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Short communication

HPTLC determination of ceftriaxone, cefixime and cefotaxime in dosage forms

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Abstract

The objective of this investigation was to develop a HPTLC method for the determination of ceftriaxone, cefixime and cefotaxime, cephalosporins widely used in clinical practice. High performance TLC of cephalosporins was performed on pre-coated silica gel HPTLC plates with concentrating zone (2.5×10 cm) by development in mobile phase ethyl acetate-acetone-methanol-water (5:2.5:2.5:1.5 v/v/v/v). A TLC scanner set at 270 nm was used for direct evaluation of the chromatograms in reflectance/absorbance mode. The calibration curves were established as dependence of peak height (linear and polynomial regression) and peak area (polynomial regression) versus ng level (125–500 ng for all cephalosporins investigated). Relative standard deviations obtained from calibration curves was compared. Precision (RSD: 1.12–2.91% (peak height versus ng) and RSD: 1.05–2.75% (peak area versus ng)), and detection limits (ng level) was validated and found to be satisfactory. The method was found to be reproducible and convenient for quantitative analysis of ceftriaxone, cefixime and cefotaxime in their raw materials and their dosage forms. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: HPTLC; Densitometry; Quantification; Ceftriaxone; Cefixime; Cefotaxime

1. Introduction

Cephem antibiotics are widely used in the treatment of bacterial infections because they are highly antimicrobial against a large number of both gram-positive and gram-negative organisms.

For the analysis of cephalosporins, several spectrophotometric methods have been reported, which are based on interaction of cephalosporins with different reagents [1–3]. Derivative spectrophotometric assay of cephalosporins in the presence of their degradation products are also reported in Refs [4,5]. A variety of chromatographic methods were introduced for the determination of cephalosporins, including conventional TLC [6,7] and HPLC [8–10]. FAB-MAS analyses of cephalosporins [11] and capillary HPLC-FAB MAS of 24 cephem antibiotics [12] have been described.

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Mostly conventional TLC of cephalosporins is followed by post chromatographic derivatization. Further optimization of all aspects of the separation process in TLC are HPTLC plates for quantitative determination because these new layers require smaller sample sizes and shorter development distances to reveal their separation potential and to provide faster separations, better resolution. HPTLC plates with concentrating zone are prepared from two layers of silica gel having different retention properties. During development, the sample migrates out of the concentrating zone and is focused at the interface as narrow band resulting in excellent chromatographic efficiency [13].

Therefore, using HPTLC plates with concentrating zone, we previously developed method for quantitative determination of cephalexine and cefaclore in pharmaceuticals [14].

Third-generation cephalosporins represent significant improvement in the β-lactam group and are considered the therapy of choice for variety of infections which develop in intensive care units. Since there is no published data concerning HPTLC determination of ceftriaxone, cefixime and cefotaxime, we used HPTLC plates with concentrating zone to develop simple, rapid and reliable HPTLC method for determination of this cephalosporins in their dosage forms.

2. Experimental

2.1. Apparatus

A TLC Sanner II with a computer system and Cats Software (V.3.15) were provided by Camag, Muttenz, Switzerland. The radiation source was a deuterium lamp. Nanomat III was used as an application device (Camag, Muttenz, Switzerland).

2.2. Reagents and materials

Pre-coated silica gel plates with concentrating zones (2.5 × 10 cm) (Merck, Darmstadt, Germany), was used without any pretreatment. The mobile phase was ethyl acetate–acetone–methanol–water (5:2.5:2.5:1.5). All chemical and solvents were of analytical grade. Ceftriaxone disodium-7-[2-(2-Aminothiazol-4-il)-2-methoxyiminoacetamido]-3-[(2,5-dihidro-6-hydroxy-2-methyl-5-oxo-1.2.4-triazin-3-il)thiomethil]-3-cephem-4-carboxilic acid, cefixime-7-[2-(2-Aminothiazol-4-yl)-2-(carboxymethoxyimino)acetamido]-3-vinyl-3-cephem-4-carboxylic acid and cefotaxime sodium-3-acetoxymethyl-7-[2-(2-aminothiazol-4-il)-2-(methoxyimino)acetamido]-3-cephem-4-carboxylate were obtained from ICN Yugoslavia (Belgrade). A formulation based on ceftriaxone, cefixime and cefotaxime were gifts as follows: Longaceph® ampule (ICN Yugoslavia), containing 1000 mg of ceftriaxone, Oroken® granule (Pharmuka) containing 40 mg of cefixime per foil packet and Tolycar® ampoule (Jugoremedia), containing 1000 mg of cefotaxime.

