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The examination of volatile oils by combined gas–liquid chromatography/thin-layer chromatography

The resolution of volatile oil components can be a particularly difficult task and as more sophisticated methods are applied, the complexity becomes more apparent. In the use of gas–liquid chromatography (GLC) for the quantitative analysis of components in volatile oils it is necessary to have an independent check on the resolution of the component of interest and it is for this particular application that our combined GLC/thin-layer chromatography (TLC) technique has mainly been developed.

The principle of using GLC to effect further resolution of components separated by TLC is widely used and there are numerous literature references to this application. Similarly the use of TLC to effect further resolution of components separated by GLC is fairly well established and was described by JANÁK in 1963[1] in relation to the separation of components in coal tar. Subsequent extensions of this work were published[2,3], but in each case, the necessary movement of the TLC plate was achieved by an independent drive mechanism. In the last paper of this series, the authors describe a logarithmic drive so the eluted homologous components from an isothermally operated GLC appeared in a linear fashion on the TLC plate.

A similar method was described by KAISER[4], in which the linearity of homologous components was achieved by temperature programming the GLC equipment. This paper also described the addition of a cooling device for the more efficient trapping of components on to the TLC plate. MINYARD *et al.*[5,6] used a combination technique to form derivatives directly on the TLC plate before final elution and they used a manual movement of the TLC plate. CURTIUS AND MÜLLER[7] applied the technique to steroid analysis and used an independent drive for the TLC plate. CASU AND CAVALLOTTI in 1962 described a method for the formation of derivatives from a GLC effluent on a TLC plate driven by a gear train from a potentiometric recorder, and no attempt was made to use the separation abilities of a TLC plate, which was used only as a means of trapping components and subsequently converting them into recognisable derivatives.

The concept of splitting the effluent from a GLC analysis and leading a portion of it to a TLC plate has the basic merit of simplicity, but automating the procedure seems to have been a more difficult task. It is evidently desirable to be able to move the TLC plate continuously and subsequently to be able to match the spots developed on the TLC plate with the peaks produced by the gas chromatograph on the recorder. It has been found that these requirements can be very easily achieved by using the chart paper from a potentiometric recorder as the driving mechanism for the TLC plate. The recorder is equipped with a smooth, flat shelf extending at least 40 cm at an angle of 45° from the point of emergence of the chart paper. The chart is allowed to run over the sloping shelf and the TLC plate is fixed to the paper with a small piece of adhesive tape. The open capillary end of a heated stream splitter is suitably positioned over the TLC plate at a distance of 2 mm from the surface. The heated transfer line in our apparatus gives a 98:2 split ratio, and a line temperature of 150° is satisfactory for most volatile oil applications, although it has been found necessary to increase this to 180° or 200° for oils containing phenols or sesquiterpene alcohols. The

