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Preliminary Studies on the Use of *n*-Butyl Chloride as an Extractant in a Drug Screening Procedure

For performance of clinical toxicological analyses, relatively few hospital or other service laboratories presently have elegant instrumentation such as gas chromatograph-mass spectrometer-computer interfaces for final measurement or mechanized devices for the prior extractions and sample transfers. Most of the small fraction of hospital laboratories providing analyses for drugs are limited to the use of "kits" using thin-layer chromatography (TLC), or ultraviolet (UV) photometric and, occasionally, gas chromatographic (GC) procedures. Most frequently the determination desired must be specified.

Because the presenting signs and symptoms of many drug overdoses are similar, screenings for a variety of drugs rather than determinations of specified items are increasingly requested. In most instances the specimens submitted are blood (or its plasma or serum) and urine. It is generally observed that in a given geographic area a small number of individual drugs account for most of the overdoses encountered. Thus, for a group of several hospitals in Dallas, Texas, in 1971 (counting barbiturate, amphetamine, and phenothiazine derivatives as single units), 15 items containing 33 structures accounted for 96 percent of the cases with positive findings. Barbiturates and carbamates were the most frequent acidic and neutral drugs detected. The most common basic drugs were chlordiazepoxide, methaqualone, phenothiazines, amphetamine and methamphetamine, propoxyphene, methadone, morphine, and codeine.

In analysis for nonvolatile drugs in small biological specimens, solvent extraction is the most common primary separational step when TLC and GC are employed for subsequent identification and quantitation. It would be convenient to have a solvent which would extract most or all of the common nonvolatile drugs encountered, so that a drug screen could be simplified in terms of manipulation but extended in usefulness. Some solvents which have been commonly used are chloroform, ethylene dichloride, ethyl acetate, ether, and hexane.

Wolen and Gruber [*1*] introduced *n*-butyl chloride for the determination of propoxyphene in plasma and this was also employed by Manno, Jain, and Forney for the same

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purpose [2]. Following some preliminary experiments, it appeared likely that *n*-butyl chloride could serve as an extracting solvent for many of the most commonly encountered drugs in a procedure which would avoid some of the time-consuming steps of evaporation and numerous manipulations with separatory funnels. The extracts ultimately obtained contained little or no materials interfering with subsequent gas chromatography. It followed that information regarding primary partitions and recoveries of individual drugs was essential in respect to detection and quantitation. It was desirable to first obtain information in respect to analysis of reference aqueous standards.

Because of the increasing demand for blood, plasma, or serum concentrations, the procedure to be described has been designed for application to 5 ml samples of serum or plasma. For many drugs this requires sensitivity of the order achieved by gas chromatography for detection and quantitation. The method permits the determination of many drugs at concentrations in excess of 50 μg/ml in 5 ml aqueous standards and detection at concentrations below 10 μg/100 ml. Recoveries from serum (or plasma) and data on protein binding will be reported subsequently.

Basic drugs are often found in very low concentrations in blood and their determination by ordinary UV photometry even at overdose concentrations may not be possible. On the other hand UV or UV-TLC, ultraviolet-infrared (UV-IR), or UV-TLC-IR methods are satisfactory for the most common acidic drugs. A much more sensitive detection system is needed to deal with basic drugs. A primary separation of basic drugs would be expected to include most neutral drugs and these are often relatively poor UV absorbers.

Materials

1. *n*-butyl chloride, redistilled (Eastman Organic Chemicals, Cat. No. 52) (1-chlorobutane)
2. Chloroform (Matheson, Coleman and Bell, Cat. No. CS1055) (suitable for use in spectrophotometry)
3. HCl, 0.2 *N*
4. NH_4OH, concentrated
5. 15-ml and 5-ml glass-stoppered conical centrifuge tubes (long taper-tip)
6. Reference drug standards, 0.2 mg/100 ml (calculated as the free acid, base, or neutral drug) in 0.2 *N* HCl
7. Gas chromatography was carried out using Glowall, Model 320; Hewlett Packard, Model 5750; and Varian Aerograph, Model 1800 gas chromatographs and the following glass columns:
   (a) 6 ft by 2 mm in diameter, 2.5 percent SE30 on 80/100 mesh Chromosorb G, A/W, DMCS
   (b) 6 ft by 3.4 mm in diameter, 5 percent Apiezon L and 5 percent KOH on 80/100 Chromosorb G, A/W, DMCS
   (c) 6 ft by 3.4 mm in diameter, 1 percent OV-1 and 1 percent OV-17 on 100/120 mesh Chromosorb W, A/W, DMCS

(Gas chromatographic conditions were adjusted to give minimum analysis times. The columns employed are designated for each compound in Tables 2–4.)

