DRUG ANALYTICAL RESEARCH
ISSN: 2527-2616 *www.seer.ufrgs.br/dar*

LC method for quantitative determination of Ciprofloxacin in ophthalmic ointment

Lionel Salem Aza-Gnandji*[a], Lívia Maronesi Bueno[b], Tércio Paschke Oppe [a], Elfrides Eva Scherman Schapoval[b]

[a]Laboratório de Controle de Qualidade Farmacêutico – LCQFar - Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS – Brasil; [b]Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Porto Alegre - RS, Brasil
**Corresponding author: brasilio@hotmail.fr*

The present study aimed to validate an analytical method for the quantification of ciprofloxacin in ophthalmic ointment by high performance liquid chromatography. The chromatographic separation of ciprofloxacin hydrochloride was achieved on a C18 reverse-phase column (Hypersil Gold (4 x 250 mm, 5 µm) using UV detection at 278 nm. The optimized mobile phase consisted of a mixture of 0.025 M phosphoric acid with a pH previously adjusted with triethylamine to 3.0 and acetonitrile (85:15, v/v). The parameters specificity, linearity, precision, accuracy and robustness were evaluated according to official guidelines. The purposed assay showed to be specific and the linearity was proved in a range of 10 - 50 µg.mL^{-1}. The RSD values obtained during precision assay (inter-day RSD = 1.38%) indicated the method reproducibility, and the accuracy testing showed good results from recovery test. Robustness assay was complementary and showed that the purposed method is adequate for drug quantitation in commercial samples. No interference from any components of the pharmaceutical dosage forms was observed.

Keywords: ciprofloxacin; ophthalmic ointment; liquid chromatography; method validation.

Article received at 14/09/2021 and accepted at 26/11/2021.
https://doi.org/10.22456/2527-2616.118365

Introduction

Ciprofloxacin acid, 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl) -1,4 dihydroquinoline-3-carboxylic, illustrated below in Figure 1, is an antimicrobial agent of the fluoroquinolone class of second generation with broad spectrum of activity against Gram-positive and Gram-negative bacteria, including Pseudomonas aeruginosa (1). The mechanism of action of antibacterial quinolones, such as ciprofloxacin, is believed to involve the inhibition of bacterial topoisomerase II (DNA gyrase) and topoisomerase IV, essential enzymes in the process of replication, transcription, recombination and repair of bacterial DNA, acting through bactericidal action (2).

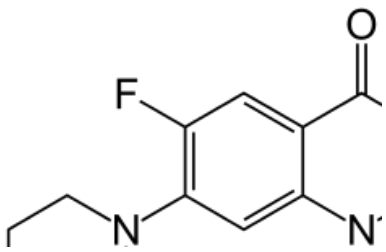

Figure 1. Chemical structure of ciprofloxacin

Ciprofloxacin hydrochloride, ciprofloxacin salt used in the production of ophthalmic ointments, is indicated for the treatment of eye infections, such as corneal ulcers and bacterial conjunctivitis, caused by susceptible microorganisms, such as the microorganism Staphylococcus epidermidis (3).

The scientific literature reports some analytical assays with ultraviolet and fluorescence determination where ciprofloxacin mixtures with other quinolones, single ciprofloxacin or in combination with other pharmaceuticals have been determined in different samples such as biological fluids (4-13) and pharmaceutical formulations (9, 13-16).

However, studies on the determination of ciprofloxacin in ophthalmic ointment are still scarce given its importance for the quality control of medicines. Therefore, in the present study we aimed to develop an LC method for quantitative analysis of ciprofloxacin in ophthalmic ointment, applying validation protocols.

Experimental

Chemicals and Reagents

Ciprofloxacin reference standard (87.33%) was purchased from Brazilian pharmacopeia. Maxiflox® containing 3.5 mg/g were purchased in the local market. The excipients used for the placebo solution (mineral oil, white petrolatum, chlorobutanol e microcrystalline wax) were acquired from different suppliers. LC grade acetonitrile were supplied from Merck (Darmstadt, Germany). All reagents used were of analytical grade. High purity water (Milli-Q® apparatus, Millipore, USA) was used to prepare the solutions.

