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Short communication

Development of a densitometric method for the determination of cephalexin as an alternative to the standard HPLC procedure[1]

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Abstract

A HPTLC-densitometric method was developed in order to obtain a reliable procedure for routine analysis of cephalexin in pharmaceutical formulations. Optimization of TLC conditions for the densitometric scanning was reached by eluting HPTLC silica gel plates in an horizontal developing chamber. Quantitation of cephalexin was performed in single beam reflectance mode by using a computer-controlled densitometric scanner and applying a five-point calibration. A linear regression has been found in the 200–1000 ng range. The setup method is precise, reproducible and accurate. Recovery was also assessed by comparison with the HPLC USP XXIII alternate method. In this case HPTLC-densitometry appears worth of consideration as being relatively inexpensive and time-saving (up to 12 samples can be determined simultaneously in less than 15 min with a solvent consumption of about 15 ml). The results suggest that the proposed method may be used in place of HPLC for the routine quantitation of cephalexin in both pure and dosage forms.

Keywords: Densitometry; Cephalexin; Capsules; Thin layer chromatography

1. Introduction

Many methods have recently been reported for the quantification of cephalexin [1–18] and the most investigated have been those based on HPLC [1,2,9,15]. Although the HPLC procedures, as the USP cephalexin analytical method [19], are accurate and effective means of assaying

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cephalexin, they are time and solvent consuming and, therefore, disadvantageous for serial estimation for a large number of samples. Consequently, there is a demand for a rapid, efficient and inexpensive analytical assay to be applied to typical cephalexin formulations during industrial process development and scale-up production. In view of the above factors, an HPTLC method was considered, being cheaper, faster and sometimes more efficient than HPLC; moreover modern densitometry, an usually underestimated technique, may be competitive with respect to HPLC-UV detection, so that a HPTLC densitometric approach should be taken into consideration as an alternative to HPLC whenever it is allowed by the sample features. Accordingly, a HPTLC-densitometric method was developed and successfully applied in routine determination of cephalexin preparations.

2. Experimental

2.1. Apparatus

Densitometry was carried out with a Camag TLC Scanner II (Camag, Muttenz, Switzerland) combined with a Merck Hitachi integrator D2500 (Merck, Darmstadt, Germany).

HPLC was performed with a Perkin-Elmer LC 200 Series apparatus consisting of a LC 200 Series Pump with Autosampler and a Perkin-Elmer 785A UV-vis detector, under the control of Turbochrom 4.1 data handling software (Perkin-Elmer, Norwalk, CT).

2.2. Materials

High purity grade (99.1%) cephalexin was a specimen of Cephalexin CRS, European Pharmacopoeia, Conseil d'Europe, Strasbourg. The assay was carried out on Cefalessina 500 mg capsules, manufactured by Stabilimento Chimico Farmaceutico Militare (Firenze, Italy), each containing 500 mg of cephalexin and 30 mg of excipients consisting in a 1:1 mixture of magnesium stearate and hydrogenated castor oil. The blank matrix was the excipient mixture.

HPTLC precoated plates, silica gel Merck 60, F254, 10×10 cm were used. All chemicals and solvents were of analytical grade.

2.3. Preparation of standard and sample solutions

The standard solution was prepared in a 1000 ml volumetric flask by dissolving 49.75 mg of Cephalexin CRS (corresponding to 49.30 mg of pure cephalexin) in 1 ml of HCl 0.01 N and diluting to volume with methanol.

2.4. Chromatography

The HPTLC plates were pre-washed by development with the mobile phase, air dried, then oven conditioned at 120°C for 1 h and let them cool down in a dessicator. The standard and sample solutions were applied bandwise (8 mm long, 15 s μl^{-1} application speed) to the plates with a Camag Linomat IV applicator and developed in a Camag horizontal developing chamber

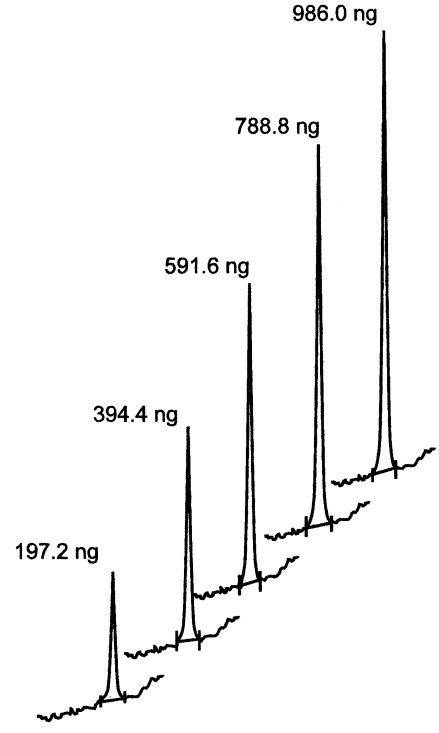

Fig. 1. Densitogram of cephalexin standard.

