (85:10:5) system of Davidow et al [*24*]. After air drying, the plate was examined under long wavelength ultraviolet (UV) light (354

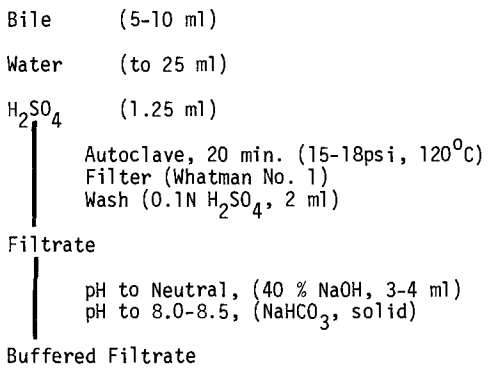

FIG. 1—*Preparation of hydrolyzed bile for extraction.*

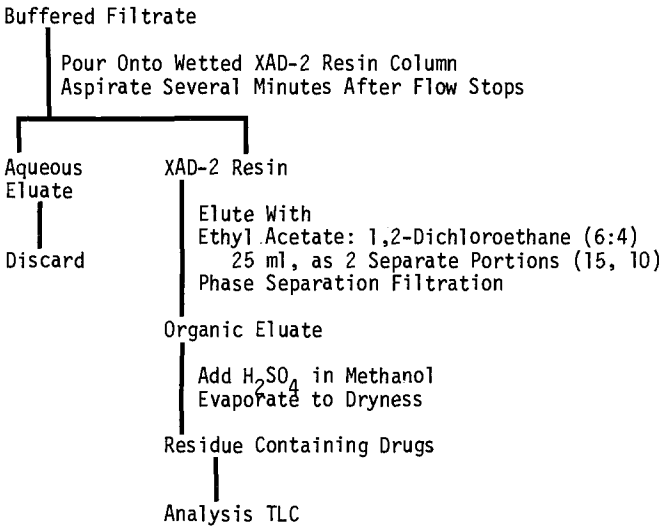

FIG. 2—*Extraction of drugs from bile using XAD-2 resin.*

nm) and any fluorescence noted. The plate was sprayed with ninhydrin (0.1% in acetone), heated at 75°C for 5 min, and again exposed to UV light for the detection of primary amines. The plate was next sprayed with diphenylcarbazone (0.1% in ethanol) followed by mercurous nitrate solution (0.3% in 0.04 nitric acid) to detect barbiturates, glutethimide, and phenytoin. The plate was then sprayed with iodoplatinate solution (0.5% in 2% potassium iodide) to detect the basic drugs. Drugs were identified by using R_f values and color reactions by comparison to known standards. The drugs identified were confirmed with UV spectrophotometric and gas chromatographic techniques as previously described [*22*]. In addition, secondary thin-layer chromatographic systems and spectrophotofluorometry were used for some drugs.

Effect of Dilution on Recovery of Drugs from Bile

Bile recovery studies were performed by adding known amounts of drugs (100 μg) to 5 ml of bile. Bile samples were hydrolyzed as described without the addition of water and with the addition of 10, 20, 40, and 80 ml of water to assess the effect of dilution on optimizing the recovery of drugs from XAD-2 resin.

Results and Discussion

Method

The analytical method is similar to that previously reported [*22,23*]. The treatment of bile with acid causes precipitation of bile salts that are easily removed by filtration. The resultant aqueous filtrate is adjusted to pH 8.0 to 8.5 and the buffered filtrate subjected to XAD-2 resin chromatography in a manner similar to urine. All drugs tested were easily detectable at a concentration of 1 μg/ml.

Effect of Dilution on Recovery of Drugs from Bile

Table 1 shows the effect of dilution on the recovery of drugs from bile. Morphine, codeine, methadone, amobarbital, and phenobarbital were studied. Recovery improved

TABLE 1—*Effect of dilution on recovery of drugs from bile (% recovered).*[a]

Drug	Volume of Water Added, ml				
	0	10	20	40	80
Morphine	58	72	81	78	70
Codeine	55	68	73	80	79
Methadone	21	44	53	70	93
Amobarbital	29	57	72	95	104
Phenobarbital	57	84	100	110	113

[a]All drugs studied in 5 ml of bile containing 100 μg drug. Recoveries corrected for determined XAD-2 and thin-layer chromatographic losses.

for each drug with the addition of increasing quantities of water. All dilutions were corrected for an estimated dilution of 25 ml, and hence some numbers exceed 100%. It was expected that increasing dilutions would improve recovery since the more dilute the solution, the longer the contact time with the resin. The longer contact time should facilitate transfer of drugs from solution to the resin. On the other hand, it is also anticipated that excessive dilution may cause a washing phenomenon and tend to reduce recovery. A recovery maximum was noted for morphine and to a much lesser extent for codeine (Fig. 3), while at the dilutions tested methadone, amobarbital, and phenobarbital tended only to increase. Increase in recovery was particularly significant for methadone and amobarbital. Recovery for these two drugs doubled when 10 ml of water was added. The improved recovery is due also to the fact that loss of drug as a result of co-precipitation with the bile salts is reduced when the sample is diluted. Although dilution of the sample is useful, in the interest of practicality of sample handling an intermediate dilution of 20 ml would be recommended.