Apparatus and Chromatographic Conditions

The liquid chromatographic system used in the present study consisted of an Agilent 1200 series LC model, equipped with a quaternary pump, an auto sampler, compartment with thermostat and diode array detector. The system used for data acquisition and analysis of results was the ChemStation software (version B03.02). A C18 reverse-phase column (Hypersil Gold (4 x 250 mm, 5 µm) was used for the separation of ciprofloxacin. An isocratic elution was achieved by using a mobile phase consisted of a mixture of 0.025 M phosphoric acid with a pH previously adjusted with triethylamine to 3.0 and acetonitrile (85:15, v/v). The flow rate was of 1.5 mL/min, injection volume of 20 µL, and the column temperature kept constant at 30 °C. The absorbance detection wavelength was 278 nm.

Standard, sample and placebo preparation

Standard solution of ciprofloxacin was prepared by dissolving of 5.60 mg of ciprofloxacin hydrochloride (5.0 mg of ciprofloxacin) in 50 mL of 0.1 M hydrochloric acid solution, obtaining stock solution at 100 $\mu g.mL^{-1}$. From this standard stock solution, 1.5 mL was transferred to a 5 mL volumetric flask, completing the volume with 0.1 M hydrochloric acid solution, obtaining the concentration of 30 $\mu g.mL^{-1}$ ciprofloxacin.

For the sample preparation, an accurately weighed amount equivalent to 750 mg of ciprofloxacin from Ophthalmic Ointment was transferred to a screw-capped tube. Add 15 mL of solvent hexane and shake vigorously until the Ophthalmic Ointment is dispersed. Loosen the cap, and heat in a water bath at 60°C for 30 min with occasional swirling. Remove from the bath, tighten the cap, and shake for 2 min while still hot. Add 12.5 mL of 0.1 M hydrochloric acid and shake vigorously for 2 min. Allow the layers to separate, and use the lower, aqueous layer. From the aqueous layer called the sample solution (60 $\mu g.mL^{-1}$), a 2.5 mL was transferred to a 5 mL volumetric flask, completing the volume with a 0.1 M hydrochloric acid solution, obtaining the final concentration of 30 $\mu g.mL^{-1}$ ciprofloxacin.

All the excipients contained on the dosage form (mineral oil, white petrolatum, chlorobutanol e microcrystalline wax), were weighed in their usual concentration according the Handbook of Pharmaceutical Excipients and prepared by the same way of sample solution (17).

Method validation

The analytical method was validated according to official guidelines (18), which establish the evaluation of the following parameters: specificity, linearity, limit of detection (LOD) and limit of quantification (LOQ), precision, accuracy and robustness.

Specificity

The specificity of the method was evaluated by the injection of the placebo solution at a theoretical concentration of 30 $\mu g.mL^{-1}$ and the chromatogram obtained was compared with the standard solution chromatogram. Besides, forced degradation of the sample solution was carried out under the following conditions: for acid, alkali and oxidative degradations. Aliquots of 2.5 mL of the sample solution were transferred to 5 mL volumetric flasks with 2.5 mL of 1M HCl, 1M NaOH or 3% H2O2. Photolytic studies were done by exposing aliquots of the sample solution to mirror chamber (100x 18 x 17 cm) equipped with UV-C (254 nm) lamp. After two hours, an aliquot of each solution, in all stress conditions, was diluted with mobile phase and injected in the chromatographic system in a concentration of 30 $\mu g.mL^{-1}$.

Linearity

The method linearity was studied by performing three independent analytical curves, within five concentration levels ranging 10-50 $\mu g.mL^{-1}$. All the points were injected in triplicate and the media of the values were used in the statistic analysis by linear regression.

Limit of detection (LOD) and limit of quantitation (LOQ)

Limits of detection and quantification of ciprofloxacin were obtained from linearity data, based on the standard deviation of the response (s) and the slope (S), and was calculated using the formulas: LOD = 3.3 s/S and LOQ = 10 s/S.