Table 1
Accuracy and precision of the method

Amount applied (ng)	Amount measured (ng)	% Average recovery	RSD ($n = 6$)
480	485	101.0	0.91
600	604	100.7	1.23
720	734	101.9	1.81

with ethyl acetate:acetic acid:water (7:2:l) mobile phase. The plates were then scanned within 24 h; afterwards a progressive optical density decay was observed.

HPLC measurements were carried out according to the USP XXIII official method by using a LichroCart (Merck) RP18, 25 cm × 4.6 mm ID, 5 μm particles, column.

2.5. Densitometric evaluation

The HPTLC plates were scanned in reflectance mode at 263 nm, deuterium lamp, monochromator bandwidth 10 nm, slit dimensions 5 × 0.3 mm, scanning speed 0.2 mm s^{-1}. Automatic scanning was performed with scan length 12 mm, distance between tracks 15 mm and three scans for each track.

2.6. Accuracy and assay procedure

Three blank matrices were spiked with 400, 500 and 600 mg of cephalexin, respectively. The mixtures were individually dissolved in 10 ml of HCl 0.01 N in 1000 ml volumetric flasks and diluted to volume with methanol. After filtering, 10 ml of each solution were further diluted to 100 ml with methanol. The concentration of the solutions obtained were 40.0, 50.0 and 60.0 ng μl^{-1}. A total of 12 μl of each solution were applied to the plates. Cephalexin capsule solution was prepared in a 1000 ml volumetric flask by dissolving one capsule content (average of 10 capsules) as above described, the resulting concentration being 50.0 ng μl^{-1}. For the assay 12 μl of this solution were applied.

3. Results and discussion

3.1. Calibration curve

Fig. 1 shows the densitogram of the standard cephalexin to be used for calibration. It was obtained after accurate optimization of the operative conditions chiefly affected by the slit dimensions and scanning speed. The calibration points were obtained in triplicate at five levels over a range of 200–1000 ng of the analyte by applying 4, 8, 12, 16 and 20 μl of the standard solution. The equation for the curve $y = 27030 + 1226x$ ($n = 15$) was calculated by linear regression analysis assuring method linearity over the mass range studied with correlation coefficient $R^2 = 0.9972$, SD = 18257, slope RSD = 0.15, intercept RSD = 32.10 and no significant day-to-day variability.

Table 2
In situ precision at 200 and 800 ng levels

Determination (six replicates)	Scanning runs	Area counts (200 ng mean)	RSD	Area counts (800 ng mean)	RSD
1	3	245 519	0.83	981 906	0.18
2	3	251 552	1.03	1 004 122	0.43
3	3	247 183	1.06	991 462	0.28
4	3	244 249	1.05	983 498	0.29
5	3	245 464	0.92	1 003 356	0.54
6	3	243 551	1.11	1 000 308	0.60
1–6	18	246 253	1.17	988 108	0.99

Table 3
Assay of 500 mg cephalexin capsules

Sample	% Average recovery (RSD, $n = 6$)	% Recovery by HPLC alternate method (RSD, $n = 6$)
Cephalexin capsule (500 mg)	102.3 (0.78)	101.7 (0.25)[a]

[a] US Pharmacopoeia XXIII ed.

3.2. Accuracy and precision

Accuracy was assessed by spiking cephalexin in blank capsule matrix over the range of 80–120% of the amount corresponding to the mid point of the curve. The precision data were available from the six-replicate analyses of the spiked samples in the accuracy study (Table 1).

The instrumental precision was also determined at all levels (six replicates and three scanning runs) giving RSD values within 0.47 (third level) and 1.17 (lowest level). Data for the lowest and middle points are reported in Table 2.

3.3. Assay

The reliability of the method for the assay of typical cephalexin dosage forms was tested by analysing 500 mg Cephalexin capsules. Recovery was also assessed by comparison with the HPLC USP XXIII as an alternative method. The statistical analysis of the results is reported in Table 3, showing a mean recovery efficiency within $100 \pm 2\%$, in agreement with the accuracy criteria for an assay method.

HPTLC-densitometry demonstrated to be relatively inexpensive and time-saving with respect to HPLC, allowing up to 12 samples simultaneously determined in less than 15 min, with a solvent consumption of about 15 ml versus a 15 min HPLC one sample analysis. Moreover HPTLC cephalexin peak showed a good chromatographic efficiency with $N = 4543$, and better simmetry, with a peak tailing factor of 0.96 compared to HPLC USP XXIII method ($N = 5842$, peak tailing factor = 0.75).

The proposed procedure fits precision and accuracy usually requested by official methods and can be used as a convenient alternative to HPLC analysis for quantitation of cephalexin in both pure and simple dosage forms.

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