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MICRO INFRA-RED SPECTROSCOPY OF GAS CHROMATOGRAPHIC FRACTIONS

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SUMMARY

A simple and effective method of obtaining infra-red spectra of gas chromatographic fractions has been developed making possible rigorous identification with microgram quantities.

After gas–liquid chromatographic injection, the separated components which emerge from the column are passed through a heated line via a stream splitter and condensed independently on a glass surface. The isolated component is then transferred to KBr for microdisc preparation. The method of sample transfer described gives a high recovery, eliminates many possible sources of contamination and yields high quality infra-red spectra for 1–10 μg samples.

The results of the technique are illustrated with a number of typical spectra.

INTRODUCTION

The introduction of gas chromatography and its rapid development has provided the forensic scientist with a convenient method of separating microgram quantities of complex organic mixtures into their different components. The technique, however, is essentially one of separation and supporting evidence is usually required to identify the separated components of the mixture. Comparison of retention times of the unknown components and simple derivatives of these unknowns with those of standards using two dissimilar stationary phases is frequently used to establish identity[1–3]. Alternatively, the column effluents may be collected and the pure components subsequently analysed by instrumental techniques such as infra-red and ultra-violet spectrophotometry, nuclear magnetic resonance spectroscopy and mass spectrometry. The measurement of other physical properties such as mobility in thin layer and paper chromatographic systems[4] has also provided supporting evidence for identification.

In many problems involving the use of gas chromatography, the component of interest is frequently a trace constituent of the sample under investigation. The injection of larger volumes into the gas chromatograph is not possible if sample economy is a necessity and is not desirable as this results in a reduced separating efficiency of the column. Consequently it is necessary to use a spectroscopic method

of analysis which is sensitive at the microgram level in combination with chromatographic separation. Organic mass spectrometry is able to analyse sub-microgram quantities of material but requires considerable expertise and the maximum amount of information is only deduced after lengthy interpretation. For many laboratories the cost of this type of instrumentation is prohibitive. I.R. spectroscopy has proved a useful and relatively inexpensive method of analysis, particularly when the spectra have been recorded in standard collections of reference spectra[5]. The technique, however, is usually employed for the analysis of sub-milligram amounts of material, but with special micro-sampling techniques and instrumental methods yields high quality spectra with as little as 1 μg of component[6,7]. GLC–I.R. combination systems in which the gas chromatograph is coupled directly to a fast scanning I.R. spectrophotometer and the separated components analysed in the gas phase as they emerge from the column is, because of the very different sampling conditions required for the two techniques, only sensitive for components in excess of 100 μg[8–10]. It is, therefore, necessary to employ an intermediate trapping system to collect microgram components for subsequent I.R. analysis.

Many methods have been reported for trapping gas chromatographic fractions for further I.R. examination[11–17]. The majority of these methods are only applicable for components in the sub-milligram range, others require elaborate and time consuming techniques or are too specific in their applications. This paper reports a simple and effective method of GLC–I.R. microanalysis which has produced rigorous identification of microgram components with only minor modifications to existing equipment. The analytical technique is of particular value in toxicological investigations in forensic science laboratories.

EQUIPMENT AND MATERIALS

The gas chromatograph employed is a Pye 104/84 fitted with a flame ionisation detector. The gas chromatograph is equipped with a stainless steel stream splitter linked to a Research and Industrial Instruments heated line. The arrangement of the equipment is shown in Fig. 1. The flow splitter, inserted between the end of the column and the flame ionisation detector, gives a split ratio of approximately 90:10. The smaller part of the effluent is allowed to enter the flame ionisation detector for monitoring the separation of the mixture on a Perkin-Elmer Hitachi 159 flat bed recorder. The larger part of the effluent gases is passed through a stainless steel capillary tube to an electrically heated exhaust assembly on the side of the chromatograph. To the stainless steel exhaust capillary is silver soldered a capillary gas line which terminates in a needle. This needle is used for piercing the silicone rubber septa enclosing the glass vessels in which the components are to be trapped. The line and needle are both resistively heated along their entire length from a low voltage transformer and variac, thus preventing premature condensation of the components and the possibility of contamination. To prevent blowback of carrier gas through the detector or ejection of the trap by the build up of gas pressure during trapping, the needle incorporates a concentric outer shell linked to an exhaust tube.

