Development and Validation of a Simple, Rapid and Stability-Indicating High Performance Liquid Chromatography Method for Quantification of Norfloxacin in a Pharmaceutical Product

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Abstract

A stability-indication high performance liquid chromatographic method has been developed for the determination of norfloxacin in tablet dosage forms. Optimum separation was achieved in less than 7 minutes using Eclipse Plus Zorbax C18 Agilent, 150 mm x 4.6 mm i.d., 5 μm particle size column. The analyte was resolved by using a mobile phase 5% acetic acid aqueous solution and methanol (80:20, v/v) at a flow rate 1.0 ml/min on an isocratic high performance liquid chromatographic system at a wavelength of 277 nm. Linearity, system suitability, precision, sensitivity, selectivity, specific, and robustness were established by International Conference Harmonization guidelines. For stress studies the drug was subjected to photoysis, oxidation, acid, alkaline and neutral conditions. The analytical conditions and the solvent developed provided good resolution within a short analysis time and economic advantages. The proposed method not required sophisticated and expensive instrumentation.

Keywords: Degradation products; HPLC-UV; Liquid chromatography; Norfloxacin; Stability-indicating method; Validation

Introduction

Norfloxacin is the first synthetic second-generation fluoroquinolone antimicrobial drug. It was developed for use in human and veterinary medicine [1]. Norfloxacin, occasionally used to treat common, as well complicated urinary tract infections, exhibits a broad spectrum of activity against Gram-positive and Gram-negative bacteria [2-4]. Chemically, it is 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid (Figure 1). The mechanism of the bacterial effect of norfloxacin is based on the primary target in bacterial enzyme DNA gyrase and topoisomerase II and IV. Inhibition of the activity of these enzymes disables DNA replication which in turn, inhibits bacterial replication [5,6].

In addition, good tolerability and a favorable safety profile make the fluoroquinolones important therapeutic options for the treatment of infections caused by antibiotic-resistant bacteria [7].

Several analytical methods for norfloxacin have been described in scientific literature, such as titrimetry UV spectrophotometry, and liquid chromatography, amongst others [8-14]. The high performance liquid chromatography (HPLC) has become an important tool for the routine determination of antimicrobial drugs, with specific emphasis on fluoroquinolones, in edible animal products, environmental, feed, pharmaceutical dosage forms.

Chemical Structure of Norfloxacin (CAS: 70458-96-7).

In the literature, there are some references about the determination of norfloxacin using HPLC methodology. Espinosa-Mansilla and coworkers reported this methodology with photoinduced fluorometric (PIF) detection, used to determine four fluoroquinolones in serum and plasma samples [20]. In another work, Patel et al. improve one method for estimation of norfloxacin and ornidazole in their combined dosage form [21]. Most of the reported methods involve troublesome mobile phase (buffers) and sample preparation.

A thorough literature search has revealed that one method was reported for separation and determination of the process related synthetic impurities of norfloxacin and one other studied of photo-stability of norfloxacin contained in directly compressible tablets and estimated the closely related ethylenediamine degrade by HPLC [17,22].

Our investigation involved the optimization of the method described above using a reliable stability indicating and one new development, as well as validating a simple, sensitive, accurate and reproducible HPLC method for the determination of norfloxacin in pharmaceutical dosage forms.

Experimental

Reagents and chemicals

Methanol and acetic acid were HPLC grade (Merck, Germany). Sodium hydroxide (NaOH), hydrogen peroxide (H₂O₂) and hydrochloric acid (HCl) were obtained from Synth (São Paulo, Brazil). Norfloxacin standard (purity 100%) and pharmaceutical product

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norfloxacin tablet with a label claim of 400 mg drug was obtained from Uniao Quimica (Pouso Alegre, Brazil). Eluents and standard solutions were prepared with high-quality water obtained from a Milli-Q system (Millipore, Milford, MA, USA). All chemicals were of an analytical grade and used as received.

**HPLC apparatus and conditions**

A Waters HPLC system, consisting in two module pumps, model 1525, a manual injector (Breeze 7725i, Rhodyne) and UV-Vis detector (Waters 2487) at 277 nm, was used. The analyses were carried out on an Eclipse Plus Zorbax C$_18$ Agilent (150 mm×4.6 mm i.d., 5 μm particle size) column as a stationary phase. The mobile phase was a mixture of 5% acetic acid aqueous solution and methanol (80:20, v/v) used in mode isocratic elution. Twenty microliters of sample was injected into the HPLC system. The overall run time was 7.0 min and the flow rate was 1.0 ml/min. All the analyses were carried out at room temperature.

**Results and Discussion**

**HPLC method development and validation**

The HPLC method carried out in this work aimed at developing a new system capable of eluting resolving norfloxacin and its degradations products, based on trial and error method, the mobile phase, which gives best possible separation and resolution, was selected and retention time was also taken in to the consideration.

During the development of this method, different compositions of mobile phase were tested. Finally the system containing 5% acetic acid aqueous solution and methanol (80:20, v/v) was found to be satisfactory and gave resolved peaks of norfloxacin (standard and tablets) and degradations products at 277 nm. The retention time for norfloxacin standard was 5.7 minutes (Figure 2).

