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ROUTINE ANALYSIS OF BARBITURATES AND SOME OTHER HYPNOTIC DRUGS IN THE BLOOD PLASMA AS AN AID TO THE DIAGNOSIS OF ACUTE POISONING

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SUMMARY

A gas–liquid chromatographic method has been devised whereby both the qualitative and the quantitative analysis of a number of hypnotic drugs in the blood may be accomplished simultaneously. Small (50-μl) plasma aliquots are required. The technique is rapid and will detect 2 mg/l concentrations of these compounds. The barbiturates glutethimide, methaqualone, and meprobamate are the principal drugs covered by the scheme. After overdose the concentrations that occur in man are at, or above, this minimum level of detection.

INTRODUCTION

The micro-extraction gas–liquid chromatographic (GLC) procedure described by Flanagan and Withers[1] has proved invaluable in our laboratory for the rapid identification and measurement of barbiturates and related hypnotics in plasma samples obtained from acutely poisoned patients. Some improvements to the technique, subsequently introduced, are described in this paper and the opportunity is taken to draw renewed attention to the advantages of micro-extraction techniques over conventional bulk-extraction methods in drug analysis.

The major innovation has been the simultaneous use of a second GLC system to supplement the information derived from extract analyses performed upon the CDMS system of the original procedure. This secondary column, based upon the Poly A 103 liquid phase, was chosen following a survey undertaken by Berry[2]. The column systems have been isothermally operated in a dual-detector chromatograph and the outputs monitored independently. The extraction efficiencies and relative retention times of some drugs not studied initially have also been included. The use of pure oxygen to support the combustion of the flames in the ionisation detectors has given greater sensitivity and accuracy with the instrument available than the use of conventional compressed air supplies.

This procedure has formed the basis of our 24-h supraregional drug analysis service designed to assist in the diagnosis of acute poisoning and has been in routine

use since 1972. The method is rapid, simple, reliable, free from interference, and gives satisfactory accuracy and reproducibility.

EXPERIMENTAL

Gas–liquid chromatography

A Pye 104 Model 24 dual-column gas chromatograph fitted with flame-ionization detectors was used throughout. This was modified so that the output from each detector could be monitored independently using separate amplifier/recorder modules. A column oven temperature of approximately 200° was used together with carrier gas (nitrogen) flow-rates of approximately 60 ml/min. The detector oven temperature was 250° and the injection port settings were 2. The hydrogen and oxygen inlet pressures were 15 and 10 p.s.i., respectively, equivalent to flow-rates of 45 and 200 ml/min. Coiled glass chromatography columns, 5 or 3 ft. in length and 0.25 in. in diameter, were silanised by immersion in 5% dichlorodimethylsilane in toluene for 1 h, rinsed in methanol and dried at 100°. The column packings were prepared by a standard evaporation technique as described by Berry[2], using 80–100 mesh HP Chromosorb W as the support material. Thermally compatible column systems were obtained by use of 5 ft. 3% w/w CDMS (Perkin-Elmer, Beaconsfield, Great Britain) and 3 ft. 3% w/w Poly A 103 (Field Instruments, Richmond, Great Britain). Use of a filtration technique, *e.g.*, as described by Supina[3], has been found to yield approximately twice the effective phase loading if the above quantities of material are used. Optimum separations were achieved with on-column injections, and these were often performed by use of a syringe fitted with an 11-cm needle.

Extraction procedure

The following reagents were used: (a) phosphate buffer, consisting of 4 mole/l sodium dihydrogen orthophosphate in distilled water, and (b) internal standard solution consisting of 10 mg/l tetraphenylethylene (TPE) in chloroform.

Sample extractions were performed in duplicate by the method of Flanagan and Withers[1].

Dreyer tubes (obtained from Scientific Supplies, Vine Hill, London, Great Britain) or their equivalents, were used as the extraction vessels. To each tube, 5 μl of phosphate buffer, 50 μl of plasma sample, and finally 50 μl of internal standard solution were added. It was found that an Everett stainless-steel needle (No. II serum) attached to a luer-fitting syringe in the dispenser used for this last addition prevented droplets of solution from being left on the needle. The contents of the tube were agitated on a Vortex mixer for 30 sec and the tube was centrifuged for 2–3 min at approximately 3000 rpm.

The mixing technique adopted was initially gentle in order to achieve an easy admixture of the two phases; following this the precipitation of plasma protein to give a definitive interface after centrifugation was ensured by more vigorous treatment.

A small quantity of air was taken into a gas chromatographic syringe and the syringe needle was passed through the lipoprotein interface into the organic phase. After slowly expelling the air, a suitable quantity of extract was taken into the syringe and the outside of the needle was wiped with a tissue prior to the injection of the ex-

tract onto a gas chromatographic column. This process was repeated to give an aliquot of extract for analysis on the second column system.

Qualitative analysis

A solution of five rapidly eluting barbiturates together with TPE was prepared in chloroform. The concentration of each of the components of this mixture was 10 mg/l and the drugs selected were barbitone, butobarbitone, amylobarbitone, pentobarbitone, and quinalbarbitone. To ensure efficient instrument performance this "Barbiturate Standard Mixture" was chromatographed upon both systems prior to the analysis of sample extracts.