"Analar" grade chloroform (British Drug Houses Ltd.) employed in the transfer of condensed components is initially dried over anhydrous calcium chloride and distilled prior to use. Evaporation of this solvent on KBr powder (50 μl/0.5 mg) and

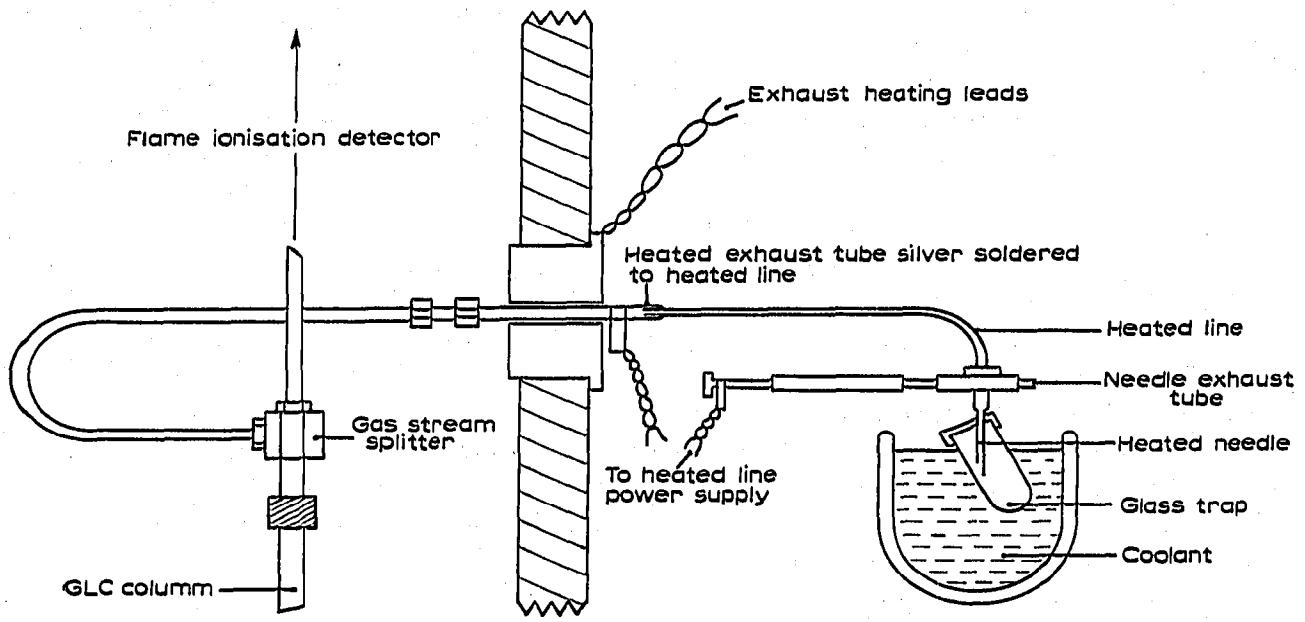


Fig. 1. Diagram indicating arrangement of trapping modifications on the Pye 104/84 gas chromatograph.

examination by I.R. spectroscopy indicated no detectable non-volatile organic residues present in the solvent. Optical grade potassium bromide (Alpha Inorganics Ltd.) is used for microdisc preparation. The potassium bromide is stored in an oven at 150° when not in use.

