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# Liquid/Solid Extraction on Diatomaceous Earth for Drug Analysis in Postmortem Blood

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**ABSTRACT:** Absorption extraction on diatomaceous earth was examined and found to be compatible with typical postmortem blood specimens encountered in forensic toxicology. The effects of solvent, solvent volume, eluate flow rate, and pH on drug recovery and extract quality were investigated. It was concluded that the best method was not necessarily that with the highest recoveries, but that considerations of extract quality were also required. The optimized method was compared with a single-step liquid/liquid extraction method and found to be superior in terms of ease of operation, extract quality, and absolute recovery. The results indicated also that although useful for screening, quantitative methods using liquid/solid extraction may be prone to error.

**KEYWORDS:** toxicology, extraction, blood

Solid-phase extraction techniques have recently gained popularity for many biochemical, clinical, industrial, and environmental applications[2] because of their ability to provide clean, efficient extracts in a single step. High recovery and selective extraction are especially important in drug screening as encountered in forensic toxicology, where the specimens which are often complex and sometimes contagious contain drugs at low concentrations.

The principle of liquid/solid absorption extraction is closely related to conventional liquid/liquid extraction. Described in 1976 by Breiter et al. [*1*], it involves the absorption of the aqueous phase onto diatomaceous earth, a porous material which acts as a support for the aqueous phase. This provides a large surface area for partition into an eluting solvent, which flows through the immobilized specimen under gravity, eluting the analytes of interest. One theoretical advantage of solid-phase extraction is that, as a continuous extraction process, a given volume of solvent would be expected to give recoveries superior to a batched liquid/liquid extraction. Other advantages include the elimination of centrifugation, aspiration and filtration steps, and the prevention of emulsion formation. The technique has been characterized for the analysis of individual compounds [*2-4*], but the factors that affect recovery and may be important in a screening procedure have not been investigated in depth. Anderson and Fuller [*4*] have described the use of diatomaceous earth for the extraction of acid/neutral drugs from postmortem blood prior to gas chromatographic (GC) analysis, and obtained good-quality extracts with high recoveries. The problems with using this approach for

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[2]Analytichem International, applications bibliography.

general drug screening in postmortem blood have not been addressed, however, and the effect of extraction conditions on the recoveries has not been considered in detail. The recovery and extract quality obtained for various drugs from a specimen of partially decomposed blood was examined under various conditions of pH, solvent, solvent flow rate, and volume to determine the optimum conditions for extraction of this group of drugs.

## Materials and Methods

All solvents were high-pressure liquid chromatography (HPLC) grade (Mallinkrodt) and were used from the bottle. Buffers were prepared in deionized water using analytical grade reagents (Mallinkrodt), including hydrochloric acid (HCl), potassium phosphate, monobasic ($KH_2PO_4$), phophoric acid ($H_3PO_4$), potassium hydroxide (KOH), and boric acid ($H_3BO_3$). GC analysis of the extracts was performed on a Hewlett Packard 5880 gas chromatograph with a flame-ionization detector (FID), using a 0.25-mm inside diameter (ID) 15-m DB-1 capillary column and operated on a temperature program from 100 to 300°C at 5°/min [5]. Absorption extraction columns were prepared in short pasteur pipettes by the popular method of closing the bottom with a plug of glass wool and filling the pipette with a diatomaceous earth material (Chem Elut, Analytichem International, Harbor City, CA) to a level 1 to 2 cm below the top, giving a bed depth of approximately 8 cm. Blood specimens (0.5 mL) were diluted with buffer or water (0.3 mL) as required, then applied to the columns and allowed to absorb for 2 to 3 min. Solvent was applied in a polypropylene reservoir and allowed to flow until the required volume had been collected. These columns were suitable for the application of up to 1.0 mL of specimen. The eluting solvent was collected in 10-mL conical centrifuge tubes, evaporated to dryness, reconstituted, and analyzed as required.

The effect of specimen pH, choice of eluting solvent, and solvent volume and flow rate were investigated. Absolute recoveries were calculated by comparison of peak heights in the extract with an appropriate unextracted standard analyzed under the same conditions.

