GAS CHROMATOGRAPHY IN ROUTINE PHARMACEUTICAL ANALYSIS

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The application of gas chromatography to the determination of various volatile constituents in a range of pharmaceutical preparations is described. The method is simple, rapid and reliable and has been used to assay camphor, menthol, diethyl phthalate and a number of volatile oils.

In pharmaceutical analysis the direct application of gas chromatography is obvious, particularly for the examination of solvents and isolates from volatile oils, or even for the determination of the main constituents of volatile oils, provided the results can be related to those obtained from the more conventional analyses now standard. There is little reference in the literature, however, to its use in this field—in a bibliography of 653 references up to July 31, 1958[1], barely a dozen of pharmaceutical interest are to be found and these are almost entirely concerned with the examination of volatile oils and flavourings.

Some modification of the recognised technique is necessary to apply it to the determination of the volatile constituents of pharmaceutical compounded preparations, particularly aqueous solutions containing considerable amounts of non-volatile compounds. Further, to make the most efficient use of the technique in the important aspect of time saving, several columns of different lengths and stationary phases maintained at various temperatures should be available for coupling when required to a single recording system. It is sometimes advantageous to have two identical columns operating so that a component with a small retention volume can be determined quickly on one column whilst unwanted components of a sample are being eluted from the other.

The application of the technique to the determination of chloroform in aqueous pharmaceutical preparations has been reported[2], and the equipment with slight modification has since been used for the determination of water in pastes, ointments and creams[3]. The purpose of this paper is to describe the extension of the technique to the determination of various volatile constituents in a wide range of pharmaceutical materials.

Experimental

Equipment

The equipment used is of conventional design, except for modifications necessitated by the fact that the non-volatile materials present in many of the preparations would quickly block the column. As frequent changing of the column cannot be tolerated for control purposes it was necessary to devise and build an injection system to overcome this difficulty. Such a system has already been described[2].

The principle adopted generally is that of using a small removable cap filled with supporting medium and positioned immediately before the chromatographic column. This cap, in which the non-volatile components are trapped, is easily removed when necessary and replaced by a freshly-filled one, the whole operation taking but a minute or so. One form of the device is used with a micropipette method of sample injection and another form utilises a conventional hypodermic syringe injection through a rubber cap. This system of removing non-volatile materials obviates any need for distillation or solvent extraction, and saves time.

The detector used is a thermal conductivity cell with platinum wires 4 in. long and 0·001 in. thick of nominal resistance 25 ohms, the wires in the two channels being matched to within 0·1 ohm. The detector current is 200 mA. and the output is recorded on a Honeywell-Brown or Sunvic recorder with 2·5 mV. full-scale deflection and chart speed of 12 in. per hour.

Calibration

Although many samples contain a number of volatile components we have in general been determining only one of these and to avoid the necessity of calibrating the detector by measuring the peak areas of each component an internal standard procedure has been adopted throughout. With such a procedure it is not necessary to ensure that a standard amount of sample is transferred to the column for each determination and this has proved a useful advantage as many of the samples are thick suspensions or viscous liquids. In all cases peaks are sharp and symmetrical and therefore heights rather than areas can be used for calibration and this is an advantage in routine applications.

Standard solutions, containing known concentrations of the component being determined are prepared and the ratios of the peak height of this material and the internal standard are measured. Calibration curves are then obtained by plotting these ratios against concentration, and working ranges have been chosen to produce linear calibration curves, passing through the origin, except in the particular case of determining water.

Despite rigorous maintenance of operating conditions the calibration curves are subject to slight changes in slope from day to day, but because of their linearity it has only proved necessary in practice to check one or two points before use.

The Determination of Camphor, Menthol and Volatile Oils

The stationary phase used in these determinations is squalane, 2, 6, 10, 15, 19, 23-hexamethyltetracosane, and a column of this material has already been operating continuously at 130° for nine months without any noticeable signs of deterioration.

Standards and samples each containing 2 per cent of ethylbenzene as the internal standard are chromatographed under the following conditions.

Column length—7 ft. Column temperature—130°. Stationary phase —20 per cent of squalane on 100–120 mesh Celite. Sample size—30 μl. Carrier gas—4 : 1 hydrogen/nitrogen mixture flowing at 100 ml./min.

The column characteristics under these conditions are as follows. Height equivalent to a theoretical plate—0·16 cm. Retention volume (ethyl benzene)—270 ml. Retention volume (camphor)—1200 ml. Retention volume (menthol)—675 ml.

The Determination of Diethyl Phthalate

As diethyl phthalate has a high boiling point (296°) it is necessary to carry out the chromatography at a high temperature and the choice of stationary phases is limited but we have found Arylan S90, sodium dodecyl benzene sulphonate, suitable for continuous use at 225°.

Standards and samples each containing 1 per cent of α-phenyl-*n*-propanol as the internal standard are chromatographed under the following conditions. Column length—6 ft. Column temperature—225°. Stationary phase—30 per cent of Arylan S.90 on 36–85 mesh Chromosorb. Sample size—30 μl. Carrier gas—4 : 1 hydrogen/nitrogen mixture flowing at 60 ml./min.

The column characteristics are as follows. Height equivalent to a theoretical plate—0·37 cm. Retention volume (α-phenyl-*n*-propanol)—695 ml. Retention volume (diethyl phthalate)—960 ml.