2.3. Standard solutions

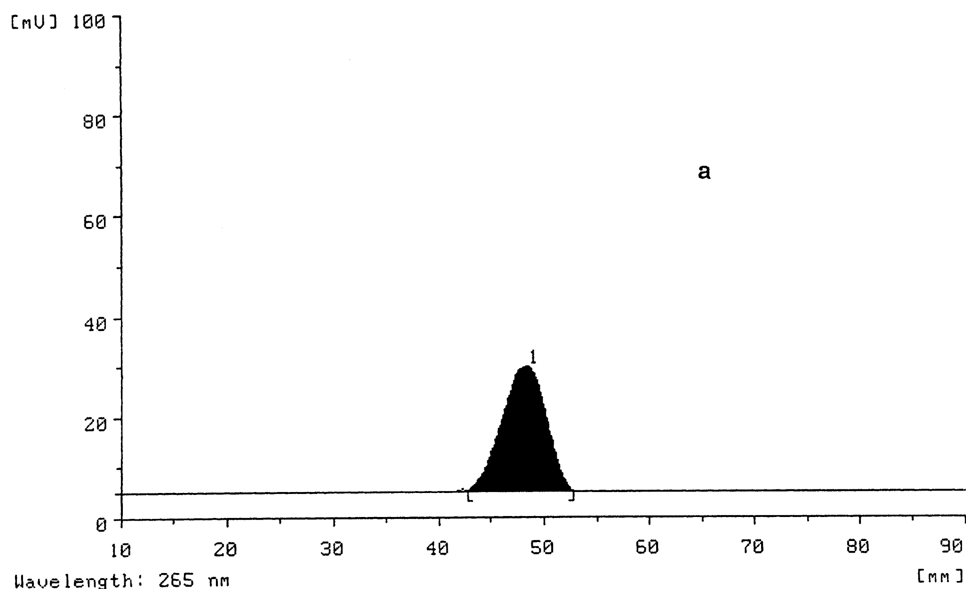
Stock standard solutions containing 1 mg ml^{-1} of ceftriaxone, cefixime and cefotaxime in methanol were freshly prepared before use. For an assay of ceftriaxone, cefixime and cefotaxime, calibration curves were prepared by diluting the stock solution to furnish solutions with final concentrations of 0.125, 0.250, 0.350, 0.425 and 0.5 mg ml^{-1} for all cephalosporins investigated.

2.4. Sample solutions

Sample solution of ceftriaxone: a quantity of Longaceph® ampoule containing 1000 mg of ceftriaxone was transferred to 100 ml calibrated flask and dissolved up to the mark with methanol. One ml of this solution was then diluted to the mark with methanol in 10 ml calibrated flasks.

Sample solution of cefixime: a quantity of Oroken® foil packet containing 40 mg of cefixime was transferred to 10 ml calibrated flask and dissolved up to the mark with methanol. 2.5 ml of this solution was then diluted to the mark with methanol in 10 ml calibrated flask.

Sample solution of cefotaxime: a quantity of Tolycar® ampoule containing 1000 mg of cefotaxime was transferred to 100 ml calibrated flask and dissolved up to the mark with methanol. One ml of this solution was then diluted to the mark with methanol in 10 ml calibrated flasks.



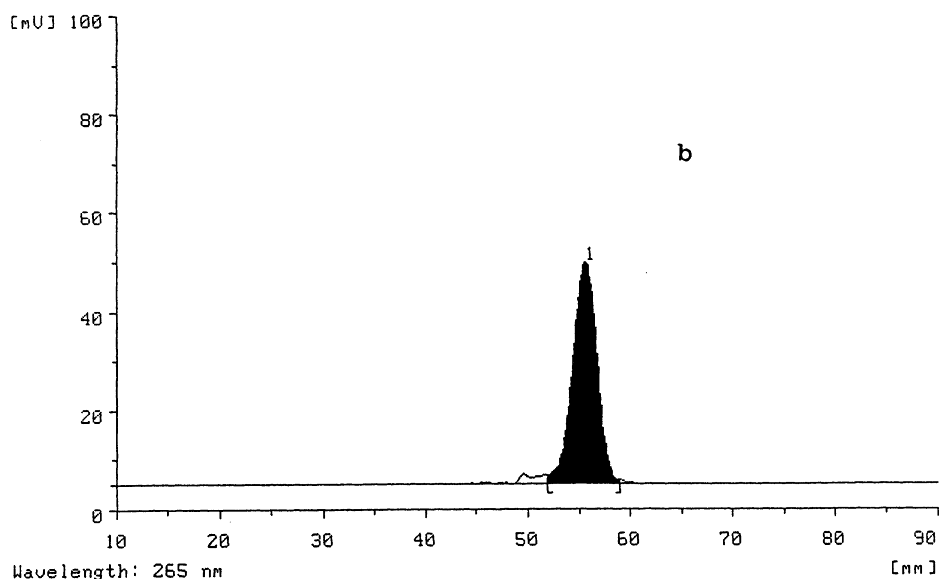


Fig. 1. Chromatograms of cephalexin monohydrate obtained on (a) HPTLC plates and (b) HPTLC plates with concentrating zone.

