CHROM. 4791

Preparative gas chromatography as a clean-up procedure in toxicology*

One basic problem inherent to toxicology is the difficulties encountered in extracting the toxic agent from tissue in amounts large enough and sufficiently pure for identification.

Several methods of purification of tissue extracts are available to the toxicologist. In general, however, those which give pure substances also give very low yields. Preparative gas chromatography (GC) has been examined as a technique for the purification of extracts and this communication relates some of its potential in toxicology.

Experimental

TABLE I

TECHNICAL DATA

Instrument:	Varian Aerograph Autoprep Model 705
Recorder:	Honeywell, 1.0 mV
Column:	3 m × 7 mm, aluminium
Support:	Chromosorb A, 45/50 mesh
Coating:	OV-1, 10% OV-17, 10%
Carrier gas:	Nitrogen; flow rate 140 ml/min, inlet pressure 60 lb.
Detector:	Flame ionisation
Split ratio:	1 : 4.3
Temperatures:	Inlet, 280° Column, varying Collector, 250°

The solution to be chromatographed was made up by dissolving the extract in ten parts of a suitable solvent, such as alcohol, chloroform or hexane. It was either injected in the conventional manner with a syringe, or where several injections were necessary, by means of the automatic injector. The latter allowed for the injection of up to 1.5 ml at one time and could be set to inject automatically at pre-determined intervals, overnight if necessary.

In most instances, the instrument was operated isothermally except where two compounds with great differences in molecular weight were run simultaneously, *e.g.* morphine and quinine or amitriptyline and perphenazine. In these cases the temperature was increased during the run so that the higher molecular weight compound was recovered in a reasonable time.

Results and discussion

Ordinarily, long columns (7–10 m) with high loading of coating material (20–

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30%) are used in preparative scale GC. In this study it was found, however, that good separations could be obtained with shorter columns (3 m) and lower loading (5–10%) in much shorter time. This also allowed the application of this method of purification to certain high molecular weight drugs (*e.g.* some of the phenothiazines), which would not elute from the high load columns at reasonable temperatures or at acceptable retention times. Another difficulty encountered with the high load columns was the contamination of the column with the extraneous material in the extract. In some cases the instrument had to run at high temperatures for 6–8 h before the column was ready for another injection. This did not occur with the shorter, lower load columns. Furthermore, contamination of the collected materials with silicone grease occurred less often with the low load columns.

Columns of a wider diameter than 7 mm were tried but since these had to be constructed so that both ends were reduced to 7 mm in order to fit the instrument, a "dead volume" was created at both these ends. This not only caused broadening of the peaks but also, in several instances, the appearance of substances injected days before as "ghost" peaks in the middle of another run. Needless to say, the recoveries in these cases were lower than usual.

Although a small part of the effluent is directed into the flame ionisation detector, the main portion is led out of the instrument and the desired material collected in specially designed bottles[1]. Most of the neutral and acidic drugs could be collected with fairly good yield without cooling of the effluent. However, nearly all of the basic drugs required cooling of the collection bottles in order to trap them in good yields. This was especially evident with the phenothiazine derivatives. This group of drugs and some others tend to form a very fine aerosol or smoke which escapes easily through the glass wool traps making them difficult to collect. In general, therefore, it is recommended that the collection bottles be cooled in order to achieve maximum recovery. An ice–water mixture will do in many cases but a dry ice–acetone mixture is more efficient and gave better recoveries.

A preparative scale chromatogram is frequently of a very poor quality. The peaks are often broad and asymmetrical and show all the characteristics of an overloaded column. This is of little importance since the tracing is needed only to show where to start and discontinue collecting; the main objective is to obtain a pure

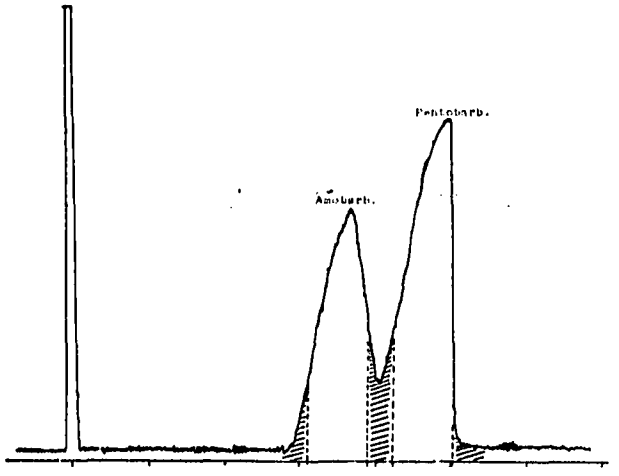

Fig. 1. Preparative GC separation of amobarbital and pentobarbital.

product. An example of this is shown in Fig. 1 for a mixture of amobarbital and pentobarbital. The collection of amobarbital was started approximately one-quarter up the first peak and stopped again before any contamination by pentobarbital could take place. The collection of the latter compound was started a short distance up the upstroke of the peak and discontinued just before the base line. The identification of the two barbiturates was accomplished by means of crystal test, analytical GC and IR spectrophotometry. Both analytical GC and TLC showed that separation of the drugs was complete.

