A New High Performance Liquid Chromatographic Analysis Method for Ciprofloxacin

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A rapid and simple high performance liquid chromatographic method for the quantitative analysis of ciprofloxacin has been developed and validated. The separation was performed on an Agilent 1100 system equipped with a Zorbax XDB-CN (100mm x 4.6mm) column using a mobile phase consisting of a mixture of phosphoric acid solution and acetonitrile (90:10, v/v), at a flow rate of 0.8mL/min. The sample volume injected was 5μL. The retention time for ciprofloxacin was 1.8 min and the overall time of the analysis was 2.2 min. The detection was done spectrophotometrically at 278nm. The limit of quantification was 0.28 μg/mL. Calibration graphs were linear for 0.28-0.70μg/mL of ciprofloxacin, with a correlation coefficient of 0.998. The average recoveries for ciprofloxacin and coefficients of variation were 99.31% and 1.30% respectively. The new method was sensitive, specific and reproducible, suitable for the quantitative analysis of ciprofloxacin in various pharmaceuticals products.

Keywords: ciprofloxacin, liquid chromatography, validation

Ciprofloxacin, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolone carboxylic acid, is a synthetic chemotherapeutic drug from the second-generation fluoroquinolone class. It kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops synthesis of both DNA and proteins [1, 2].

Several assay methods are available for ciprofloxacin, including HPLC-UV and LC-MS/MS methods [3-11].

The present study reports a simple and sensitive HPLC method using UV detection for the quantitative determination of ciprofloxacin when dissolved in a *p*H 6.8 buffer solution. The analytical method is suitable for through dissolution tests of ciprofloxacin from various pharmaceutical products.

Experimental part

All analyses were performed on an Agilent 1200 system. The system components included an Agilent 1200 Degasser, an Agilent 1200 Binary Pump, an Agilent 1200 Autosampler and an Agilent 1200 UV Detector. The Agilent Chem 32 software was used for system control and data acquisition. An analytical balance Mettler-Toledo XP56, a Sigma 2-16 K centrifuge and a Vibramax 110 shaker were used for the sample preparation. The separation was performed using a reverse phase column Zorbax XDB-CN, (100mm x 4.6mm, 3.5μm) supplied by Agilent, USA.

The standard ciprofloxacin hydrochloride used in this study was supplied by Hiran Orgochem Ltd. (batch number FPCPF070979) with a purity of 99.50%. All solvents and other chemicals were HPLC grade provided by Merck, Germany.

The mobile phase consisted in a mixture of phosphoric acid solution (*p*H = 3.0) and acetonitrile 90:10 (v/v), at a flow rate of 0.8mL/min.

A stock solution of ciprofloxacin hydrochloride with a concentration of 1.4mg/mL was prepared by dissolving an appropriate amount of ciprofloxacin hydrochloride reference substance in the mobile phase. This solution was kept at 5±3°C. In those conditions, it was found to be stable for at least 7 days.

For the study of linearity response, six ciprofloxacin solutions were prepared in *p*H 6.8 buffer solution, covering the concentration range between 0.28÷0.70μg/mL. The theoretical concentrations of ciprofloxacin calibration standard solutions were 0.28, 0.42, 0.49, 0.56, 0.63 and 0.70μg/mL respectively. The quality control samples of ciprofloxacin theoretical concentrations of 0.350μg/mL (low-QC1), 0.525 μg/mL (medium–QC2) and 0.595μg/mL (the high–QC3) were considered to be appropriate to be used in order to validate the analytical method [12, 13].

Results and discussions

The analytical method for the assay of ciprofloxacin dissolved in *p*H = 6.8 buffer solution was validated. The parameters usually examined in the validation process were selectivity/specificity, linearity, limit of quantification, accuracy and precision, recovery and stability [14-20].

Selectivity/Specificity study: A placebo mixture containing all the non-active ingredients of tablets containing 20mg ciprofloxacin was carried through the extraction procedure and chromatographed to determine the extent to which non active ingredients could interfere with the assay of ciprofloxacin. No significant interferences were observed in 3 different extracted samples.

The study of the linearity of the method: Calibration curves were found to be consistently accurate over the calibration range between 0.28 and 0.70μg/mL (fig. 1).

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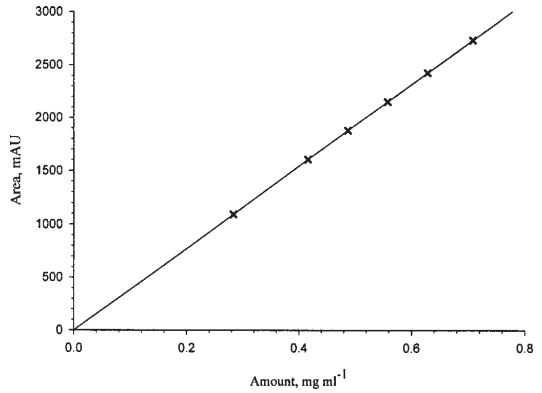

