RP-HPLC METHOD FOR DETERMINATION OF CIPROFLOXACIN IN PHARMACEUTICAL DOSAGE FORMS

V. Maslarska[1], S. Bozhanov[1], B. Tsvetkova[2], L. Peikova[2], N. Zareva[2]

Medical University – Sofia, Faculty of Pharmacy,
[1]Department of Chemistry,
[2]Department of Pharmaceutical chemistry,
2 Dunav str., 1000 Sofia, Bulgaria

Abstract: In this study, reversed phase high performance liquid chromatographic method have been developed and validated for the determination of ciprofloxacin in tablets. Separation was achieved with a C_{18} (250 mm x 4.6 mm, 5 μm) column, temperature of 33ºC with isocratic mode with mobile phase containing 0.3% orto-phosphoric acid and acetonitril (65:35). UV detection was performed at 290 nm. The flow rate was 0.8 ml/min. The retention time of ciprofloxacin was found to be 3.10 min. The response was linear (R2 0.9999) in the range of 12.5 – 100 μg/ml. The % recovery was estimated as 98.96±2.14. No chromatographic interference from the tablet excipients was found. The results of the studies showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which can be applied for the routine analysis of ciprofloxacin in tablet dosage forms.

Key words: liquid chromatography, validation, ciprofloxacin, tablet dosage form, quality control

Introduction

Fluoroquinolones are an important group of broad spectrum synthetic antibacterial agents used for the treatment of different bacterial infections in both human and veterinary medicine. Ciprofloxacin (1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid) is an important drug because of its broad range of therapeutic indications, particularly active against gram-negative pathogens and against a few gram-positive cocci. It inhibits bacterial DNA gyrase, an enzyme responsible for counteracting excessive supercoiling of DNA during replication or transcription [1]. The drug was first patented in 1983 by Bayer A.G. and then accepted by the United States Food and Drug Administration in the year 1987 [2]. Ciprofloxacin is used to cure a wide variety of infections, including infections at joints and bones, endocarditis, gastroenteritis, respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, and chancroid [3]. USP 2014 and BP 2014 described RP- HPLC methods for estimation of this antibacterial agent as substance and included in tablets [4, 5]. The literature survey revealed that the most methods for determination of ciprofloxacin in tablets and injection formulations include separation techniques [6-17], electrochemical procedures [18-23], spectrophotometric and spectrofluorimetric methods [24-40].

The objective of the present study is to develop a simple, specific and precise liquid chromatographic method for determination of ciprofloxacin in several commercially available tablet dosage forms.

Materials and methods

Reagents and chemicals

Three ciprofloxacin products containing 500 mg active compound from different pharmaceutical companies were obtained commercially and analysed by chromatographic method proposed. Ciprofloxacin HCl reference substance was obtained by Sigma Aldrich. LC-grade acetonitrile was supplied from Merck (Germany). All other chemical reagents were of analytical grade.

Instrumentation

A Shimadzu HPLC system was utilized consisting of the following components: LC – 20 AD pump, vacuum degasser unit DGU – 20 A5 and a UV/VIS variable detector SPD – 20 A. Separation was carried out on a LiChrosorb C 18 column (250 x 4.6 mm, particle size 5 μm) under reversed phase partition chromatographic conditions. The equipment was

controlled by a PC installed properly with the chromatographic software.

Chromatographic Conditions

The mobile phase was 65:35 % v/v mixture of 0.3% o-phosphoric acid:acetonitrile. The mobile phase was filtered through 0.45 μm membrane filter and degassed by using sonicator for about 10 min before use. The sample solutions were also filtered using 0.45 μm membrane filters. The mobile phase was delivered isocratically at a flow rate 0.8 ml/min. The column was maintained at 33°C temperature. The injection volume was 20 μl and the total run time was 5 minutes. The detection was carried out at 290 nm.

Preparation of reference solutions

Reference solution (A): The solution was prepared by dissolving 50.00 mg of accurately weighed ciprofloxacin RS in 100.0 ml diluent (0.3% o-phosphoric acid:acetonitrile 80:20 v/v).

Preparation of standard solutions for evaluation of linearity

Standard solutions used in estimation of linearity were prepared as follows (Table 1). The dilutions were made with diluent mentioned above.

Sample preparation

Ten tablets from each product were accurately weighed (to obtain the average mass of tablets) and finally powdered. Weight equivalent to 500 mg of ciprofloxacin (1 tablet) was taken and transferred into a 100 ml volumetric flask. Approximately 70 ml of diluent were added and the mixture was manually shaken for 10 minutes. The sample was then diluted to volume with diluent. The solution was then filtered off through a 0.45 μm filter discarding the first few ml of filtrate. 5.00 ml of this sample were diluted to 50.00 ml with diluent to produce the final concentration 50 μg/ml.