Aliquots of the primary sample extract were analysed simultaneously on both systems. It was found that quantitative phenobarbitone analyses could be conveniently performed by injection of an aliquot of the second extract onto the CDMS system immediately after the elution of TPE from the first. (Cyclobarbitone, phenylbutazone,

TABLE I

RETENTION TIMES OF THE DRUGS UNDER STUDY ON BOTH CHROMATOGRAPHIC SYSTEMS

The compounds are listed in order of elution on the Poly A 103 column.

Drug	*Retention time (relative to TPE)*		*Retention time (min)*	
	Poly A 103	*CDMS*	*Poly A 103*	*CDMS*
Barbitone	0.21	0.29	3.1	3.8
Glutethimide	0.29	0.49	4.3	6.4
Aminopyrine*,**	0.30	0.41	4.4	5.3
Phenacetin*				
Allobarbitone***	0.32	0.49	4.7	6.4
Hexobarbitone	0.36	0.52	5.3	6.8
Butobarbitone	0.37	0.48	5.5	6.2
Allylbarbitone§	0.38	0.51	5.6	6.6
Amylobarbitone	0.43	0.52	6.4	6.8
Phenazone*	0.45	0.77	6.5	10.0
Meprobamate	0.48	1.00	7.1	13.0
Pentobarbitone	0.49	0.59	7.3	7.7
Vinylbarbitone§§	0.53	0.69	7.8	9.0
Quinalbarbitone	0.61	0.74	9.0	9.6
Methaqualone	0.68	1.16	10.1	14.8
TPE	1.00	1.00	14.8	13.0
Cyclobarbitone	1.58	2.06	23.4	26.8
Phenylbutazone	1.77	1.94	26.2	25.2
Heptabarbitone	2.32	2.81	34.3	36.5
Phenobarbitone	2.46	3.62	36.4	47.1
Primidone§§§	7.30	10.2	108	133
Phenytoin§§§	19.2	25.2	284	328

* These compounds gave characteristic (tailing) peaks on both systems.

** Amidopyrine, amidophenazone.

*** 5,5-Diallylbarbituric acid.

§ Butalbital, 5-allyl-5-isobutylbarbituric acid.

§§ 5-Vinyl-5-(1-methylbutyl)barbituric acid.

§§§ Primidone and phenytoin are included for information; they obviously played no part in the analyses.

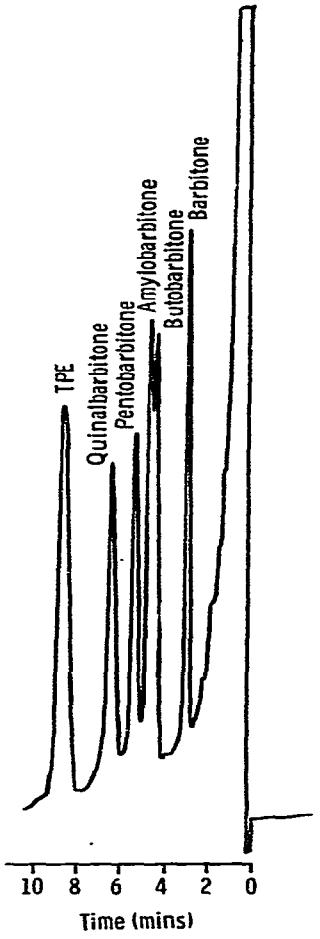
TABLE II

DRUG STANDARD SOLUTIONS AND CALIBRATION GRADIENTS

Drug	*TPE (mg/l)*	*Calibration gradient (l/mg)*		*Standard solutions of drug available (mg/l)*													
		Poly A	*CDMS*	*5*	*10*	*15*	*20*	*25*	*30*	*40*	*50*	*70*	*75*	*100*	*150*	*200*	*250*	*300*
Allobarbitone	10	0.123	0.066	×	×	×	×	×	×	×	×							
Allylbarbitone	10	0.129	0.078	×	×	×	×	×	×	×	×							
Amylobarbitone	10	0.086	0.086	×	×	×	×	×	×	×	×							
Barbitone	20	0.060	0.063		×		×		×	×	×	×		×				
Butobarbitone	10	0.072	0.081	×	×	×	×	×	×	×	×	×		×				
Cyclobarbitone	10	0.026	0.022	This compound is unstable in chloroform solution														
Glutethimide	10	0.162	0.132	×	×	×	×	×	×	×	×							
Heptabarbitone	10	0.020	0.019	×	×	×	×	×	×	×	×							
Hexobarbitone	10	0.133	0.099	×	×	×	×	×	×	×	×							
Meprobamate	10	0.025	"nil"		×		×		×	×	×	×		×				
Methaqualone	10	0.120	0.078	×	×	×	×	×	×	×	×							
Pentobarbitone	10	0.054	0.061	×	×	×	×	×	×	×	×							
Phenobarbitone	5	0.035	0.028	×	×	×	×	×	×	×	×	×		×				
Phenobarbitone	10	0.019	0.015					×			×		×	×	×	×	×	×
Phenylbutazone	10	0.024	0.025		×		×		×	×	×	×		×				
Quinalbarbitone	10	0.062	0.058	×	×	×	×	×	×	×	×							
Vinylbarbitone	10	0.083	0.054	×	×	×	×	×	×	×	×							
"Tuinal"*	10	**	**	×	×	×	×	×	×									

* Each standard contained the specified quantity of amylobarbitone and quinalbarbitone.

