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An HPTLC method for the determination and the purity control of ciprofloxacin HCl in coated tablets

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Abstract

A high-performance thin-layer chromatographic method (HPTLC) has been developed for the determination and the purity control of a synthetic fluoroquinolone antibiotic ciprofloxacin hydrochloride in coated tablets when desfluoro compound, ethylene diamine compound, by-compound A and fluoroquinolonic acid are considered as impurities. Silica gel F_{254} was used as a stationary phase and a mixture of acetonitrile, ammonia 25%, methanol and methylene chloride (1:2:4:4, v/v/v/v) as a mobile phase. The method was validated in terms of linearity (range), selectivity (placebo and related compounds), precision, accuracy (Recovery), system suitability (repeatability, peak symmetry, resolution) and impurities limit of detection (LOD). The analysis of variance (ANOVA) and *t*-test were applied to correlate the results of ciprofloxacin hydrochloride determination in coated tablets by means of the HPTLC method and the official British Pharmacopoeia (BP 1999) high-performance liquid chromatographic (HPLC) method.

Keywords: Ciprofloxacin hydrochloride coated tablets; HPTLC; Quantitative analysis; Purity control; Method validation; HPTLC versus HPLC; ANOVA; *t*-test

1. Introduction

Ciprofloxacin hydrochloride (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid hydrochloride) is a synthetic fluoroquinolone antibiotic with a broad antimicrobial activity [1]. According to BP 1999 [2] and USP 24 [3] an official method for determination of ciprofloxacin hydrochloride (CHCl) and its related substances such as desfluoro compound (1-cyclopropyl-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylicacid), ethylenediamine compound (7-[(2-aminoethyl)amino]1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid) and by-compound A (7-chloro-1-cyclopropyl-1,4-dihydro-4-oxo-6-(piper-

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azin-1-yl)(piperazin-1-yl)quinoline-3-carboxilic acid) is high-performance liquid chromatography (HPLC), while thin-layer chromatography (TLC) is an official method for its identification and limit test of fluoroquinolonic acid (7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinoline-3-carboxylic acid). TLC method was reported for monitoring qualitatively and quantitatively CHCl photodegradation in aqueous solution [4]. The aim of this work was to develop and validate in compliance with ICH guideline [5] a high-performance TLC (HPTLC) method for quantitative analysis of CHCl in coated tablets, as well as for limit test of its related compounds. The analysis of variance (ANOVA) and t-test were applied to correlate the HPTLC method and the official HPLC method [6].

2. Experimental

2.1. Materials and reagents

CHCl, fluoroquinolonic acid, desfluoro compound, ethylenediamine compound and by-compound A were chemical reference compounds (CRC) supplied by European Pharmacopoeia (Council of Europe, European Pharmacopoeia, Strasbourg Cedex, France). Cifloxinal® coated tablets, each containing 291 mg of CHCl, were product of PRO.MED.CS (Prague, Czech republic). Methylene chloride and ammonia 25% were analytical grade purity, while methanol and acetonitril were gradient grade purity (Merck, Darmstadt, Germany). As a stationary phase HPTLC silicagel $60F_{254}$ precoated plates 20×10 cm (Art. no. 1.05548, Merck, Darmstadt, Germany) were used.

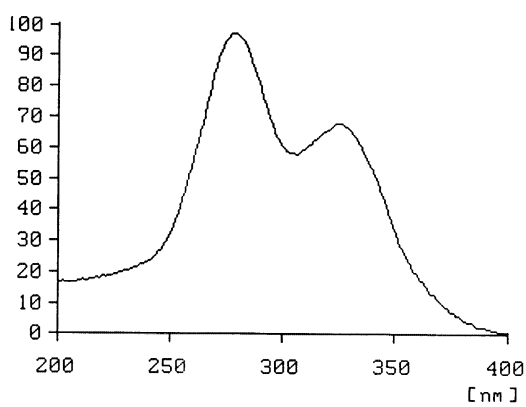

Fig. 1. In situ spectrum of ciprofloxacin hydrochloride measured from 200 to 400 nm.