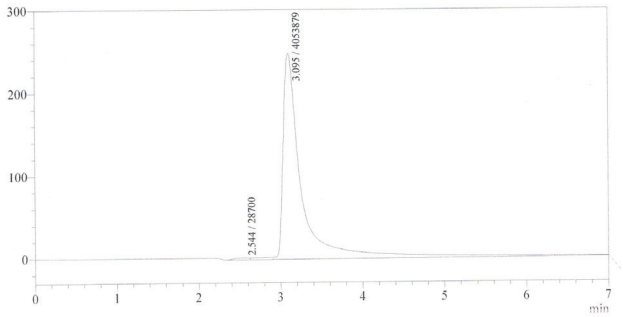

Figure 1. Chromatogram of analysis of ciprofloxacin hydrochloride RS

Results and discussion

In this work an LC method with UV detection for analysis of ciprofloxacin in tablet formulations was developed and validated. From the chromatogram shown in Figure 1, it is evident that, under the proposed chromatographic conditions ciprofloxacin was eluted with short retention time – t_r =3.10 min.

The specificity analysis revealed the HPLC method did not suffer from interference by the formulation excipients, since there was not another peaks on the retention time of the drug of interest.

Method validation

The proposed method was validated with respect to linearity, precision, accuracy, limit of quantitation (LOQ) and limit of detection (LOD).

Linearity

Calibration curve was constructed in the range of 12.50-100.0 μg/ml for ciprofloxacin to encompass the expected concentration in measured samples. The corresponding linear regression equation was

Table 1. *Preparation of standard solutions for linearity.*

%	Concentration (μg/ml)	Volume pipetted of solution A (ml)	Volume to made up (ml)
25	12.5	5.00	200.0
50	25	5.00	100.0
100	50	5.00	50.00
150	75	15.00	100.0
200	100	5.00	25.00

Table 2. Precision of the HPLC method

Amount claimed (mg/tablet)	Amount found (mg/tablet)	Percentage purity obtained	Mean± S.D.	RSD %
500.00	485.2	97.04	98.96± 2.141	2.163
	487.7	97.54		
	488.3	97.66		
	502.1	100.4		
	492.6	98.52		
	512.8	102.6		

y=73875.7x-23662.9 with square of correlation coefficient R^2 of 0.9999. An excellent correlation existed between the peak areas and concentration of drug of interest. The limit of quantitation and limit of detection were calculated from the standard deviations and slopes of the responses using a signal-to-noise ratio and found to be 125 ng and 25 ng, respectively.

Precision

The precision of the method was evaluated by performing six independent determinations of the sample preparation and calculating RSD (%). The RSD value measured during assessment of precision was <3.0% for ciprofloxacin, confirming the method is precise (Table 2).

Accuracy

Accuracy was determined by applying the proposed method to synthetic mixtures of the drug product components to which known quantities of ciprofloxacin substance were added (corresponding to 50, 100 and 150 % of the label claim of the drug). The accuracy was expressed as the percentage of analyte recovered by the assay. The results indicated good accuracy of the method for the determination of analyzed drug as revealed by mean recovery data (Table 3).

Analysis of tablet formulations

The capability of the described method for accurate and precise determination of ciprofloxacin was demonstrated in assay of drug in tablets manufactured by three different companies presented by product codes CIP-A, CIP-B and CIP-C, respectively. The results obtained were presented in Table 4.

Table 3. Results from study of accuracy

Drug	Level (%)	Theoretical concentration (μg/ml)	Observed concentration (μg/ml)	Mean recovery (%) ± SD	RSD (%)
Ciprofloxacin	50	24.90	24.56	99.11±1.31	1.32
			24.43		
			25.06		
	100	49.25	49.52	99.81±0.642	0.64
			49.10		
			48.87		
	150	74.72	73.95	99.63±0.620	0.62
			74.85		
			74.51		

Table 4. Analysis of ciprofloxacin in different tablet formulations

Product code	Ciprofloxacin, mg/tablet – label claim	Ciprofloxacin, mg/tablet – obtained quantity	Results, %
CIP-A	500	484.6	96.92
CIP-B	500	475.1	95.02
CIP-C	500	488.3	97.66

Conclusion

The statistical data have been proven that developed HPLC procedure for determination of ciprofloxacin was found to be accurate, precise, and sensitive. Therefore the proposed method can be applied for routine quality control analysis.

AKNOWLEDGMENT

The present study was kindly supported by Project № 34/2015 from Medical Science Council.