** These values are given under the individual drugs.



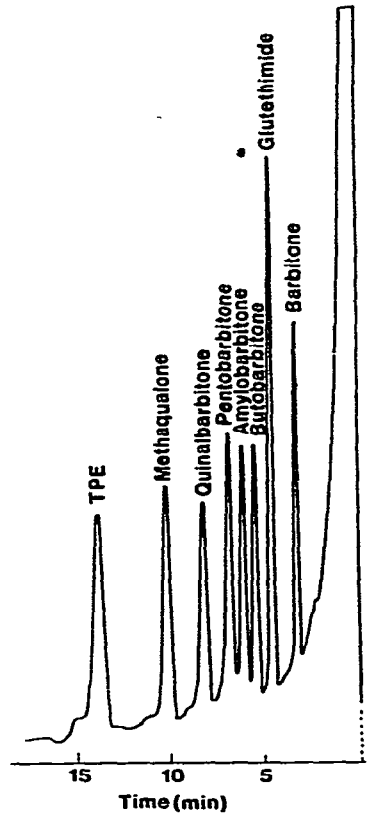


Fig. 1. Analysis of the "Barbiturate Standard Mixture" on the CDMS system. The concentration of all components was 10 mg/l; 3-μl injection.

Fig. 2. Analysis of glutethimide and methaqualone together with some barbiturates on the Poly A 103 system. The concentration of all components was 10 mg/l; 3-μl injection.

and heptabarbitone, all of very rare occurrence in overdose samples, were in this case indicated from the Poly A 103 chromatogram.) The compounds present were identified by their retention times relative to TPE, either from the standard mixture chromatogram, or from the data presented in Table I. The separation of this standard mixture on the CDMS system is shown in Fig. 1 and the chromatography of a similar solution (but also containing glutethimide and methaqualone at concentrations of 10 mg/l) on the Poly A 103 system is illustrated in Fig. 2.

Quantitative analysis

Instrument calibration. Standard drug solutions, each containing a known concentration of TPE (usually 10 mg/l), were prepared in chloroform. The range of drug concentrations used was that commonly encountered in overdose; Table II lists the

standard solutions available for each drug together with their TPE concentration. These solutions were stored in the dark at room temperature and were found to be stable for at least one year (exception: cyclobarbitone).

The ratio of the peak height of the compound to the peak height of TPE was linearly related to the concentration of each drug over the range studied. Typical calibration gradients (*i.e.*, peak height ratio/drug concentration) are also shown in Table II.

Sample drug concentrations. The concentrations of compounds identified during the qualitative stage of this procedure were measured by comparison of peak height ratios of sample and standard chromatograms. To ensure similarity at least two standard solutions containing the identified drug(s) were chromatographed and their calibration gradient(s) compared to those listed in Table II. Normally, the CDMS system was used for quantitative analyses; the Poly A 103 system had obviously to be used, however, for meprobamate. If the difference between the duplicates was greater than 10%, the extractions and analyses were repeated. Sample drug concentrations were calculated by dividing the calibration gradient into the sample peak height ratio. Correction of this result for extraction losses was made by reference to the data presented in Table III.

TABLE III

RECOVERY FACTORS

Recovery of added drug from heparinised bovine plasma; mean ± S.D. for 15 extracts.

Drug	*% Recovery*	*S.D.*	*"Recovery factor"*
Allobarbitone	76	8	1.32
Allylbarbitone	100	8	1.00
Amylobarbitone	91	8	1.10
Barbitone	43	5	2.33
Butobarbitone	83	5	1.20
Cyclobarbitone	89	7	1.12
Glutethimide	100	7	1.00
Heptabarbitone	87	8	1.15
Hexobarbitone	94	4	1.10
Meprobamate	64	8	1.56
Methaqualone	88	5	1.14
Pentobarbitone	94	5	1.10
Phenobarbitone	78	6	1.28
Phenylbutazone	80	9	1.25
Quinalbarbitone	85	8	1.18
Vinylbarbitone	100	5	1.00

RESULTS AND DISCUSSION

We feel that GLC with flame-ionisation detection is the method of choice for the analysis of the drugs described here in the acute overdose situation. With the present technique, both qualitative and quantitative results could be obtained upon a minimal sample with maximal speed and accuracy. Identification as well as measurement is imperative if a worthwhile interpretation of the analysis is to be derived.

