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SHORT COMMUNICATION

A novel and rapid microbiological assay for ciprofloxacin hydrochloride

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Abstract The present work reports a simple, fast and sensitive microbiological assay applying the turbidimetric method for the determination of ciprofloxacin hydrochloride (CIPRO HCl) in ophthalmic solutions. The validation method yielded good results and included excellent linearity, precision, accuracy and specificity. The bioassay is based on the inhibitory effect of CIPRO HCl upon the strain of *Staphylococcus epidermidis* ATCC 12228 used as the test microorganism. The results were treated statistically by analysis of variance (ANOVA) and were found to be linear ($r=0.9994$, in the range of 14.0–56.0 μg/mL), precise (intraday RSD %=2.06; interday RSD%=2.30) and accurate (recovery=99.71%). The turbidimetric assay was compared to the UV spectrophotometric and HPLC methods for the same drug. The turbidimetric bioassay described on this paper for determination of ciprofloxacin hydrochloride in ophthalmic solution is an alternative to the physicochemical methods disclosed in the literature and can be used in quality control routine.

1. Introduction

Ciprofloxacin hydrochloride (CIPRO HCl), namely 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid hydrochloride (Fig. 1), is a second generation fluoroquinolone antimicrobial with a wide spectrum of activity against Gram-positive and Gram-negative bacteria, including *Pseudomonas aeruginosa* [1]. The mode of action of fluoroquinolones involves interactions with both DNA gyrase, the originally recognized drug target, and topoisomerase IV, a related type II topoisomerase [2].

The drug is official in British Pharmacopoeia [3] presenting an HPLC assay for CIPRO HCl tablets and ciprofloxacin lactate intravenous infusion. In Brazilian Pharmacopoeia [4] three methods are proposed to determine CIPRO injection, CIPRO HCl tablets and ophthalmic solution; an UV spectrophotometric, an HPLC and a microbiological diffusion agar methods. The United States Pharmacopoeia [5] describes an HPLC method for CIPRO and CIPRO HCl assay in bulk, CIPRO injection, ophthalmic ointment, ophthalmic solution and tablets.

Despite most methods presented in official compendia are physicochemical assays, these methods do not represent the potency of antimicrobials neither can predict the loss of activity.

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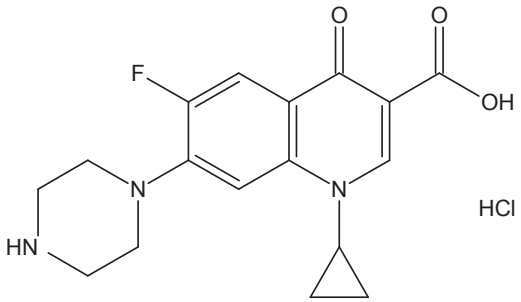

Fig. 1. Chemical structure of ciprofloxacin hydrochloride.

Furthermore, the low cost and simple procedures of bioassays have allowed them to become an alternative methodology for drug potency assessment in pharmaceutical formulations.

The literature has reported microbiological assays by agar diffusion method for determination of others fluoroquinolones in pharmaceutical formulations, such as norfloxacin [6], sparfloxacin [7], ofloxacin [8], enrofloxacin [9], lomefloxacin [10], gatifloxacin [11] and orbifloxacin [12]. However, no microbiological assay using turbidimetric method for the determination of quinolones has been reported yet. This assay is faster than agar diffusion method and presents easier management. The microbiological assay can reveal subtle changes not demonstrable by chemical methods and it gives the possibility to evaluate the potency of this substance, which is very important for the analysis of antibiotics. Bioassay is an ecological technique because it is not a residue or solvent producer. Moreover, microbiological assay requires no specialized equipment or toxic solvents [13].

In this paper, a novel, rapid, simple and sensitive turbidimetric bioassay method is described for determination of CIPRO HCl in ophthalmic solution as an alternative to the physicochemical methods described in the literature.

2. Materials and methods

2.1. Chemicals and instruments

CIPRO HCl reference standard (assigned purity 100%) was kindly supplied by EMS Sigma Pharma Group (São Paulo, Brazil). Pharmaceutical dosage form (ophthalmic solution) containing CIPRO HCl was obtained commercially and claimed to contain 3.5 mg/mL of drug and boric acid, sodium citrate, dissodium edetate (EDTA), benzalconium chloride and purity water as excipients.

All chemicals and reagents used were of analytical grade. High purity water was prepared using Millipore Milli-Q purification system (Millipore, Bedford, MA, USA). The absorbances were carried out in spectrophotometer Beckman model DU® 530 (California, USA).

2.2. Microorganism and inoculum

The cultures of *Staphylococcus epidermidis* ATCC 12228 were cultivated on Casoy agar and maintained in the freezer as stock. The cultures were pealed to brain heart infusion (BHI) broth (24 h before the assay) and kept at 36 ± 1 °C. A culture broth of $25 \pm 2\%$ turbidity (transmittance) was obtained at 530 nm, using a suitable spectrophotometer and a 10 mm diameter test tube as absorption cells against BHI broth as blank.

2.3. Preparation of the standard solutions

Accurately weighed 100 mg of CIPRO HCl reference standard was transferred to a 100 mL volumetric flask and dissolved in water (final concentration of 1000 μg/mL). Aliquots of this solution were diluted in water at concentrations of 14.0, 28.0 and 56.0 μg/mL, which were used in the assay.

2.4. Preparation of the sample solutions

Aliquots (40, 80 and 160 μL) of CIPRO HCl ophthalmic solution (3500 μg/mL) were transferred volumetrically into 10 mL volumetric flasks and added water to give a final concentrations of 14.0, 28.0 and 56.0 μg/mL.

2.5. Turbidimetric assay

1.0 mL of the inoculated BHI broth was added in tubes containing 10.0 mL of sterile BHI broth. Aliquots of 200 μL of CIPRO HCl reference standard and sample solutions were added in the respective tubes. Twenty tubes were used to carried out parallel lines 3×3 design, three tubes for each concentration of standard and sample, one tube for positive control (broth and inoculum), without addition CIPRO HCl and one for negative control (only broth).

After incubation at 35 ± 2 °C for 4 h in shaker incubator, the bacteria growing was discontinuous through adding 0.5 mL of 12% formaldehyde aqueous solution. The absorbance was determined in each tube using a spectrophotometer at 530 nm employing the negative control as blank.

2.6. Calculation

The potency of CIPRO HCl in ophthalmic solution was calculated by Hewitt equation [14]. The assay was statistically treated by the linear parallel model and by linear regression analysis. Analysis of variance (ANOVA) was also used to verify the validity of the method.

2.7. Method validation

The method was appropriately validated by determination of the following parameters: linearity, precision, accuracy, specificity and robustness.

2.7.1. Linearity

The calibration curve was obtained with three doses of the reference standard. The linearity was evaluated by linear regression analysis, which was calculated by the least squares regression method.

2.7.2. Precision

The precision of the assay was determined by repeatability (intra-assay) and intermediate precision (inter-assay). Repeatability was evaluated by assaying the samples in the same concentration and same day. The intermediate precision was studied by comparing the assays on three different days. The results were expressed in relative standard deviation (RSD%).

2.7.3. Accuracy

The accuracy was determined by % recovery of known amounts of CIPRO HCl reference standard added (4.5, 24.5 and 44.5 μg/mL) to the samples at the beginning of the process. Aliquots of 30 μL