References

1. Goodman LS and Gillman AG, The Pharmacological Basis of Therapeutics, 9th edition (McGraw-Hill, NewYork), 1996; 1065.
2. U S Food and Drug Administration (2009) Cipro Labeling Revision 04/06/2009 Supplement 073. Retrieved 8 September 2009.
3. Ciprofloxacin Hydrochloride. The American Society of Health-System Pharmacists, 2011.
4. British Pharmacopoeia (2014), the stationery office, London, with the monographs of the seventh edition of the European pharmacopoeia monograph.
5. The United State Pharmacopoeia 37 (2014), the National Formulary 32, United State Pharmacopoeial Convention
6. Singh B, Parwate D, Srivastava S, Shukla S. High performance thin-layer chromatographic selective and stability indicating method for assay of ciprofloxacin in pharmaceuticals. Der Pharma Chemica 2010; 2 (4): 178-188.
7. Adam E, Elaziz M, Saeed A. Stability study of ciprofloxacin hydrochloride under stress conditions using reverse phase-high performance liquid chromatography method. Der Pharmacia Sinica 2012; 3 (2): 217-223.
8. Sibinovic P, Smelcerovic A, Palic R, Dordevic S, Marinkovic V. Ruggedness testing of an HPLC method for the determination of ciprofloxacin. Journal of Serbian Chemical Society 2005; 70 (7): 979-986.
9. Witte B, Dewulf J, Demeestere K, Ruyck M, Langenhove H. Critical points in the analysis of ciprofloxacin by high-performance liquid chromatography. Journal Chromatogr. A 2007; 1140: 126-130.
10. Ali a, Mmuo C, Abdulraheem R, Abdulkareem S, Alemika E, Musa A, Sani M, Ilya M. High performance liquid chromatography (HPLC) method development and validation indicating assay for ciprofloxacin hydrochloride. Journal of Applied Pharmaceutical Sciences 2011; 01: 239-243.
11. Qureshi M, Rahman I, Marwat. Comparative analysis of ciprofloxacin in different pharmaceutical products by high performance liquid chromatography. Science, Technology and Development 2012; 31: 69-73.
12. Shihn-Sheng W, Chih-Yuan C, Yeh-Hsia W. Analysis of ciprofloxacin by a simple high performance liquid chromatography method. Journal of Chromatographic Science 2008; 46: 490-495.
13. Kassab N, Singh A, Kedor-Hackmam E, Santoro M. Quantitative determination of ciprofloxacin and norfloxacin in pharmaceutical preparations by high performance liquid chromatography. Brazilian Journal of Pharmaceutical Sciences 2005; 41 (4): 507-513.
14. Thoppil S, Amin P. Stability indicating reversed-phase liquid chromatographic determination of ciprofloxacin as bulk drug and in pharmaceutical formulations. Journal of Pharmaceutical and Biomedical Analysis 2000; 22: 699-703.
15. Joshi S. HPLC separation of antibiotics present in formulated and unformulated samples.

Journal of Pharmaceutical and Biomedical Analysis 2002; 28: 795-809.

