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Development of a Simple, Sensitive and Rapid Quantitative Analytical Method for Lomefloxacin by High Performance Liquid Chromatography

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Original Research Article

ABSTRACT

An attempt has been made to develop a simple, sensitive and rapid high performance liquid chromatographic (HPLC) method of analysis for lomefloxacin as in pharmaceutical dosage form using 0.025 M phosphoric acid and acetonitrile (80:20) as mobile phases. The mobile phase was used as solvents to dissolve lomefloxacin and 0.0122 mg/mL stock solution was prepared. Lomefloxacin solution was scanned with UV-spectrophotometer and the absorption maximum (λmax) was found to be 287 nm. This method was successfully applied to five eye drop dosage forms of lomefloxacin encoded as pp1, pp2, pp3, pp4 and pp5 marketed by five different pharmaceutical companies and the result was found to be satisfactory and reproducible. The method was validated by spiked recovery experiments and shown to be linear for lomefloxacin. The method can be used for routine analysis in both research laboratories, and pharmaceutical and chemical industries to analyze the drugs and chemicals without any interference by the excipients.

***Key words*:** Lomefloxacin, HPLC, Analysis, Reproducible.

INTRODUCTION

Lomefloxacin is one of the third generation fluoroquinolones with some specific activity in upper respiratory tract infections and community acquired pneumonia. It is also used in meningitis, osteomyelitis, urinary tract infections, sexually transmitted diseases, bacteraemia, nosocomially acquired infections, gastrointestinal infections and in combination with other agents in the treatment of tuberculosis. It is an INN drug and as such it has not been yet included in the BP or USP (Goodman and Gilman, 1996). A number of analytical methods (Tozo *et al.*, 2006; Santoro *et al.*, 2006; Lyon *et al.*, 1994; Wright *et al.*, 1998.) has been developed for the analysis of lomefloxacin for research purposes. Of these the most widely used method for the analysis of lomefloxacin is based on HPLC with specific mobile phase composition for a particular condition. This is because HPLC has become the fastest growing analytical tool during the last decade. The purpose of the present study thus was to develop handy and easily operable HPLC method for the analysis of lomefloxacin in pharmaceutical dosage form which would be simple, rapid, cost-effective and reproducible. We introduced a mobile phase by a long trial and error method. We, therefore, proposed a HPLC method which is simple, sensitive and rapid for the estimation of lomefloxacin in pharmaceutical preparations (as eye drops) that can also be used for quantitative estimation in research laboratory for research purpose and in pharmaceutical industries for routine analysis of lomefloxacin without any interference by the excipients.

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Table 1. Estimation of lomefloxacin in eye drops by proposed HPLC method (*Data are expressed as mean ± SD, where, n = 5).

Name of sample	Concentration (mg/mL)	Average retention time (min)*	Potency (%)*
Standard	0.01-0.03	9.14 ± 0.002	99.99
pp1	0.03	9.13 ± 0.002	98.92 ± 0.068
pp2	0.03	9.13 ± 0.004	106.00 ± 1.581
pp3	0.03	9.15 ± 0.004	101.65 ± 1.318
pp4	0.03	9.15 ± 0.004	101.65 ± 1.318
pp5	0.03	9.13 ± 0.003	98.54 ± 0.370

MATERIAL AND METHODS

Drugs and Chemicals

Standard lomefloxacin hydrochloride (potency 99.99%) was a kind gift from Eskayef Pharma Ltd. It was collected in an air tight vial, stored in a cool & dry place and was used without further purification. Acetonitrile was of HPLC grade, (E. Merck, Germany). *Ortho*-phosphoric acid, sodium dihydrogen phosphate and other reagents were of analytical grade (E. Merck, Germany) and de-mineralized water was used throughout the experiment.

