ANALYSIS OF ESSENTIAL OILS

PART I. INJECTION SYSTEM FOR A GAS–LIQUID CHROMATOGRAPHY APPARATUS

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Essential oils contain a diversity of organic compounds which may be liquid or solid at room temperature and whose boiling points therefore fall in a wide temperature range. Many of these compounds have closely related structures, similar boiling points and similar retention volumes, as well as being thermally unstable and readily undergoing intramolecular rearrangements. Much progress has been made in the gas–liquid chromatographic analysis and separation of essential oil components[1,2], in ascertaining the gas–liquid chromatographic behaviour of terpenoid compounds and in the selection of the most suitable column materials and operating conditions[3–6]. Several workers[7] have stressed the importance of the technique of sample injection on to the chromatographic column due to its effect on separation and resolution, and a number of methods of sample introduction have been described[8–10]. The system developed in this laboratory allows the introduction of solids and liquids with equal facility under readily standardised conditions, but it also provides considerable flexibility in adjusting temperatures and pressures to the conditions most suitable for the chromatographic handling of thermally unstable compounds of higher boiling point. Moreover, if desired, consecutive injections may be made from the same sample, this frequently having been found to be particularly helpful in the separation and resolution of essential oil mixtures.

EXPERIMENTAL

The inlet system is shown in Fig. 1 and was constructed of Pyrex capillary tubing, standard high vacuum taps and ball and socket joints. The right arm of the U-tube D was of ordinary pyrex tubing. All inlet tubing from tap A to the column was wound with "Electrothermal" heating tape capable of maintaining the inlet system at temperatures up to 350°. The U-tube D of which each arm was fitted with a ball and socket joint was wound with a separate heating tape, allowing the removal of this section and also its rapid heating after changing sample vials. At the temperatures necessary for the volatilisation of higher boiling point constituents of essential oils

the use of standard vacuum greases on the ground joints was not desirable and it was found that reduced pressures within the system could be satisfactorily maintained using thin silicon rubber washers with a very thin coating of Apiezon T grease. This also reduced to a minimum the problem of volatile components dissolving in the

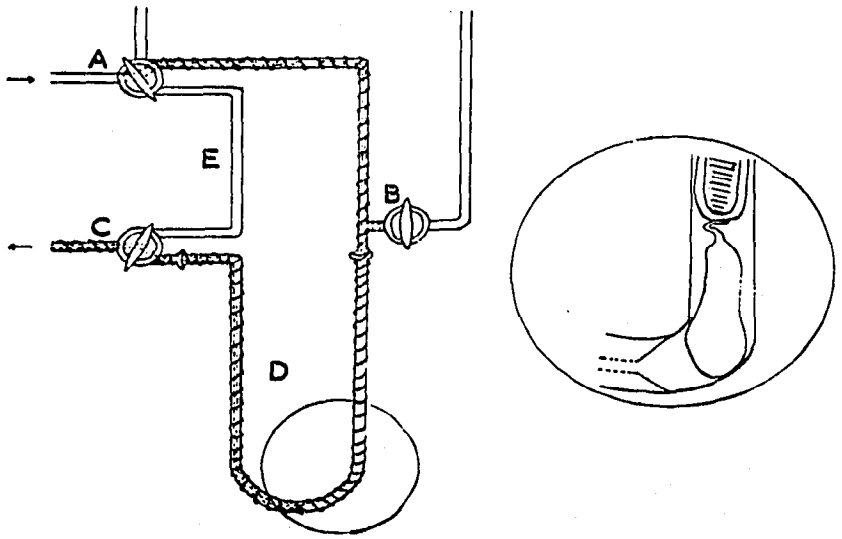

Fig. 1. Inlet system. Inset: sample vial arrangement.

vacuum grease. The minimum amount of Apiezon T was used to grease the taps. Taps A, B and C allowed the passage of the carrier gas, helium, through either the inlet U-tube D or the by-pass tube E, while tap B was used for the reduction of pressure to that of the column outlet after the insertion of a sample vial into the U-tube. The inlet U-tube was also interchangeable with corresponding U-tubes used as fraction collection traps on the column outlet so that a separated fraction of a sample could be recycled if desired.

A measured amount of the solid or liquid (usually 5 μl) was sealed in the sample vial on the tip of which a fine capillary hook was drawn (inset Fig. 1). The vial, together with a magnetic hammer consisting of a piece of steel sealed in glass tubing, were placed in the right arm of the sample U-tube. This was then attached to the system and heated by means of the heating tape, to the desired temperature. While the helium carrier stream flowed through by-pass E, the pressure in the inlet chamber was reduced through tap B to that on the outlet side of the column. This reduction in pressure had the secondary effect of considerably reducing the time required to flush the air and all traces of the previous sample from the system. After closing B, taps A and C were opened cautiously to avoid a surge in the gas flow thus allowing the helium carrier to thoroughly flush the inlet system. The helium flow was directed through the by-pass E again while the sample vial was crushed with the magnetic hammer. The vapour of the sample was then swept on to the column by changing the helium carrier gas flow path via the inlet system for a known period of time, usually one to five seconds.