The quality of the extract was assessed according to the appearance of nondrug GC peaks present in an extract of drug-free postmortem blood and also on the basis of a visual inspection of the physical appearance of the extract. The drugs investigated are listed in Table 1. They were chosen to represent drug groups commonly encountered in forensic toxicology casework, namely barbiturates, phenothiazines, stimulants, benzodiazepines, and tricyclic

TABLE 1—*Mean recovery (n = 3) for each of eleven compounds using ether, ethyl acetate, methylene chloride, and n-butyl chloride as eluting solvent. Specimens were extracted at pH 9.*

| | Solvent | | | |
|---|---|---|---|---|
| Compound | Ether | Ethyl Acetate | Methylene Chloride | *n*-Butyl Chloride |
| Pentobarbital | 77 | 69 | 105 | 90 |
| Meperidine | 80 | 65 | 78 | 70 |
| Meprobamate | 90 | 77 | 100 | 30 |
| Phenobarbital | 95 | 74 | 78 | 15 |
| Cocaine | 77 | 64 | 63 | 90 |
| Imipramine | 70 | 59 | 30 | 61 |
| Desipramine | 86 | 57 | 39 | 65 |
| Promethazine | 7 | 76 | 22 | 51 |
| Diazepam | 86 | 65 | 39 | 70 |
| Alprazolam | 95 | 63 | 70 | 98 |
| Thioridazine | 0 | 41 | 12 | 43 |

antidepressants. It is stressed, however, that the behavior of a particular drug in this investigation should not be used to infer the extraction behavior of other members of the same class.

Drug-free postmortem blood was collected, pooled, and spiked at a concentration of 5 μg/mL of each drug. One half millilitre of blood, representing 2.5 μg of drug, was analyzed in each extraction.

## Results and Discussion

The calculation of recovery in each case was based on the mean of two determinations and was calculated by comparison with an unextracted standard. Reproducibility varied with absolute recovery, but, under optimized conditions, for absolute recoveries over 15%, the coefficient of variation (CV) was between 4 and 12% for all compounds examined. Some studies of extraction procedures have measured recoveries by ultraviolet (UV) absorbance [*1,6*] in order to eliminate error resulting from transfer, filtration, evaporation, and reconstitution steps. It was noted that non-drug material coextracted from typical postmortem blood specimens gave considerable variation in background UV absorbance. Reliable assessment of recovery could be made only after separating the compounds of interest from the coextractants. Analyzing these extracts by GC allowed qualitative assessment of extract purity. The decision to measure recoveries by GC was also based on the fact that most toxicological screening procedures involve GC.

### *Effect of Solvent on Recovery*

Because extracts obtained by absorption extraction on diatomaceous earth proved to be equally compatible with both GC and HPLC, this approach was investigated in more detail. The HPLC analysis of these extracts will be described elsewhere.[3]

Four solvents, diethyl ether, ethyl acetate, *n*-butyl chloride, and methylene chloride, were examined. These were selected because of their widespread use in liquid/liquid extraction protocols [*1.2.4-10*]. Certain other solvents, notably mixed solvents containing alcohols, were considered but proved to be incompatible with absorption extraction columns because changes in the sample due to protein precipitation restricted the flow of the eluent through the matrix.

The effect of pH on recovery is considered later. The results described here were obtained at pH 9. Recovery patterns for each solvent are given in Table 1 and shown graphically in Fig. 1.

The solvent which allowed the highest recovery for the greatest number of compounds was diethyl ether, giving recoveries of greater than 70% for all the compounds examined with the exception of the phenothiazines (thioridazine and promethazine), for which the recoveries were 0 and 7%, respectively. The efficiency with which ether will extract a large number of compounds from tissue is well known; however, ether is less commonly used now due to its high volatility and potential health and fire risks.

Ethyl acetate gave slightly poorer recoveries than ether for most compounds, ranging from 40% (thioridazine) to 77% (meprobamate). This ability to extract a wider range of compounds, however, made ethyl acetate preferable to ether as a solvent for drug screening.

Methylene chloride gave the lowest general recoveries for basic compounds although most were still above 30%. However, it did give the highest recoveries, greater than 78%, for the acid/neutral compounds phenobarbital, pentobarbital, and meprobamate.

*N*-Butyl chloride gave higher absolute recoveries than methylene chloride for most basic compounds, ranging from 43 to 98%, but gave notably poor recoveries for the acid/neutral compounds phenobarbital and meprobamate.

[3]Manuscript in preparation.