Precision

Precision was determined by repeatability (intraday) and intermediate precision (interday). Repeatability was evaluated by assaying six samples solutions at 30 $\mu g.mL^{-1}$ during the same day, and the intermediate precision was studied by comparing the assays on two different days. The analyses were done in triplicate and results were expressed as the relative standard deviation (RSD) of the analytical measurements. Samples were prepared as previously described.

Accuracy

Accuracy was determined based on the recovery of known amounts of ciprofloxacin reference standard added to samples at the levels of 5, 10 and 15% of the sample concentration (30 $\mu g.mL^{-1}$). The accuracy was calculated as the percentage of the drug recovered and expressed as the relative standard deviation (RSD) between the measurements.

Robustness

Robustness was determined by through small modifications in the established analytical conditions. The main modifications were with respect to the mobile phase proportion, flow rate and temperature.

Results and Discussion

The present study describes a simple, accurate, and reproducible LC method for the determination of ciprofloxacin in ophthalmic ointment. This method has some advantages over the previously reported method (19). The mobile phase is simple, does not deteriorate the column and the analysis time is short.

The LC conditions were optimized in order to provide an adequate separation of ciprofloxacin hydrochloride. Mobile phase and flow rate were selected according to peak parameters (peak height, tailing, plate number), baseline smoothness, run time, easy preparation of mobile phase. The liquid chromatographic system with the isocratic mobile phase and 1.5 $mL.min^{-1}$ flow rate proved quite robust. A C18-column had been recommended because of its demonstrated ruggedness and reproducibility in this assay. Injection volume was set to 20 µL for an assay requiring a higher sensitivity. The retention time was 4.39 min, approximately, for ciprofloxacin (figure 2).

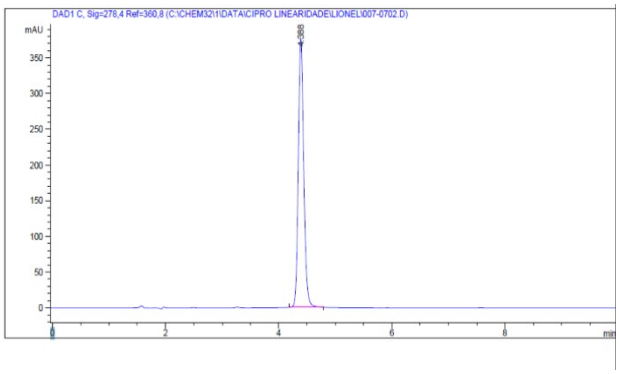

Figure 2. Representative chromatogram of the injection of ciprofloxacin 30 $\mu g.mL^{-1}$. Chromatographic conditions: mobile phase H3PO4: ACN (85:15, v/v), flow rate 1.5 $mL.min^{-1}$, injection volume 20 µL. Column: hypersil gold (4 x 250 mm, 5 µm), 30°C.

The specificity of the method was proved by the absence of any significant interference from placebo and degradation products on ciprofloxacin determination. Figure 3 shows representative chromatograms of placebo and ciprofloxacin under acid, alkaline, oxidative and UV-C degradations. After 2 hours, was observed that the drug was susceptible to degradation in acid, alkaline, oxidative environments and in UV-C radiation, originating new chemical entities. However, the products formed do not interfere with the quantification of ciprofloxacin.

The scientific literature reports that ciprofloxacin is susceptible to photolytic degradation, suffering decarboxylation degradation reactions or cyclopropane opening of its chemical structure, however it has thermal stability (20).

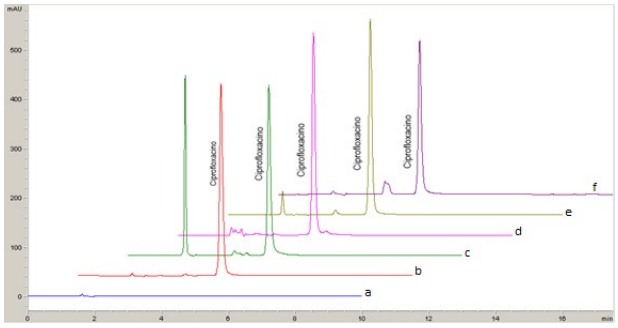

Figure 3. Representative chromatograms of the placebo, sample and degradation studies of ciprofloxacin in ophthalmic ointment 30 $\mu g.mL^{-1}$, under the following conditions: placebo (a); undegraded sample (b); oxidative degradation (c); sample in alkaline medium (d); sample in acidic medium (e); sample in the presence of UVC radiation (f).

