QUANTITATIVE ANALYSIS BY THIN-LAYER CHROMATOGRAPHY USING A FLAME IONIZATION DETECTOR

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INTRODUCTION

Several methods exist for the direct quantitative interpretation of thin-layer chromatographic (TLC) separations which do not involve scraping off the plate that portion of the adsorbent containing the zone. Measurement of spot area, densitometry, ultraviolet absorption and radiation-counting are representative of the techniques available[1,2]. Many of these methods fail to define the exact distribution of the zone on a thin-layer plate owing to diffusion of the sample material into the adsorbent layer, and some are limited to particular applications. Arising from work on the adaptation of a detector, normally used for gas–liquid chromatography (GLC), to liquid chromatography[3], a method has been developed whereby spots are successively vaporized from the TLC plate and burned in a flame ionization detector (FID). The method only requires that the compounds to be determined can be volatilized from the plate and is otherwise reasonably independent of the physical and chemical properties of the sample material.

APPARATUS

A quartz tube, of rectangular cross-section, fitted with a removable end cap sealed with a Neoprene gasket, contains a quartz TLC plate (Fig. 1). The cap is fitted with a nitrogen inlet, and a heated outlet from the other end of the tube goes to the FID. The FID is connected through an amplifier to a 2.5 mV recorder. The furnace fits closely round the tube and a water-cooled shield is attached at the leading edge to effect a sharp heat front which enhances the resolution of zones close together on the TLC plate. The heaters for the furnace are contained in Pyrex tubes embedded in fireclay cement supported in a three-sided stainless steel shell; the fourth side is left open to clear the outlet from the quartz tube to the FID. The furnace is mounted on an electrically powered trolley driven through a worm gear and rack and pinion along rails to traverse the tube countercurrent to the nitrogen flow within 10, 20 or 40 min as required.

For a given analysis, a speed was chosen at which material from one zone was completely vaporized before the next zone was reached, i.e. the fastest speed could be used for relatively volatile compounds and the slowest for compounds which were difficult to vaporize.