2.2. Quantitative analysis

2.2.1. Sample preparation

An accurately weighted amount of powdered Cifloxinal® coated tablets containing the equivalent of 291 mg of CHCl, and cornstarch, hydroxypropylmethyl cellulose, polyvinylpyrrolidone, titanium dioxide and macrogolum as non-medicinal ingredients, was transferred to a 100-ml volumetric flask, dissolved in water and sonicated for 10 min. The suspension was diluted to the volume and filtered through a membrane filter Nylon, pore size 0.45 μm, diameter 25 mm (Sigma-Aldrich, Dorset, Great Britain). The transparent solution was diluted 1:25 with water.

2.2.2. Ciprofloxacin hydrochloride CRC solution

An accurately weighed amount of CHCl CRC was dissolved in water to obtain a solution containing ~ 0.116 mg ml^{-1}.

2.2.3. Chromatography

A Camag TLC system composed of an automatic TLC sampler III, TLC Scanner 3 and CATS4 software (Camag, Muttenz, Switzerland) was used for sample application and quantitative evaluation. The samples and CHCl CRC solution were band applied (2 mm length) at 5 mm intervals under nitrogen stream on an HPTLC layer. The applied volume was 400 nl per band. Number of bands per plate was 36. The layer was exposed to ammonia vapour for 10 min and than developed over a path of 50 mm in a twin-trough developing chamber 20×10 cm (Camag) using a mixture of acetonitrile–ammonia 25%-methanol–methylene chloride (1:2:4:4, v/v/v/v). Chromatograms were evaluated via peak area after scanning in absorbency mode at 278 nm.

Table 1
Two-way ANOVA test of CHCl determination in six independent samples in duplicate by HPTLC and HPLC

Sample	HPTLC[a]		HPLC[a]	
	1st sampling	2nd sampling	1st sampling	2nd sampling
1	97.90	99.42	99.63	96.78
2	97.24	99.56	98.12	98.43
3	100.16	98.98	97.54	96.32
4	97.16	98.07	96.43	96.20
5	103.48	103.25	105.03	104.92
6	97.45	96.15	95.74	95.93

Anova: two-factor with replication

Summary	HPTLC	HPLC	
(1)			
Count	6	6	12
Sum	593.39	592.49	1185.88
Average	98.898333	98.748333	98.823333
Variance	6.2899367	11.303257	8.0030424
(2)			
Count	6	6	12
Sum	595.43	588.58	1184.01
Average	99.238333	98.096667	98.6675
Variance	5.4398967	11.970187	8.2691477
Total			
Count	12	12	
Sum	1188.82	1181.07	
Average	99.068333	98.4225	
Variance	5.3632697	10.694657	

ANOVA

Source of Variation	SS	df	MS	F[b]	P-value	F crit
Sample	0.1457042	1	0.1457042	0.01665035	0.89861783	4.35125
Columns	2.5026042	1	2.5026042	0.28598513	0.5987007	4.35125
Interaction	1.4751042	1	1.4751042	0.16856755	0.68575329	4.35125
Within	175.01638	20	8.7508192			
Total	179.1398	23				
				$F < F$ crit		

[a] The results are presented as [%] of declared amount of CHCl per coated tablet.
[b] $F < F$ crit

2.2.4. HPLC measurements

The official BP 1999 method was applied on CHCl HPLC determination in tablets. Samples were prepared according to BP 1999. The measurements were carried out on Lichrospher 100 RP-18 column (250 × 4.0 mm I.D., 5 μm particle size, Merck, Darmstadt, Germany) using an integrated system of Thermo Separation Products (pump P2000, autosampler AS1000 with a 20 ml injection loop) with a UV detector 1000 and a data station Optiplex XL 5100. The mobile phase was a mixture of 13 volumes of acetonitrile and

Table 2
Average results of CHCl determination by HPTLC and HPLC and their correlation by paired *t*-test