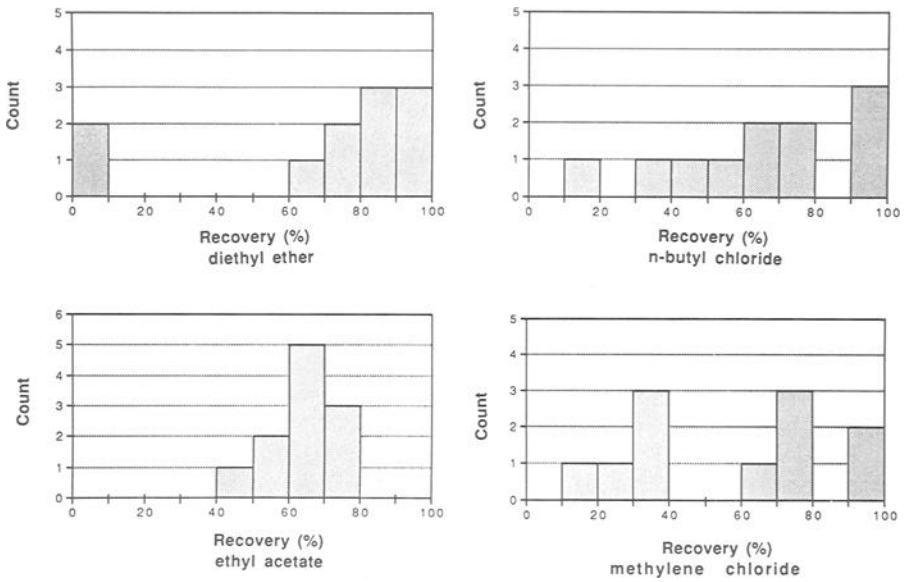


FIG. 1—*Distribution of absolute recoveries for all eleven compounds with each solvent examined. Histograms show number of compounds recovered (count) within each 10% increment. This illustrates general extracting ability of each solvent.*

The results showed that no single solvent could be used to give optimum recovery of all compounds, even within one class. When they were ranked on the basis of recovery and ability as a general solvent, it was concluded that of these four solvents, the optimum eluent for drug screening on liquid/solid absorption extraction columns was ethyl acetate, followed by diethyl ether, *n*-butyl chloride, and methylene chloride.

### *Effect of pH on Recovery*

The effects of pH on recovery at pH 2, 7, and 9 were investigated. Hydrochloric acid (0.01 *M*) was used to adjust the sample pH to 2, the pH 7 buffer was 0.1 *M* phosphate buffer, and the pH 9 buffer was 0.1 *M* borate buffer.

The results for ether are given, (Table 2) and similar patterns were observed for the other three solvents. The absolute recovery of most compounds (for example, cocaine, meperidine, desipramine, alprazolam) showed some reliance on the pH of the extraction. The ionic condition of the analyte will influence its partition coefficient between two phases during liquid/liquid extraction procedures, and recovery can be maximized in this way. Because solid-phase absorption extraction is a nonequilibrium elution process, the partition coefficient was not expected to play as great a role in the recovery of these compounds; consequently, the small changes in partition coefficient with pH should be less significant. The observed differences in recovery at pH 7 and 9 indicated, however, that differences in partition coefficient with pH did have a significant effect, illustrating that the partition coefficient was still a significant factor in liquid/solid extraction.

The effect of pH on the recovery of several other compounds (diazepam, pentobarbital, phenobarbital, and meprobamate) was considerably less, and the absolute recovery showed greater dependence on the solvent being used. It was observed that those compounds which were recovered with highest efficiency were recovered relatively independent of pH for a given solvent.

TABLE 2—*Variation in mean (n = 3) recovery of eleven drugs from postmortem blood. Solvent used was ether, and pH was varied by addition of an appropriate buffer at pH 2, 7, and 9.*

| | pH | | |
|---|---|---|---|
| Compound | 2 | 7 | 9 |
| Pentobarbital | 56 | 63 | 76 |
| Meperidine | 0 | 24 | 80 |
| Meprobamate | 63 | 73 | 90 |
| Phenobarbital | 63 | 74 | 95 |
| Cocaine | 0 | 42 | 77 |
| Imipramine | 0 | 25 | 69 |
| Desipramine | 0 | 36 | 86 |
| Promethazine | 0 | 13 | 7 |
| Diazepam | 59 | 67 | 86 |
| Alprazolam | 6 | 69 | 95 |
| Thioridazine | 0 | 4 | 0 |

On a practical level, the use of acid to adjust the pH of the sample led to the denaturation and precipitation of blood proteins. Although this is observed in liquid/liquid extraction, its effects are more significant when solid-phase extraction is used, in which the precipitation of proteins prevented the passage of the eluent through the column, resulting in much longer elution times.

Of note in these results was the relative independence of the extraction efficiency for acid/neutral compounds with pH. The implication of this finding was that the use of pH 9 would allow the extraction of acid/neutral and basic compounds in one step if an appropriate solvent were used. The limited effect of pH on absolute recovery of these compounds suggested that liquid/solid extraction might be used as a screening technique for acid/neutral and basic compounds, performed on a single specimen buffered at pH 9.