The direct-extraction technique employed is simple and reproducible. It

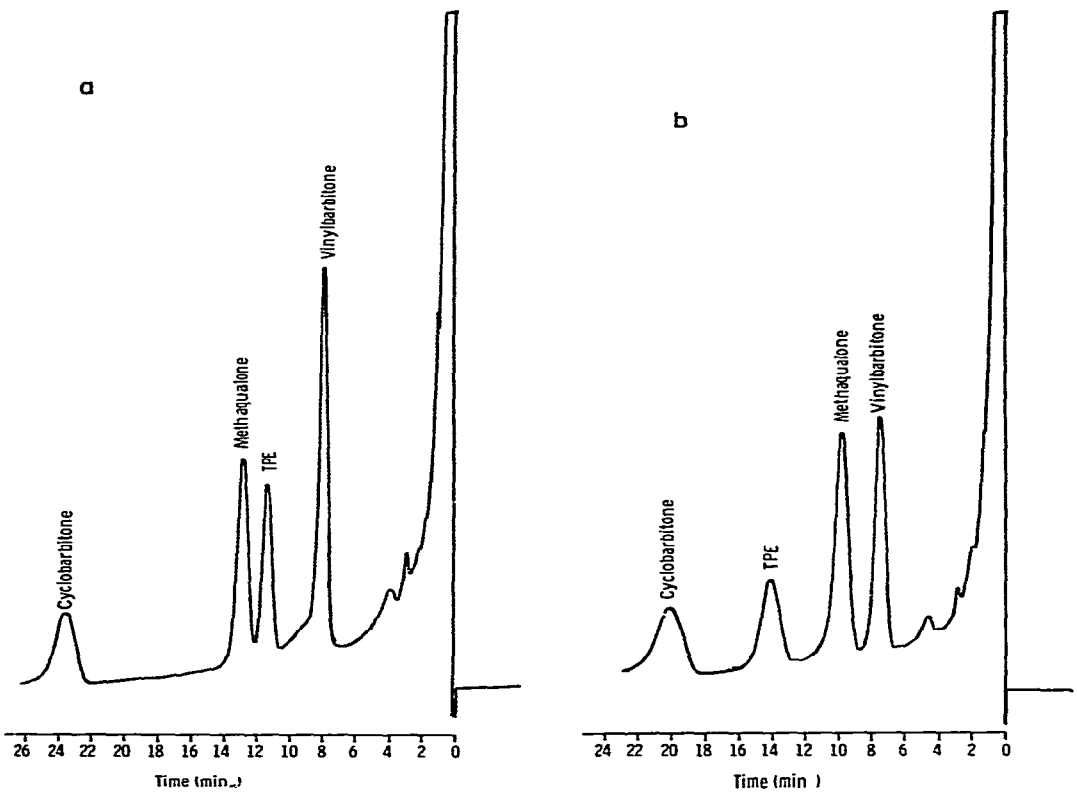


Fig. 3. Analysis of sample extracts from a patient after a multiple drug overdose; 3-μl injection. (a) CDMS system; (b) Poly A 103 system.

offers a further advantage over procedures applicable to barbiturates only in that some commonly encountered non-barbiturate hypnotics may be analysed simultaneously. This is well illustrated by the chromatograms shown in Figs. 3a and b, which were obtained from extracts of plasma from a 26-year-old female who had ingested (amongst other drugs) methaqualone, cyclobarbitone, and vinylbarbitone.

Choice of a second column system

The second column system to supplement the information obtained from the CDMS system originally described was introduced to remove the need for complementary TLC analyses. Relative retention times on the second system served to confirm drug identities and to differentiate between the few drugs which co-chromatographed on the CDMS system. A Poly A 103 system has proved an ideal complement, its particular advantages being:

(i) The barbiturate drugs commonly prescribed in Great Britain can be differentiated using this system and their order of elution is identical to that on the CDMS system. These drugs are barbitone, butobarbitone, amylobarbitone, pentobarbitone, quinalbarbitone, cyclobarbitone, heptabarbitone, and phenobarbitone.

(ii) Phenobarbitone had a shorter retention time than on the CDMS system. In practice, phenobarbitone analyses upon the samples in question were performed

isothermally with the other drug analyses described in this paper and not as originally recommended at a slightly higher temperature. This was because changing the oven temperature did not result in an overall decrease in analysis times, especially if the technique of "doubling up" of injections was adopted.

This lowered temperature for phenobarbitone analyses did, however, result in an increase in the limit of sensitivity of the technique to this compound. 10 mg/l was considered to be the limit as opposed to 5 mg/l when the columns were operated at approximately 220°.

(iii) Methaqualone and glutethimide were fully resolved from TPE and amylobarbitone, respectively, compounds with which they tended to co-chromatograph on the CDMS system. This was especially important in the case of glutethimide, although if the CDMS system alone was available, the chromatographic pattern given by this drug and its metabolites was characteristic. The chromatography of sample extracts from glutethimide overdose patients will be discussed in detail in the following section.

(iv) Certain uncommon drug mixtures, *e.g.*, butobarbitone/amylobarbitone or glutethimide/butobarbitone, could be analysed easily on this system.

(v) On the Poly A 103 system meprobamate was resolved from TPE, whereas these compounds had virtually identical retention times on the CDMS system. Very high meprobamate concentrations in the sample have given rise to a greatly heightened internal standard peak and evidence of thermal degradation of the drug on this latter system. Thus, the presence of meprobamate in overdose would not go undetected if the CDMS system was being used alone.

(vi) Both Poly A 103 and CDMS systems could be operated simultaneously and isothermally in a suitable chromatograph. Careful matching of column pairs was essential to ensure that optimum chromatographic performance was achieved. This was accomplished by preparing batches of column packing approximating to the loadings quoted and then adjusting column lengths and carrier gas flow-rates until the systems were compatible; the quoted conditions usually proved satisfactory.

Interpretation of chromatograms from cases of glutethimide overdosage

The fact that chloroform extracts of plasma obtained from patients who have taken large quantities of glutethimide contain compounds in addition to the parent drug was reported by Sunshine *et al.*[4]. These compounds, which were thought to be metabolites, were present on chromatograms obtained during analyses on an SE-30 column system, a substance eluting after glutethimide being present in apparently higher concentrations than glutethimide itself. Similar findings using diethyl ether extracts of plasma were reported by Ambre and Fischer[5].