Sample	HPTLC[a]	HPLC[a]
1	98.66	98.21
2	98.40	98.28
3	99.57	96.93
4	97.61	96.32
5	103.35	104.98
6	96.79	95.84
Average	99.06	98.43

t-test: paired two sample for means

	Variable 1	Variable 2
Mean	99.06333333	98.42666667
Variance	5.303026667	11.27670667
Observations	6	6
Pearson correlation	0.942970226	
Hypothesized mean	0	
Df	5	
***t* Stat**	**1.10395772**	
$P(T \leq t)$ one-tail	0.159942882	
t Critical one-tail	2.015049176	
$P(T \leq t)$ two-tail	0.319885763	
***t* Critical two-tail**	**2.570577635**	

t Stat < *t* Critical

[a] The results are presented as [%] of declared amount of CHCl per coated tablet.

87 volumes of a 0.245% w/v solution of orthophosphoric acid the pH of which was adjusted to 3.0 with triethylamine. Detection wavelength was 278 nm and the column temperature was 40°C.

2.3. Limit test

2.3.1. Sample preparation

An accurately weighed amount of powdered Cifloxinal® coated tablets containing the equivalent of 100 mg of CHCl was transferred to a 10-ml volumetric flask, dissolved in water and sonicated for 10 min. The suspension was diluted to the volume and filtered through a membrane filter Nylon, pore size 0.45 μm, diameter 25 mm (Sigma-Aldrich).

2.3.2. Sample spiked with related substances

An accurately weighed amount of powdered tablets containing the equivalent of 100 mg of CHCl was transferred to a 10-ml volumetric flask and an aliquot of 1 ml of fluoroquinolonic acid, desfluoro compound, ethylenediamine compound and by-compound A solution in water containing 0.2 mg per ml of each single compound was added. Further preparation was as described under Section 2.3.1.

2.3.3. Chromatography

The samples were band applied (7 mm length) at 11 mm intervals. Number of bands per plate was 17. The applied volume was 4000 nl per band. The other chromatographic conditions were as described above. A developing path was 80 mm and the plate was scanned in absorbency mode at 254 and 366 nm, respectively.

3. Results

3.1. Quantitative analysis

The method was validated in compliance with IHC guideline [5]. The following parameters were validated: linearity, accuracy (trueness), precision, range, selectivity, system suitability (repeatability, peak symmetry, resolution) and robustness.

3.1.1. Linearity

The linearity of the method was tested by applying aliquots from 50 to 1000 nl of CHCl CRC solution. The calibration dependence was polynomial from 6 to 116 ng per spot. The linear part of the calibration was from 23 to 70 ng per spot. The correlation coefficient (r) and the relative standard deviation (RSD) were 0.9964 and 2.0%, respectively.

3.1.2. Accuracy (trueness) and range

Ten model samples were prepared by mixing Cifloxinal® coated tablets placebo and CHCl CRC to test the accuracy and the range of the method. Six samples contained the declared ratio of CHCl and placebo, two samples contained $\sim$80% and two $\sim$120% of declared amount of