8. Vortex mixer

Method

The flow diagram (Fig. 1) gives details of the procedure for basic drugs in aqueous reference standards.

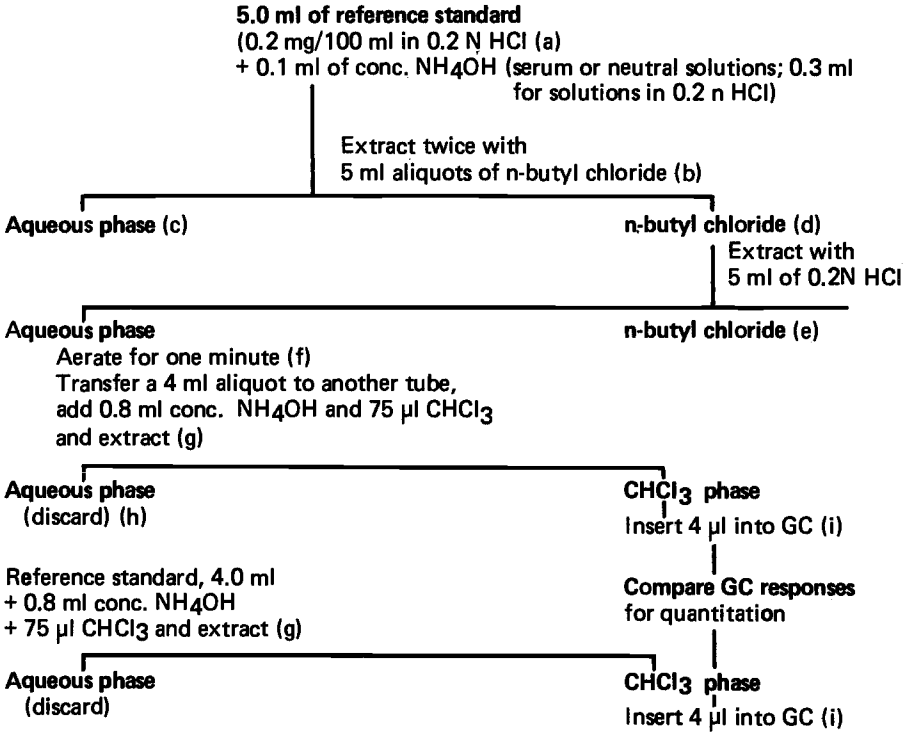

FIG. 1—*Flow diagram for extraction of basic drugs.* (a) *In a glass-stoppered centrifuge tube with a long conical tip,* (b) *transfer the supernatant to another centrifuge tube with a Pasteur pipet.* (c) *If the sample is serum, save for acidic drug extraction,* (d) (*usually circa 9.8 ml*); (e) *if the sample is serum, save for the neutral drug determination. Transfer the supernatant with a Pasteur pipet* (f) *with air via the tip of a Pasteur pipet to remove the remaining* n-*butyl chloride.* (g) *Mix in a Vortex mixer, 1 min, followed by centrifugation, 5 min, 3000 rpm, 8-in. head.* (h) *Remove almost all before sampling the chloroform.* (i) *Presumptive identification by retention time is confirmed by immediate injection of a reference standard when serum is analyzed.Further confirm by repeating injection with altered column conditions or by using a different parameter* (*for example, TLC* R_F, *etc*) *if necessary.*

In practice, serum or plasma is substituted for the 5 ml of reference standard, 0.2 mg/100 ml.

The extraction of acidic drugs from aqueous reference standards, or the aqueous phases following basic drug extraction are shown in Fig. 2. In practice, serum or plasma would substitute for the 5 ml of aqueous reference standard.

Figure 3 shows the flow diagram for the extraction of a neutral drug from an aqueous reference solution (or a serum sample).