**System suitability**

This test was performed by collection of data from a standard solution containing 22 μg/ml of norfloxacin that was injected six times of standard resolution solution [25]. The parameters measured were tailing factor, capacity factor, theoretical plates, retention time and peak
to retain unaffected by small, but deliberate variations in method conditions and provides an indication of its reliability during normal usage [24]. To determine the robustness of the proposed method, the following variations were made in the analytical method: percentage of methanol in the mobile phase (18% and 22%), wavelength (275 and 279 nm), flow rate (0.8 and 1.2 ml/min) and column was altered to a Luna C18 Phenomenex (150×4.6 mm, 5 μm). The robustness of the method indicates the stability [23]. A stability indicating method accurately measures the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities.

Robustness

The robustness of the analytical method was determined by the consistency of the peak height and peak shape with the deliberately small changes in the experimental conditions. It is a measure of its capacity to retain unaffected by small, but deliberate variations in method conditions and provides an indication of its reliability during normal usage [24]. To determine the robustness of the proposed method, the following variations were made in the analytical method: percentage of methanol in the mobile phase (18% and 22%), wavelength (275 and 279 nm), flow rate (0.8 and 1.2 ml/min) and column was altered to a Luna C18 Phenomenex (150×4.6 mm, 5 μm). The robustness of the method shows %RSD value 0.67%, that there were no marked changes in the chromatographic conditions, which demonstrates that the method developed is robust.

Selectivity/specificity

Through the forced degradation study, was evaluate the specificity of the method indicate the stability [23]. A stability indicating method accurately measures the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities.

The specificity of the development method was determined by injecting sample solutions (22 μg/ml) which were prepared by stress conditions, like UV light, neutral, base, acid, and oxidative agent. In two stress conditions, (UV light and neutral) peak of norfloxacin was found to be 1.98% with a corresponding percentage recovery value of 103.93%.

Table 1: Results of recovery study by standard-addition method.

| Amount of standard norfloxacin added (µg/ml) | Total found (µg/ml) | Recovery (%) | Mean Recovery (%) |
|---------------------------------------------|---------------------|--------------|-------------------|
| R1 7.6                                      | 7.62                | 101.75       | 103.96            |
| R2 12.0                                     | 12.53               | 104.29       |                   |
| R3 16.4                                     | 17.58               | 105.85       |                   |

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Accuracy

To make certain the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%) described in table 2. Percent RSD for area. The tailing factor showed less than 2, the capacity factor was more than 2 and the theoretical plates were more than 2000. The average of retention time was 5.5 minutes and the %RSD of peak area was 0.93%. The values for system suitability parameters showed feasibility of this method for routine pharmaceutical application. All results are shown in the table 1.

Table 2: Data of system suitability of developed method to norfloxacin.

| Injection Number | Retention Time (min) | Peak area of norfloxacin | Tailing factor | Theoretical plates | Capacity factor |
|------------------|-----------------------|--------------------------|----------------|-------------------|-----------------|
| 1                | 5.58                  | 2995654                  | 0.75           | 2200.19           | 3.29            |
| 2                | 5.61                  | 2997951                  | 0.75           | 2223.92           | 3.31            |
| 3                | 5.61                  | 3020060                  | 0.72           | 2223.92           | 3.31            |
| 4                | 5.61                  | 2994350                  | 0.74           | 2223.92           | 3.29            |
| 5                | 5.58                  | 3048035                  | 0.72           | 2200.19           | 3.29            |
| 6                | 5.59                  | 3058041                  | 0.74           | 2208.09           | 3.30            |
| Mean             | 5.59                  | 3019015                  | 0.73           | 2213.37           | 3.29            |
| %RSD             | 0.26                  | 0.93                     | 1.85           | 0.59              | 0.29            |

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Figure 2: Typical chromatogram of norfloxacin standard (A), norfloxacin dosage form (B) and placebo solution (C).

Figure 3: Plot showing the changes % norfloxacin area peak vs. time. Mean values.

The specificity of the development method was determined by injecting sample solutions (22 μg/ml) which were prepared by stress conditions, like UV light, neutral, base, acid, and oxidative agent. In two stress conditions, (UV light and neutral) peak of norfloxacin was found to be 1.98% with a corresponding percentage recovery value of 103.93%.

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product, with a good selectivity and resolution the compounds, these results seem to suggest that HPLC is a selective and specific method for the analysis of norfloxacin samples from stability studies.

Assay of norfloxacin

The developed method applied for the determination of norfloxacin content in market formulation (tablets 400 mg). The result of the assay yielded 102.84% (RSD=0.11%). The assay result showed that this method was sensitive and specific for the quantitative analysis of norfloxacin in dosage form. No significant interference was observed from excipients commonly used in the formulation.

Conclusions

The proposed stability indication HPLC method is found to be simple, sensitive, accurate, precise, linear, robust and specific for quantitative estimation of norfloxacin dosage forms. The developed chromatographic method was validated using ICH guidelines. The analytical conditions and the solvent developed provided good resolution within a short analysis time and proved to be economical. The proposed method does not need sophisticated and expensive instrumentation.

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