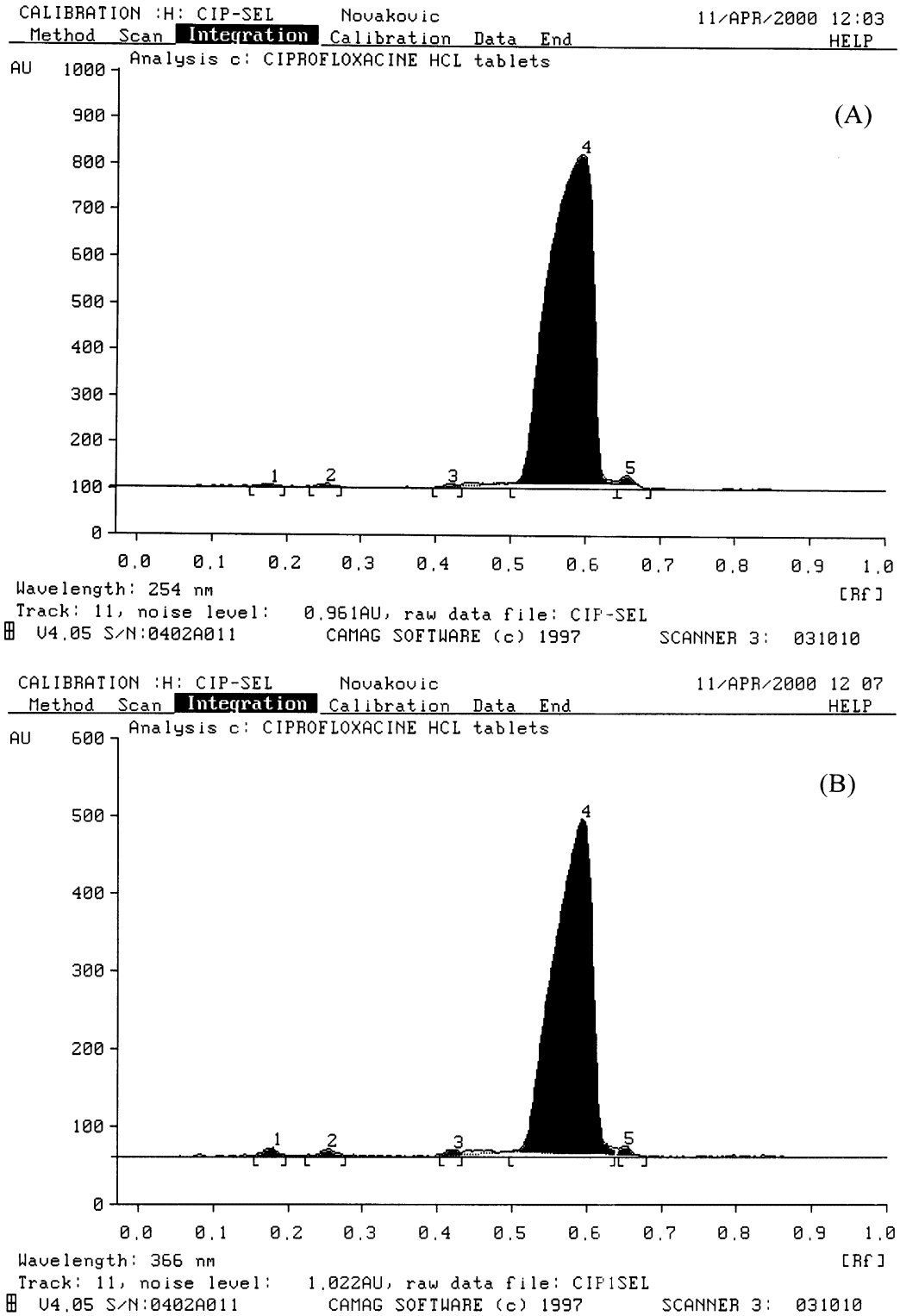

Fig. 2. Densitogram of Cifloxinal® coated tablets measured at 254 nm (A) and at 366 nm (B). In situ amount of ciprofloxacin hydrochloride is 40 μg.

CHCl. The Recovery was from 99.28 to 103.71%, respectively.

3.1.3. Precision and range

The analytical method procedure was applied repeatedly by sampling 10 times different amounts (six times 100%, two times 120%, and two times 80% of the average coated tablet mass) of one homogenous sample of Cifloxinal® coated tablets to test precision and range of the method. The average found amount of CHCl per tablet was 291.287 mg. The results varied from 100.10 to 104.16% of the declared amount and the RSD was 1.02%.

3.1.4. Selectivity and system suitability

Placebo of Cifloxinal® coated tablets was prepared and chromatographed as described under II.2. There were no peaks detected on the densitogram of placebo. The resolution, the peak symmetry and repeatability were tested to approve the system suitability. The resolution between CHCl (0.3 mg ml^{-1}) and fluroquionolonic acid (0.02 mg ml^{-1}) was 1.1, and the peak and the symmetry factor of CHCl measured at 5% of peak height was 1.0. The volume applied to the plate was 400 nl.