The I.R. spectra were recorded on a Perkin-Elmer 225 Spectrophotometer using a slit programme of 6–7 and a scan rate of 1.5 min/micron. To illustrate that satisfactory spectra can equally well be obtained from less sophisticated instrumentation, the spectra of the isolated components were subsequently recorded on the Unicam SP200 I.R. spectrophotometer. In all cases the spectra of microdiscs were recorded using a Research and Industrial Instruments 4× KBr lens refracting beam condenser to increase sample transmission. Energy balance between the reference and sample beams of the spectrophotometers was obtained with a Research and Industrial Instruments AT02 reference beam attenuator. In the majority of cases, ordinate scale expansion was found to be unnecessary for recording good spectra of 1 μg components trapped in the manner described in this communication.

EXPERIMENTAL

Choice of split ratio

The ability of the detector to resolve and monitor the separated components emerging from the column is dependent on the design of the chromatograph and the flow rate of the effluent gas entering the flame ionisation detector. High split ratio's (*e.g.* 95:5, 99:1) give rise to a substantial reduction in gas flow to the detector and result in broadening and distortion of the peaks. This phenomenon can to a certain extent be overcome by the injection of more concentrated solutions onto the column. This is not always possible in forensic science investigations. Attempts to remove these dead volume effects of the detector by the addition of a purge gas (oxygen free

nitrogen) between the detector and the splitter were unsuccessful. Low split ratios (*e.g.* 50:50, 75:25) give the required sensitivity but reduce the proportion of component available for I.R. examination.

The minimum quantities of a mixture of five barbiturates necessary for a satisfactory separation to be monitored with different stream splitters are indicated in Table I.

It can be seen that in order to trap 1 μg of one of these barbiturates and also have the separation simultaneously monitored on a recorder, a split ratio of 90:10 is best employed.

TABLE I

MINIMUM QUANTITIES OF 5 BARBITURATES (BARBITONE, BUTOBARBITONE, PENTOBARBITONE, AMYLOBARBITONE AND SECOBARBITONE) WHICH WERE READILY RESOLVED BY THE FLAME IONISATION DETECTOR FOR DIFFERENT STREAM SPLITTERS*

Stream splitter approximate split ratio	*Minimum quantity of each barbiturate in the mixture required for a satisfactory separation to be monitored* (μg)	*Quantity of each barbiturate passing through the FID* (μg)
No splitter	0.02	0.02
90:10	0.4	0.04
95:5	1.8	0.09
99:1	50	0.50

* A Pye 10% SE30 column was used for this study.

Collection of GLC effluents

Two main methods have been described for trapping gas chromatographic effluents. These are (i) the trapping of the fraction in a tube containing normal gas chromatography column packing[12], and (ii) allowing the components to condense in glass or teflon capillary tubing or in glass trapping vessels[18,19]. Trapping efficiencies approaching 100% are obtainable by trapping on column packing but to date, the trapped component has to be subsequently concentrated, transferred and converted to a form suitable for I.R. examination. This is achieved either by subliming the component directly on to a KBr microdisc[13] or eluting the chromatographic fraction into silver chloride I.R. solution cells cooled in liquid nitrogen[14]. We found that the procedure of trapping on column packing and transferring the trapped components by these two methods was relatively unsuccessful for 1–10 μg quantities of barbiturates and alkaloids.

The trapping efficiency of a simple condensing system depends upon a number of factors. These include the temperature and geometry of the trapping vessel, the flow rate of the effluent gases emerging from the chromatograph trapping capillary, the time the sample resides in the trap and the vapour pressure of the sample under the trapping conditions employed. Some materials present the additional problem that they form aerosols as they emerge from the exhaust of the gas chromatograph, particularly when drastic cooling conditions are employed. For this reason condensing in glass or teflon capillary tubing is inefficient for many components. We obtained trapping efficiencies at the 1–10 μg level of 20–35% for barbiturates and 5–10% for

amphetamine and nicotine using this method of trapping. However, by employing a carefully aligned glass trap in the manner described in this communication with moderate cooling conditions (cold water) trapping efficiencies up to 70% for barbiturates were consistently recorded. For components with high vapour pressures such as nicotine and amphetamine, drastic cooling with liquid nitrogen or a solid CO_2–acetone mixture was found to be essential for recoveries in excess of 50%.