### *Considerations of Extract Quality*

The extracts obtained with each of the four solvents were then considered on the basis of cleanliness. This was assessed in two ways, first by a visual inspection and description of the extract, (for example, visible residue, oily/crystalline) and second by the appearance of coextracted material in a chromatogram of an extract from a blank postmortem blood specimen.

Extraction of the diatomaceous earth itself produced no significant extraneous peaks with any of the solvents when analyzed by GC.

A visual inspection of the extracts before reconstitution showed that ethyl ether and ethyl acetate gave the greatest amount of visible material in the extracts. This appeared as a brown/green film on the inside of the tube in the case of ethyl acetate and as an oily film in the case of ethyl ether. With each of these solvents, during the evaporation step, a small persistent residue of water was observed, causing an increase in time required for evaporation. The appearance of the residue was considerably worse when the extraction was carried out at pH 7 and 9, when the residue contained large amounts of crystalline material, assumed to be the phosphate or borate buffer salts used to control the pH. There was no visible residue at any pH when methylene chloride or *n*-butyl chloride were used as elution solvent. This degree of coextraction observed correlates closely with recoveries achieved using these solvents. The significant water solubility of ether (1.2% at saturation) and ethyl acetate

(3.3% at saturation) [*11*] results in the coextraction of a large amount of water soluble material, notably salts, whose presence in an extract intended for GC is extremely undesirable.

This subjective visual assessment of extract quality is perhaps of more significance than the subsequent interference assessment, since material which does not elute from the chromatographic column will have a deleterious effect on the chromatography and also on the lifetime of the column and injection liner.

The second assessment of extract quality was based on the number of nondrug peaks which appeared in an extract from a drug-free blood specimen. It was found that the greatest amount of interference was encountered when using ether or ethyl acetate, the least with methylene chloride and *n*-butyl chloride. This is also ascribed to solvent properties as discussed above. Extracts obtained using each of the four solvents contained a peak corresponding to cholesterol at both pH 7 and 9.

An overall assessment of recovery and extract quality for all conditions of pH and solvent investigated showed that ether and ethyl acetate were the best general solvents. This made them the least specific of the solvents, however, extracting not only extraneous fatty/oily/lipid and other organic contaminants from the sample, but also water and water soluble crystalline material. The quality of these extracts could be improved by partitioning the extracts between *n*-hexane and the acetonitrile reconstitution solvent as described by Anderson and Fuller [*4*]. In contrast to the acid neutral drugs, however, it was found that the recoveries of some of the basic drugs were reduced by up to 35% following a single hexane/acetonitrile partition.

As noted above, the use of *n*-butyl chloride and methylene chloride gave poorer absolute recovery of the drugs, but it was found here that these solvents were most selective in terms of extract quality. The use of pH 9 resulted in less selective extracts than at pH 7, but did result in the greatest recovery for the greatest number of basic compounds without significantly reducing the recovery of the acid/neutral compounds. Thus the use of pH 9 was favored. Consideration of these factors led to the further investigation of *n*-butyl chloride and methylene chloride at pH 9 in an effort to determine if changes in elution conditions might improve the absolute recovery of the drugs while retaining the high quality of the extract.

### *Effect of Elution Conditions on Recovery*

*Flow Rate*—Under normal eluting conditions, the flow rate of the eluent through the column was fairly uniform, with 10 mL of eluent being collected in approximately 10 to 15 min. To investigate the effect of slowing the flow rate, several columns had glass beads added above the glass wool, which slowed the flow rate from the 0.7 to 1.0 mL/min normally encountered, to values between 0.5 and 0.1 mL/min. Other experiments were performed at higher flow rates by eluting the solvent under positive pressure at faster flow rates. The mean recovery for all compounds achieved in 10 mL of eluent was calculated (Fig. 2) and found to be insignificantly different from recoveries achieved at the normal flow rate, up to flow rates of greater than 2 mL/min, at which point the recovery was observed to drop significantly. The slowest extractions also were found to contain a greater amount of coextracted material as a result of breakthrough of specimen from the column matrix. This suggests that there may be a maximum flow rate of about 2 mL/min, which would still allow optimum recovery without unduly lengthening the extraction procedure or interfering with the extract quality.

Under normal gravity flow elution, the physical properties of the absorbed specimen on the solid phase resulted in a flow rate of no greater than 0.7 mL/min, which was well within the range in which optimum recovery was achieved. Having selected the optimum conditions for elution on the basis of the above results (namely pH 9 and the use of *n*-butyl chloride or methylene chloride as the eluting solvent), the elution profile of the drugs in each solvent was investigated.