Plasma samples obtained from similar sources also gave these additional peaks when analysed by the present technique, although in no cases were any of these compounds present in much higher concentrations than glutethimide. However, it is unlikely that the hydroxy metabolite suggested by Ambre and Fischer[5] to give rise to this large peak would be efficiently extracted and chromatographed close to glutethimide under the conditions of this assay. Similarly, this metabolite is unlikely to penetrate to the central nervous system to exert hypnotic effect; in our experience the course of glutethimide poisoning follows the plasma decay of the parent drug.

We have made no attempt to further identify the compounds observed, or to definitively characterise their retention times on both systems. However, Figs. 4–6

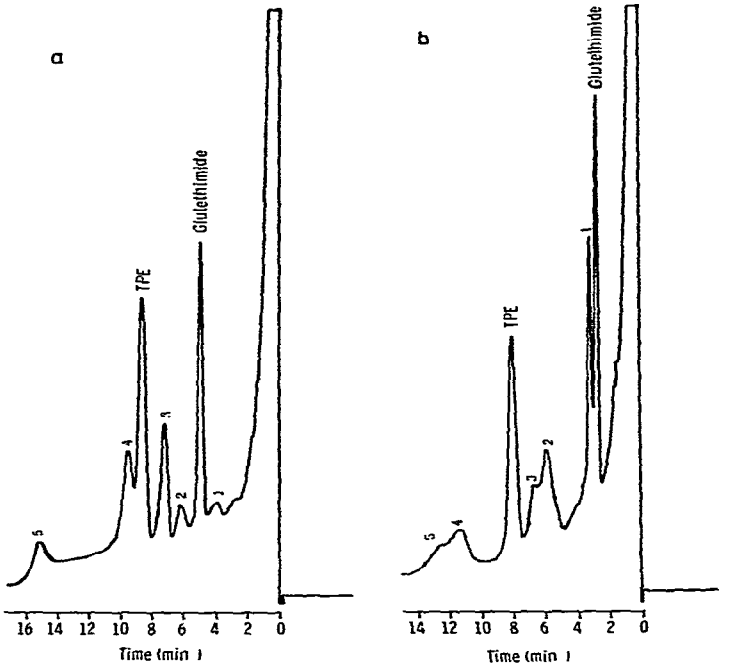


Fig. 4. Analysis of sample extracts from a single glutethimide overdose patient at approximately 6 h after ingestion of the drug. 3-μl injection. (a) CDMS system; (b) Poly A 103 system. 1–5 are metabolites (*cf.* Table IV).

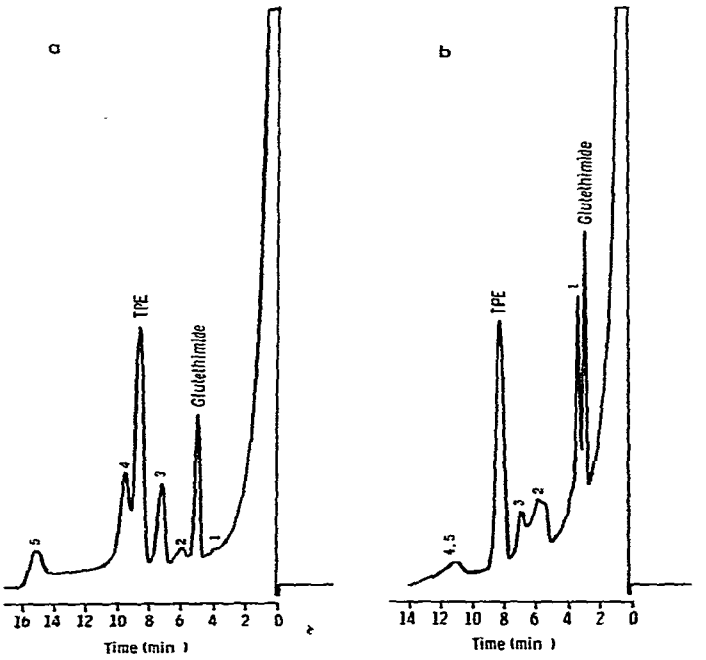


Fig. 5. Analysis of sample extracts from a single glutethimide overdose patient at approximately 12 h after ingestion of the drug. 3-μl injection. (a) CDMS system; (b) Poly A 103 system. 1–5 are metabolites (*cf.* Table IV).