Chromatographic Conditions

The HPLC system consisted of a solvent delivery system, an injector with 20 µL sample loop, a variable wavelength detector, a stainless steel column (C-18 column, 25 cm long with i.d. 4.6 mm) packed with silica gel, the average particle size being 5 µm. The column was always kept vertical with guard column. The column was washed with the mobile phase for about an hour before sample injection. Mobile phase was developed by long trial and error method and finally it was composed of 0.025 M phosphoric acid and acetonitrile as a ratio of 80:20 and the pH was maintained as 3.0. Before use, mobile phase was degassed by filtering through 0.2 µm filter paper (Shimadzu, Japan) in a degasser unit (Shimadzu, Japan). Flow rate was adjusted as 1.0 mL/min. Oven temperature was maintained at 40°C. Thermal printer plotting was used as method of recording and at a chart speed of 25 mm/min.

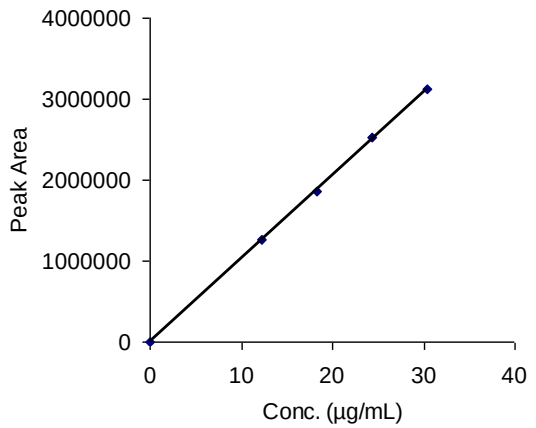

Figure 1. Standard curve of lomefloxacin.

Preparation of Stock Solution

About 12.2 mg of standard Lomefloxacin was accurately weighed and taken in a 100mL volumetric flask containing 50 mL of mobile phase. It was dissolved and made up to the mark with the mobile phase. This was solution of 0.122 mg/mL and it was used as stock solution for the subsequent experiments. The pH of the preparation was adjusted to 3.0 with phosphoric acid.

Determination of wavelength of maximum absorption (λ_{max})

The stock solution (0.122mg/mL) was diluted to 10 times to give a solution of 0.0122 mg/mL or 12.2 µg/mL solution and 5mL of this solution was taken in a cuvette and scanned from 200 to 400 nm with Shimadzu Double Beam UV-VIS 160A Spectrophotometer. The mobile phase was used as the blank. Lomefloxacin was found to absorb maximum radiation at 287 nm.

Calibration Curve

The series of standard solutions prepared by diluting the stock solution with mobile phase and the concentrations were 0.0122 mg/mL, 0.0183 mg/mL, 0.0244 mg/mL and 0.035 mg/mL.

Table 2. Statistical analysis for recovery experiment for lomefloxacin by the proposed method.

Sample no. (N)	Active added (mg) (X)	Active recovered* (mg) (Y)	% of recovery*
1	0	100.85 ± 0.35	100.85 ± 0.55
2	10	108.75 ± 0.58	98.86 ± 0.65
3	20	116.98 ± 0.88	97.48 ± 0.88
4	30	135.44 ± 0.25	104.18 ± 0.48
5	40	137.76 ± 0.75	98.40 ± 0.89

*Data are shown as mean ± SD, where n = 5

20 µL of each of the lomefloxacin preparation was injected into the sample-loading chamber and the peak areas were determined. The calibration curve was constructed by plotting peak area versus concentration (Figure 1).

Assay in the Dosage Form

Five different marketed lomefloxacin eye drops formulations (Coded as pp1, pp2, pp3, pp4 and pp5) were selected for analysis. 1 mL (equivalent to 3 mg lomefloxacin) of each pharmaceutical product was taken in 100 mL volumetric flask and then the volume was made up to the mark by adding mobile phase so that the final concentration became 0.03 mg/mL (30 µg/mL). The solution was filtered with milli pore filter paper. 25 mL of the solution was taken and processed as per the procedure under calibration curve. 20 µl of the sample solution was injected into the HPLC column and chromatogram taken (Figure 2). The potency of the five different marketed lomefloxacin was then determined from the calibration curve (Table 1).