Micro I.R. spectrophotometry

To achieve maximum sensitivity in the I.R. examination of microgram quantities of material it is necessary to confine the component to the smallest cross sectional area. For this reason, micro KBr pellet techniques proved to be more sensitive than solution or gas cell methods with the presently available equipment[20]. Further advantages of the micro pellet technique include high optical transmission by the KBr matrix and good heat dissipation, thereby lessening the likelihood of sample deterioration. Attenuated total reflectance techniques also proved to be less sensitive than the KBr pellet methods.

The difficulty with the KBr method of sample presentation is the transfer of minute amounts of material to a small quantity of KBr with a minimum of manipulative losses. Grinding techniques on such small samples proved to be most unsatisfactory. Addition of the solution containing the component to KBr in a mortar and lightly grinding after the solvent has evaporated resulted in partition between the mortar and KBr and generally introduces impurities that far exceed the small amount of sample present. Similarly, although the addition of the solution to finely ground powder in the die was simpler, the sample loss was very great. The lyophilization technique[21,22] gave essentially quantitative sample transfer but was difficult with small amounts of KBr, proved time consuming and because of large contributions by the lyophilization blank made identification of the spectra difficult. The syringe technique described in this communication resulted in substantially quantitative recovery of high boiling components. Incorporation of components with high vapour pressures in KBr by all the methods described resulted in a very substantial loss of sample. We found that for amphetamine or nicotine, an injection of 10 μg of these compounds on the column was necessary to obtain acceptable I.R. spectra. An alternative procedure for positively identifying these amines is to convert the bases into simple less volatile derivatives (*i.e.* Schiff's bases for primary amines) either before injection or on the gas chromatographic column itself, when 1 μg will suffice if the Hamilton syringe technique is used.

METHOD OF TRAPPING GAS CHROMATOGRAPHIC COMPONENTS FOR I.R. EXAMINATION

The gas chromatograph is adjusted to give maximum recorder response for an injection of 1μg of a material similar to the components under investigation. For the Pye 104/84 gas chromatograph the optimum operating conditions for the identification of barbiturates were:

(a) 5 ft. × ¼ in. O.D. glass column packed with 10% SE30 on silanized Chromosorb W, 100/120 mesh, maintained at 175°.
(b) Carrier gas — oxygen free nitrogen at 50 ml/min.
(c) Hydrogen flow rate to the detector was 5 ml/min (adjusted to give maximum

recorder response on injection of 1 μg of barbitone). Air flow was approximately 120 ml/min.

(d) The injection and detector temperatures exceeded the oven temperature by 20°.

For trapping gas chromatographic fractions, the variac is adjusted to give a heated line temperature approximating to that of the chromatographic oven. All chromatographic columns employed must be well aged before use to minimise column bleed.

In the system described, the sample, in this case a mixture of four barbiturates in ethanol, is injected into the gas chromatograph in the orthodox manner and the components of the mixture separated on the column. As each component emerges it is simultaneously recorded and passed along the heated line via the flow splitter to the heated needle and condensed on a glass surface.

The trapping cell is connected to the heated effluent outlet by piercing the serum cap with the heated needle. For high trapping efficiencies it is important to align the trap so that the point of the heated needle is very close to and at an angle of 30–45° to the sides of the trapping vessel (Fig. 1). On emergence of the component from the column, the trap is immediately immersed in a suitable coolant while the peak is being eluted. When the recorder has indicated that the component of interest has been completely eluted from the column, the trap is quickly removed and a second assembly attached to the needle to trap the next peak as required. This removal and replacement of glass traps takes less than 30 sec.