*Solvent Volume*—Extraction columns were set up as before, and the eluting solvent was

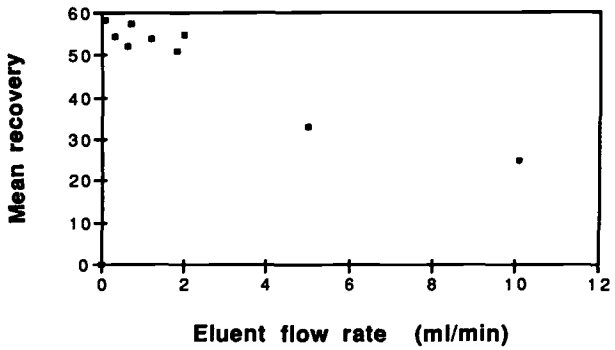


FIG. 2—*Plot of overall mean recovery for all eleven compounds as function of flow rate of eluting solvent (n-butyl chloride) through the extraction column.*

collected in 1-mL fractions, which were evaporated, reconstituted, and analyzed individually. The absolute recovery in each aliquot was calculated, as was the fraction of the total amount recovered.

Methylene chloride, which had previously been shown to elute all eleven compounds with intermediate efficiency, showed similar elution profiles for each drug. Some examples are shown in Fig. 3. The drugs eluted rapidly, with the largest amount appearing in the first millilitre. For all compounds, greater than 80% of the total amount recovered was collected in 5 mLs, and greater than 95% in 7 mLs. It was calculated by linear regression from the elution profile of the slowest eluting compound, diazepam, that a further 20 mL of solvent would be required to increase the yield by a further 5%. This represents an additional 200% volume with corresponding addition in time required for solvent collection and evaporation, for a negligible gain in terms of sensitivity. It thus appeared that for methylene chloride, the collection of 10 mL of eluent represents an optimum amount in terms of absolute recovery, specimen handling, and time of analysis.

The elution profiles for *n*-butyl chloride differed in one important respect (Fig. 4). For those compounds which were found to have a high recovery, the elution profile was similar to that observed for all compounds using methylene chloride; but for those compounds for which *n*-butyl chloride was shown to be a poor solvent, notably phenobarbital and meprobamate, the elution profile differed considerably. There was little or no elution of these drugs in the first 2 to 4 mL. As the drug began to elute, however, it did follow the profile observed for the other compounds, reaching greater than 95% of total recovered within 4 mL and reaching a plateau soon after. Increasing the volume of *n*-butyl chloride eluent did not permit an increased recovery of these compounds, but it did lead to increased contamination of the extract by coextracted material.

*N*-Butyl chloride and methylene chloride had been found to provide cleaner extracts than ether or ethyl acetate, but *n*-butyl chloride was preferred because it allowed a higher recovery of most basic compounds. However, the elution profiles showed that, even using larger volumes, *n*-butyl chloride could not be used to recover a significant fraction of the two common acid/neutral compounds phenobarbital and meprobamate. This made *n*-butyl chloride less than ideal as a solvent for a total drug screen. The poor recovery achieved for some basic compounds with methylene chloride would also prevent the use of that solvent as a general drug-screening solvent.

The expected advantages of using this continuous, nonequilibrium extraction procedure as discussed above were not realized for compounds with very poor recoveries. It is proposed that this is so because those compounds which have very low partition coefficients will have a