To test repeatability 400 nl of CHCl CRC solution prepared as described under II.2 was applied at the position 1, 7, 15, 22, 29 and 36 of an HPTLC plate. The RSD of the corresponding peak areas was 1.6%.

3.1.5. Robustness and method optimisation

In situ spectrum of CHCl was measured from 200 to 400 nm (Fig. 1). The optimum wavelength for quantitative analysis was at 278 nm.

Stability of CHCl was tested: (a) in the water solution (6 h); (b) on an HPTLC plate after application (30 min); and (c) in situ after development (the plate was re-scanned in 20 min intervals during 60 min). The samples were protected from light during preparation, application to the plate and development. Under given conditions there were no indications of CHCl degradation.

The plate exposition before development to ammonia vapours for 10 min had influence on the layer activity and consequently reduced tailing and improved CHCl peak symmetry.

3.1.6. HPTLC versus HPLC

Six different samples taken during in-process control of Cifloxinal® coated tablets manufacturing were determined simultaneously by HPTLC and HPLC methods. Each sample was analysed in duplicate. To test differences between the proposed HPTLC method and the official HPLC method statistical tests were performed for the level of confidence 95% ($P = 0.05$). Two-way ANOVA was applied to test both method-sample interactions (interaction variation) and differences in the methods precision (columns variation). Since the within cell variation (residual variation) is greater than interaction variation as well as the columns variations, the method-sample interaction and the differences between the methods are not significant. To test means (averages) a paired t-test was applied. The test removes any variations between samples [6]. The obtained value of t_{stat} is lower than two-tail $t_{critical}$, which leads to the conclusion that there is no significant difference between the means. The results of two-way ANOVA and paired t-test are given in Tables 1 and 2, respectively.

3.2. Limit test

Densitograms of Cifloxinal® coated tablets and Cifloxinal® coated tablets spiked with limit concentration of CHCl related substances measured at 254 and 366 nm are given in Figs. 2 and 3, respectively. In situ amount of CHCl was 40 μg, while the amount of the each single related substance was 0.08 μg per band, which corresponds 0.2% of CHCl amount. Signal-to-noise ratios (S/N) of ethylenediamine compound (R_F 0.41), desfluoro compound (R_F 0.45), by-compound A (R_F 0.48), and fluoroquinolonic acid (R_F 0.66) were 13, 10, 25 and 45 at 254 nm, and 18, 15, 15, and 8 at 366 nm, respectively. Two unidentified impurities were detected (R_F 0.18 and 0.26, respectively). Limit of detection (LOD) of ciprofloxacin related substances was 0.04 μg per spot ($S/N > 5$), which corresponds 0.1% of CHCl in situ amount.