To obtain an I.R. spectrum of the isolated component, the condensate on the sides of the trap is immediately dissolved in 25 μl of chloroform; care being taken to completely wash the internal walls of the trap with the solvent. This solution is then removed from the trap with a 25 μl Hamilton syringe equipped with a multiple repeating dispenser and 0.5 μl of solution "dispensed" to the tip of the needle. A small quantity (approximately 0.5 mg) of KBr can then be taken up by dipping the needle into finely ground KBr powder. On removal of the needle a further 0.5 μl of solution is dispensed to the KBr residing at the needle point and the chloroform allowed to evaporate under a table lamp (Fig. 2). The procedure of releasing 0.5 μl volumes of the solution from the syringe to the KBr powder and allowing the solvent to evaporate is continued until the Hamilton syringe is empty and the chromatographic component completely transferred to the 0.5 mg of KBr at the point of the needle. The dry powder obtained is transferred to a Perkin-Elmer KBr Ultra Micro Die and a 0.5 mm disc pressed by applying vacuum ($<$ 10 mm Hg) for a few minutes. The spectrum is recorded using a beam condenser and a reference beam attenuator. To correct for impurities present in the KBr matrix and possible effects of column bleed, the procedure is repeated without sample injection on the gas chromatographic column and a blank KBr disc prepared.

RESULTS AND DISCUSSION

The majority of GLC–I.R. systems of microanalysis have many disadvantages. Poor trapping efficiencies and additional manipulative losses give rise to low recoveries and necessitate quantities well in excess of 1 μg for identification. A further disadvantage frequently results from the introduction of extraneous materials in the