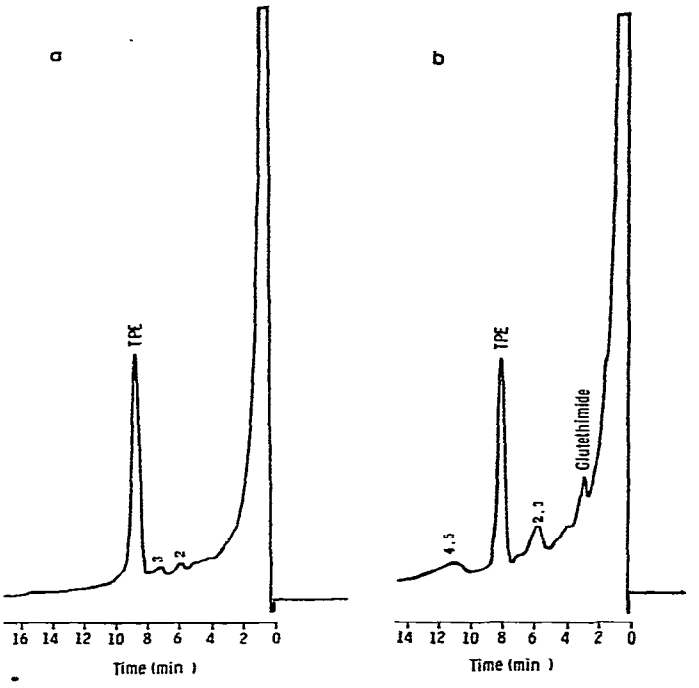


Fig. 6. Analysis of sample extracts from a single glutethimide overdose patient at approximately 60 h after ingestion of the drug. 3-μl injection. (a) CDMS system; (b) Poly A 103 system. 1–5 are metabolites (*cf.* Table IV).

show the chromatograms obtained on the analysis of samples obtained at different times from the same glutethimide overdose patient. A similar number of peaks appear in the chromatograms from each column system indicating that glutethimide does no co-chromatograph with any of these compounds. (The peak designated No. 2 on the Poly A 103 system may arise from two compounds.) Similarly, sample extracts performed using chloroform but without TPE did not reveal additional metabolites which could co-chromatograph with the internal standard. Both column systems gave similar quantitative glutethimide results also making it unlikely that a metabolite chromato-

TABLE IV

RETENTION TIMES AND FREQUENCY OF OCCURRENCE OF GLUTETHIMIDE METABOLITES (*cf.* FIGS. 4–6)

C = Common; O = occasional; R = rare.

CDMS			*Poly A 103*		
Metabolite No.	*Retention time (relative to TPE)*	*Frequency*	*Metabolite No.*	*Retention time (relative to TPE)*	*Frequency*
1	0.44	R	1	0.39	C
2	0.72	R	2	0.73	C
3	0.84	C	3	0.83	R
4	1.09	C	4	1.40	O
5	1.74	O	5	1.58	R

graphs with the parent drug. The occurrence of these compounds and their relative concentration is, however, highly variable from patient to patient. Table IV gives the retention times of these compounds relative to TPE on both systems together with an indication of their frequency in overdose samples.

Emulsion formation

The frequency of emulsion formation with human plasma samples has been estimated to be less than 1 % when the procedure outlined earlier was used. If this did occur, it was found that a rapid re-mixing of the contents of the tube after the initial centrifugation followed by a brief (*ca.* 1-min) re-centrifugation served to break up the emulsion and yield a clear extract. The extraction efficiency might vary in this situation, however, although this extract would be adequate for a qualitative analysis. It is important to ensure an easy mixing of the two phases initially as this gives a reproducible extraction and reduces the risk of emulsion formation. Rat, mouse, or rabbit plasmas have not given rise to problems when analysed by this technique during drug metabolism studies, while bovine plasma has recently been troublesome in this respect.

Instrument conversion to dual flame ionization detection

All GLC-based drug analyses are at present performed isothermally in our laboratory. Since a large majority of our Pye 104 instruments are fitted with dual-column attachments designed for temperature-programming applications, this idle capacity was exploited. A simple "splitting" of the detector outputs, coupled with investment in an additional amplifier/recorder system, gave in effect two chromatographs, provided always that temperature-compatible columns are available. Should future requirements be for temperature-programmable instruments, back-conversion would be a simple matter.

Supplementary instrumentation

Quantitative phenobarbitone analyses can be performed, as has been stated, with adequate speed using the dual-column system in the isothermal mode by careful timing of sample injections. However, where extra instrument capacity is at hand, it is useful to have an additional column system specifically for this purpose. Glass columns, 5 ft. in length, packed with 1 % Poly A 103 or 3 % OV-17 on HP Chromosorb W (80–100 mesh) are suitable; phenobarbitone has a retention time of 1.2 relative to TPE on the OV-17 system. Corroborative retention time data were always sought on a second or subsequent system. Columns based upon the phase OV-225 have been poorly reproducible and therefore are no longer to be recommended for barbiturate analyses.

Use of oxygen

This work was facilitated by the use of pure oxygen instead of air to support the combustion of the flames in the ionisation detectors. The advantages are:

(i) Sensitivity is increased by a factor of approximately 5 (Jones and Green[6]). This means that the instrument can be operated at a higher attenuation, thereby reducing instrument noise.

(ii) Running costs are reduced for, although pure oxygen is more expensive

than compressed air on a volume-to-volume basis, less is used in the operation of the detectors. Consequently fewer cylinders are rented and "downtime" due to cylinder changeover is minimised.

(iii) Complete combustion of the solvent (chloroform) is achieved, thus preventing the deposition of soot on the detectors and increasing the period of operation between maintenance.

Dedicated instrumentation

Our workload has justified the dedication of a dual-column instrument to this analytical system. These requirements are composed of patient and "quality control" samples, teaching commitments and, when possible, research and development. This dedication has resulted in improved instrument performance (both reduced "downtime" and increased practicable sensitivity), and in constant readiness for emergency work.