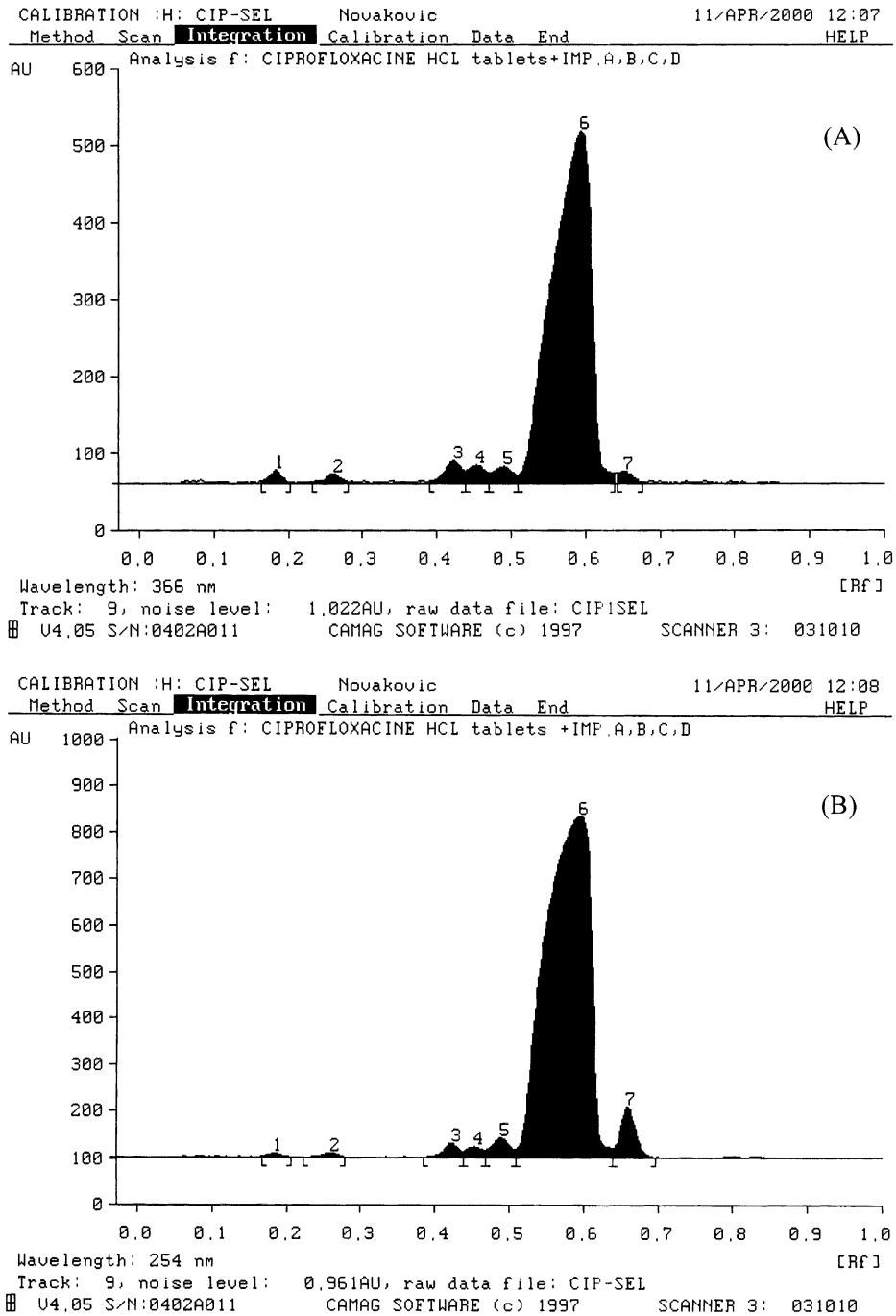

Fig. 3. Densitogram of Cifloxinal® coated tablets spiked with limit concentration of ciprofloxacin hydrochloride related substances measured at 254 nm (A) and 366 nm (B), respectively. The peak identification: (1) unidentified impurity; (2) unidentified impurity; (3) ethylenediamine compound; (4) desfluoro compound; (5) by-compound A; (6) ciprofloxacin hydrochloride; (7) fluoroquinolonic acid. In situ amount of ciprofloxacin hydrochloride is 40 μg (100%), while the amount of the each single related substance is 0.08 μg (0.2%) per band.

4. Conclusion

The HPTLC method for the determination of CHCl in coated tablets was validated in terms of linearity (range), precision, accuracy, selectivity, system suitability (repeatability, peak symmetry and resolution) and robustness. Six real samples of Cifloxinal® coated tablets were determined simultaneously by the HPTLC and by the official HPLC method and the results were correlated. Statistical tests indicate that the proposed HPTLC method is comparable to the official HPLC method and can be used for the determination of CHCl in coated tablets. Due to large sample throughput and short analysis time, the HPTLC method was developed predominantly for in-process control and content uniformity test of finished dosage form.

The HPTLC method is suitable for the purity control (limit test) of ciprofloxacin hydrochloride coated tablets when ethylenediamine compound, desfluoro compound, by-compound A, and fluoroquinolonic acid are considered as impurities with the limit of 0.2% each.

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