Fig. 2 shows the UV spectra obtained before and after a preparative GC clean-up

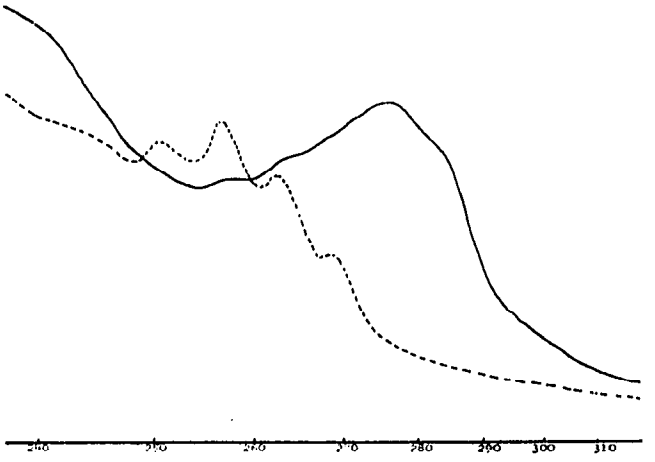

Fig. 2. UV spectrum of glutethimide. Case No. 884-67 LC liver. Solvent: ethanol. ———, Neutrals from 5-way split; – – – –, neutrals after preparative GC clean-up.

of a neutral fraction from a routine five-way extraction of a liver. The history of the case indicated suicide by ingestion of glutethimide (Doriden). Due to partial decomposition of the organ the resulting extract was very impure and no satisfactory thin-layer or gas chromatogram could be obtained. Only the UV spectrum gave a slight indication of the presence of the drug in the extract. The clean-up resulted in a crystal-

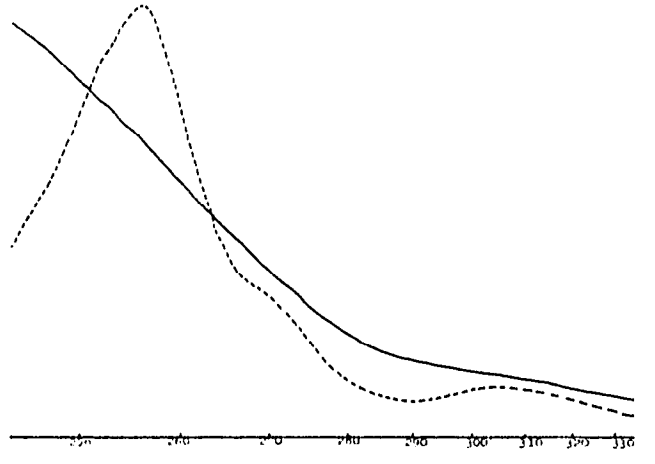

Fig. 3. UV spectrum of perphenazine. Case No. 1546-69 JK liver. Solvent: ethanol. ———, Phenothiazine extraction; – – – –, after preparative GC clean-up.

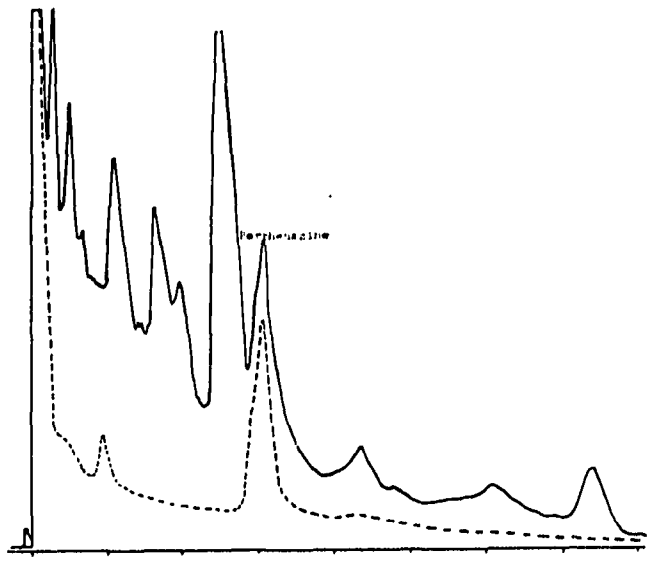

Fig. 4. Gas chromatogram of perphenazine. Case No. 1546-69 JK liver. OV-1, 250°, FID. ———, Phenothiazine extraction; – – – –, after preparative GC clean-up.

line colourless material, which could easily be identified by conventional, analytical methods.

