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Note

An extension of the programmed multiple development (PMD) technique

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The interaction between different chromatographic techniques is well known and its effects are strongly stimulating each and every area of the separation sciences. Many technical details utilized for high-pressure liquid chromatography (HPLC) originate, for instance, from the earlier developed gas–liquid chromatography (GLC). Furthermore, the physical principles of thin-layer chromatography (TLC) and liquid column chromatography (LC) are essentially identical. Investigators in the field of HPLC often recommend TLC for optimizing developing solvent systems prior to the actual HPLC[1]. These interactions almost imperatively urge the investigators' mind to search intentionally for still missing links between different chromatographic techniques.

Such an unexplored counterpart of TLC in HPLC is the programmed multiple development (PMD)[2] technique. PMD allows the re-concentration of the chromatographic zones due to the fact, that the solvent front passes through the fractions after every evaporation of the solvent. Drying out a liquid chromatographic column however, would not improve the separation and detection of the components chromatographed, since the formation of gas bubbles and inhomogeneity of the column packing would override the results of a zone reconcentration with certainty.

The present study was performed on TLC systems in such a way, that the results can feasibly be transferred to HPLC systems.

MATERIALS AND METHODS

The TLC plates were of E. Merck (Darmstadt, G.F.R.) quality on glass backing (pre-coated silica gel 60, 20 × 5 cm, 0.25 mm thick). The steroids were purchased from Steraloids (Pawling, N.Y., U.S.A.). The solvents and reagents were delivered by Fisher Scientific (Fair Lawn, N.J., U.S.A.). The densitometric scannings were performed on a Zeiss Chromatogram Spectrophotometer (C. Zeiss, Oberkochen, G.F.R.) as described earlier[3].

Two standard mixtures of steroids were chromatographed containing 10 μg of each component; mixture I: estriol (E3), estradiol (E2), and estrone (E1); mixture II: testosterone (T), and estrone, both as 2,4-dinitrophenylhydrazone (2,4-DNPH) derivatives prepared as previously described[4] except, that trichloroacetic acid was replaced by the highly volatile trifluoroacetic acid.

Both mixtures were separated on the first plate in ascending chromatography in the solvent system chloroform–dioxan (94:6)[4] also containing acetone and acetic acid (0.2% each) (A) in order to remove the excess of the 2,4-DNPH reagent forming the very non-polar acetone 2,4-DNPH.

In the second experiment mixture I was developed in the solvent system chloroform–acetone–acetic acid (98:1:1) (B) in descending chromatography during 6 h. Mixture II was separated similarly, except that the solvent system chloroform–acetone–acetic acid (99.5:0.25:0.25) (C) was used.

In the third experiment each mixture was developed in descending chromatography under retaining the previous mixture–system combinations (I–B, II–C). However, after 90 min development time, the plates were quickly transferred, without any drying, into another chamber containing petroleum ether (D) and the descending chromatography was continued for 90 min. Then, the plates, once again without any drying, were returned into their original system for the second cycle. This manipulation was carried out all together four times.

After the final development the plates were dried. Mixture I was treated with nitrous gases, subsequently with ammonia gas. The resulting nitroso derivatives were scanned at 415 nm. Mixture II containing the bright yellow fractions of the 2,4-DNPH derivatives was always scanned at 385 nm.

RESULTS AND DISCUSSION

In comparison of the densitometric scans the complete separation of the components in all three experiments can be noted. However, only the second and third experiment separated T-2,4-DNPH into its *syn* and *anti* isomers. Earlier attempts[5] to separate both isomers were only successful, if chromatographic systems based on benzene were used. Systems based on chloroform gave consistently a single spot for T-2,4-DNPH.

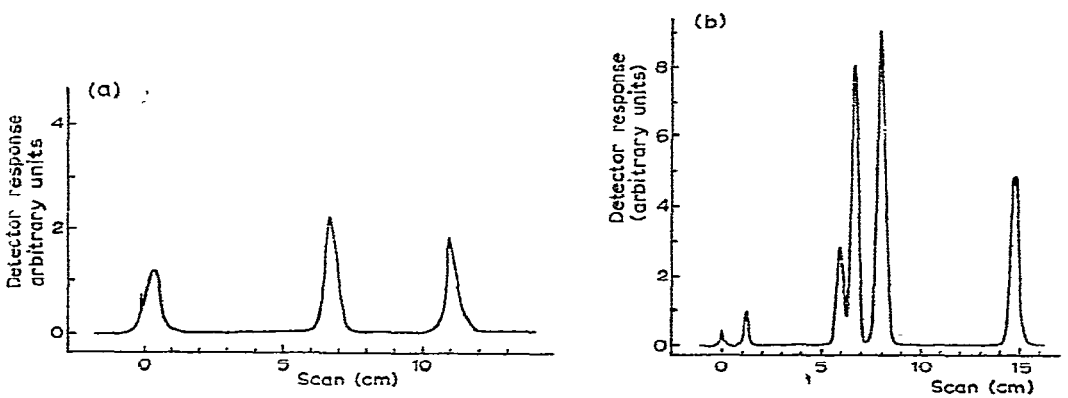

Fig. 1. Densitometric charts after alternating development in descending chromatography in (a) system B (4 × 90 min) and system D (3 × 90 min); E3 (at 0.4–1 cm), E2 (at 6.2–7.4 cm) and E1 (at 10.4–11.5 cm) scanned at 415 nm; (b) Development in system C (4 × 90 min) and system D (3 × 90 min); T-2,4-DNPH (double peak reasonably resolved at 5.4–6.3 cm and 6.3–7.3 cm) and E1-2,4-DNPH (at 7.3–8.8 cm), and 2,4-DNPH by-products scanned at 385 nm.

