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THIN-LAYER CHROMATOGRAPHIC TRANSPORT DETECTOR FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

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SUMMARY

A procedure for the detection and quantitation of compounds in high-performance liquid chromatography (HPLC) is described. Solutes from the column are transferred on to a moving chromatoplate and subsequently detected according to well established thin-layer chromatographic methods. The performance compares favourably with existing methods of detection in HPLC and in particular has a universal and more linear response to organic compounds.

INTRODUCTION

Despite a great deal of research, detectors still remain the weakest component of high-performance liquid chromatography (HPLC). One approach has attempted to devise a means of interfacing the liquid chromatograph with a well-proven gas chromatographic detector such as the flame-ionisation or electron-capture detector. In most instances some form of transport device, *e.g.* a moving wire[1], belt[2] or disc[3] is involved.

Segura and Gotto[4] recently described a general-purpose fluorescent technique for detection of compounds separated by thin-layer chromatography (TLC) at the nanogram level. This prompted us to explore TLC plates as a transport mechanism for HPLC. The device described here resembles in principle the one devised by Janák[5] for gas chromatography but differs in that it is applied to column liquid chromatography.

EXPERIMENTAL

Chromatograph–chromatoplate interface

The most crucial factor determining band spreading lies in the extent to which the solutes may be confined to their point of application on the chromatoplate. It is therefore necessary to remove the solvent as rapidly as possible once it has made contact with the adsorbent, without removing any of the solute material.

* *Editor's note*: The performance of the assembly seems to be in the order of the new high-performance thin-layer chromatographic (HPTLC) method. One asks oneself therefore, why the authors do not chromatograph directly on the new fast thin layers. *Editor of J. Chromatogr.*

Authors' reply: The comment about using the new HPTLC plates from Merck appears to us to be irrelevant. The device we describe is an interface for column liquid chromatography.

For this purpose, the eluent was transferred with a fine steel capillary tube (0.5 mm O.D. × 0.25 mm I.D.) to the surface of the chromatoplate. The actual gap between the tube and adsorbent was adjustable in the region of 0.25 mm. Regular application of the eluent to the plate could be achieved only by maintaining a continuous eluent bridge between the capillary and the adsorbent surface. It was necessary to decide upon a compromise among a number of parameters: sample size, length and speed of the chromatoplate, the solvent flow-rate, volatility and surface tension, the temperature of the plate, the rate at which the solvent vapour was to be removed and the amount of solute material, if any, lost in the process.

Standard 20 × 5 cm chromatoplates were used as they are readily available and a plate speed of 2 cm/min was chosen so as to provide 8–10 min in which to record the chromatogram. For most applications a flow-rate of 1 ml/min would thus be

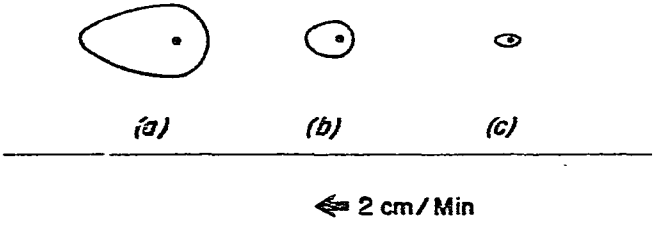

Fig. 1. Effect of temperature on band spreading. Temperature: (a) ambient; (b) 50°; (c) 90°.