2.5. Chromatography

A 1 μl loading of each standard and sample solution was spotted on the HPTLC plate with concentrating zone by means of a Nanomat III. The chromatogram was allowed to develop during 20 min to a height of about 90 mm in a twin-trough chamber previously saturated with the mobile phase. The measurement of each spot was carried out in situ at 270 nm using absorbance/reflectance mode.

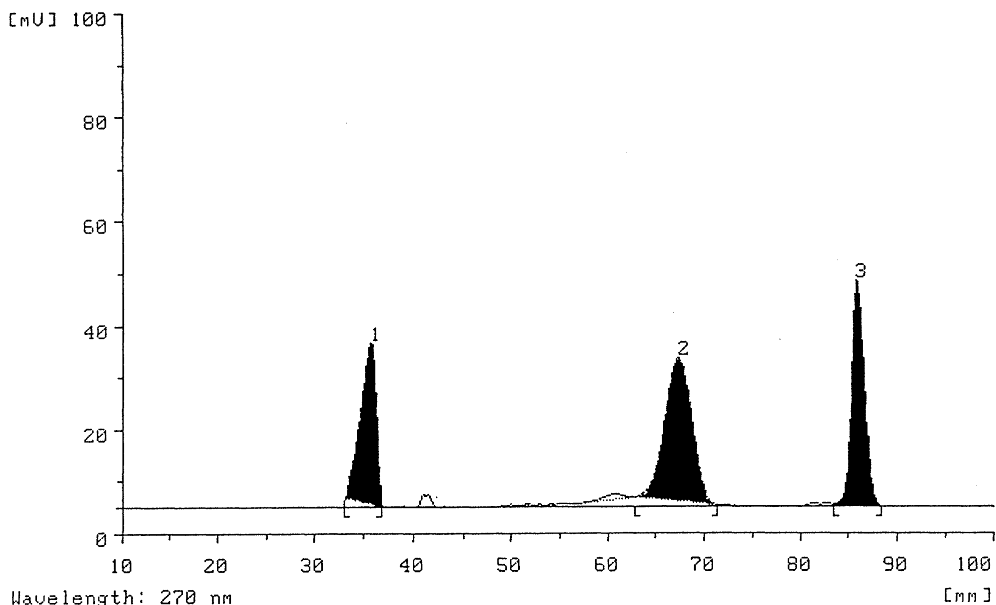


Fig. 2. Typical chromatogram of ceftriaxone, cefixime and cefotaxime obtained by HPTLC method.

3. Results and discussion

The chromatograms of cephalexin monohydrate obtained on (a) HPTLC plate and (b) HPTLC plate with concentrating zone (Fig. 1) shows that HPTLC plates with concentrating zone maximize resolution and are more suitable for quantitative evaluation of cephalosporins [14]. Therefore, quantitative determination of ceftriaxone, cefixime and cefotaxime carried out on HPTLC plates with concentrating zone. Typical chromatograms obtained for compounds investigated are shown in Fig. 2.

A mixture ethyl acetate–acetone–methanol–water (5:2.5:2.5:1.5 v/v/v/v) gave a good separation with minimum tailing. Migration distances ($\pm$SD, n = number of determination) were 34.7 ± 0.78 ($n = 9$), 66.9 ± 0.67 ($n = 8$) and 85.1 ± 0.56 ($n = 9$) for ceftriaxone, cefixime and cefotaxime, respectively. The differences in chemical structures of cephalosporins at C_3 and C_7 provided different retention behavior of cephalosporins. Stronger retention was observed for the ceftriaxone possibly due to the formation of intramolecular hydrogen bonds between heterocyclic nitrogen hydrogen at C_3 and primary amino group at C_7. Consequently, specific interaction with polar eluent are weakened and nonspecific interactions with the hydrophobic adsorbent are increased. Cefotaxime, with lipophilic ester functional group gives the highest R_f value. The effect of the concentration of methanol in the mobile phase on the retention of compounds was investigated. The better separation of compounds was performed with content of methanol as organic modifier between 30 and 50%.