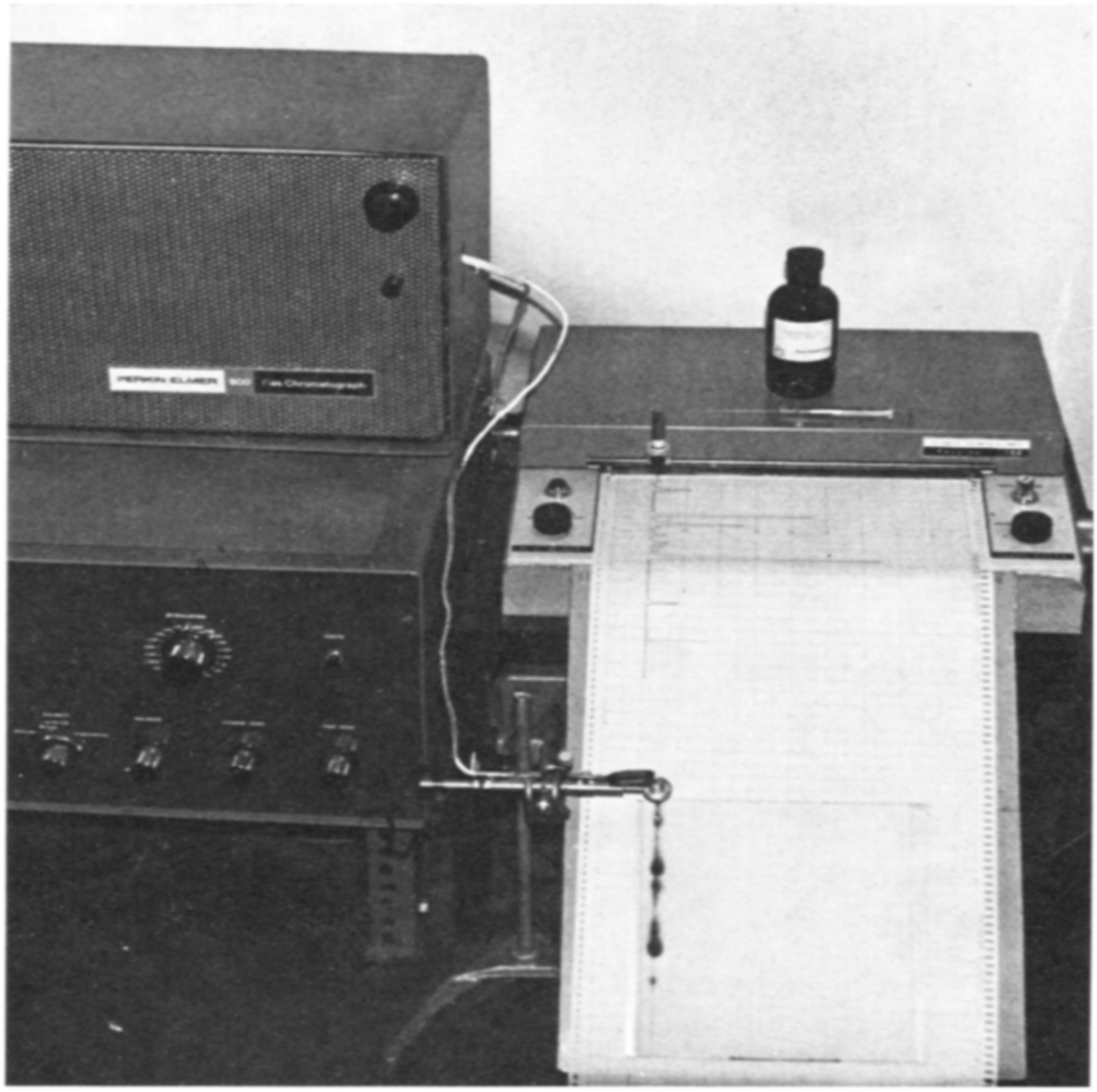

Fig. 1. A general view of the combined GLC/TLC arrangement.

transfer line is heated electrically by means of a separate power supply, using the stainless steel capillary itself as the resistance element. The maximum electrical requirements are 8 A, 12 V, and in our original experiments the transfer line was bolted directly to the stream splitter inside the gas chromatograph and the electrical connections were made using the outer end of the line and an earth return through the chassis of the chromatograph. The power supply was 0–12 V a.c. supplied from a Variac transformer. This was found to be satisfactory for a short time, but noise was gradually developed on the recorder and this was attributed to the gradual breakdown of the capacitors in the input filter circuit due to a.c. being picked up by the detector. The power supply was then rectified using four silicone diodes and a smoothing capacitor, which reduced the problem but did not eliminate it entirely. Using this rectified supply, the transfer line was then electrically isolated from the gas chromatograph by making the connection to the stream splitter with a short length of PTFE tubing. This entirely eliminated the problem and the arrangement has now worked

satisfactorily for several months. When the recorder chart is set in motion, the TLC plate moves with it and the trapped spots are thus kept in perfect phase with the peaks drawn by the recorder. The general arrangement is shown in Fig. 1. At the end of the run, the TLC plate is removed from the chart paper and eluted with a suitable solvent system. The separated components are detected with spray reagents, which may be specific in nature, thereby converting the TLC system into a specific detector. Alternatively, a specific reagent may be used before the TLC plate is eluted. Particular applications of this method are the formation of 2,4-dinitrophenylhydrazones with ketones and aldehydes and 2,4-dinitrobenzoates with alcohols. However, when the technique is applied to volatile oils, preparation of derivatives is not always feasible, because many terpenoid components do not readily form derivatives. Two well known examples are camphor and fenchone, which only form DNP derivatives with difficulty. When dealing with volatile oil components on a TLC plate, the R_F values can be a good guide to the nature of the components, and the colours which they form on condensation with vanillin or other aromatic aldehydes can also be helpful.