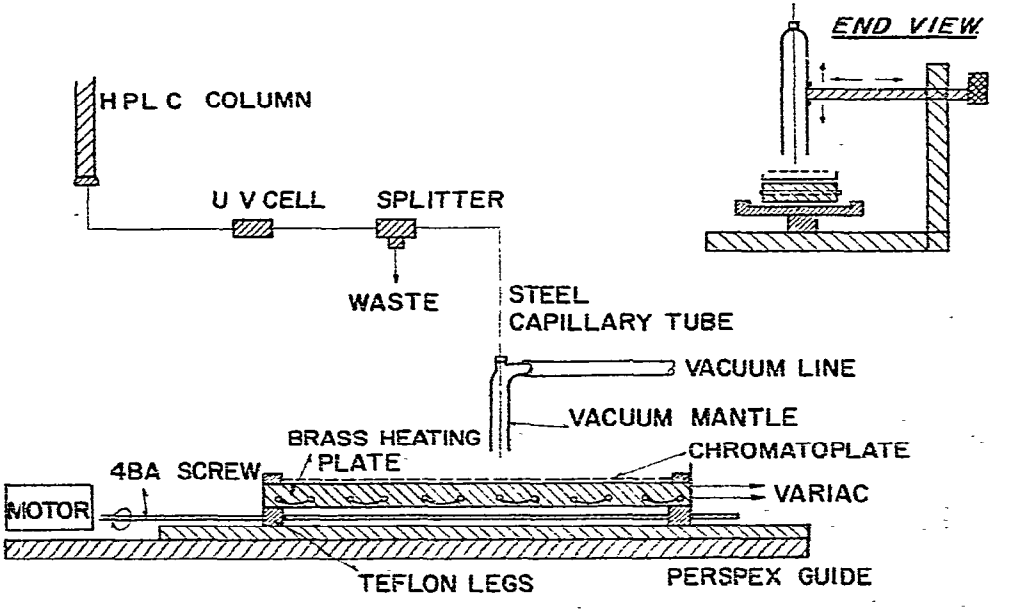

Fig. 2. Diagram of apparatus.

required and, as this could not be coped with efficiently, an eluent splitter was introduced.

The chromatoplate was evenly heated on a brass plate, whose temperature was regulated with a 5-A Variac. An indication of the effect of chromatoplate temperature on the degree of solvent spreading in the adsorbent material may be gained from Fig. 1.

The solvent vapour was removed from the adsorbent by positioning a glass tube of 8 mm diameter concentrically about the capillary, at a height of 6 mm above the chromatoplate. A vacuum line connected this tube to a strong venturi pump. The latter facility is not only essential to prevent band spreading but also avoids a possible health hazard.

The chromatoplate and heating carriage were driven by a small reversible and variable-speed motor, which was coupled to a 25-cm long 4 BA screw, which was in turn threaded to the Teflon legs on the carriage assembly (Fig. 2).

Chromatoplates. Pre-coated 20 × 5 cm silica gel plates with a 0.25-mm coating, both with and without F254 fluorescent indicator, were obtained from E. Merck (Darmstadt, G.F.R.). Anasil®-H chromatoplates were obtained from Analabs (North Haven, Conn., U.S.A.). An attempt was made to remove possible organic impurities by continous development of the plates with methanol for 12 h.

Fluorescence measurements. These measurements were made after the compounds on the chromatoplates had been derivatized according to Segura and Gotto's method. In this method the chromatoplates (without fluorescent indicator) containing the eluted compounds were placed on a plate holder which was transferred to a 4-gal capacity tank to which 6 g of ammonium hydrogen carbonate had been added. The tank was placed into an oven and heated at 110–150° for 3–12 h. Steroids usually form the required fluorescent derivatives after 3 h at 110°.

A relatively inexpensive TLC scanning instrument, constructed in our laboratory, was used to measure the chromatoplates. Working in the absorption mode, plates containing F254 were measured directly. Only UV-active compounds are detectable in this manner (Fig. 3a).

For chromatoplates treated according to Segura and Gotto's method, the derivatized products were activated at 360 nm and the subsequent fluorescent emission, with a maximum close to 450 nm, was detected and recorded with a photomultiplier and a strip-chart recorder (Fig. 3b).

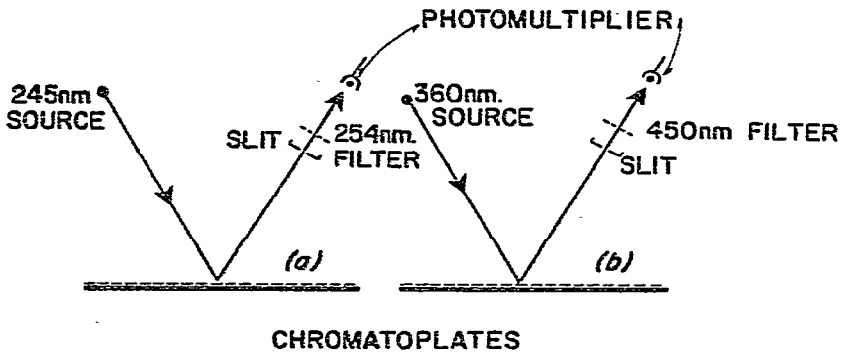

Fig. 3. Optics for plate scanning: (a) absorption mode; (b) emission mode.

