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The Screening of Blood by Gas Chromatography for Basic and Neutral Drugs

The screening of urine for the presence of drugs has received considerable attention [*1-3*] but methods for screening blood for basic and neutral drugs have not received the same attention, with the result that the methods which are available lack sensitivity [*4.5*]. A system that can detect drugs at a blood level of at least 0.1 mg per 100 ml of blood (1 μg/ml) would be considered reasonable, although this sensitivity is not required for all drugs.

From an instrumental point of view, the method of Adams et al [*3*] would appear to be the most sophisticated published to date. It uses a twin-column system (3% OV-1 and 3% OV-17) with temperature programming, solvent-free injection, and automatic data reduction. The use of the solvent-free injection system increases the range of drugs that can be chromatographed on a given column; however, this range can also be extended by derivatization and the use of suitable liquid phases [*6*] without the necessity of solvent-free injection. A screening procedure based on such a system is described here.

Experimental

Instrumentation

The gas chromatographic (GC) system consists of two 1.2-m U-shaped glass (0.6-cm outside diameter) columns, one containing 3% Poly A-103 on Gas-Chrom Q, 80-100 mesh (Applied Science Lab.) and the other 3% OV-1 on Gas-Chrom Q, 80-100 mesh. Both columns are set in the oven of a Varian 2100 (Varian, Walnut Creek, Calif.) equipped with two flame ionization detectors, a dual electrometer, and twin-pen recorder. The oven temperature is set at 215°C and the injector and detector temperatures at 260°C with the initial flow rates (approximately 50 ml/min of N_2) adjusted so that acetylated tranylcypromine has a retention time of approximately 6 min on the Poly A-103 column and codeine a retention time of 5 min on the OV-1 column.

Materials

All solvents used are of analar reagent grade. The acetylating solution consists of 0.1 ml of acetic anhydride in 10 ml of chloroform. "Spiked" samples were prepared by

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adding standard drugs at room temperature to blood obtained from a hospital blood bank. The prepared blood samples were left to stand for at least 30 min.

Procedure

A 2-ml blood sample is extracted by allowing the blood to drip into a vortexing mixture of di-isopropylether (IPE) (6 ml) and NaOH (saturated solution) (0.2 ml). The mixture is allowed to settle and the IPE decanted. A second 2-ml blood sample is processed in the same way.

One of the processed blood samples is reextracted with IPE (6 ml), the second IPE extract is combined with the first, and this mixture is back-extracted with 1.5 ml of 0.5*N* H_2SO_4. The IPE layer is pipetted off, reduced to dryness at 50°C with N_2, and the residue taken up in 50 μl of chloroform/*t*-butanol (19:1). This is the neutral fraction. The 0.5*N* H_2SO_4 solution is transferred to a 5-ml centrifuge tube and 0.3 ml of 4*N* NaOH is added, followed by 50 μl of the chloroform/*t*-butanol mixture. The mixture is shaken on the vortex mixer for 30 s and then centrifuged. Five microlitres of the acetylating mixture are added to the chloroform layer with a syringe and the mixture left to stand for 10 min before being shaken on the vortex mixer and centrifuged. This chloroform/*t*-butanol layer constitutes the basic Fraction A.

The second 2 ml of blood, after initial IPE extraction, are reextracted with 6 ml of *n*-butyl chloride (*n*-bu-cl), the *n*-bu-cl extract is added to the IPE extract, and the mixture is treated in a similar manner as above but not acetylated. The final chloroform/*t*-butanol layer constitutes the basic Fraction B.

The fractions are injected into the GC in the following sequence:

(1) 5 μl Fraction A Poly A-103 at 215°C
    5 μl Fraction B, OV-1 at 215°C
(2) 5 μl neutrals, Poly A-103 at 215°C
    5 μl neutrals, OV-1 at 215° C
(3) 5 μl Fraction B, OV-1 at 245°C

A schematic diagram of the extraction steps is shown in Fig. 1.