Method Validation and Recovery Experiment

The experiment was carried out according to Wahed *et al.,* 2007. The linearity of the analytical method is its ability to elicit test results that will be directly or by a well-defined mathematical transformation, proportional to the concentration of the analyte in samples within a

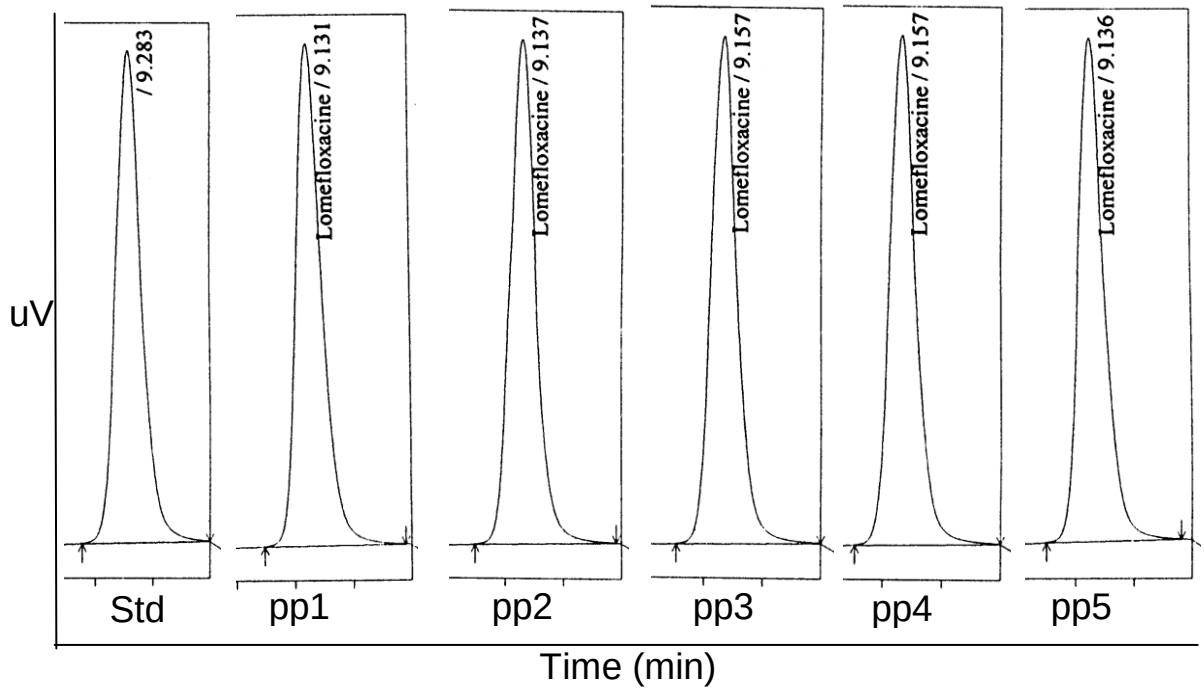

Figure 2. Chromatogram of standard and pharmaceutical dosage form (eye drops) of Lomefloxacin by proposed HPLC method. X-axis indicates retention time in minutes and Y-axis indicates area under the curve. (Std=Standard preparation, pp1=Product of Pharmaceutical Industry-1, pp2= Product of Pharmaceutical Industry-2, pp3= Product of Pharmaceutical Industry-3, pp4= Product of Pharmaceutical Industry-4, pp5= Product of Pharmaceutical Industry-5).

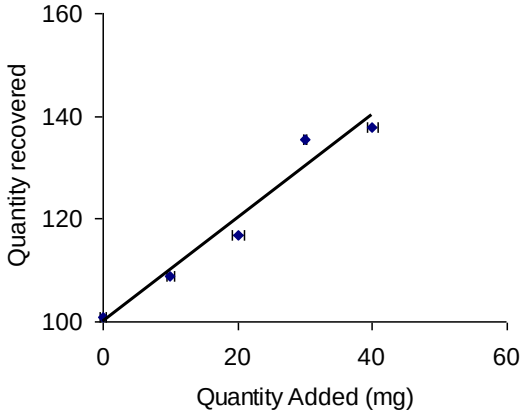

Figure 3. Data of the recovery experiment of lomefloxacin.