RESULTS

As a rule, eluent flow-rates in excess of 0.6 ml/min on to the chromatoplate resulted in band spreading, due mainly to sporadic backwashing of the excess of solvent across the saturated adsorbent.

Increasing the adsorbent thickness caused problems with detection owing to the tendency of the solute to advance with the solvent front. Although heating of the chromatoplate had a marked effect on band spreading, care was necessary not to reduce the volume of solvent between the capillary and chromatoplate as this bridge had to be maintained and, further, the passage of solutes across this bridge caused visible boiling. This was a particular problem with solvents of low surface tension or when excessive air was present in the solvent supply.

It was found that washing the chromatoplate with methanol improved the stability of this solvent bridge. This improvement is possibly due to the displacement of water from the adsorbent particles, thus permitting a miscible interface. Chromatoplates were therefore washed by continuous development with methanol for 12 h, allowed to dry at room temperature and activated at 80° for 15 min *in situ* prior to use.

Because the solutes tend to advance with the solvent front, they tend also to be deposited on the adsorbent at the solvent perimeter. The result of this effect is that the chromatoplates appear as depicted in Fig. 4a.

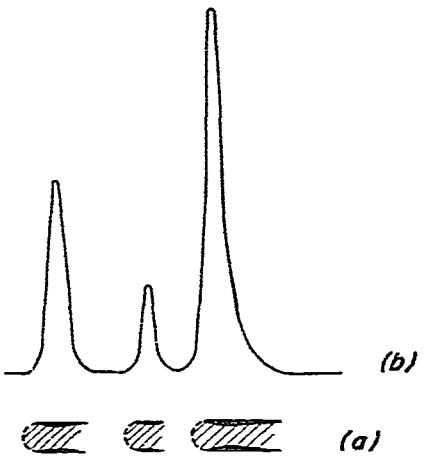

Fig. 4. (a) Appearance of chromatographic spots. (b) Strip-chart recording of (a).

The strip-chart recording in Fig. 4b was obtained by scanning the plate with a spectrofluorimeter with a 1 cm × 1 mm slit image at the plate.

The analysis of a synthetic mixture of steroids is shown in Fig. 5. A 10-mg amount of each of progesterone, testosterone, cortisone and 17β-estradiol were diluted to give solute concentrations of 100 ng/μl. A 2-μl volume of this solution was introduced on to a 25-cm Partisil (5 μm) column and eluted with dichloromethane–methanol (95:5, v/v) at a flow-rate of 1 ml/min. The eluent was split in the ratio 2:1 after passing through a UV detector. A 66-ng amount of each solute was thus deposited on the chromatoplate. The plate temperature was 80°.