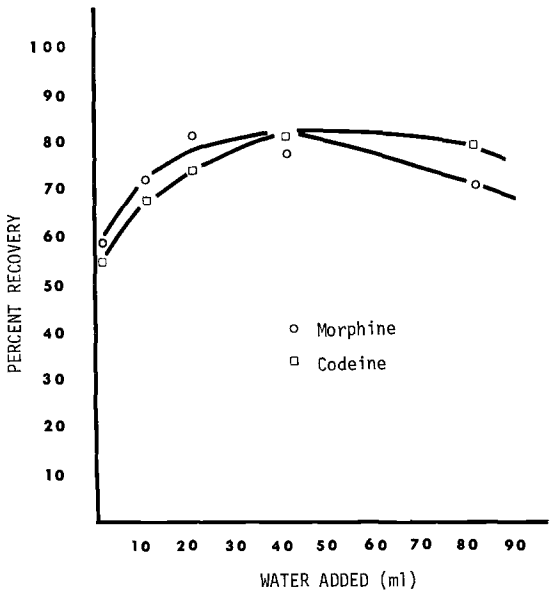

FIG. 3—*Effect of dilution on recovery of morphine and codeine from bile.*

Medical Examiner's Cases

The following drugs were detected in medical examiner's cases (one-year period) by using the described procedure: aminophylline, amobarbital, butabarbital, chlordiazepoxide, chlorpheniramine, chlorpromazine, codeine, diazepam, doxepin, flurazepam, hydromorphone, methadone, methaqualone, methapyrilene, methorphan, morphine, pentazocine, pentobarbital, phenobarbital, propoxyphene, quinine, salicylic acid, secobarbital, and thioridazine.

Table 2 shows blood and bile concentrations as well as the blood/bile ratio for a group of medical examiner's cases in which the cause of death was acute barbiturate intoxication. Blood concentrations were determined by the Broughton modification of the Goldbaum procedure [*25*] and compared to bile concentrations determined by the XAD-2 resin method. The blood/bile ratios averaged 3.2 (range, 2.1 to 4.4). Irey [*26*] reported an average ratio of 1.4 in 27 barbiturate death cases. These ratios are also consistent with Sunshine's tissue distribution study [*14*].

The ability to use bile in toxicologic investigations is important since many drugs are excreted into the bile and undergo enterohepatic circulation. Biliary excretion can affect toxicity by causing certain drugs to persist in the body or by causing modified toxicity from normal concentrations of drugs when the biliary route becomes unavailable for drugs requiring it for elimination. The procedure described permits rapid analysis of bile specimens, is useful for screening, and may be used quantitatively by utilizing reference standards.

Summary

The ability of bile to concentrate drugs and metabolites coupled with its general availability make it suitable for analysis and often the fluid of choice in postmortem cases requiring drug screening. Bile (5 to 10 ml) was diluted with water, sulfuric acid was added, and the mixture was autoclaved. The precipitated bile salts were easily removed by filtration and the filtrate (pH adjusted to 8.0 to 8.5) extracted with XAD-2 resin. Drugs were eluted with a mixture of ethyl acetate/1,2-dichloroethane and analyzed with thin-layer chromatography. Varying the dilution of bile improved the recovery of morphine, codeine, methadone, amobarbital, and phenobarbital. Excessive dilution, however, caused a washing phenomenon and reduced recovery of some drugs, as shown with morphine and codeine. The procedure described is useful for the rapid screening of bile specimens for drugs.

TABLE 2—*Barbiturates detected in blood and bile in medical examiner's cases (acute barbiturate intoxications).*

Case	Barbiturates	Concentration, mg/100 ml		Blood/Bile Ratio
		Blood	Bile	
1	pentobarbital	3.30	0.79	4.2
2	amobarbital, pentobarbital, and secobarbital	1.22	0.28	4.4
3	amobarbital and secobarbital	2.40	1.11	2.2
4[a]	pentobarbital and secobarbital	3.50	1.64	2.1
5	amobarbital and secobarbital	3.93	1.24	3.2

[a]Blood ethanol concentration, 0.03%.

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