16. Nandipati S, Vanka K, Uba S. The separation and quantitative determination of ciprofloxacin in a pharmaceutical formulation by ultra performance liquid chromatograph. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5 (3): 312-317.
17. Faria A, deSouza M, deOliveira A. Validation of a capillary zone electrophoresis method for the determination of Ciprofloxacin, Gatifloxacin, Moxifloxacin and Ofloxacin in pharmaceutical formulation. Journal of Brazilian Chemical Society 2008; 19 (3): 389-396.
18. Zhang S, Wei S. Electrochemical determination of ciprofloxacin based on the enhancement effect of sodium dodecyl benzene sulfonate. Bulletin of Korean Chemical Society 2007; 28 (4): 543-546.
19. Rezk M, Riad S, Khattab F, Marzouk H. Electrochemical determination of ciprofloxacin hydrochloride in pharmaceutical formulation, aquatic environment and in fish tissues. International Journal of Biological and Pharmaceutical Research 2013; 4 (6): 390-396.
20. Faridbod F, Taheren P, Parviz N, Mohammad G. Ciprofloxacin nano-composite carbon paste and PVC membrane potentiometric sensors. International Journal of Electrochemical Science 2012; 7: 3693-3705.
21. Mandil H, Sakur A, Nasser B. Potentiometric determination of gatifloxacin and ciprofloxacin in pharmaceutical formulations. International Journal of Pharmacy and Pharmaceutical Science 2012; 4 Suppl 4: 537-542.
22. Abdala A, Salah S, Abeer M, Sheikha M. Differential electrolytic potentiometric titration method for the determination of ciprofloxacin in drug formulations. Talanta 2003; 61: 239-244.
23. Uslu B, Bozal B, Kuscu M. Anodic voltammetry of ciprofloxacin and its analytical applications. The Open Chemical and Biomedical Methods Journal, 2010, 3, 108-114.
24. Kanakapura B, Paregowda N, Bankavadi C, Veeraiah R. Spectrophotometric and titrimetric determination of ciprofloxacin based on reaction with cerium (IV) sulphate. Science Asia 2006; 32: 403-409.
25. El-Brashy A, El-Sayed M, El-Sepai F. Spectrophotometric and atomic absorption spectroscopic determination of some fluoroquinolone antibacterials by ion-pair complex formation with Bismuth (III) tetraiodide. Journal of the Chinese Chemical Society 2005; 52: 253-262.
26. Eboka C, Aigbavboa S, Akerele J. Colorimetric determination of the fluoroquinolones. Journal of Antimicrobial Chemotherapy 1997; 39: 639-641.
27. Cazedey E, Bonfilio R, Araujo M, Salgado H. A first-derivative spectrophotometric method for the determination of ciprofloxacin hydrochloride in ophtalmic solution. Physical Chemistry 2012; 2 (6): 116-122.
28. Bhongade B, Talath S, Dhaneshwar S. A validated method for the quantitation of ciprofloxacin hydrochloride using diffuse reflectance infrared fourier transform spectroscopy. International Journal of Spectroscopy 2014; http://dx.doi.org/10.1155/2014/294612
29. Pandey S, Pandey P, Tiwari G, Tiwari R, Rai A. FTIR Spectroscopy: A Tool for Quantitative Analysis of Ciprofloxacin in Tablets. Indian Journal of Pharmaceutical Sciences 2012; 74(1): 86–90.
30. Adegoke O, Balogun B. Spectrophotometric determination of some quinolones antibiotics following oxidation with cerium sulphate. International Journal of Pharmaceutical Sciences Review and Research, 2010; 4 (3): 1-10.
31. El-Brashy A, El-Sayed M, El-Sepai F. Spectrophotometric determination of some fluoroquinolone antibacterials by ion-pair complex formation with Cobalt (II) tetrathiocyanate. Journal of the Chinese Chemical Society 2005; 52: 77-84.
32. Cazedey E, Salgado H. Spectrophotometric determination of ciprofloxacin hydrochloride in ophthalmic solution. Advances in Analytical Chemistry 2012; 2(6): 74-79.
33. Igboasouiui A, Attih E, Ofoefule S, Umoh E, Udoh O. Spectrophotometric determination ciprofloxacin using charge-transfer complexation technique. International Journal of innovative research and development 2014; 3 (11): 177-182.
34. Rekha K, Santosh J, Dilshadbee T, Ashpak T. Estimation of ciprofloxacin hydrochloride in bulk and formulation by derivative UV-spectrophotometric methods. International Journal of Advances in Scientific Research 2015; 1(03): 137-144.

35. Zuhair A-A Khammas, Noora S. Mubdir. An Eco-Friendly Method for Extraction and Determination of Ciprofloxacin in Blood Serum and Pharmaceuticals. Science Journal of Analytical Chemistry 2014; 2 (5): 47-54.
36. Ragab G, Amin A. Atomic absorption spectroscopic, conductometric and colorimetric methods for determination of fluoroquinolone antibiotics using ammonium reineckate ion-pair complex formation. Spectrochimica Acta Part A 2004; 60: 973–978.
37. Ramanna P, Saritha B, Reddy T. A validated second derivative visible spectrophotometric procedure for the assay of ciprofloxacin in pharmaceutical dosage forms using Fe (III) in buffer media. International Journal of Pharmaceutical Research and Bio-Science, 2014; 3 (5): 50-61.
38. Abdel-Hay M, Hassan E, Gazy A, Belal T. Kinetic spectrophotometric analysis and spectrofluorimetric analysis of ciprofloxacin hydrochloride and norfloxacin in pharmaceutical preparations using 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl). Journal of the Chinese Chemical Society 2008; 55: 818-827.
39. Shah J, Jan M, Ullah I, Shah S. Sensitive spectrofluorimetric method for determination of fluoroquinolones through charge-transfer complex formation. American Journal of Analytical Chemistry 2013; 4: 521-530.
40. Sarr S, Ndiaye S, Fall D, Diedhiou A, Diop A, Ndiaye B, Diop Y. Development and validation of a simple and economical spectrofluorimetric method for estimation of ciprofloxacin in pharmaceutical dosage forms. International Journal of Analytical and Bioanalytical Chemistry 2013; 3(3): 72-77.

✉ **Corresponding author**
Assoc. Prof. Lily Peikova, PhD
Department of Pharmaceutical chemistry
Faculty of Pharmacy, Medical University – Sofia
2 Dunav str., 1000, Sofia, Bulgaria
Tel: +3599236538; Fax: +3599879874
E-mail: lily_peikova@yahoo.com