Results

Preliminary experiments indicated that a considerable and variable fraction of chloroform was dissolved in the aqueous phase after the final extraction prior to gas chromatography. This variation, which would correspondingly affect drug recovery, was largely eliminated by aerating the aqueous phase to remove dissolved *n*-butyl chloride before adding the chloroform. Pertinent typical data are shown in Table 1. These and other

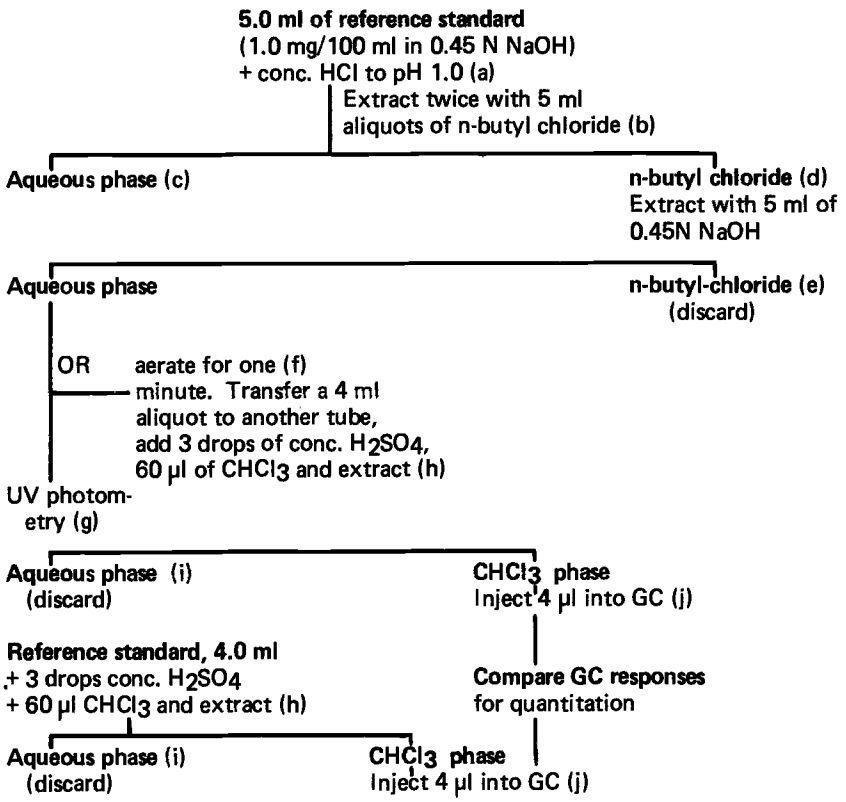

FIG. 2—*Flow diagram for extraction of acidic drugs.* (a) *In a glass-stoppered centrifuge tube with a long conical tip,* (b) *transfer the supernatant to another centrifuge tube with a Pasteur pipet.* (c) *If the sample is serum, save if the acidic drug extraction is first; otherwise discard* (d) *(usually circa 9.8 ml).* (e) *If the sample is serum, save for the neutral drug extraction if the acidic extraction is first; otherwise discard* (f) *with air via the tip of a Pasteur pipet to remove the remaining* n-*butyl chloride.* (g) *If preferred, a UV scan may be made versus an* n-*butyl chloride saturated blank prior to proceeding to gas chromatography. (A correction must be made for any change in volume as in the case of a 2 pH barbiturate determination.)* (h) *Mix in a Vortex mixer, 1 min, followed by centrifugation, 5 min, 3000 rpm, 8-in. head,* (i) *Remove almost all before sampling the chloroform.* (j) *Presumptive identification from retention time is confirmed by immediate injection of a reference standard when serum is analyzed. Confirm by repeating the injection with altered column conditions or by using a different parameter for example, TLC* R_F *if necessary.*

experiments show that the partition of chloroform is reproducible within a range of 1 to 2 percent when the *n*-butyl chloride has been removed by aeration.

Table 2 shows the recoveries from aqueous reference solutions of the basic drugs mentioned above, with the exception of the phenothiazines and chlordiazepoxide which are currently being studied. Even though the recovery of methaqualone is very poor, the detection limit in a plasma sample would be adequate because of the relatively high concentrations achieved with therapeutic and abuse dosages. The increased recovery by use of stronger acid suggests that methaqualone is acting as a very weak base and that much of it will be found in the neutral fraction. Use of a correction factor established for each drug should permit accuracy in the range of 90 to 110 percent to be obtained in routine analysis for bases other than methaqualone.