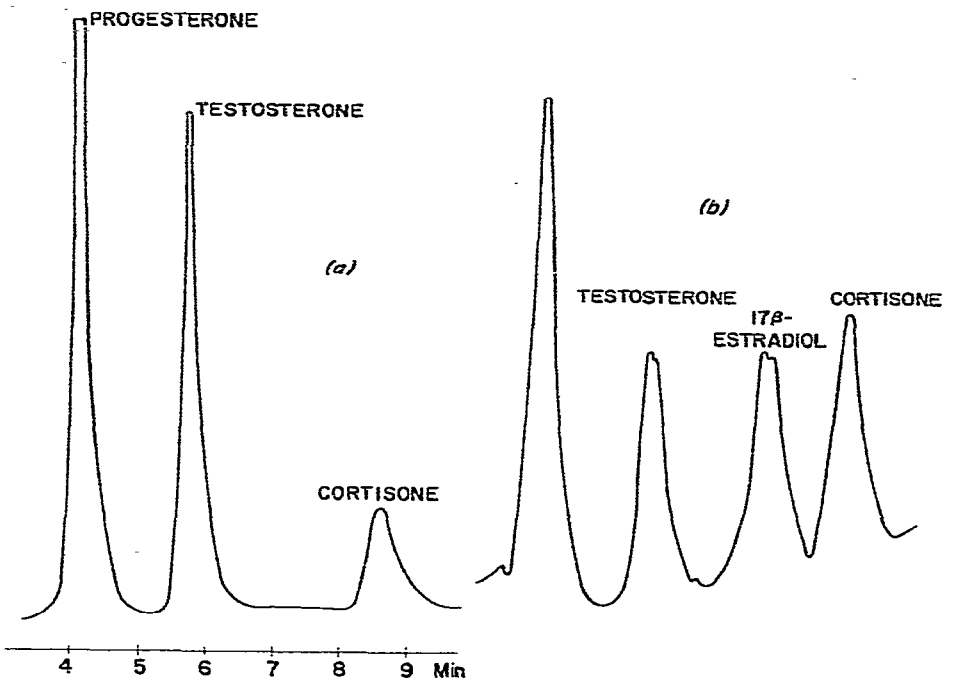

Fig. 5. Synthetic steroid mixture: (a) recording from UV photometric detector, (b) fluorescence emission recording obtained from HPLC–TLC.

Fluorescent products were prepared according to Segura and Gotto's method. Three of the four compounds had absorption maxima at 240 nm and were strongly UV active ($E^{1\%}_{1\,cm}$ = 390–540), while 17β-estradiol was UV inactive at 254 nm (λ_{max} = 280 nm, $E^{1\%}_{1\,cm}$ = 50). Progesterone was very soluble in the solvent system while the other three steroids were only slightly soluble.

The analysis of a mixture of nine steroids is shown in Fig. 6. The same conditions were used as in the previous example except that the solvent system was chloroform–methanol (97:3, v/v), the splitting ratio was 1:1 and the flow-rate was 0.6 ml/min.

The analysis of insecticides produces unexpected difficulties relating to Segura and Gotto's method. Although organochlorine compounds produced excellent fluorescent products, the organophosphorus compounds examined did not. In such instances, F254 chromatoplates, or alternatively some other method of detection such as esterase inhibition, can be used. The analysis of a mixture of three insecticides is shown in Fig. 7. The chromatoplate contained F254 indicator. A 25-cm Partisil (5 μm) column was used, the solvent was pentane–chloroform (1:1, v/v), the flow-rate was 0.7 ml/min and the splitting ratio was 1:1.

Sensitivity

Methoxychlor was diluted to give a solution of 1 ng/μl concentration, 2 μl (2 ng) were then introduced on to a 25-cm Partisil (5 μm) column and the column was eluted with chloroform–methanol (95:5, v/v). The effluent was split after passing through the UV photometer cell in the ratio 1:2. The chromatoplate thus contained

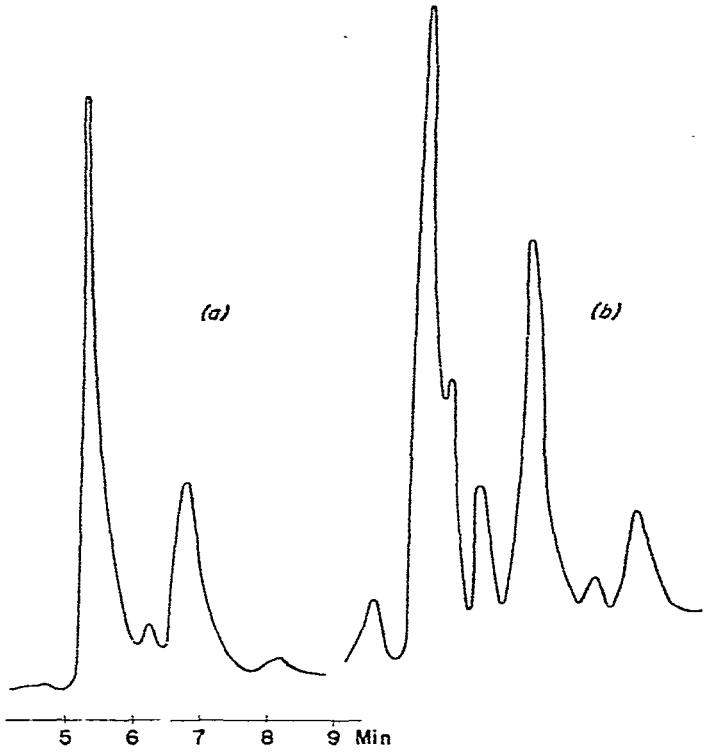

Fig. 6. Complex steroid mixture: (a) UV detector recording; (b) HPLC–TLC recording.