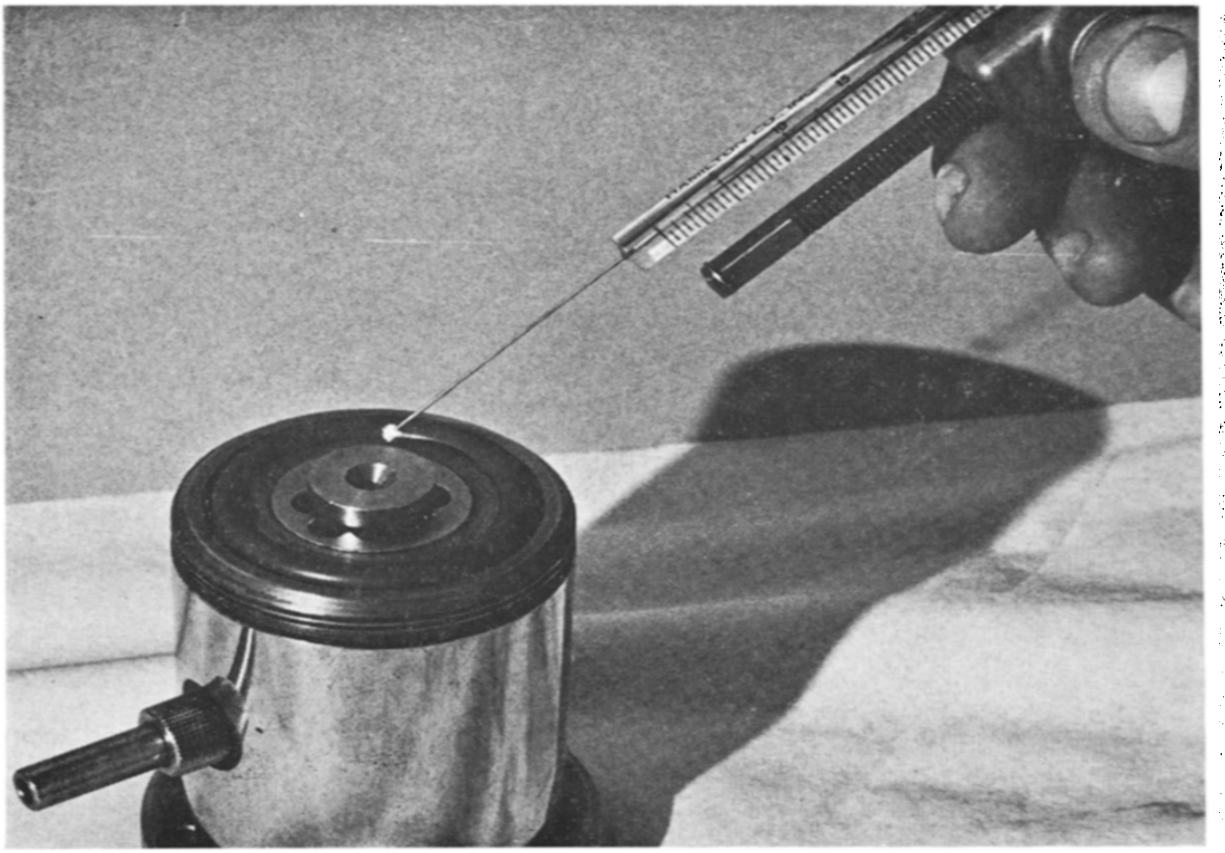


Fig. 2. Syringe technique for transferring microgram components to KBr powder.

analytical procedure and gives rise to large blanks which may obscure the sample spectrum and make identification difficult.

The GLC–I.R. method of microanalysis described in this paper yields good quality spectra for 1 μg of high boiling components. For liquid compounds with high vapour pressures such as nicotine and amphetamine, quantities of up to 10 μg are necessary to produce satisfactory spectra. Provided well aged gas chromatography columns with substrates of low vapour pressure such as SE30 are used, small blanks are obtained (Fig. 4) and unequivocal identification of gas chromatographic fractions

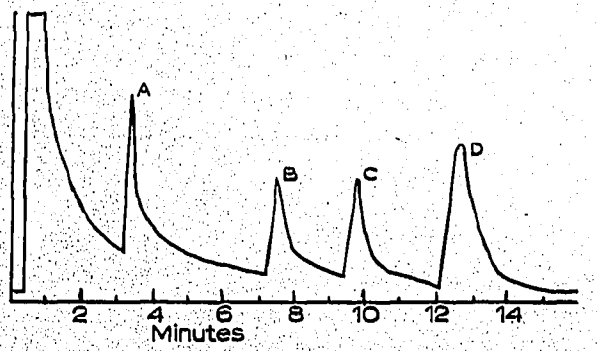


Fig. 3. Gas chromatogram obtained for the micro-analysis of 1 μl of an ethanol solution containing (A) barbitone (1 μg), (B) amylobarbitone (1 μg), (C) quinalbarbitone (1 μg) and (D) hexobarbitone (1 μg).

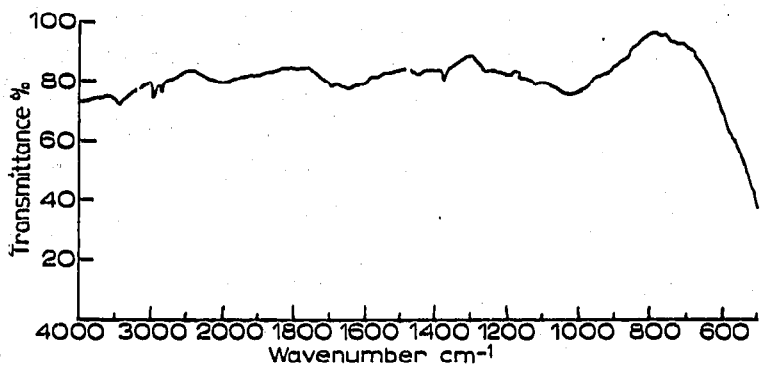
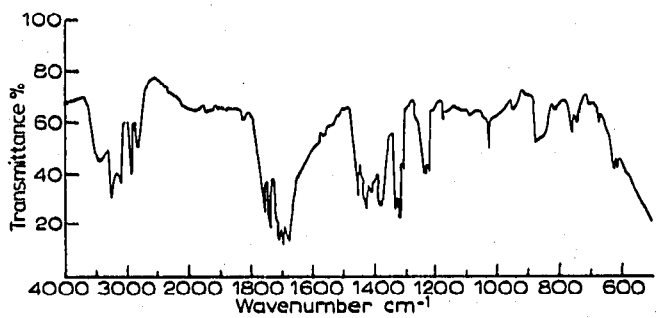


Fig. 4. Typical I.R. blank obtained using a 10% SE30 column operating at 175°.