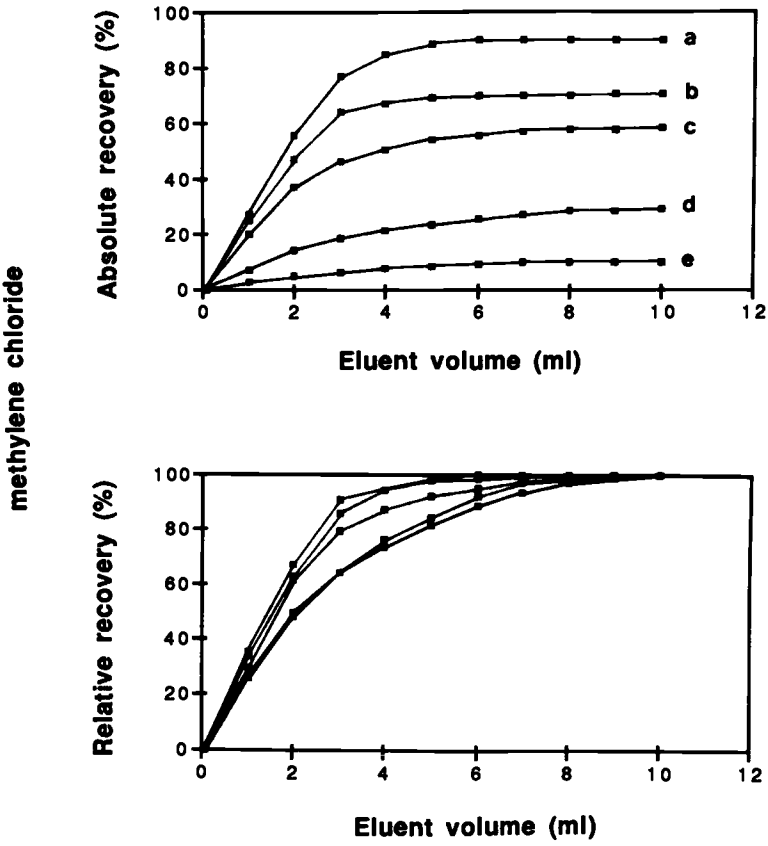


FIG. 3—*Elution profiles for selected drugs using methylene chloride as a solvent:* (top) *cumulative absolute recoveries;* (bottom) *elution profiles showing cumulative recovery in each fraction as a percentage of total amount recovered. (*a = *meprobamate,* b = *phenobarbital,* c = *cocaine,* d = *imipramine,* e = *thioridazine.)*

very much lower equilibrium constant (Keq) and at low concentrations would require very large elution volumes or long elution times to achieve complete recovery. The advantages of a continuous extraction procedure, therefore, are only realized for compounds with high partition coefficients, as evidenced for example with cocaine, diazepam, alprazolam, and pentobarbital, with *n*-butyl chloride as the solvent, or phenobarbital, pentobarbital, and meprobamate with methylene chloride.

Another observation made from the elution profiles concerns the use of absorption extraction as a quantitative method. The dependence of recovery on the volume of solvent collected would be an important factor in the precision of any quantitative method. The use of an internal standard may improve this, but the internal standard must be carefully selected to have an elution pattern as similar as possible to that of the analyte.

### *Comparison of Liquid/Solid and Liquid/Liquid Extraction Methods*

On the basis that *n*-butyl chloride was a better general solvent than methylene chloride, a comparison of equivalent liquid/solid and liquid/liquid procedures was made, using identical solvent and specimen volumes and extracting at pH 9. A blood specimen spiked with

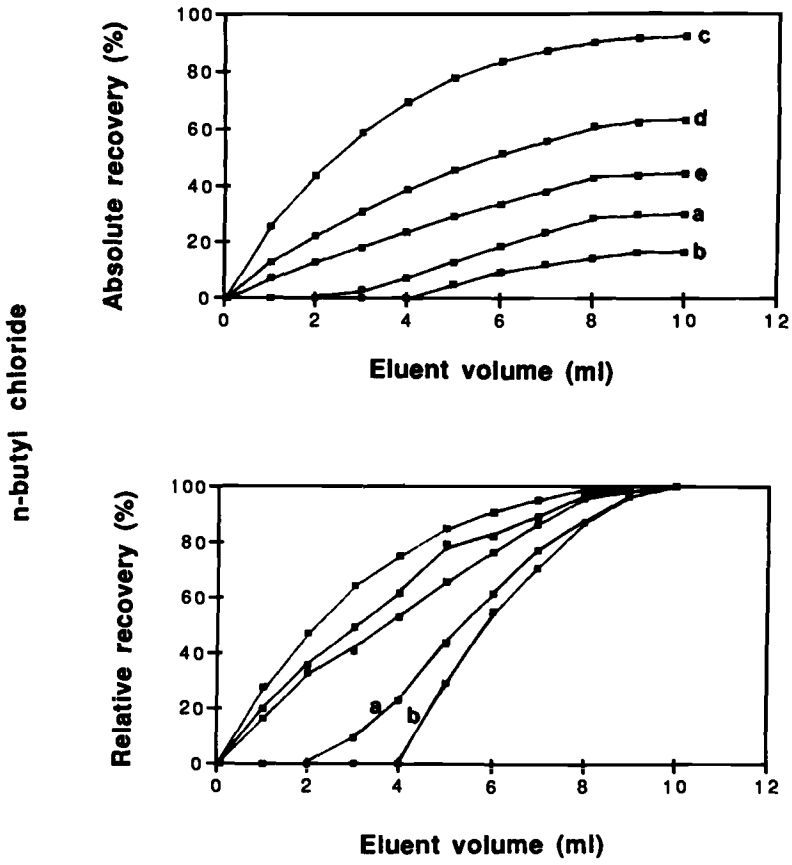


FIG. 4—*Elution profiles for selected drugs using* n-*butyl chloride as a solvent:* (top) *cumulative absolute recoveries:* (bottom) *elution profiles showing cumulative recovery in each fraction as percentage of total amount recovered.* (a = *meprobamate,* b = *phenobarbital,* c = *cocaine,* d = *imipramine,* e = *thioridazine.*)

