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## Note

# Simple method for the separation of cephalosporins using silica gel 60 as the stationary phase in thin-layer chromatography and in high-performance liquid chromatography

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Cephalosporin antibiotics have gained increasing importance recently. For testing the purity and checking the synthesis of these compounds, chromatographic methods are frequently used. Both reversed-phase materials[1–3] and hydrophilically bound carriers have been used[4] as stationary phases in high-performance liquid chromatography (HPLC). The individual substances are usually determined quantitatively on the basis of the individual peaks in the chromatograms.

Reversed-phase pre-coated thin-layer chromatographic (TLC) plates modified with dodecylalkyl groups have been used[5] for the separation of cephalosporins. The elution conditions can be extrapolated approximately for application in HPLC using an RP-18 column[6]. However, direct optical evaluation of the separate spots of the substances on the reversed-phase thin-layer pre-coated plates used is inadequate because of the coarse adsorbent material used.

The objective of this investigation was to develop a TLC method for the separation of cephalosporins which permits direct optical evaluation of the spots of the substance on the plate and also the direct extrapolation of the chromatographic conditions for application in HPLC.

## EXPERIMENTAL

### *Chemicals*

Adipic acid, extra pure (E. Merck, Darmstadt, G.F.R.; Cat. No. 90) and water for chromatography, LiChrosolv® (E. Merck; Cat. No. 15333), were used for the preparation of the mobile phase for the chromatographic separation.

### *Test substances*

Cephalosporin C, deacetoxycephalosporin C and deacetylcephalosporin C were kindly provided by Ciba-Geigy (Basle, Switzerland). Solutions in water (0.1 %, w/w) and mixtures of 0.1 % (w/w) of each of the individual substances in water were prepared.

### *Thin-layer chromatography*

HPTLC pre-coated plates (10 × 20 cm) with silica gel 60 $F_{254s}$, extra pure

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(suitable for aqueous eluents) (E. Merck; Cat. No. 15552) were used for the thin-layer trials. Volumes of 10 $\mu$l of the substance solutions were applied in streaks and the plates were developed by ascending elution without chamber saturation. Water containing 2% (w/w) of adipic acid proved to be a particularly good eluent. The direct optical evaluation of the HPTLC pre-coated plates was carried out at a wavelength of 254 nm using a TLC/HPTLC scanner from CAMAG (Muttenz, Switzerland).

*Column chromatography*

The HPLC system consisted of an SP 8000 liquid chromatograph from Spectra-Physics (Darmstadt, G.F.R.) and a Hibar RT 250-4 column pre-packed with LiChrosorb Si 60 with a mean particle size of 5 $\mu$m (E. Merck; Cat. No. 50388). The eluent used in the TLC was also used as the mobile phase here (2%, w/w, aqueous solution of adipic acid). A Schoeffel 770 photometer was used for detection at 250 nm. The amount of sample introduced into the column was 10 $\mu$l of the solutions described above in each instance.

## RESULTS

The cephalosporins investigated are highly polar compounds and differ only in the substitution of the six-membered heterocyclic ring. Complete separation of the three cephalosporin derivatives on an HPTLC pre-coated plate silica gel 60 $F_{254s}$, extra pure "waterproof" (suitable for aqueous eluents), is achieved within a migration distance of 10 cm within 38 min (Fig. 1). The elution sequence of the substances in order of increasing $R_F$ values is deacetoxycephalosporin C, cephalosporin C and deacetylcephalosporin C.

The same elution sequence and similar selectivity can be obtained using a Hibar RT 250-4 column pre-packed with LiChrosorb Si 60, 5 $\mu$m (Fig. 2). In this

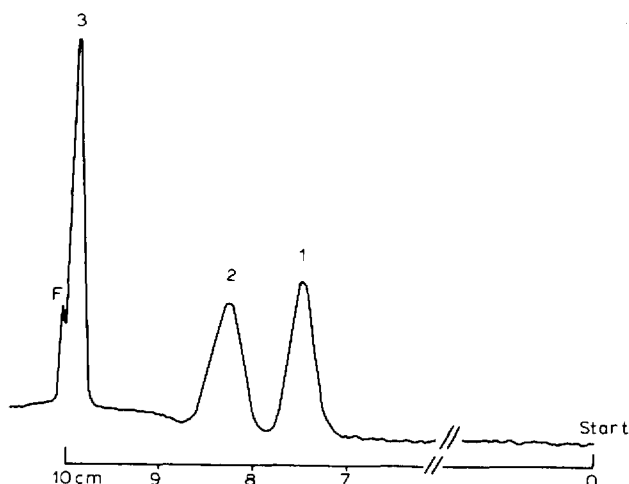


Fig. 1. TLC separation of cephalosporins. HPTLC pre-coated plate, silica gel 60 $F_{254s}$, extra pure (suitable for aqueous eluents). Eluent: Water containing 2% (w/w) of adipic acid. Migration distance: 10 cm. Standard chamber: without chamber saturation. Detection: UV (254 nm). Peaks: 1 = deacetylcephalosporin C; 2 = cephalosporin C; 3 = deacetoxycephalosporin C; F = eluent front.

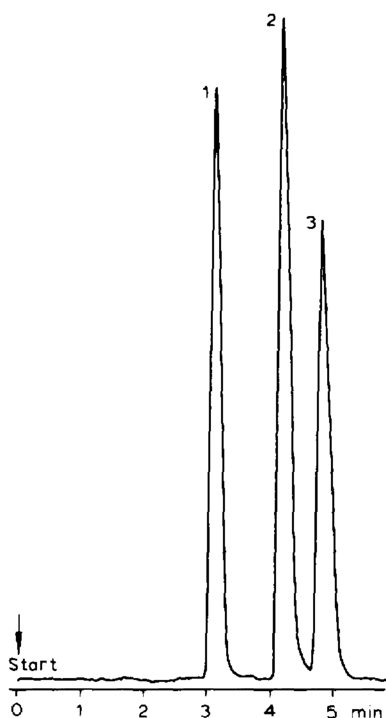


Fig. 2. Separation of cephalosporins using HPLC. Hibar RT 250-4 column pre-packed with LiChrosorb Si 60, 5 μm. Eluent: water containing 2% (w/w) of adipic acid. Flow-rate: 1 ml/min. Detection: UV (250 nm). Peaks: 1 = deacetylcephalosporin C; 2 = cephalosporin C; 3 = deacetoxycephalosporin C.

instance too, deacetylcephalosporin C is eluted first, followed by cephalosporin C and deacetoxycephalosporin C. The separation takes 6 min at a flow-rate of 1 ml/min.

The chromatograms in Figs. 1 and 2 show clearly that the chromatographic conditions for the plate also apply to the column. Further, Fig. 1 shows that because of the homogeneity of the layer on the HPTLC pre-coated plate and the resulting extremely low baseline noise, direct optical evaluation of the spots of the substances is possible without problems.

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