The limits of detection and quantification of the drug were obtained through the linearity curve, by the standard deviation of the response (s) and the slope (S). The results founded for ciprofloxacin were 0.34 $\mu g.mL^{-1}$ (LOD) and 1.04 $\mu g.mL^{-1}$ (LOQ). These results were considered satisfactory according to the official Guidelines and indicate that the method has adequate sensitivity for the determination and quantification of ciprofloxacin in ophthalmic ointment.

The linearity of the method was evaluated by linear regression analysis of the ciprofloxacin hydrochloride and the results were all satisfactory (Table 1). The calibration curve of for the ciprofloxacin hydrochloride showed determination coefficient (r^2) ≥ 0.99 and the residual analysis indicated absence of atypical samples.

Table 1. Linearity results obtained from validation of LC method for quantitative determination of ciprofloxacin in ophthalmic ointment.

Parameter	Results
Calibration range ($\mu g.mL^{-1}$)	10.0 – 50.0
Regression equation	y= 84.447x – 9.5361
Determination coefficient (r^2)	0.9999
F	101160.3191
Significance F	6.85394×10^{-08}
Lower 95%	-37.52541071
Upper 95%	18.52541071

As illustrated in Table 2, the data of precision studies, obtained from the intra-day (repeatability) and inter-day (intermediate precision) results. The RSD values were calculated, and all are ≤ 2%. These results were confirmatory for the expected reproducibility in this experimental purpose. Accuracy was studied by applying the recovery test, which was performed by adding the reference standard to the sample solution and analyzing the total drug content in the final matrix, expressing the results as percentage of drug recovered. As described in Table 3, the proposed method by LC method showed to be accurate for ciprofloxacin determination, presenting an average recovery between 96.90% and 97.68%, being considered satisfactory. The method was also shown to be robust, considering the experimental modifications made in order to evaluate this parameter. Testing mobile phase composition, flow rate and temperature, the drug content was not influenced, demonstrating that the method supports little variations during analysis.

Table 2. Results obtained for repeatability and intermediate precision parameters studied during validation of LC method for quantitative determination of ciprofloxacin in ophthalmic ointment.

	Repeatability[a]			
	Day 1	Day 2	Intermediate[b]	RSD
Amount (%)	104.45	101.27	102.96	1.38
	103.21	101.42		
	103.92	102.96		
	104.25	101.27		
	104.54	100.84		
	104.47	102.98		
Mean (%)	104.14	101.79		
RSD	0.49	0.92		

[a] mean of two determinations.
[b] mean of the determinations obtained in two days of analysis.

Table 3. Results obtained from recovery testing studied for validation of LC method for quantitative determination of ciprofloxacin in ophthalmic ointment.

	Theoretical concentration ($\mu g.mL^{-1}$)	Experimental concentration ($\mu g.mL^{-1}$)	Recovery (%)	Mean (RSD) (%)
Level 1	35	35.01	98.24	97.68 (1.98)
			99.29	
			95.52	
Level 2	40	40.01	97.20	96.90 (0.92)
			95.90	
			97.61	
Level 3	45	45.01	96.90	97.63 (1.40)
			96.78	
			99.21	

Conclusions

A simple, fast and reliable LC method for quantitative analysis of ciprofloxacin in ophthalmic ointment was developed and validated. The proposed method presented adequate performance for the intended analysis, demonstrating to be sensitive, precise and accurate. The method developed proved to be simpler than the method described in United States Pharmacopeia, especially when comparing the components of mobile phases. Thus, it constitutes a positive collaboration for quality control of the analyzed product, as well as collaborates for the preparation of a monograph of the Brazilian Pharmacopoeia on ciprofloxacin ophthalmic ointment.