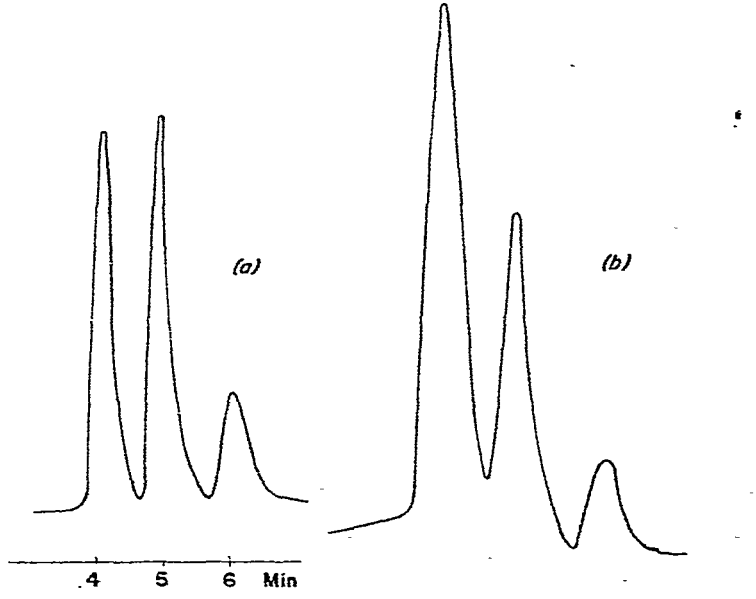

Fig. 7. Organochlorine insecticide mixture: (a) UV detector recording; (b) absorption recording obtained from HPLC–TLC (F254).

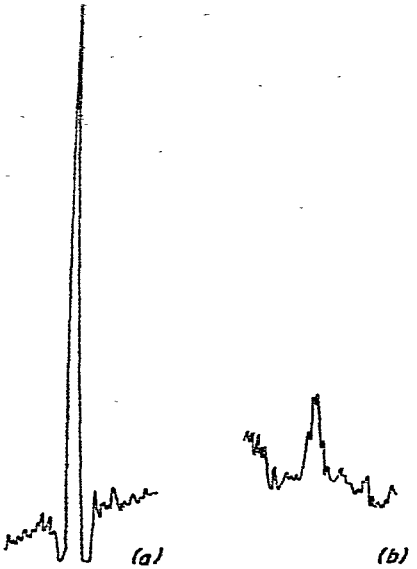

Fig. 8. Recording of methoxychlor: (a) 2-ng sample recorded with a UV detector, sensitivity 0.05 a.u.f.s.; (b) 0.66-ng fluorescence emission recording from chromatoplate, recorded at maximum available sensitivity, 1 mV f.s.d.

0.66 ng of methoxychlor. The UV response was recorded at 0.05 a.u.f.s. (Fig. 8a) and the chromatoplate was treated according to Segura and Gotto's method with ammonium hydrogen carbonate for 10 h at 110°. The spectrofluorimeter recording was obtained at the maximum available sensitivity on a 1-mV f.s.d. strip-chart recorder (Fig. 8b). The maximum available sensitivity of the UV photometric detector was 0.01 a.u.f.s. and a recorder of 1 mV f.s.d. was used. As the signal to noise ratios were of a similar order, these results indicate the UV photometric method to be one order of magnitude better in sensitivity for methoxychlor ($\lambda_{max} = 220$ nm, $E^{1\%}_{1\,cm} = 460$) than that obtained for the fluorescent method.

CONCLUSION

It has been demonstrated that solutes eluted in HPLC can be transferred to a chromatoplate without serious loss of resolution and that suitable methods are available for subsequent detection at the lower nanogram level.

In particular, when this approach is used in conjunction with Segura and Gotto's method, sensitivities of the same order as those found for the UV photometric method are possible and, in addition, a wider range of compounds respond favourably to detection.

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