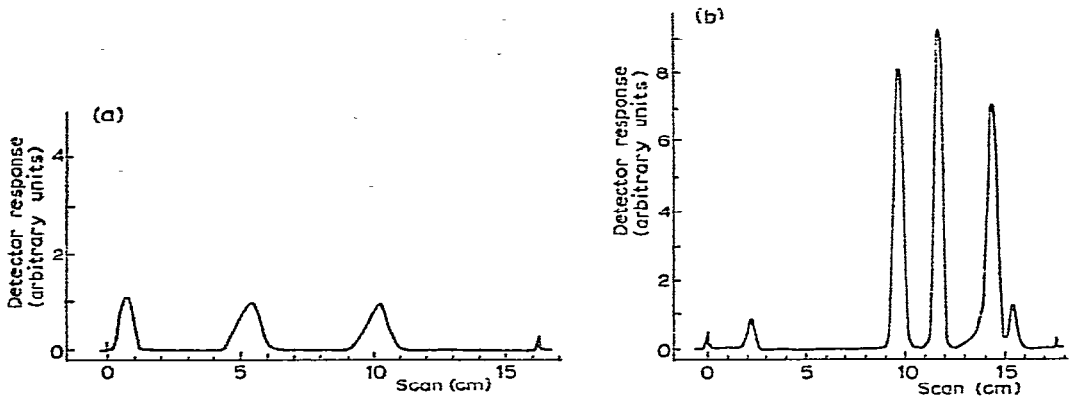

Fig. 2. Densitometric charts after development in system A in ascending chromatography. (a), Nitroso derivatives of E3 (at 0–1.2 cm), E2 (at 4.3–6.2 cm) and E1 (at 9–11 cm) scanned at 415 nm; (b), 2,4-DNPH derivatives of T (at 9–10.5 cm) and E1 (at 11–12.4 cm), and reaction by-products of the 2,4-DNPH reagent scanned at 385 nm.

The second observation is that the alternating development (Figs. 1a and b) actually reconcentrates the chromatographic zones (*cf.* Figs. 2a, 2b, 3a, 3b), demonstrating a similar effect as that of PMD. The double peak of E3 and E2 (Fig. 3a) is due to the fact, that the sample application onto the plate resulted in an accumulation of the dissolved material in a circle around the origin.

Considering the experimental results one can reasonably assume, that PMD can actually be applied to HPLC also. The experiment for HPLC, in analogy to that described above, should consist of the alternating use of a developing solvent and a non-developing solvent. Such solvent combinations can easily be found in various areas of HPLC. For the adsorption chromatography two examples were presented above. Investigations on the distribution coefficients (in many cases available from

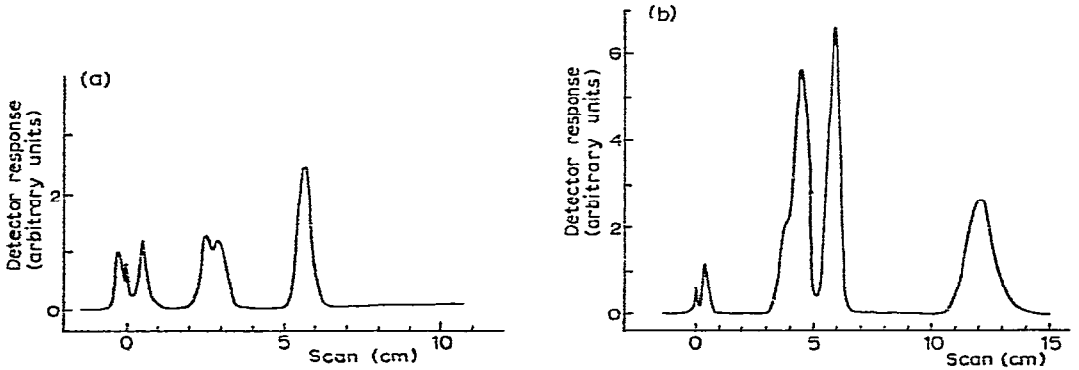

Fig. 3. Densitometric charts after development in descending chromatography in (a) system B during 6 h; the nitroso derivatives of E3, E2 and E1 appear at –0.5–1 cm, 2–3.5 cm and 5–6.3 cm, respectively, scanned at 415 nm. (b) Development in system C during 6 h; the 2,4-DNPH derivatives of T (the double peak only partially resolved) and E1 at 3–5.2 cm and 5.2–6.6 cm, respectively, scanned at 385 nm.

the literature) would also provide similar information for the liquid–liquid partition chromatography. For ion-exchange chromatography distilled water would be an obvious choice of the non-developing solvent.

The technique presented above can evidently be used for TLC of compounds sensitive to elevated temperature or oxidation, as demonstrated convincingly here. Nevertheless, it is felt, that the present results give us preliminary data to a previously unexplored link between different chromatographic techniques, such as TLC, HPLC, and possibly GLC. Investigations on PMD (or tentatively "Programmed Alternating Development") in HPLC are now in progress and the results will be published in due course.

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