drugs listed in Table 1 was diluted 3:5 with pH 9 0.05 *M* $KBO_3/H_3BO_3$ as before. Drug-free postmortem blood was also extracted by both methods to investigate the cleanliness of the extract. Of the specimen, 0.8 mL was extracted under the optimum conditions for liquid/solid extraction described above (that is, flow rate less than 2 mL/min, 0.5-mL blood, 0.3-mL 0.1 *M* pH 9 borate buffer, collecting 12 mL of solvent) using *n*-butyl chloride as the eluting solvent.

An equal volume of buffered blood was extracted into 12 mL of *n*-butyl chloride by shaking thoroughly for 5 min. The sample was centrifuged for 5 min, and the solvent was aspirated and evaporated to dryness. The extracts were compared in terms of quality and recovery. This comparison was performed in triplicate for each drug, and the mean recovery along with the coefficient of variation are given in Table 3. Comparative chromatograms from a blank extract are shown in Fig. 5.

Both by visual inspection and on a comparison of nondrug peaks, the liquid/solid extract was superior. As the same chemical conditions pertained in both situations, the chromatograms would be expected to be similar. The extra material in the liquid/liquid extract was assumed to have arizen from micellar/particulate material carried over by aspiration.

TABLE 3—*Mean recovery and CV (n = 3) for each compound in comparative liquid/solid and liquid/liquid extractions. Extraction conditions were: sample volume 0.5 mL, buffer (pH 9 0.1M $KBO_3$/$HB_3O_3$) volume 0.5 mL, solvent (n-butyl chloride) volume 12 mL. Concentration of drugs in blood was 5 μg/mL each (2.5 μg extracted).*

| | Recovery | |
|---|---|---|
| Compound | Liquid/Liquid (CV) | Liquid/Solid (CV) |
| Pentobarbital | 40 (6.6) | 41 (4.8) |
| Meperidine | 21 (8.4) | 25 (9.0) |
| Meprobamate | 0 | 4 (40)[a] |
| Phenobarbital | 0 | 0 |
| Cocaine | 44 (15.5) | 58 (8.6) |
| Imipramine | 48 (10.4) | 51 (12.5) |
| Desipramine | 59 (12.6) | 54 (10.1) |
| Promethazine | 42 (8.4) | 60 (11.3) |
| Diazepam | 60 (17.1) | 70 (7.6) |
| Alprazolam | 73 (12.3) | 74 (9.5) |
| Thioridazine | 20 (19.1) | 41 (12.6) |
| MEAN | 37 (12.3) | 48 (9.5) |

[a]Not included in calculation of mean CV.

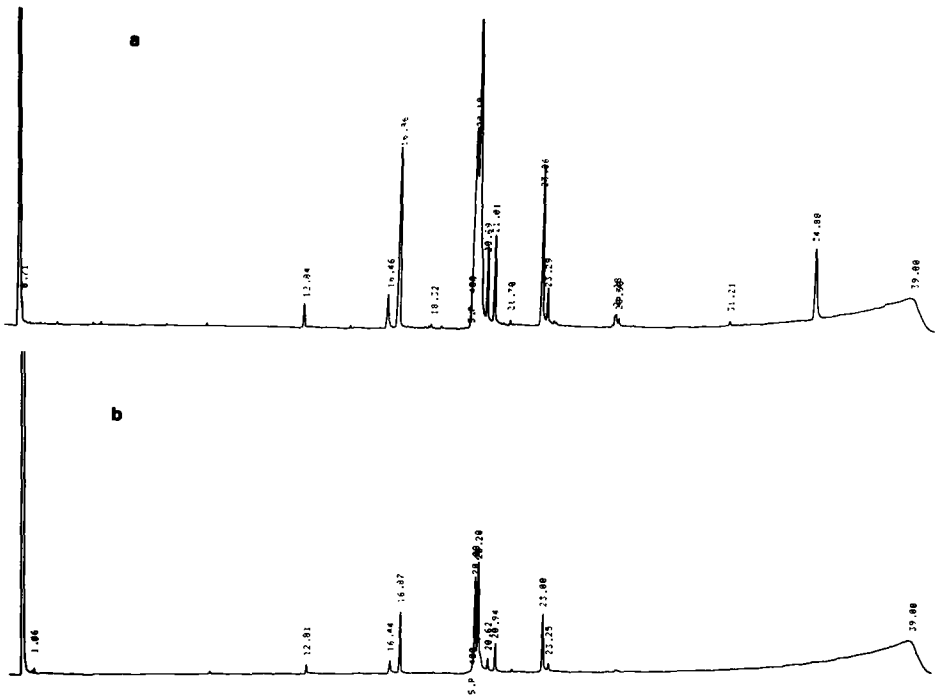


FIG. 5—*Chromatograms showing comparative extracts from identical samples using* (a) *liquid/liquid extraction and* (b) *liquid/solid absorption extraction. Extraction conditions in text.*