The nitrogen carrier gas and the hydrogen and air for the FID are supplied
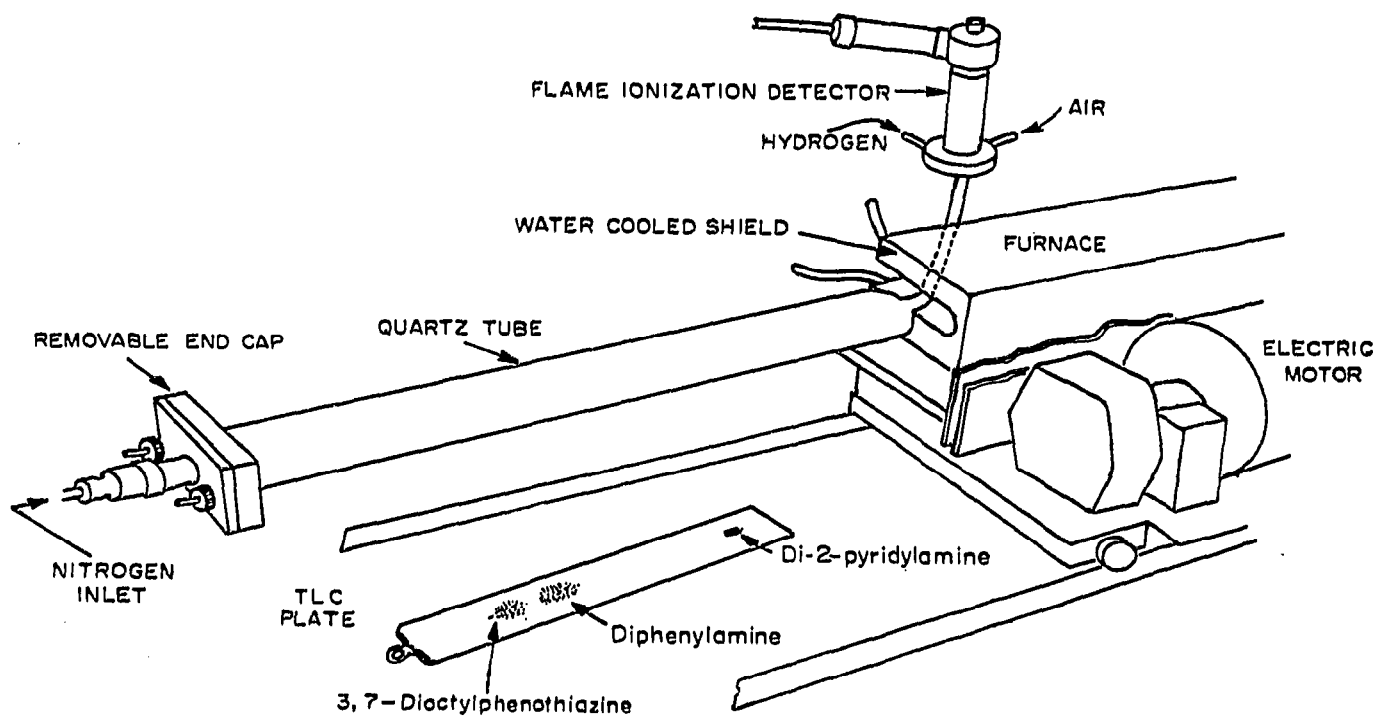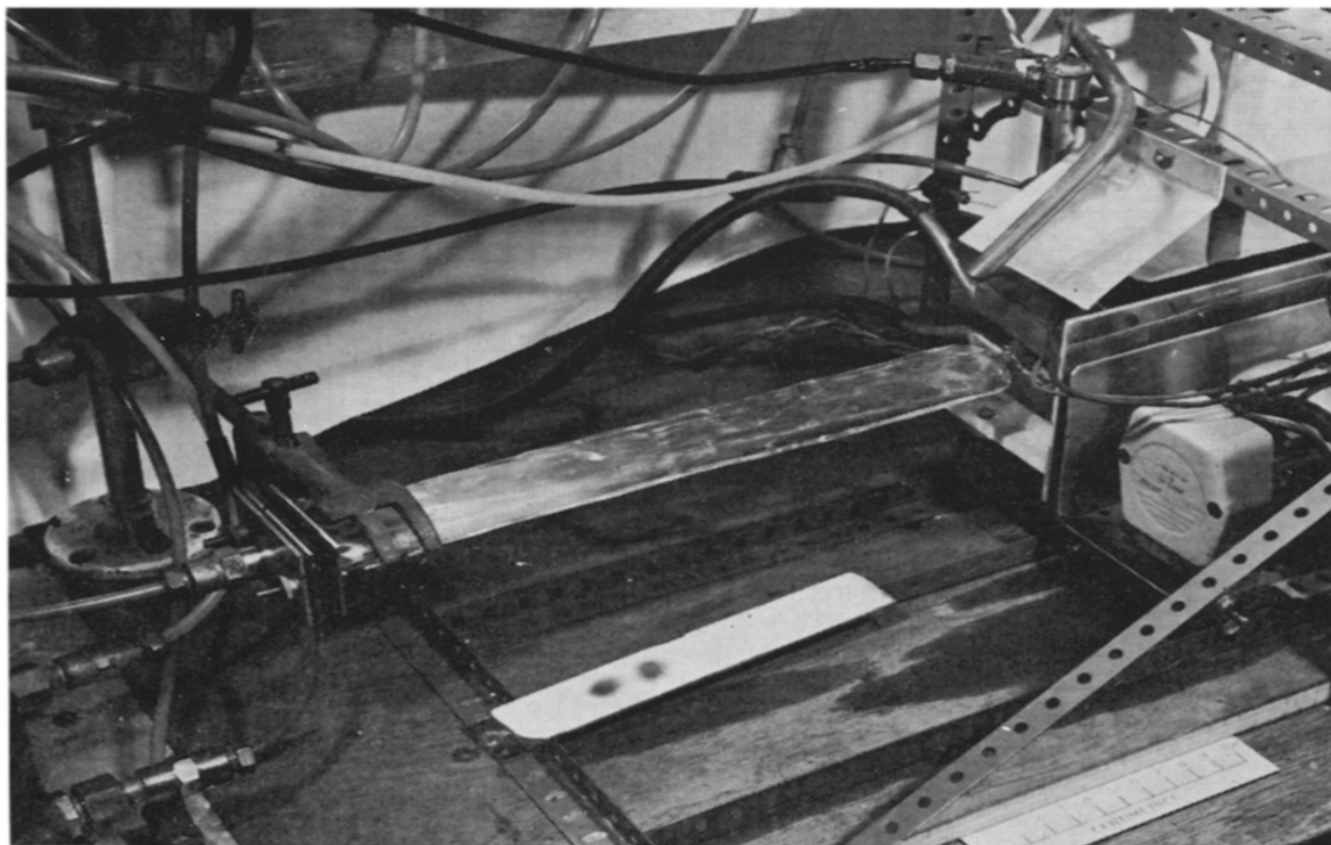
Fig. 1. Apparatus for quantitative thin-layer chromatography using a flame ionization detector.