Results and Discussion

Di-isopropylether (IPE) was found to be the most useful solvent since emulsion formation was rare and the extracts obtained were relatively free of interfering peaks (Figs. 2-7). This was particularly true of the neutral fraction (Fig. 6), although after three consecutive injections, blood artefacts begin to elute and it becomes necessary to raise the temperature to clear the columns of the interference. Fractions A and B do not produce any significant interfering peaks. *N*-butyl chloride is a useful solvent [7], but the neutral fraction obtained from the combined IPE and *n*-bu-cl extraction gave too many interfering peaks on the chromatogram. Other solvents tried were chloroform and acetone/ether (1:1) and heptane/*t*-butanol (19:1). Chloroform and acetone/ether frequently gave rise to emulsions and the heptane/*t*-butanol, although a useful solvent system, was not superior overall to IPE or IPE/*n*-bu-cl. Sodium hydroxide was used to alkalize the blood in preference to borate buffer [1], ammonia, or potassium carbonate, all of which, under the conditions of extraction, were more prone to lead to emulsion formation.

The combination of the two columns, Poly A-103 and OV-1, was chosen so that as large a range of drugs as possible could be screened at a given time [6].

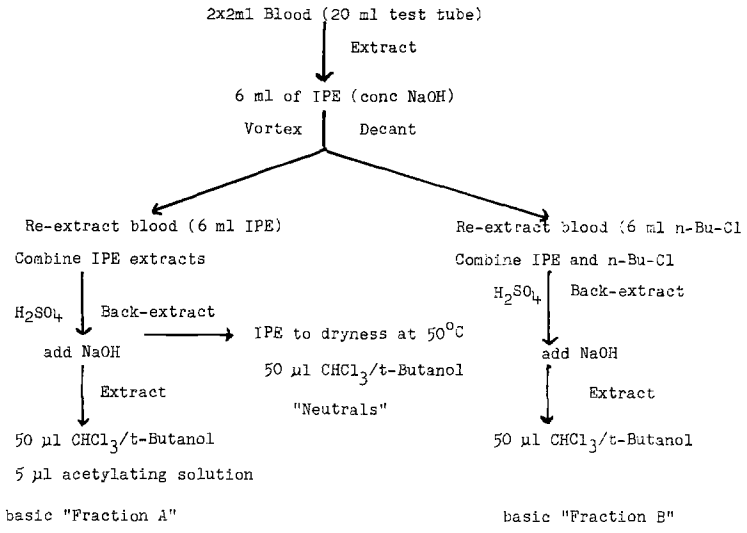

FIG. 1—*Extraction procedure.*

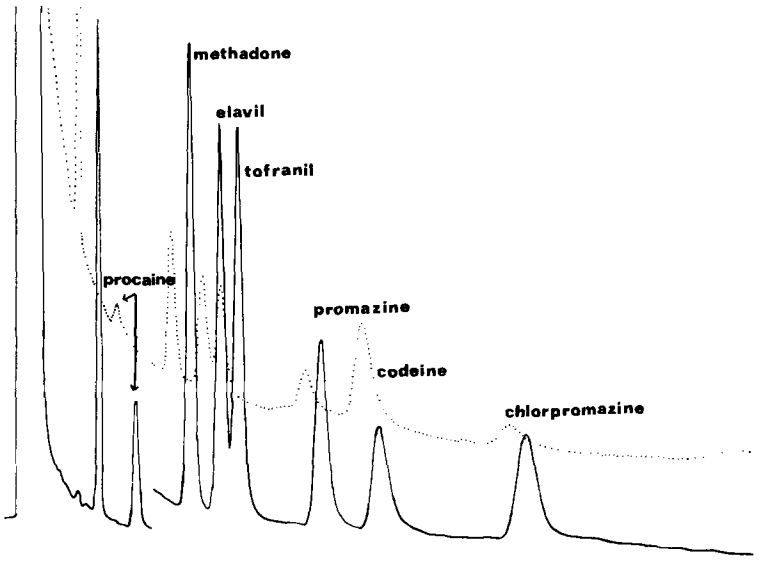

FIG. 2—*The solid line represents a chromatogram (on 3% OV-1 at 215°C) of a direct IPE extract of drugs from aqueous alkaline solution. The dotted line (displaced from the solid line for comparison) represents a chromatogram of Fraction B of a "spiked" blood sample extracted as in the section on Procedure and containing procaine (0.1 mg%), methadone (0.1 mg%), amitriptyline (Elavil®, 0.1 mg%), imipramine (Tofranil®, 0.1 mg%), promazine (0.1 mg%), codeine (0.1 mg%), and chlorpromazine (0.1 mg%).*

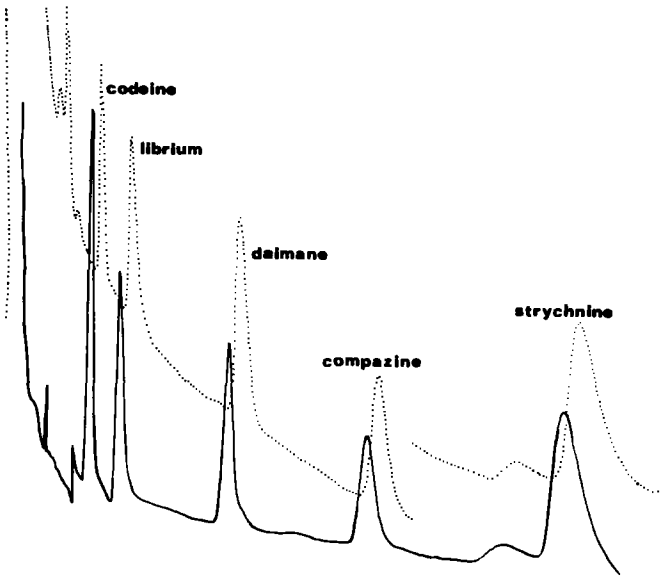