Figs. 3, 4 and 5 show the results of chromatographing basic fractions from a putrefied fatty liver. Since suicide by means of Etrafon D (a mixture of perphenazine and amitriptyline) was indicated, the isolation of perphenazine according to the method of CURRY[2] was attempted. This produced a very impure extract as indicated by the UV spectrum and gas chromatogram in Figs. 3 and 4. During this run it was essential to cool the collection flask in a dry ice–acetone bath to avoid perphenazine escaping as a fine aerosol. Pure amitriptyline was isolated from the basic fraction of the regular five-way extraction (Fig. 5).

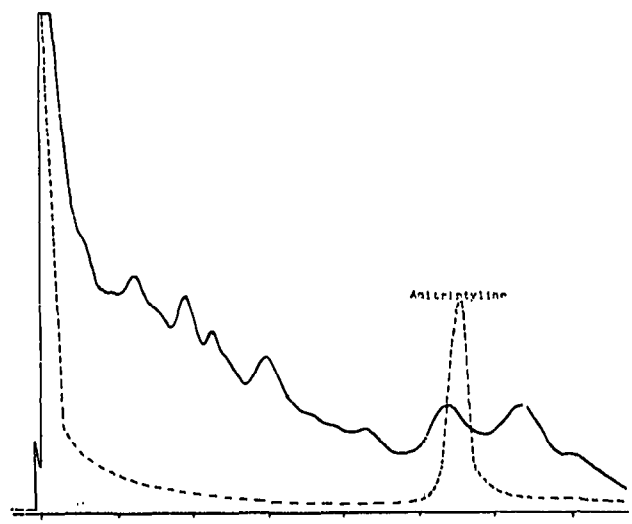

Fig. 5. Gas chromatogram of amitriptyline. Case No. 1546-69 JK liver bases. OV-17, 220°, FID. ———, Bases from 5-way split; – – – –, bases after preparative GC clean-up.

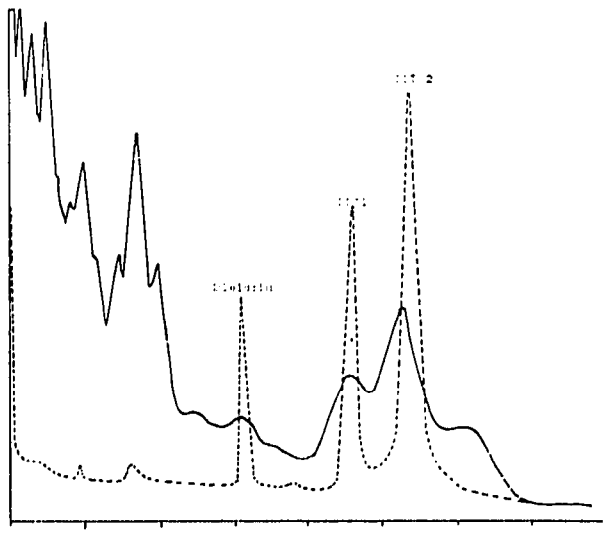

Fig. 6. Gas chromatogram of Dieldrin and DDT. Case No. 1144-67 RS liver neutrals. SE-30, 190°, EC. ———, Conventional clean-up; – – – –, preparative GC clean-up.

Fig. 6 shows the results of a clean-up of another neutral fraction of a putrefied fatty liver. Conventional purification procedures with an activated alumina column and dimethylformamide partition[3] gave only a slight indication of the pesticides suspected.

In none of the cited cases was any quantitative estimation of the poison encountered attempted. Work along these lines is in progress. Preliminary recovery studies from the injection of 1 mg quantities are given in Table II.

TABLE II

RECOVERY DATA

Drug	*% Recovery*
Barbiturates	95–98
Doriden	90–95
Meprobamate	90–95
Librium	82–85
Phenothiazine	70–75

The losses of material are mainly due to the column and the collection technique. The data do not represent recovery from tissue, but give only the recovery when a known amount of the substance is injected and collected.

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1 K. P. DIMICK, *G.C. Preparative Separations*, Varian Aerograph, Walnut Creek, Calif., 1966, p. 27.
2 A. S. CURRY, *Poison Detection in Human Organs*, C.C. Thomas, Springfield, Ill., 1963, p. 93.
3 M. J. DE FAUBERT MAUNDER, H. EGAN, E. W. GODLY, E. W. HAMMOND, J. ROBURN AND J. THOMSON, *Analyst*, 89 (1964) 168.

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