through control systems commonly used for temperature-programmed GLC. A by-pass supplies nitrogen to the FID to prevent overheating of the jet tip when the tube is opened for inserting a plate.

EXPERIMENTAL AND DISCUSSION

The early work on this project was carried out with 150 mm × 12 mm plates and a correspondingly sized tube and furnace; this work was largely directed to improving the separate vaporization of individual spots close together on a plate coated with silica gel. A major factor in this respect is the sharpness of the moving heat front. The water-cooled shield at the leading edge of the furnace made a significant contribution to ensuring the complete vaporization of one spot before the next was affected. Spots of cetane of 3-4 mm spot diameter and 12 mm apart, measured from the spot centre, were clearly depicted as separate peaks and spots with centres less than 6 mm apart were detected as partly separated peaks. To obtain a more sharply defined heat front, an optical furnace, as used for zone melting[1,5], was constructed from two 18 cm spotlight reflectors at each end of a 23 cm long cylinder. Light from a bulb held in the first reflector was delivered in a parallel beam to the second reflector which focused it back to a point inside the quartz tube which entered the furnace through the bulb socket in the second reflector. A water-cooled shield mounted on the second reflector fitted round the tube adjacent to the focused hot zone. When a standard 12 V 48 W transverse filament prefocused bulb over-run at 15 V was used, a temperature of 600° was reached. With a 12 V 100 W quartz-iodine bulb, the temperature reached 700° whilst the temperature within the shield remained below 100°. A slight improvement in the separate vaporization of cetane spots was achieved with this furnace but when an attempt was made to vaporize glycol spots from a plate, it appeared that the sample vapour was being readsorbed by the silica gel after the hot beam had passed over it. This effect persisted even though the temperature in the body of the furnace was raised to 400° with auxiliary heaters. Induction heating was also considered but this too would have required auxiliary heating after the initial hot area. In addition, it would have been necessary to provide the TLC plate with a metal backing formed in transverse strips to prevent heat flow along the plate. The benefits of these systems did not warrant the extra apparatus complications entailed and they were therefore abandoned in favour of the more simple electric furnace, which maintains a high temperature throughout its length. With this furnace operating at 700°, a temperature of 600° was reached inside the quartz tube. The operating conditions were:

Outlet pipe temperature: 300°
Nitrogen carrier gas flow rate: 4 l/h
Hydrogen flow rate to FID: 2.5 l/h
Air flow rate to FID: 7 l/h