Interference

Many types of samples were analysed by this extraction technique over a period of four years. The interference observed may be classified as follows:

(i) Non-drugs. Extracts of neonatal plasma have given complex chromatograms and it was necessary to resort to TLC analysis of urine to confirm the identities of some compounds. Generally, plasma samples collected into lithium heparin are free from non-drug sources of interference. Cholesterol, although present in the extracts, has a very long retention time on both systems and did not interfere. Both serum and grossly haemolysed samples gave analogous results to plasma. Specimens obtained from patients undergoing intravenous infusion showed interference, however, as did samples collected into citrate anticoagulant. Extraneous peaks in samples collected into rubber-stoppered or -sealed tubes, which presumably arose from compounds leaching out of the rubber, were also observed.

(ii) Drugs. The system has proved effective in the differentiation from the drugs discussed thus far of three barbiturates not prescribed in Great Britain. These were allobarbitone, allylbarbitone, and vinylbarbitone. Their retention times are given in Table II and recovery factors in Table III; peak shape proved an important consideration when assigning barbiturate status to these compounds. Phenazone (antipyrine), phenacetin, and aminopyrine, the latter drugs rarely encountered in Great Britain, were extracted and chromatographed upon the column systems, but gave characteristic tailing peaks from sample extracts. Their retention times are included in Table II, but in our experience these may vary slightly even between columns containing the same liquid phase. This is probably due to the polar interactions with the support that also cause peak tailing. Aminophenazone pure substance gives a symmetric peak so it is possible that a metabolite is present which contributes to the peak observed in sample extracts. Fig. 7 illustrates the chromatography of phenazone and phenacetin on both systems at concentrations of 20 mg/l relative to 10 mg/l TPE.

Sensitivities

When only TPE was present upon the sample chromatograms, it was concluded that drugs were not present in the sample at concentrations of 2 mg/l or greater (10 mg/l for phenobarbitone and meprobamate). The use of effectively deactivated sup-

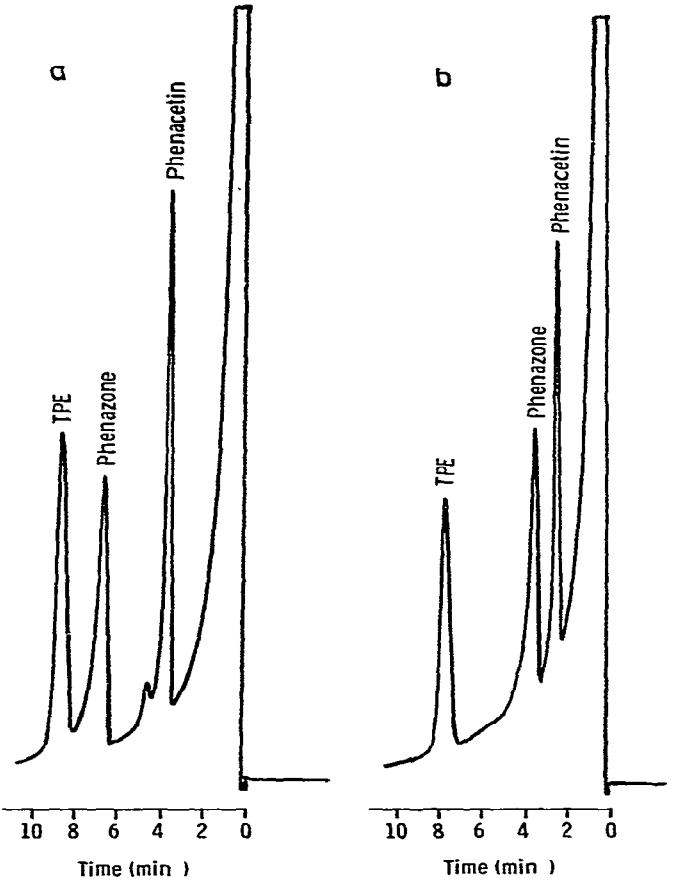


Fig. 7. Analysis of phenacetin and phenazone at concentrations of 20 mg/l relative to 10 mg/l TPE. 3-μl injection. (a) CDMS system; (b) Poly A 103 system.

port material is essential if adsorption of drugs on this material is to be avoided, for should this phenomenon occur, it would decrease the sensitivity attained. Although this work was performed using column systems based upon HP Chromosorb W, problems attributable to batch variations in this support have recently been experienced. Another material found to be satisfactory in this respect, not only for barbiturates but also for work with other drugs, was Varaport 30.

The quality of the support used could also influence the extent to which meprobamate was thermally degraded during the analysis. All Poly A 103 column systems were tested to ensure adequate sensitivity to this compound before acceptance for use. CDMS systems were similarly checked for their chromatography of phenylbutazone.

Quality control

Although this procedure was designed to yield maximal reliability with regard to both the identification and measurement of compounds, it was felt obligatory to

incorporate additional safeguards. Thus, a "quality control" scheme was initiated whereby prepared specimens were periodically analysed "blind" during sample runs.

Plasma solutions of the various drugs at overdose concentrations were obtained as follows:

A 1 g/l drug solution was prepared in chloroform; a Hamilton dispenser was used to add multiples of 50-μl aliquots (equivalent to 50 μg) of this drug solution to 10-ml volumetric flasks. (More than one drug solution was added in some cases, *e.g.*, amylobarbitone and quinalbarbitone to give a "Tuinal" sample). The solvent was removed under a stream of compressed air and 10 ml of heparinised bovine plasma were added. After time had been allowed for the drug to dissolve, the standard was ready for use. It was convenient to store these samples in 0.5-ml aliquots at —20° until required, although glutethimide was found to be unstable when stored in this way for periods longer than approximately six months.