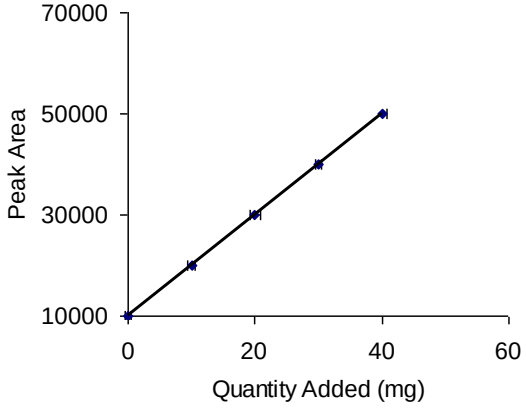

Figure 4. Data of the recovery experiments of lomefloxacin.

given range. The linearity is important to demonstrate that the response of the measurement of detector system is linear over the range of interest of the method. This was determined by means of calibration graph using increasing amounts of a standard solution of lomefloxacin.

These standards were tested five times in agreement with the International Conference on Harmonization (ICH) (ICH, Q2B, 1995). A calibration curve was constructed and the proposed method was evaluated by statistical method as described in the equation. The accuracy of the assay method was measured by analyzing five spiked samples of lomefloxacin (standard addition of 0 mg, 10 mg, 20 mg, 30 mg and 40 mg of lomefloxacin). Accuracy was determined by means of recovery experiments, by spiked addition of active drugs. The amount recovered was plotted against the theoretical amount which produced a straight line of slope 1 and intercept zero. The accuracy was expressed as mean ± SD (Figure 3 and Figure 4).

Recovery experiments

Method validation and recovery experiments were conducted following our previous paper[6] and that of other investigators (ICH, Q2B, 1995; Rahman and Ahmad, 2007, Paul *et al.*, 2002,). Eye drops solution equivalent to 100 mg were taken in five 100 mL volumetric flask and 0.0, 10, 20, 30 and 40 mg standard lomefloxacin were added to the volumetric flasks, respectively. Then the content of the each of the volumetric flask were diluted with mobile phase and the potency was determined by the proposed method. The data of the recovery experiment were statistically analyzed to study the reproducibility and validity of the proposed method (Table 2) and for this the following equation was used:

$$\%\,\mathrm{Recovery} = \frac{N\Sigma XY - \Sigma X.\Sigma Y}{N\Sigma X^2 - \Sigma X.\Sigma X} \times 100$$

Statistical Analysis: The results are expressed as mean ± SD, where n=5.

RESULTS AND DISCUSSION

Lomefloxacin is slightly soluble in water and insoluble in alcohol. So, sodium dihydrogen phosphate was used as solvent and after long trial and error method 0.025 M phosphoric acid and acetonitrile as a ratio of 80:20 was established as mobile phase. The proposed method is simple, rapid and handy because the mobile phase is a binary mixture and easy to prepare. It does not require any complex calculation. The standard calibration obtained by plotting known concentrations of lomefloxacin against corresponding area values was found to be linear (Figure 1). Beer's law was found to be obeyed in the concentration range of 10 to 50 µg/mL. The proposed method has been successfully applied for the estimation of lomefloxacin in commercial eye drop preparations (coded as pp1, pp2, pp3, pp4 and pp5), the result of which were represented in

Table 1. In order to confirm the reproducibility and validity of the proposed method recovery experiments were conducted following our previous papers and that of other investigator (Rahman and Ahmad, 2007; Paul *et al.*, 2002). The recovery was almost 100% (99.14%) which showed that the method developed suffered no interference from common excipients used in the formulation (Table 2). The lower values of standard deviation reflect the validity and reproducibility of the proposed method. The values of different statistical parameters indicate that the proposed method is accurate enough to give a valid and acceptable result. The percent recovery was calculated by followed equation described in the text.

CONCLUSION

The present method thus offers several advantages in terms of simplicity, rapidity and accuracy over many of the known procedures and can be applied for the quality control analysis of lomefloxacin in pharmaceutical preparations.

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