FIG. 3—*The solid line represents a chromatogram (on 3% OV-1 at 245°C, codeine RT 1.5 min) of a direct IPE extract of drugs from aqueous alkaline solution. The dotted line (displaced from the solid line for comparison) represents a chromatogram of Fraction B of a "spiked" blood sample extracted as in the section on Procedure and containing codeine (0.1 mg%), chlordiazepoxide (0.2 mg%), flurazepam (Dalmane®, 0.1 mg%), compazine (0.2 mg%), and strychnine (0.2 mg%).*

Tables 1 and 2 list the drugs that were detectable in blood to which known quantities had been added. Detectability means the production of a signal that is at least 10 times the background noise. Figures 2-4 illustrate the detectability of drugs from blood and, as can be seen, most of the peaks obtained are more than adequate. The majority of the drugs listed are easily detectable at the 0.1 mg per 100 ml of blood level; however, notable exceptions are thioridiazine, nortriptyline, and desipramine. The poor recovery of the latter two is probably pH dependent. The poor recovery of some of the neutral drugs is not a problem, as the therapeutic levels of these drugs are relatively high [8].

Although the data from "spiked" blood are useful, the true test of any procedure is its performance with case samples. Fifty-nine cases have been completed to date. In 22 of these no drugs were detected, in 20 only one drug was indicated, in 12 two drugs, and in 5 cases three drugs or more were found; 23 different drugs were indicated in all. Emulsions did occur in two of the cases and were corrected by adding more solvent. One badly putrefied blood sample gave a few background peaks, particularly on the Poly A-103 column. Figures 5 and 6 illustrate a case where propoxyphene was indicated together with phenacetin (neutral fraction); the second peak in Fig. 5 is believed to be norpropoxyphene amide [9], since blank blood "spiked" with norpropoxyphene and treated as under the section on Procedure gave a peak with the same retention time. The amide has been found in six of the eight positive propoxyphene cases. The flexibility of the two-column system is illustrated in Fig. 4, where the separation of amphetamine and meperidine is not possible unless the sample is rechromatographed on the OV-1 column. It should be remembered that for those drugs that acetylate, the nonacetylated drug is available in Fraction B if additional confirmatory GC is required.