Under normal circumstances, a back extraction would be used; however, since this was a comparison of single-step extraction methods, this was not done. A back extraction step would however also be expected to decrease the recoveries through specimen loss, solvent mixing, and incomplete aspiration.

Ironically, the best method found for cleaning up the organic phase from the liquid/liquid extract was to filter it through a short column of diatomaceous earth, the material used in the liquid/solid extraction columns. This filtration, however, resulted in some loss of recovery.

In terms of absolute recovery of the drugs from postmortem blood, solid-phase extraction was the superior technique. This was attributed in part to the nature of solid-phase extraction as a continuous, nonequilibrium process as opposed to a batch liquid/liquid extraction method. Because solid-phase absorption extraction is a nonequilibrium elution process, the partition coefficient should not play as great a role in the recovery of these compounds as it would in liquid/liquid extraction. Solvent entering the column reaches equilibrium with the immobilized aqueous phase and is immediately replaced with fresh solvent. Since this is a continuous process, real equilibrium is never achieved, and in theory 100% recovery should be possible if sufficient solvent is allowed to pass. The improved recovery obtained in the liquid/solid extract in this study may be due in part to this effect. The recovery of the liquid/liquid extraction method can be improved by using a second batch of solvent, but in theory can not be expected to exceed that achieved by the constant flow extraction method. The reproducibility was generally good, with a CV of between 4.8 and 12.5% for the drugs examined (Table 3). Efforts were made to keep the consistency of the specimen uniform throughout this study, but the nature of the specimen is that it is not homogeneous and the extent to which this influences variation in recovery is not known. The variation observed in recovery and the differences in CV are of more importance in quantitative analysis than in drug screening and are currently under investigation.

## Conclusions

The aim of this study was to examine the applicability of liquid/solid extraction as a specimen preparation technique for drug screening of postmortem blood prior to GC analysis and to determine the factors influencing recovery and extract quality. It was found that the use of an organic solvent with a low water solubility gave the cleanest extracts. The selectivity of the solvent, that is, those compounds which it preferentially dissolves, is not significantly different from that achieved with liquid/liquid extraction. Consequently, the solvent should be carefully selected to suit the group of compounds of interest in any particular application, but the recoveries achieved with a solvent in a liquid/solid extraction procedure are expected to be similar and will probably be enhanced due to easier specimen handling.

The effect of pH on the recovery of the compounds investigated showed that with liquid/solid extraction pH had an effect similar to that observed in liquid/liquid extraction, with basic drugs being recovered most efficiently at alkaline pH. With the use of a suitable solvent, however, acid/neutral drugs could also be recovered at this pH. The effect of flow rate of the solvent through the column on absolute recovery was found to be significant only at very high flow rates. Flow rates normally encountered under gravity elution conditions were in the correct range for optimum recovery.

It was found that the theoretical 100% recovery was not achieved for many compounds. This shows that partition coefficients still play an important role in liquid/solid extraction and emphasizes the care required in selection of an appropriate solvent for a given application. The liquid/solid extraction procedure, however, did give increased recovery over an equivalent liquid/liquid extraction method because of the combined effects of nonequilibrium extraction and easier specimen/solvent manipulation.

The results make it very clear that the behavior of a particular drug in this investigation should not be used to infer the extraction behavior of other members of the same drug class. Absorption extraction on a diatomaceous earth material provided clean, efficient extracts of sufficient quality to be analyzed directly by GC without the risk of contamination of the chromatogram or of the instrument.

On a practical basis, solid-phase absorption extraction has many advantages over a similar liquid/liquid extraction, including higher recoveries and cleaner extracts as a result of the elimination of emulsification, micelle formation, and carryover during aspiration. Other advantages include "hands-off" extraction, which could be readily performed in a fume hood. With this method, no shaking or centrifugation was required and, consequently, more rapid analysis was possible. In addition, there was less operator contact with potentially infectious specimens or toxic solvents.

It is hoped that the results of this investigation will encourage more use of liquid/solid extraction techniques in forensic toxicology and will foster an awareness of the need to optimize procedures and measure recoveries of compounds in any new extraction method before its use for drug screening or quantitative analysis is advocated.

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