The TLC separations were first carried out on 12 mm wide plates but the chromatographic separation of sufficient sample (0.3 mg) to enable detection resulted in edge effects on the plate, thus giving diffuse zones. A diffuse zone on vaporization and detection gives a poor chart trace which is quite unsuitable for quantitative interpretation. It was then decided to use 25 mm wide TLC plates and the apparatus (furnace etc.) was reconstructed to accommodate the wider plates. When the wider plates were used no difficulties were experienced with the chromatographic separations.
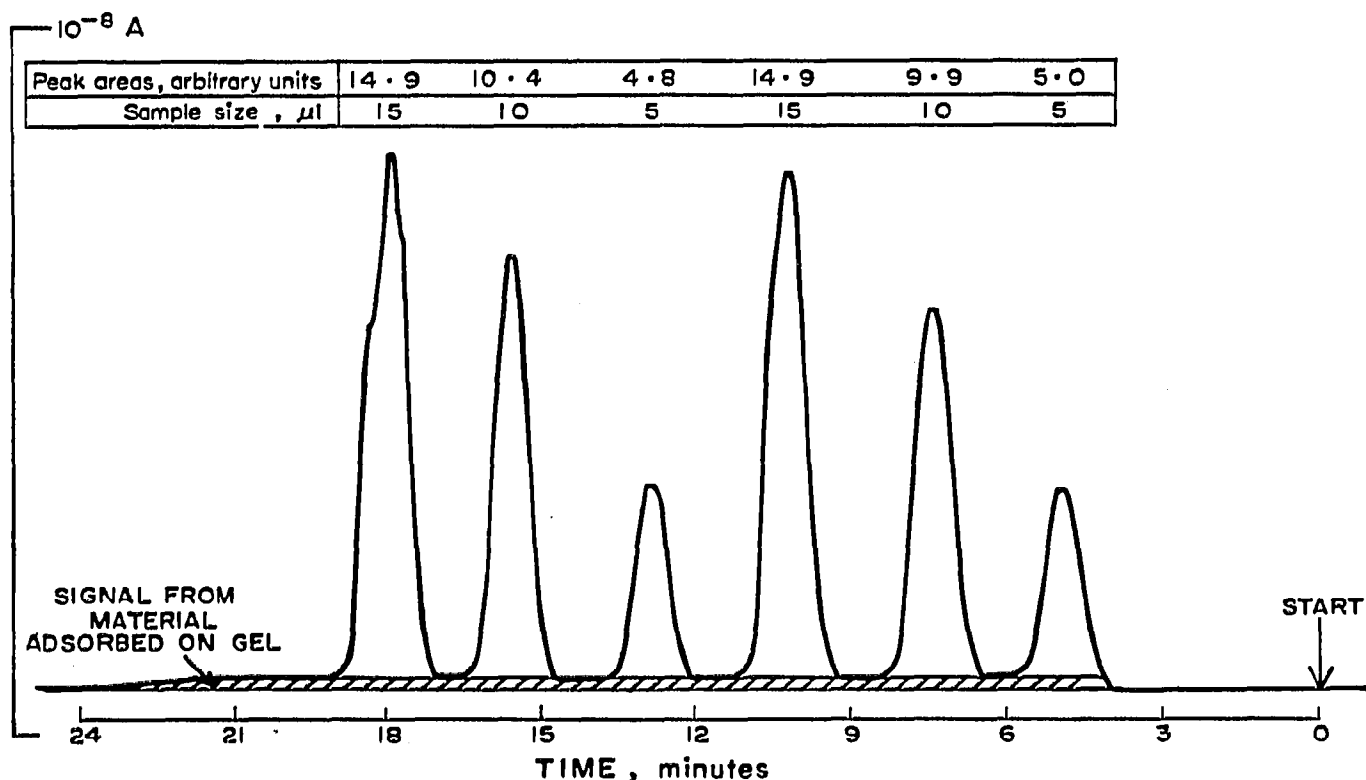
Fig. 2. Chart record obtained from a preheated Silica Gel H plate (12 mm) spotted with a 1 % solution of cetane in light petroleum spirit.

The plates were prepared by pouring a 3:1 water–silica gel slurry onto the plate and allowing it to stand for a few minutes before it was activated at 120° for 30 min. The silica gel used was Merck Kieselgel H. Although the chromatographic separations were satisfactory, when the furnace was moved over the plate a strong signal was obtained which persisted for the length of time taken for the furnace to pass over the plate, making it impossible to detect the compounds separated by the chromatography. This signal is probably caused by organic material in the silica gel. A considerable reduction in the background signal was achieved by heating the activated plates in the quartz tube at the operating temperature (600°) before use.

It has been shown[6] that when silica gel is heated up to 400° the activity of the gel increases owing to the loss of physically bound water. Above this temperature the gel loses its activity because of loss of water from the silanol groups. However, we have found that the length of time for which the plates are preheated does not subsequently affect the chromatographic separation to any marked degree. It is essential to carry out the chromatographic separation immediately after the furnace treatment of the plate; if the coated plate is allowed to stand for any length of time, a high signal is obtained, probably caused by organic material picked up by the silica gel from the atmosphere.