TABLE 1—*Relative retention times (RRT) of common drugs on 3% Poly A-103 at 215°C extracted from blood and detectable at a level of 0.1 mg%.*

Fraction A	RRT	Neutrals	RRT
Methamphetamine Ac	0.35	Meprobamate[a]	0.39
Amphetamine Ac	0.38	Methyprylone[a]	0.47
Meperidine	0.40	Glutethimide[a]	1.49
Phenylethylamine Ac	0.45	Phenacetin[a]	1.34
Methyprylone[a]	0.47		
Diphenhydramine	0.61		
Phencyclidine	0.68		
Phenmetrazine Ac	0.87		
Lidocaine	0.94		
p-Methoxyamphetamine Ac	0.98		
Tranylcypromine[a] Ac	1.00 (6 min)		
Phenacetin[a]	1.34		
Methadone	1.42		
MDA Ac	1.63		

[a] 0.5 mg%.

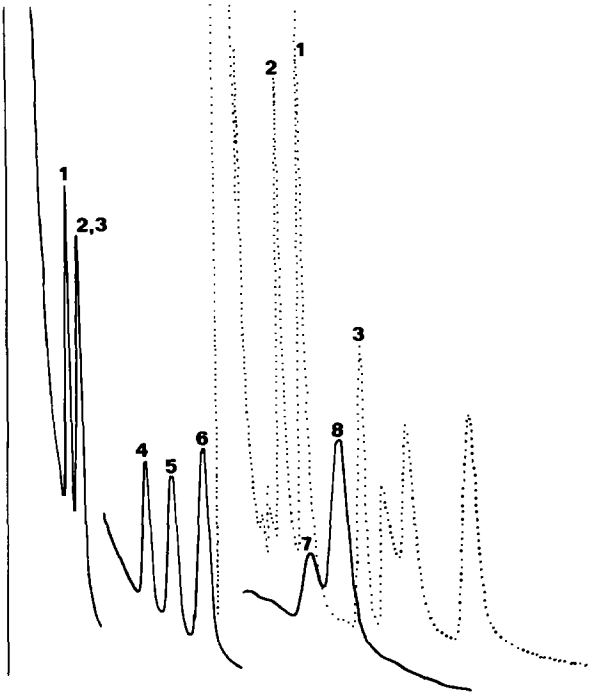

FIG. 4—*The solid line represents a chromatogram (on 3% Poly A-103 at 215°C) of Fraction A of a "spiked" blood sample extracted as in the section on Procedure and containing* (1) *methamphetamine (0.1 mg%),* (2) *amphetamine (0.1 mg%),* (3) *meperidine (0.1 mg%),* (4) *phencyclidine (0.1 mg%),* (5) *phenmetrazine (0.1 mg%),* (6) p-*methoxyamphetamine (0.1 mg%),* (7) *methadone (0.1 mg%), and* (8) *methylenedioxyamphetamine (0.1 mg%). The dotted line (displaced from the solid line for comparison) represents a chromatogram of the same sample chromatographed on the OV-1 column at 170°C. Meperidine RT approximately 4.9 min.*