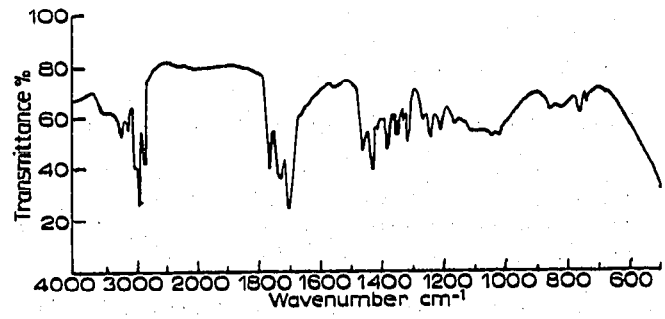
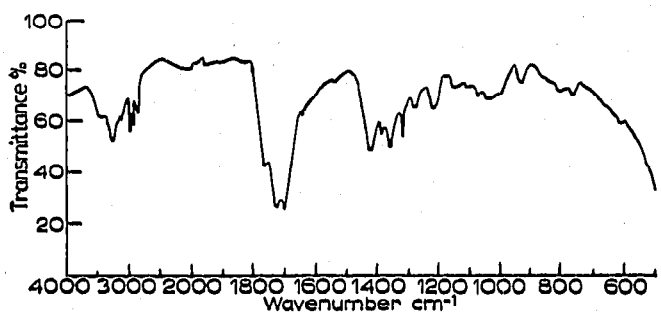


Fig. 5. I.R. spectrum of barbitone recovered from the gas chromatographic effluent of peak A.

Fig. 6. I.R. spectrum of amylobarbitone recovered from peak B.



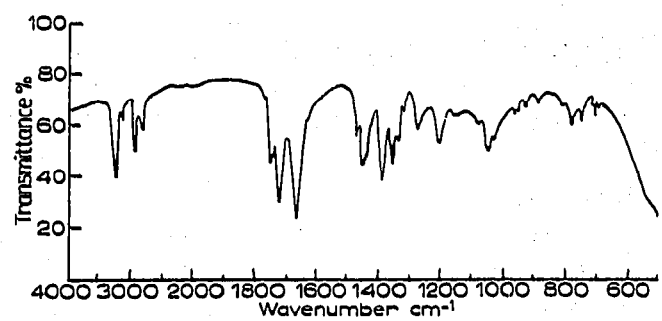


Fig. 7. I.R. spectrum of quinalbarbitone recovered from peak C.

Fig. 8. I.R. spectrum of hexobarbitone recovered from peak D.

is possible. The use of this GLC–I.R. procedure may be best illustrated by the analysis of a synthetic mixture of barbiturates. Samples of the peaks of the chromatogram (Fig. 3) were collected using the procedure described and the spectra recorded. These spectra are indicated in Figs. 5–8. Using this technique we have found no evidence of cross contamination between peaks in close proximity to each other. However, the use of interrupted elution chromatography[23] would be advantageous for trapping peaks following closely on each other.

Each of the spectra indicated in Figs. 5–8 represent 0.5–0.6 μg of barbiturate

in the sample beam of the spectrophotometer; full scale spectra being obtained for less than 1 μg of these compounds in the KBr microdisc. The use of ordinate scale expansion would enable the sample in the KBr microdisc to be further reduced probably to about 0.1 μg. The utilisation of computer addition techniques could further extend the detection limit to approximately 0.01 μg[24]. However, with these further increases in sensitivity an appreciable KBr impurity problem arises.

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