PROCEDURE

The plates are heated at 600° until the recorder indicates a low signal; this usually takes 5–20 min. They are then cooled in the furnace under nitrogen, removed
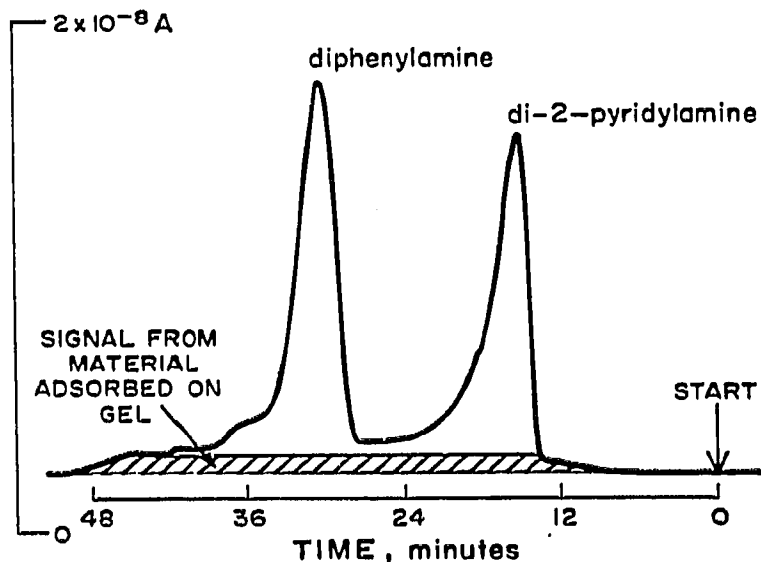
Fig. 3. Chart record for the thin-layer chromatographic separation of two antioxidants on a 25 mm plate with toluene.

from the tube and immediately "spotted" with the compounds to be solvent-developed and separated.

Initially we have examined the separation of nitrogen-containing antioxidants since these compounds are of current interest to us. We have chromatographed single antioxidants and mixtures of two and three antioxidants; this has been done in the usual way, a lined tank being used with toluene or toluene–hexane as developing solvents.

After chromatography the developing solvent is evaporated from the plate in an oven at 120° and the plate is then placed in the quartz tube to vaporize the separated components. For the quantitative interpretation of the chart record, the areas of the peaks are measured and expressed as a percentage of the total area.

RESULTS

Some experiments were carried out with the 12 mm plates to ascertain the quantitative aspects of the method of vaporizing material from a plate coated with silica gel. The plate was first cleaned in the quartz tube, cooled and spots of a 1 % solution of cetane in light petroleum spirit were placed along its length from a syringe. The solvent was evaporated in the oven and the plate returned to the quartz tube. The trace and results, expressed as a ratio of peak areas, are shown in Fig. 2. The areas correspond with the amount of sample present to within about 5 %. The shaded area on the trace is the signal obtained from organic material adsorbed on the gel during the "spotting" and drying processes. The separation and detection of two antioxidants on a 25 mm plate are shown in Fig. 3. This was carried out by the procedure previously mentioned with toluene as the developing solvent. About 0.3 mg of each component was present and the zone centres were about 80 mm apart. The results of duplicate determinations on this blend are shown in Table I.