In the planar chromatography, when the analytes are adsorbed on the highly active polar surface of silica sorbent layer in the presence of air, substances can decompose more easily than, for example, in HPLC [15]. Considering that the cephalosporins undergo the degradation reactions and isomerisation on silica gel plates to Δ^2 cephalosporins [16], stability of cephalosporins were tested while standing in solution, and on chromatographic plate for different time periods prior to densitometry. Analyte were tested against a freshly prepared/spotted standard solutions at each time interval and following results were obtained: (a) studies of the stability of analytical solutions from the standard and sample preparations at temperature 4°C demonstrated a good

Table 1
Statistical data for calibration curves of cephalosporins (125–500 ng per spot)

Calibration function											
Substance	$Y = a + bX$			$Y = a + bX + cX^2$							
	height			height				area			
	a	b	RSD	a	b	c	RSD	a	b	c	RSD
Ceftriaxone	−0.92	0.06	2.64	0.09	0.05	$1.3E^{-5}$	2.1	−237.6	3.4	$-6.5E^{-4}$	1.9
Cefixime	3.54	0.03	2.12	4.83	0.02	$1.6E^{-5}$	1.8	238.0	0.83	$-3.0E^{-4}$	1.4
Cefotaxime	2.57	0.11	1.94	3.40	0.10	$1.1E^{-5}$	1.7	28.26	1.38	$-4.6E^{-4}$	1.4

Table 2
Precision of HPTLC determination of ceftriaxone, cefixime and cefotaxime

Cephalosporin	ng	Peak height	RSD	Peak area	RSD
Ceftriaxone	125	7.2	2.91	234.6	2.75
	350	14.7	1.12	498.1	1.14
Cefixime	125	8.1	1.96	334.9	1.55
	350	20.9	1.27	811.7	1.12
Cefotaxime	125	16.1	2.18	193.7	2.08
	350	39.9	1.93	478.3	1.97

stability of the analytes over a period of 48 h; (b) no decrease in the concentration of cephalosporins on plate was observed within 3 h; (c) no decomposition of cephalosporins was observed during chromatogram development; and (d) the loss of cephalosporins of 3% 3 h after the development, and even 42% 2 days after the development was observed. Therefore, zone of cephalosporins were scanned within 2 h after the development of chromatograms.

Measuring in absorbance–reflectance mode the polynomial second grade regression better fit than linear one [13]. According to ICH regulation for validation of analytical method the linear function is required. Therefore we tested both linear and polynomial regression. Using plates with concentrating zone, linearity was obtained measuring peak height vs amounts of substances applied.

The calibration curves were established as dependence of peak height (linear and polynomial regression) and peak area (polynomial regression) in concentration range 125–500 ng per spot, for all cephalosporins analysed. The regression coefficients obtained are summarized in Table 1. Correlation coefficients were $r > 0.997$ for linear regressions and $r > 0.996$ for polynomial regressions.

The precision of the method was determined by running replicate samples, each containing 350 ng per spot of ceftriaxone, cefixime and cefotaxime (Table 2). The relative standarde deviations obtained for both peak height and peak area were of analytical levels. Insignificant differences were found using peak height and peak area for quantitative evaluation of ceftriaxone, cefixime and cefotaxime.

The limit of detection (LOD) and quantification (LOQ) were determined by fitting interday, back calculated standard deviations of each calibration standard. The LOD was defined as 3 SD_0 and LOQ as 10 SD_0. The LOD for ceftriaxone, cefixime and cefotaxime was found to be 19.1, 18.4 and 16.7 ng, and LOQ 63.73, 61.33 and 55.67 ng, respectively. Experimental LOD and LOQ were found to be 60–100 ng, respectively, for all cephalosporins investigated.

Even in planar chromatography, the fact is that fluorescence detection shows increased sensitivity

Table 3
HPTLC determination of ceftriaxone, cefixime and cefotaxime in its dosage forms

Dosage form	Taken (mg)	Found (height vs. ng) (mg)	RSD	Found (area vs. ng) (mg)	RSD
Longaceph® ampule	1000	1015.38	1.2	1014.72	1.05
Oroken® granule	40	41.82	2.31	41.66	1.82
Tolycar® ampule	1000	1016.60	1.38	1015.1	1.05

over absorbance detection [17]. Using absorbance detection and HPTLC system, the obtained results show the similar detection limits for cephalosporins, compared with in situ fluorescence detection using OPA (0.017–0.019 vs. 0.020–0.04 μg) [18]. On the other hand, in situ fluorescence detection using fluorescamine, even on conventional TLC gave 10 times lower detection limits than measuring absorbance on HPTLC plate. (0.002–0.003 vs. 0.017–0.019 μg) [18,19].

The accuracy of the densitometric method was proved by the determination of ceftriaxone, cefixime and cefotaxime from the laboratory made dosage forms. Mean recovery were 98.1% ($n = 8$), 101.4% ($n = 7$) and 98.8% ($n = 6$) for ceftriaxone, cefixime and cefotaxime, respectively.

The results of quantitative determination of ceftriaxone, cefixime and cefotaxime in their dosage forms are presented in the Table 3. Relative standard deviations obtained from determination of cephalosporins confirmed insignificant differences between results obtained from peak height and peak area.

The proposed method was found to be simple, rapid and precise and, therefore, suitable for routine control of cephalosporins investigated.

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