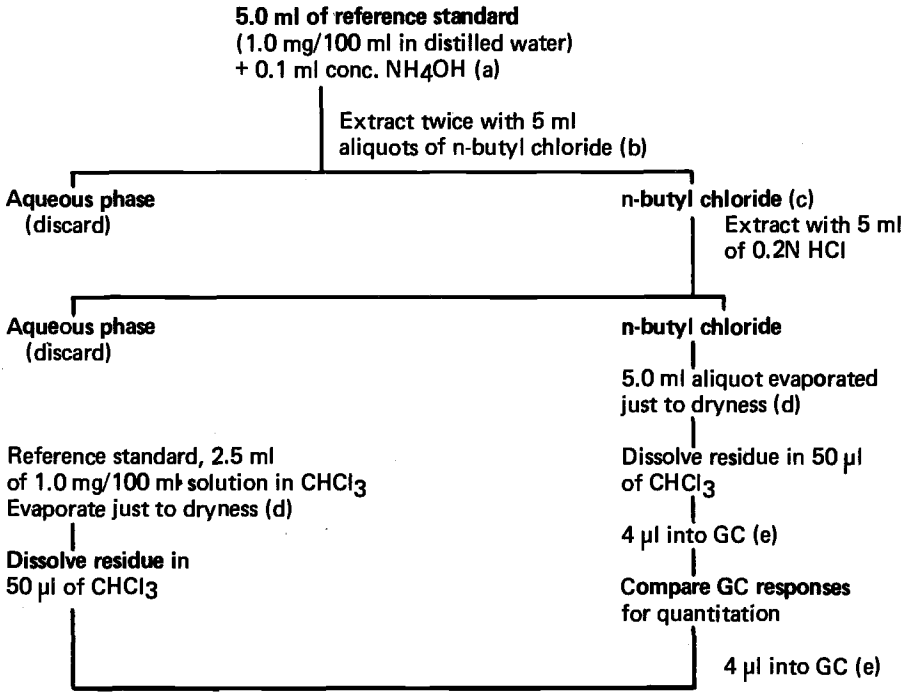

FIG. 3—*Flow diagram for extraction of neutral drugs.* (a) *In a glass-stoppered centrifuge tube with a long conical tip,* (b) *transfer the supernatant to another centrifuge tube with Pasteur pipet* (c) (*usually circa 9.8 ml*), (d) *with a current of air via a Pasteur pipet with the lower part of the tube immersed in warm (60–70°C) water.* (e) *Presumptive identification from retention time is confirmed by immediate injection of a reference standard when serum is analyzed. Confirm by repeating the injection with altered column condition or by using a different parameter for example TLC* R_F *if necessary.*

TABLE 1—*Volume of chloroform phase after equilibration of 75 µl with 4.8 ml of aqueous phase.*

Aqueous Phase	$CHCl_3$, µl
0.2 *N* HCl, 4 ml + 0.8 ml conc. NH_4OH	43.0
0.2 *N* HCl, 4 ml + 0.8 ml conc. NH_4OH	43.0
0.2 *N* HCl, 4 ml + 0.8 ml conc. NH_4OH, saturated with *n*-butyl chloride	46.0
0.2 *N* HCl, 4 ml + 0.8 ml conc. NH_4OH, saturated with *n*-butyl chloride	46.0
0.2 *N* HCl, 4 ml + 0.8 ml conc. NH_4OH, aerated 1 min prior to equilibration	42.5
0.2 *N* HCl, 4 ml + 0.8 ml conc. NH_4OH, aerated 1 min prior to equilibration	42.5

The following basic drugs have been found to be extractable from biological samples by the procedure so that they are easily detectable, and percentages of recovery are being determined: ethoheptazine, methylphenidate, mephentermine, phentermine, nicotine, cocaine, phenylethylamine, diphenhydramine, amitriptyline, and lidocaine. In addition, metabolites of propoxyphene, diphenhydramine, methadone, methaqualone, and lidocaine have been identified.

Recoveries of some acidic drugs are shown in Table 3.