The detection of the individual zones of a three-component mixture has proved more difficult and an allowance has had to be made on the chromatogram for partial
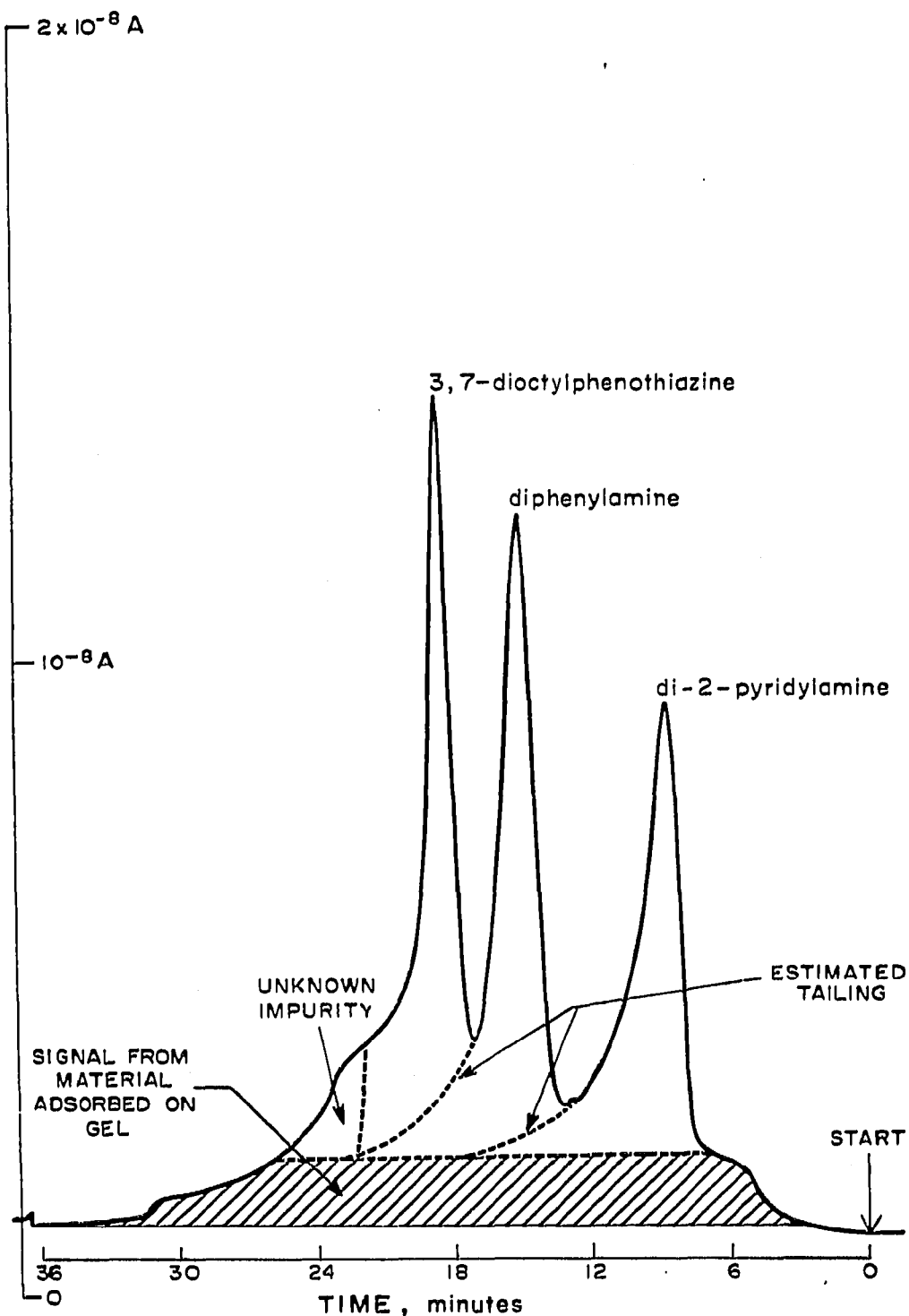
Fig. 4. Chart record for the thin-layer chromatographic separation of three antioxidants on a 25 mm plate with toluene–hexane (4:6).

TABLE I

ANALYSIS OF TWO ANTIOXIDANTS

Component	Actual composition (% wt.)	Peak areas (%)	
		Run 1	Run 2
Di-2-pyridylamine	48.5	48	46
Diphenylamine	51.5	52	54

overlapping of the peaks (Fig. 4). The results of duplicate determinations on a 25 mm plate developed with toluene–hexane are shown in Table II. A similar plate sprayed with antimony pentachloride shown in Fig. 1 indicates the relative positions of the zones. (See also the photograph of Fig. 1, in which the di-2-pyridylamine spot does not show up.)

TABLE II

ANALYSIS OF THREE ANTIOXIDANTS

Component	Actual composition (% wt.)	Peak areas (%)	
		Run 1	Run 2
Di-2-pyridylamine	40	36	37
Diphenylamine	30	34	38
3,7-Dioctylphenothiazine	30	30	25

CONCLUSION

The quantitative response of the apparatus to easily volatilized materials is satisfactory, as has been shown by means of the plate spotted with cetane. Good results have also been obtained with an actual TLC separation of more complex materials, i.e. the two antioxidants. Improved quantitative interpretation of the record from the three antioxidants would be possible if the peaks were better separated and certain minor modifications to the apparatus are in hand in an attempt to effect this.

The results so far obtained have been encouraging and we shall investigate the possibility of making quantitative many of the TLC separations of interest to us in this laboratory; these include glycols, phenols, amines and acids. The overall technique should be of wide application in TLC and the use of equipment with alternative detectors should be possible.

SUMMARY

A method has been developed in which sample components separated on a plate by thin-layer chromatography are successively vaporized, by a slow-moving furnace, into a stream of nitrogen which passes into a flame ionization detector. Each zone is recorded on a strip chart as a peak having an area proportional to the amount of material present.

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