TABLE V

QUANTITATIVE RESULTS OF A "QUALITY CONTROL" SCHEME

Drug	*No. of analyses*	*Drug concentration (mg/l)*		
		Prepared	*Measured*	*S.D.*
Amylobarbitone	8	5	4.8	0.7
	5	10	11.0	1.3
	4	15	12.6	0.7
	5	20	19.7	1.8
	5	30	27.6	2.4
	6	45	39.8	4.4
	6	50	47.0	4.0
Butobarbitone	5	10	9.7	1.6
	8	20	19.2	2.3
	5	40	40.1	4.4
	5	80	80.5	2.6
Glutethimide	2	10	9.3	1.7
	2	25	20.0	0.7
	3	50	34.8	1.7
Methaqualone	4	5	5.1	0.9
	3	15	15.3	1.5
	4	25	22.5	3.7
Pentobarbitone	2	5	4.6	0.3
	3	10	8.6	1.0
	5	20	18.4	2.2
	4	40	40.5	6.4
	5	45	43.7	4.4
Phenobarbitone	4	15	14.2	0.9
	6	35	32.5	3.6
	5	70	59.9	10.0
	6	130	112.4	9.2
	4	150	141.0	23.5
Quinalbarbitone	3	5	5.2	0.6
	10	10	10.0	1.6
	5	15	17.0	2.1
	11	25	25.6	3.0

Twenty-six laboratory personnel of all grades participated in our initial scheme, over a period of approximately two years. Both the qualitative and the quantitative aspects of the technique were tested and, in all, 144 samples were analysed. This gave rise to a total of 161 possible quantitative measurements (since some samples contained two drugs) and of these 153 (95%) were correctly identified; a summary of the results obtained is presented in Table V.

In addition five "blanks" were correctly "identified" together with a further eight glutethimide samples which were found to have lost some drug through decomposition and are therefore not included in Table V. Finally, of three samples containing phenazone at a concentration of 50 mg/l, two were correctly identified while one was mistaken for quinalbarbitone.

CONCLUSIONS

The work described has shown (a) that this direct extraction procedure performed upon 50-μl plasma samples is suitable for routine laboratory use and (b) that the operation of a gas–liquid chromatograph with flame-ionisation detectors in this mode enables the identification and measurement of as little as 10 ng of some of the named hypnotics "on-column" and without prior derivatisation. In effect this meant that, when analysing specimens from suspected drug overdose patients, a preliminary acid/neutral thin-layer chromatographic screen of urine was dispensed with, since virtually simultaneous identification and measurement of the plasma concentration of all drugs detectable upon the thin-layer chromatographic system was obtained with the GLC technique. In the rare instances when the GLC analysis was inconclusive, supplementary information was needed to aid in the interpretation of the chromatograms, and the capacity to perform thin-layer chromatographic analyses was therefore retained.

The principle embodied in this procedure is one of rapid solvent extraction followed by analysis of this extract without prior concentration. Its main advantages are as follows:

(a) Economy

Little time, reagents, or apparatus are required. This has obvious advantages in the emergency situation, but is also valuable where large numbers of samples are to be analysed.

(b) Sample requirements

The small quantity of plasma required has proved advantageous where samples from young children are analysed or when other analyses were performed on the same sample. This feature has also proved invaluable when the procedure was used for work in small animals.

(c) Sources of interference

GLC techniques are liable to show interference from a number of origins. Impurities may be present in the extracting solvent or arise from the apparatus used in the analysis; they may also be normal, "endogenous" plasma constituents which are

extracted under the conditions used. Procedures in which large volumes of solvent and sample, prolonged extraction times, and multiple transfers of extracts between vessels are used emphasize these problems. A final extract evaporating stage serves to concentrate the impurities present as well as the compound to be measured.

The present technique eliminates these potential sources of interference by employing a 1:1 ratio of sample to solvent, thereby avoiding any concentration stage, a short extraction time, and using only one glass vessel.

(d) Accuracy and reproducibility

The limited number of steps in the procedure —addition of reagents, mixing, centrifugation, extract withdrawal, and analysis and measurement of peak retention times and heights— minimised the potential sources of error.

This direct extraction and analysis principle has been successfully applied to the plasma measurement of other compounds and groups of compounds within our laboratory. Most notable is the measurement of benzodiazepine drugs (particularly diazepam) using electron capture detection (D. M. Rutherford, unpublished results). The hypnotic ethchlorvynol is assayed by a similar procedure (R. J. Flanagan and D. J. Berry, unpublished results) to that described here for barbiturates but with the use of a different internal standard and a lower column temperature. Phenazone (R. J. Flanagan, unpublished results) and acetanilide (F. Harvey, unpublished results) are measured routinely by direct-extraction techniques, as are ethotoin and methyprylone (D. J. Berry, unpublished results).

Finally, the current method has been operational for our 24-h supraregional drug analysis service for the last four years. It has proved to be extremely reliable and easy to operate, since in order to achieve a rapid throughput of samples in an emergency, the instrument is maintained at a constant degree of readiness. In our opinion, no better system for the analysis of the drugs we have described in the acute overdose situation has so far been evolved.

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