Results and Discussion

Methods for the determination of camphor in galenicals usually depend on the optical rotation of natural camphor, or on its ketonic nature and have several disadvantages. We have shown that gas chromatography offers a reliable and speedy alternative, an assay taking about 20 minutes. It has been possible to determine camphor in a variety of samples at concentrations greater than 1 per cent, the normal working range being 1 to 10 per cent. The replacement of the thermal conductivity method of detection by an ionisation detector[4,5] should enable as little as 0·1 per cent to be assayed.

Table I shows the results obtained on a variety of samples and the good agreement between these and the chemical results or theoretical concentrations shows the reliability of the gas chromatographic procedure. The chromatographic results are the mean of duplicate determinations, the average divergence from the mean being $\pm$ 1·5 per cent.

Menthol, like camphor, can only be determined at present at concentrations greater than 1 per cent and the use of the technique is therefore limited. In preparations where the concentration of menthol is less than 1 per cent it has proved possible to identify it but not to determine it quantitatively but here again the introduction of the ionisation detector should overcome this difficulty. Liniment of Methyl Salicylate and Eucalyptus B.P.C. and Compound Ointment of Methyl Salicylate B.P.C. contain 5 per cent and 10 per cent of menthol respectively, but the methyl salicylate has a similar retention volume under the conditions employed and completely masks the peak due to the menthol. We have

determined menthol in Oil of Peppermint, and our results agree very well with those obtained by the official B.P. method. Also the amount of oil of peppermint in a number of flavouring compounds, such as one containing equal amounts of oil of peppermint and oil of aniseed has been determined. In these cases the menthol peak was used for measurement but because of the variable amount of menthol in oil of peppermint it was necessary to use as standard the actual batch of oil used in manufacture.

In simple formulations such as Spirits of Aniseed we have determined the volatile oil content fairly accurately using as standard the batch of oil used in the preparation. The need to use the actual manufacturing batch of oil is a disadvantage in applying this technique but is unavoidable

TABLE I

COMPARISON OF CHEMICAL AND GAS CHROMATOGRAPHIC RESULTS AND THEORETICAL VALUES

Sample	Theory	Camphor per cent	
		By gas chromatography	Chemical
Camphor Water Conc. (*a*)	4·0	3·85	3·91
(*b*)		4·13	
Chloral with Camphor	57·1	57·6	59·0
Cold Sore Remedy (*a*)	3·0	2·88	
(*b*)		3·05	
(*c*)		3·02	
Compound Ointment of Capsicum	10·0	10·3	9·8
Hard Ointment of Camphor	6·0	5·95	6·05
Liniment of Belladonna Meth.	5·0	4·3	6·5
Liniment Camphor	20·0	21·2	20·6
Liniment of Camphor Ammon.	12·5	13·0	12·3
Liniment Soap	4·0	3·9	
Liniment Turpentine Acetic	8·2	8·0	
Nasal Drops Chlorbutol	1·37	1·2	
Nasal Drops Chlorbutol and Menthol	1·37	1·3	
Solution Camphor and Oil Aniseed	5·0	4·86	4·95
Spirits of Camphor	10·0	9·98	9·72

because of the variable nature of volatile oils. We have prepared accurately, samples of Spirits of Aniseed, Cinnamon, Juniper, Nutmeg and Rosemary, each with 10 per cent of volatile oil and examined them under the same conditions as for camphor. The average divergence from the theoretical concentration was 1 per cent with a maximum divergence of 3 per cent.

In many toilet waters and allied preparations where the perfume essential oils or the perfume synthetic chemicals or both amount to less than 5 per cent, diethyl phthalate is commonly added as a denaturant, usually at a concentration of 1 per cent v/v. The chemical determination of this compound is a lengthy procedure but it can be determined by gas chromatography in about 20 minutes.

We have examined a number of samples, including lavender water, eau de Cologne, and an oily brilliantine, with theoretical concentrations of 1·00 per cent to 1·02 per cent and the concentration found has varied from 0·99 per cent to 1·05 per cent. To obtain an idea of the reproducibility of the method one particular sample was examined in triplicate and results of 0·99, 1·02, 0·98 per cent were obtained, for which the mean is 1·00 per cent, and the coefficient of variation is 2 per cent. A 1 per cent standard solution when examined on 4 separate days gave results of

0·69, 0·68, 0·67, 0·68 for the ratio of the peak heights of the diethyl phthalate to the internal standard, α-phenyl *n*-propanol.

It has also proved possible to determine diethyl phthalate in solidified perfume sticks. The procedure adopted was to melt sufficient to produce about 10 ml. of liquid and transfer this to a 10 ml. graduated flask containing 0·1 ml. of internal standard, the flask being slightly warmed so as to keep the sample in the liquid state. The micropipette injection system was used and the pipette was again slightly warmed. In this way the sample itself plus the internal standard was transferred directly to the column and no extraction of the diethyl phthalate was required. For two sticks containing theoretical amounts of 0·99 per cent and 1·07 per cent respectively the results obtained were 1·0 per cent and 1·07 per cent.

REFERENCES

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3. Elvidge and Proctor, *Analyst*, 1959. In the press.
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5. McWilliam and Dewar, in *Gas Chromatography*, edit. Desty, Butterworth's Scientific Publications, London, 1958, p. 142.

After Mr. Proctor presented the paper there was a DISCUSSION. The following points were made.

Amplification of the output of the thermal conductivity detector was suggested as a better means of increasing sensitivity than the use of an ionisation detector, which was considered too sensitive, although the latter had the advantage in not being so sensitive to fluctuations in gas flow rate. Background interference could originate from columns that had been used for some time.