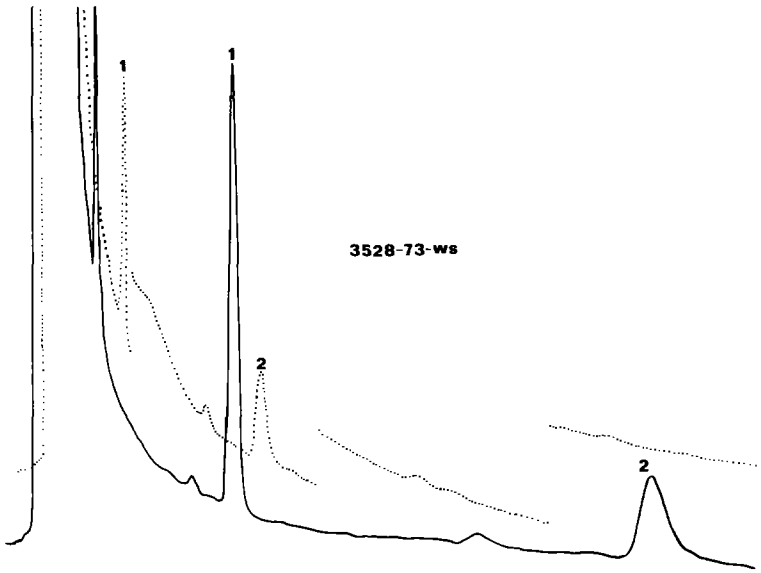

FIG. 5—*The solid line represents a chromatogram (on 3% OV-1 at 215°C) of Fraction B of a case blood sample extracted as in the section on Procedure. The blood contains* (1) *propoxyphene (0.2 mg%) and* (2) *norpropoxyphene amide. The dotted line (displaced from the solid line for comparison) represents a chromatogram of the same sample chromatographed at 245°C on the OV-1 column. (Codeine RT 1.5 min).*

TABLE 2—*Relative retention times (RRT) of common drugs on 3% OV-1 at 215 and 245°C extracted from blood and detectable at a level of 0.1 mg%.*

Fraction B at 215°C	RRT	Neutrals at 215°C	RRT	Fraction B at 245°C	RRT
Methadone	0.48	Glutethimide [a]	0.21	Codeine	1.00 (1.5 min)
Propoxyphene	0.56	Meprobamate [b]	0.19	Trifluperazine	2.05
Amitriptyline	0.58	Methaqualone	0.49	Flurazepam	2.52
Trimipramine	0.60			Quinine [b]	2.90
Imipramine	0.62			Anileridine	3.19
Doxepine	0.64			Chlorpromazine sulph [b]	3.33
Nortriptyline [a]	0.64-1.64			Haloperidol [b]	4.10
Desipramine [a]	0.73-1.89			Compazine [b]	4.35
Artane	0.68			Thioridazine [a]	6.20
Promethazine	0.71			Strychnine [b]	6.20
Pentazocine	0.74				
Promazine	0.85				
Diphenidol	0.90				
Cyproheptadine	1.00				
Codeine	1.00 (5 min)				
Diazepam [b]	1.19				
Chlorpromazine	1.40				
Levomepromazine [b]	1.40				
Chlordiazepoxide [b]	1.48				
Norpropoxyphene amide	1.67				

[a] 0.5 mg%.
[b] 0.2 mg%.