Fig. 1. Calibration curve for ciprofloxacin dissolved in *p*H 1.2 buffer

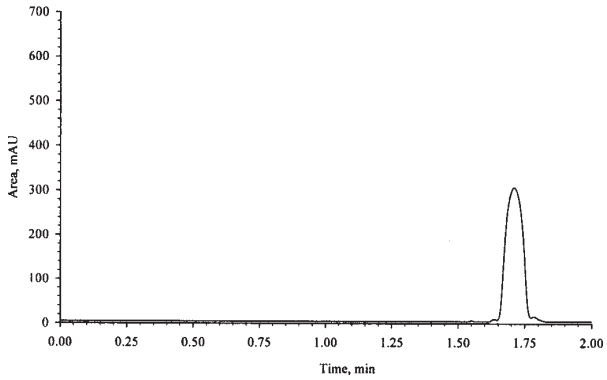

Fig. 2. The chromatogram recorded for a 0.28μg/mL ciprofloxacin sample

The coefficients of correlation were greater than or equal to 0.99993.

The lower limit of quantification, i.e. the lowest standard level with a coefficient of variation less than 2% and a signal to noise ratio higher than or equal to 5, was 0.28μg/mL. The analytical method proved to be sensitive, allowing a precise quantification of concentrations as low as 0.28μg/mL (fig. 2). Results are presented in table 1.

Accuracy and Precision: Intra-day accuracy and precision evaluations were performed by analyzing replicate concentrations of ciprofloxacin. The run consisted of a calibration curve and a total of 18 quality control samples, 6 replicates of each of low (QC1), medium (QC2) and high (QC3) quality control samples. The intra-day coefficient of variation ranged between 0.401 and 1.868%. The intra-day percentages of nominal concentration ranged between 99.036 and 100.142%. Results are presented in table 2.

*The inter-day accuracy and precisi*on were assessed by the repeated analysis of quality control samples containing different concentrations of ciprofloxacin on separate occasions. A single run consisted of a calibration curve and 6 replicates of the low (QC1), medium (QC2) and high (QC3) quality samples for ciprofloxacin. The inter-day coefficient of variation ranged between 1.697 and 2.269%. The inter-day percentages of nominal concentrations ranged between 98.537 and 100.497%. Results are presented in table 3.

The Recovery of ciprofloxacin was evaluated by comparing analyte response of three extracted samples of low, medium and high quality control samples to those of three appropriately diluted standard solutions. Mean recovery values for ciprofloxacin were 101.471, 101.713 and 99.637% at low, medium and high quality control levels, respectively. Results are presented in table 4.

	Ciprofloxacin 0.280mg/mL		
	Concentration (mg/mL)	Nominal (%)	Signal/Noise Ratio
N = 3	0.28421	101.503	213.200
	0.28771	102.753	232.500
	0.28536	101.915	260.000
Mean	0.28576	102.057	235.233
SD (±)	0.002		
CV (%)	0.624		

Table 1
LOWER LIMIT OF QUANTIFICATION

	QC1 0.350mg/mL		QC2 0.525mg/mL		QC3 0.595mg/mL	
N = 3	Found (μg/mL)	Nominal (%)	Found (μg/mL)	Nominal (%)	Found (μg/mL)	Nominal (%)
Mean	0.347	99.036	0.526	100.142	0.616	103.521
SD (±)	0.003		0.002		0.012	
CV (%)	0.756		0.401		1.868	
Acceptance criteria: 67% Total QCs must be 100 ± 15% nominal values; 50% QCs per level must be 100 ± 15% nominal values; Mean % nominal 100 ± 15%; CV(%) ≤ 15%.						

Table 2
INTRA-DAY PRECISION AND ACCURACY

	QC1 0.350mg/mL		QC2 0.525mg/mL		QC3 0.595mg/mL	
N = 15	Found (μg/mL)	Nominal (%)	Found (μg/mL)	Nominal (%)	Found (μg/mL)	Nominal (%)
Mean	0.347	99.165	0.517	98.537	0.598	100.497
SD (±)	0.006		0.009		0.014	
CV (%)	1.697		1.796		2.269	
Acceptance criteria: 67% Total QCs must be 100 ± 15% nominal values; 50% QCs per level must be 100 ± 15% nominal values; Mean % nominal 100 ± 15%; CV(%) ≤ 15%.						

Table 3
INTER-DAY PRECISION AND ACCURACY

Samples	R_QC1		R_QC2		R_QC3	
	Unextracted	Extracted	Unextracted	Extracted	Unextracted	Extracted
Mean	1281.51	1300.37	1924.56	1957.53	2200.15	2192.17
SD (±)	2.723	2.497	5.291	41.295	13.470	26.540
CV (%)	0.212	0.192	0.275	2.110	0.612	1.211
C (mg/mL)	0.0350	0.0350	0.0525	0.0525	0.0595	0.0595
Mean Recovery (%)	101.471		101.713		99.637	
Acceptance criteria: 95 ≤ Mean Recovery (%) ≤ 105; CV(%) ≤ 5.						