The TLC plate, duly developed by one or other of the foregoing methods, is then placed alongside the GLC tracing in such a way that the spots correspond to the peaks. In the case of volatile oils the correlation is usually self evident but the start points

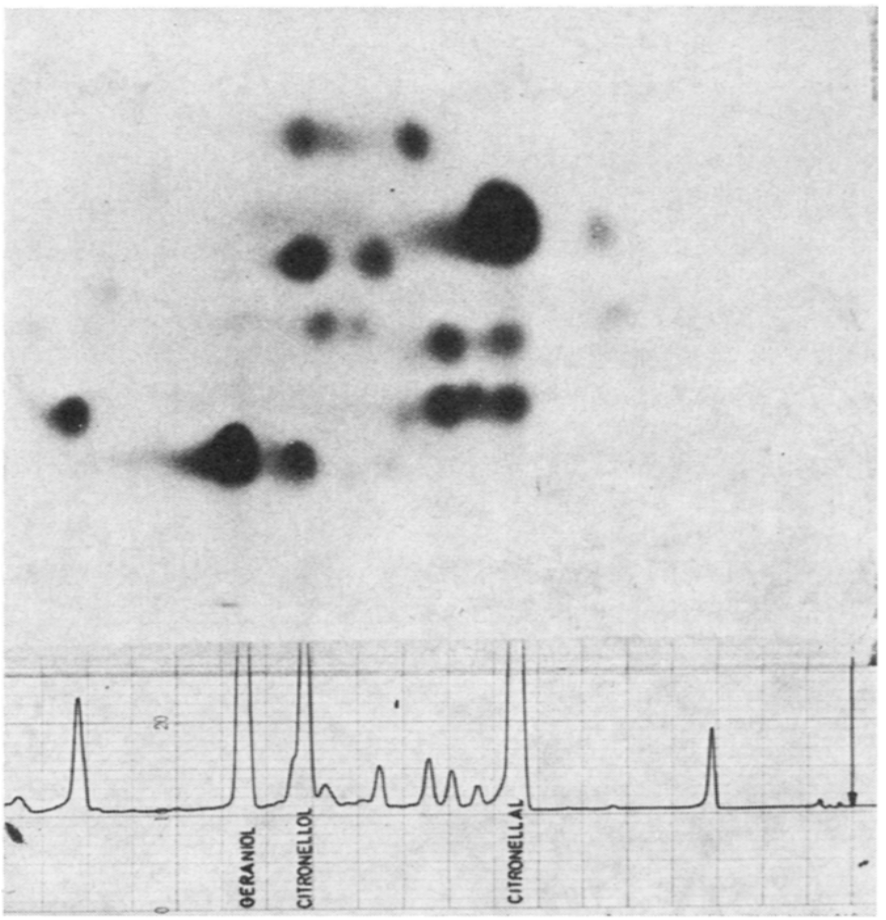

Fig. 2. An examination of citronella oil (Java type) by combined GLC/TLC. Sample applied, 1 μl. Conditions for GLC: 6-ft. PEG 20 M column; temperature 75°–225°, 3.3°/min. Conditions for TLC: adsorbent, Silica Gel HR; solvent, 5% ethyl acetate–methylene chloride.

of the GLC and TLC are best marked, so that no doubt can occur. Examination of the spots on the TLC plate will show whether the GLC peaks represent pure compounds and, if not, can often give information as to the nature of the accompanying compounds.

As an example of the results which may be obtained, our work on citronella oil has shown that the GLC peaks assigned to citronellal and citronellol contain more than one compound although the geraniol peak appears to be specific (see Fig. 2). Samples of pure citronellal and citronellol, when examined under the same conditions, each give a single TLC spot and it was therefore concluded that the interfering compounds in the citronella oil were not artefacts but genuine components of the oil.

Although the GLC was run under programmed temperature conditions it is apparent that this column (6 ft. × $\frac{1}{8}$ in. Carbowax 20M 15% on Chromosorb W, 80/100 mesh) would be satisfactory for the determination of geraniol in citronella oil under isothermal conditions.

We feel that the general principles of a combined GLC/TLC technique have wide application and the simplicity of the arrangement allows results to be obtained very rapidly.

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