TABLE 2—*Recovery of some basic drugs from aqueous reference solutions.*

Drug	Column Employed	% Recovery[a]
Propoxyphene	(c)	89
Pentazocine	(c)	90
Amphetamine	(b)	93
Methamphetamine	(b)	96
Meperidine	(c)	92
Methadone	(c)	97
Codeine	(c)	88
Methaqualone[b]	(c)	6

[a] Average of two determinations.
[b] If the normality of the HCl is increased to 1.0 the recovery is 38 percent.

TABLE 3—*Recovery of some acidic drugs from aqueous reference solutions.*

Drug	Column Employed	% Recovery[a]
Pentobarbital	(a)	86
Phenobarbital	...	45
Secobarbital	...	97
Salicylic acid	...	89
Diphenylhydantoin	...	84
Phenylbutazone	...	96

[a] Average of two determinations.

Recoveries of phenobarbital, secobarbital, and salicylate were determined by UV photometry [Fig. 2 (g)]. The recovery of diphenylhydantoin was determined by UV photometry following oxidation with $KMnO_4$ by the procedure (modified) of Wallace [*3*]. The reason for poor recovery of phenobarbital is not presently understood.

A few recoveries of neutral drugs are given in Table 4.

Meprobamate is not extracted from aqueous solutions by *n*-butyl chloride in significant quantity. Fortunately, carbamates may be quickly ruled out or detected by making a chloroform extraction of the first discarded aqueous phase (Fig. 3). The residue from this may be employed for TLC (furfural-HCl as a locating agent) for detection and identification and GC for detection [Fig. 3 (a)], identification, and quantitation.

TABLE 4—*Recovery of some neutral drugs from aqueous reference solutions.*

Drug	Column Employed	% Recovery[a]
Glutethimide	(a)	94
Diazepam	(a)	81
Methyprylon	(a)	58
Meprobamate	(a)	0

[a] Average of two determinations.

Discussion

These findings indicate that *n*-butyl chloride may be a useful extractant in a screening procedure for commonly abused drugs. The procedure described is designed so that it also may be employed for a specified drug analysis with a response time of about 1 h. A screening may require 3 to 4 h. The sensitivity is such that it may be applied to a 5-ml sample of serum or plasma. Application to urine, stomach content filtrates, and solid tissues has not yet been studied.

Alternate rapid detection procedures are available for those common drugs which the procedure does not detect because of inextractability (for example, carbamates, see above), relative volatility (for example, ethchlorvynol), or remarkably low plasma concentrations (for example, certain phenothiazines, morphine). Thus, ethchlorvynol may be detected by a color test applied to a trichloracetic acid filtrate of plasma, or to urine [*4*],[3] with subsequent quantitation by GC [*5*] or photometry [*4*]. Phenothiazines may be detected and excluded by applying the FPN[4] test [*6*]; however, derivative identification may present considerable difficulty. Morphine in urine may be detected or excluded by use of various fluorometric procedures [*7*]. Free morphine in blood or serum also may be determined fluorometrically [*8*].

Detailed further studies of recoveries from plasma or serum, and solid tissues will be reported subsequently. These will provide the correction factors necessary for quantitation.

Summary

1. A screening procedure for the detection and quantitation of the most common nonvolatile drugs encountered in blood specimens referred to the clinical chemistry laboratory, has been described. The procedure employs *n*-butyl chloride as an extractant and requires only limited GC capability, UV photometry, and TLC in addition to the usual equipment in the laboratory.

2. Recovery data for 18 acidic, basic, and neutral drug structures in aqueous reference solutions have been presented. Most of these structures had been previously found in *n*-butyl chloride extracts of biological samples. Comparable recoveries from biological specimens will be reported subsequently. Establishment of the percentage recovery of each drug will permit quantitation by use of a correction factor.

3. The procedure is applicable to specified drug analysis as well as to screening.

4. The response time for a screen or specified analysis is considered to be within tolerable limits.

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[*4*] Finkle, B. and Bath, R. in *Manual of Analytical Toxicology*, I. Sunshine, Ed., The Chemical Rubber Co., Cleveland, Ohio, 1971, pp. 150, 152.

[*5*] Kananen, G. in *Manual of Analytical Toxicology*, I. Sunshine, Ed., The Chemical Rubber Co., Cleveland, Ohio, 1971, p. 154.

[3] The test may be adapted to spot plate performance.

[4] Ferric chloride, 5 percent W/V; perchloric acid, 20 percent W/W; and nitric acid, 50 percent W/W, in the ratio of 5:45:50.

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