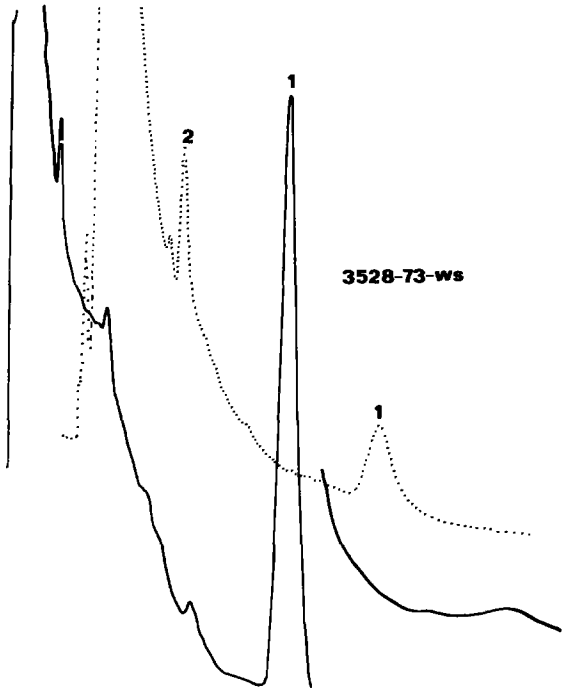

FIG. 6—*The solid line represents a chromatogram (Poly A-103 at 215°C) of an extract (neutral fraction) of a case blood sample treated as in the section on Procedure. The blood contained* (1) *phenacetin. The dotted line (displaced from the solid line for comparison) represents a chromatogram (on 3% Poly A-103 at 215°C) of Fraction A of the same blood sample containing* (1) *phenacetin and* (2) *phenylethylamine.*

A limited number of case urine specimens have been carried through the procedure and satisfactory results were obtained. Using "spiked" urine samples, detection limits were lower (typically a drug level of 0.05 mg% was detectable using 4 ml of urine) than for blood. All the drugs listed in Tables 1 and 2 are detectable in urine.

Using the present system it has been possible for one person to process up to five samples per day. The efficiency obviously could be increased by the use of an automatic injection system. Work is continuing with case samples in an effort to build up a more complete picture of the utility of the system.

Summary

A GC procedure is described for the screening of biological fluids for the presence of basic and neutral drugs. Extracts are chromatographed simultaneously on a two-column system designed so that a wide range of drugs can be detected. A majority of the drugs is readily detectable in blood in concentrations of 0.1 mg per 100 ml. The procedure is illustrated with examples of casework as well as examples of "spiked" blood samples.

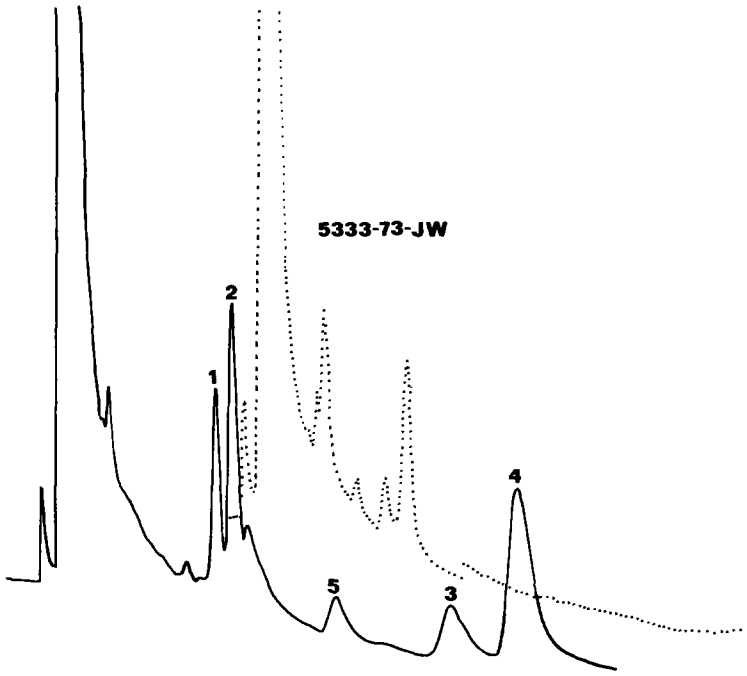

FIG. 7—*The solid line represents a chromatogram (on 3% OV-1 at 215°C) of Fraction B of a case blood sample extracted as in the section on Procedure. The blood contains* (1) *propoxyphene,* (2) *imipramine,* (3) *chlordiazepoxide,* (4) *norpropoxyphene amide, and probably* (5) *codeine. The dotted line (displaced from the solid line for comparison) represents a chromatogram of the same sample chromatographed on the OV-1 column at 245°C.*

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