This particular system has a number of advantages in the analysis of essential oil components. Solid and liquid samples can be inserted with equal facility, without modification to the apparatus or changes in technique.

The inlet system can readily be maintained at any point within the wide temperature range required for the manipulation of essential oils. Other workers[11–13] have emphasized the need to heat the inlet system to temperatures considerably in excess of the column temperature. This can be readily achieved since the heating of the sample inlet system and of the inlet tubing between tap C and the column may be independently controlled.

For higher boiling compounds unstable at high temperatures the pressure within the apparatus was reduced[14–17]. The reduction of pressure in the inlet system is advantageous in ensuring rapid and adequate vaporisation of the sample at lower temperatures than would be necessary at atmospheric pressure, without the risk of sample decomposition. The complete chromatographic apparatus was also maintained at reduced pressure, which in particular avoids bad surges in flow which occur when there is a large pressure drop between the inlet system and the column; it also has advantages when trapping out higher boiling point compounds in the outlet traps, and in increased sensitivity of the thermal conductivity cel helpfull, at higher temperatures. At the reduced pressures used (usually 100–150 mm mercury) careful selection of the flow-rate is necessary not only to satisfy the requirements of the cell and column, but also those of the inlet system. The flow rate must be sufficient to sweep an adequate vapour sample on to the column in the minimum period of time. When sample vapour in high concentration, obtained by controlling the temperature and pressure of the inlet apparatus as already described, is swept on to the column in the minimum time period thus using a very small quantity of sample, optimum separation and resolution are obtained. Injection periods of 1–5 sec gave the most satisfactory results and those of over 10 sec gave markedly poorer resolution. This is shown in Fig. 2. The size of the sample is of considerable importance for good separation and resolution[4,9] and the present apparatus is very versatile for such selection. Also, if desired, introduction of extremely small samples suitable for certain types of column, is possible.

The value of successive injections from the same sample in the qualitative analysis of essential oils can also be seen from Fig. 2. After several injections from the one sample, the peaks produced by the major components can be proportionately sufficiently reduced in intensity to reveal the trace component peaks. Moreover, by a suitable adjustment of the sample inlet temperature, major and minor components of quite different volatilities can be swept on to the column in a number of successive injections from the one sample. The apparatus therefore provides considerable versatility in the analysis of complex mixtures.

A simple calibration would make the system suitable for quantitative analysis. Other workers in describing various inlet systems[10] have not sufficiently emphasized that quantitative sweeping of the sample on to the column is only possible with (a) inordinately long injection periods or (b) a relatively large volume for the inlet system,

both of which result in very poor separation and resolution, or (c) very small quantities of sample, allowing complete vaporisation thus ensuring that the sample vapour is below saturation point at the selected pressure and temperature. Providing these conditions are met quantitative use of the inlet system would require simple calibration.

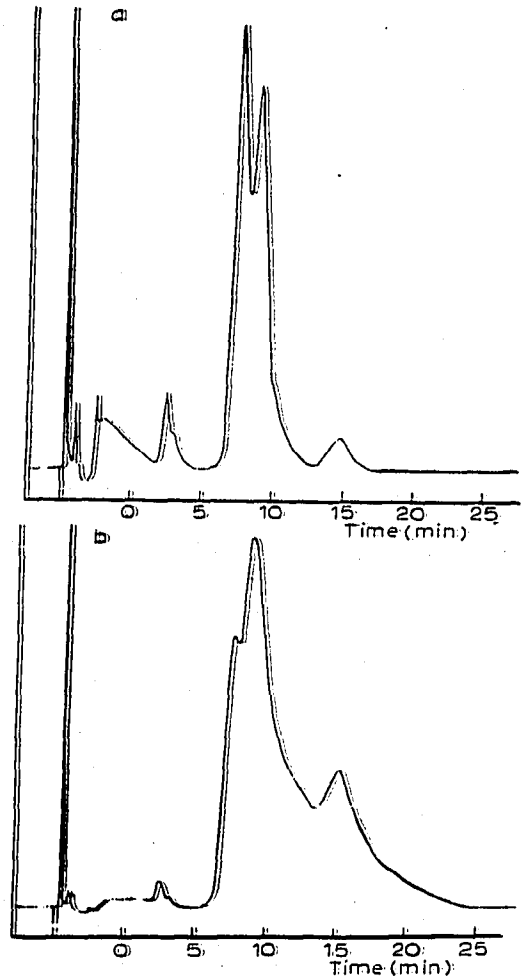

Fig. 2. Chromatograms of commercial terpinolene showing the effect of successive injections, and of different injection periods, from the same sample. (a) Injection period 5 sec. (b) Injection period 15 sec.

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SUMMARY

A method for sampling both solid and liquid essential oil components in such a way as to assist in their qualitative analysis has been described. Even though optimum column conditions of temperature, flow-rate and other relevant factors have been

chosen, unless the sample injection technique is also carefully considered, good separation and resolution will not be achieved. The apparatus described provides versatile control of the various factors involved in sample introduction, particularly of the temperature and pressure of the inlet system, and of the injection period thus controlling the sample concentration and size, and has proved very satisfactory in the introduction of essential oils.

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