References

1. Campoli-Richards DM, Monk JP, Price A, Benfield P, Todd PA, Ward A. Ciprofloxacin a Review of its Antibacterial Activity, Pharmacokinetic Properties and Therapeutic Use. Drug Evaluation. 1988; 35:373-447.
2. Andriole VT. The Quinolones: Past, Present, and future. Supplement Article. 2005; 41:S113-9.
3. Silva P. Pharmacology. 6th ed. Rio de Janeiro: Guanabara Koogan; 2002.
4. Weber A, Chaffin D, Smith A, Opheim KE. Quantitation of Ciprofloxacin in Body Fluids by High-Pressure Liquid Chromatography. Antimicrob Agents Chemother. 1985 Apr; 27(4):531-534.
5. Basci NE, Bozkurt A, Kalayci D, Kayaalp SO. Rapid liquid chromatographic assay of ciprofloxacin in human aqueous humor. J Pharm Biomed Anal. 1996; 14:353-356.
6. Ba BB, Ducint D, Fourtillan M, Saux MC. Fully automated high-performance liquid chromatography

of ciprofloxacin with direct injection of plasma and Mueller–Hinton broth for pharmacokinetic /pharmacodynamic studies. J Chromatog B. 1998;714: 317-324.
7. Kamberi M, Tsutsumi K, Kotegawa T, Nakamura K, Nakano S. Determination of ciprofloxacin in plasma and urine by HPLC with ultraviolet detection. Clin Chem. 1998; 44(6):1251-1255.
8. Maya MT, Gonçalves NJ, Silva NB, Morais JA. Simple high-performance liquid chromatographic assay for the determination of ciprofloxacin in human plasma with ultravioleta detection. J Chromatog B. 2001; 755: 305-309.
9. Zotou A, Miltiadou N. Sensitive LC determination of ciprofloxacin in pharmaceutical preparations and biological fluids with fluorescence detection. J Pharm Biomed Anal. 2002; 28:559-568.
10. Imre S, Dogaru MT, Vari CE, Muntean T, Kelemen L. Validation of an HPLC method for the determination of ciprofloxacin in human plasma. J Pharm Biomed Anal. 2003; 33:125-130.
11. Sowinski KM, Kays MB. Determination of ciprofloxacin concentrations in human serum and urine by HPLC with ultraviolet and fluorescence detection. J Clin Pharm Ther. 2004; 29:381-387.
12. Vybíralová Z, Nobilis M, Zoulova J, Kvetina J, Petr P. High-performance liquid chromatographic determination of ciprofloxacin in plasma samples. J Pharm Biomed Anal. 2005; 37:851-858.
13. Wu SS, Chein CY, Wen YH. Analysis of Ciprofloxacin by a Simple High-Performance Liquid Chromatography Method. J Chromatogr Sci. 2008 Jul; 46: 490-495.
14. Husain S, Khalid S, Nagaraju V, Rao RN. High-performance liquid chromatographic separation and determination of small amounts of process impurities of ciprofloxacin in bulk drugs and formulations. J Chromatog A. 1995; 705:380-384.
15. Lacroix PM, Curran NM, Sears RW. High-pressure liquid chromatographic methods for ciprofloxacin hydrochloride and related compounds in raw materials. J Pharm Biomed Anal. 1996; 14:641-654.
16. Santoro MIRM, Kassab NM, Singh AK, Kedor-Hackmam ERM. Quantitative determination of gatifloxacin, levofloxacin, lomefloxacin and pefloxacin fluoroquinolonic antibiotics in pharmaceutical preparations by high-performance liquid chromatography. J Pharm Biomed Anal. 2006; 40:179-184.
17. Rowe RC, Sheskey PJ, Owen SC. Handbook of Pharmaceutical Excipients. 5th ed. London: Pharmaceutical Press, 2006.
18. Agência Nacional de Vigilância Sanitária (BR). Resolução RDC n° 166 de 27 de julho de 2017. Dispõe sobre a validação de métodos analíticos e dá outras providências. Brasília, DF: Diário Oficial da União; 2017.
19. The United States Pharmacopeia. USP 42-NF 37. Rockville: United States Pharmacopeia Convention, 2019.
20. Nudelman, N.E.S. Estabilidad de medicamentos. Buenos Aires: El Ateneo, 1975.