Table 4
RECOVERY OF CIPROFLOXACIN

N = 3	QC1 0.350µg/mL			QC3 0.595µg/mL		
	Comparison Samples		Stability Samples	Comparison Samples		Stability Samples
	Measured	Nominal (%)	Measured	Measured	Nominal (%)	Measured
Mean	0.352	100.560	0.351	0.592	99.468	0.582
SD (±)	0.003		0.003	0.013		0.006
CV (%)	0.730		0.812	2.129		1.031
Change (%)		-0.371			-1.685	
Acceptance criteria: CV(%) ≤ 5%; % Change ± 5%.						

Table 5
STABILITY AT ROOM TEMPERATURE

N = 3	QC1 0.350mg/mL			QC3 0.595mg/mL		
	Comparison Samples		Stability Samples	Comparison Samples		Stability Samples
	Measured	*Nominal (%)*	*Measured*	*Measured*	*Nominal (%)*	*Measured*
Mean	0.353	100.801	0.354	0.594	99.913	0.602
SD (±)	0.001		0.001	0.009		0.002
CV (%)	0.393		0.316	1.541		0.329
Change (%)		0.346			1.241	
Acceptance criteria: CV(%) ≤ 5%; % Change ± 5%.						

Table 6
SHORT TERM STABILITY IN DISSOLUTION MEDIUM

1.4000mg/mL	Comparison Samples	Stability Samples
Analyte Response	2609.11621	2637.44922
	2618.85327	2647.31128
	2619.25488	2645.86621
N	3	3
Mean	2615.741	2643.542
SD (±)	5.741	5.326
Corrected Mean	-	2643.542
CV (%)	0.219	0.201
Change (%)	1.063	
Acceptance criteria: CV(%) ≤ 5%; % Change ± 5%.		

Table 7
SHORT TERM STABILITY AT 5 ± 3°C

Concentration, 1.4000mg/mL	Comparison Samples	Stability Samples
	2584.48706	2646.09888
Analyte Response	2595.25903	2656.83862
	2589.53320	2651.19336
Mean	2589.760	2651.377
SD (±)	5.390	5.372
CV (%)	0.208	0.203
Change (%)	2.379	
Acceptance criteria: CV(%) ≤ 5%; % Change ± 5%.		

Table 8
LONG TERM STABILITY AT 5±3°C

Stability of Analyte after Sample Processing at Room Temperature: Samples prepared at low (QC1) and high (QC3) quality control levels were submitted to the extraction procedure and kept at room temperature. A calibration curve and 3 replicates of low and high quality control samples (comparison samples) were freshly processed and analyzed with 3 replicates of the stability samples in a single run. Concentrations were calculated to determine % change over time. Ciprofloxacin was found to be stable for 73 h at room temperature after sample processing with changes of -0.371 and -1.685%. Results are presented in table 5.

Short Term Stability of Analyte in Dissolution Medium at Room Temperature: Samples were prepared at low (QC1) and high (QC3) quality control levels. Three replicates of low and high quality control samples were left at room temperature for approximately 1 h (stability samples). A calibration curve and 3 replicates of low and high quality control samples (comparison samples) were freshly processed with the stability samples and analyzed in a single run. Concentrations were calculated to determine % change over time. Ciprofloxacin is found to be stable in dissolution medium for 1 hour at room temperature with % changes of 0.346 and 1.241%. Results are presented in table 6.

Short Term Stability of Ciprofloxacin in Solution at 5 ± 3°C:

The short term stability of ciprofloxacin in the mobile phase consisting a mixture of phosphoric acid solution (*p*H 3) and acetonitrile (90:10, v/v) was evaluated.

A stock solution of ciprofloxacin was prepared in mobile phase, aliquoted and stored at 5±3°C (stability samples). Replicates of stability sample and comparison sample (freshly prepared stock solution) were diluted at approximately the same analyte concentration and analyzed in a single run. Analyte responses were used to determine % change over time.

Ciprofloxacin was found to be stable in mobile phase for 4 h at 5 ± 3°C with % changes of 1.063. Results are presented in table 7.

Long Term Stability of Ciprofloxacin in Solution at 5±3°C

The short term stability of ciprofloxacin in the mobile phase consisting a mixture of phosphoric acid solution (*p*H = 3.0) and acetonitrile (90:10, v/v) was evaluated.

A stock solution of ciprofloxacin was prepared in mobile phase, aliquot and stored at 5±3°C (stability samples). Replicates of stability sample and comparison sample (freshly prepared stock solution) were diluted at approximately the same analyte concentration and analyzed in a single run. Analyte responses were used to determine % change over time.

Ciprofloxacin was found to be stable in mobile phase for 7 days at 5±3°C with % changes of 2.379%. Results are presented in table 8.

Conclusions

A reversed phase HPLC method has been developed and validated for the determination of ciprofloxacin dissolved at *p*H 6.8. This chromatographic assay fulfilled

all the requirements for being a reliable method, including accuracy and precision, linearity, selectivity/specificity and stability. The assay has proven to be sensitive, specific and reproducible and it can be used for the assay of ciprofloxacin in various pharmaceuticals products.

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