

Validation of UV Spectrophotometric Method for Determination of Lomefloxacin in Pharmaceutical Dosage Form

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SUMMARY. A simple and reproducible method was developed for the assay of lomefloxacin in tablets. The excipients in the commercial tablet preparation did not interfere with the assay. Beer's law is obeyed in the range 2.0 - 9.0 $\mu\text{g}\cdot\text{mL}^{-1}$ at λ_{max} 280 nm. The molar absorptivity was calculated. Six triplicate analyses of solutions containing six different concentrations of the examined drug were carried out and gave a mean correlation coefficient 0.9997. The proposed method was applied to the determination of the examined drug in coated tablet and the results demonstrated that the method is equally accurate, precise and reproducible as the official methods.

RESUMEN. "Validación del método por espectrofotometría UV para la determinación de Lomefloxacin en la dopaje de formas farmacéuticas". Fue desarrollado un método simple y reproducible para el ensayo de lomefloxacin en comprimidos. El excipiente en la preparación del comprimido comercial no interfirió con el ensayo. La ley de Beer se cumple en el rango de 2,0-9,0 $\mu\text{g}\cdot\text{mL}^{-1}$ a una λ_{max} de 280 nm. Se calculó la absorptividad molar. Se llevaron a cabo seis análisis por triplicado de soluciones conteniendo seis diferentes concentraciones de la droga examinada y se obtuvo un coeficiente de correlación promedio de 0,9997. El método propuesto fue aplicado a la determinación de la droga en examen en comprimidos recubiertos y los resultados mostraron que el método es igualmente seguro, confiable y reproducible como los métodos oficiales.

INTRODUCTION

The quinolone antimicrobials are the class of inhibitors of bacterial topoisomerases that has been developed most fully for clinical use in human medicine. Initial members of the class had their greatest potency against Gram-negative bacteria, but newly developed members have exhibited increased potency against Gram-positive bacteria and soon agents will be available with additional activity against anaerobic bacteria. Lomefloxacin is highly efficient and safe in the treatment of infections of the urinary tract and respiratory tract ¹.

The 4-quinolone nucleus possessing a nitrogen at position 1, a carboxyl group at position 7, and a ketone at position 4 is responsible for the antibacterial action of the quinolones. Fluoroquinolones, quinolones with at the fluorine attached at the 7 position, have an expanded spec-

trum of activity and increased antibacterial potency compared to non-fluorinated compounds.

Chemically lomefloxacin (CAS 98079-52-8) is a third generation fluoroquinolone (1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolone (Fig. 1).

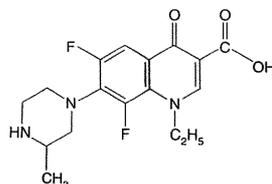


Figure 1.
Chemical structure
of lomefloxacin (LFLX).

No official method is available for the assay for pure drug and its formulations. Some methods such as high-performance liquid chromatography (HPLC) with UV detection for the determination of lomefloxacin in human plasma ²,

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high-performance liquid chromatography for determination of lomefloxacin in biological fluids ³, spectrophotometric method based on the formation of complex with PrCl₃ in acid media ⁴ were developed for determination of lomefloxacin.

Spectrophotometric assay for determination for other fluoroquinolones, as sparfloxacin ⁵, levofloxacin ⁶, and gatifloxacin ⁷ has been described, but no method for lomefloxacin as raw material and in pharmaceutical formulations had been previously described.

The aim of this work was to develop a simple and reproducible spectrophotometric procedure for the determination of lomefloxacin in raw material and coated tablets.

EXPERIMENTAL

Samples

The lomefloxacin reference substance (assigned purity 99.6%) and coated tablets containing lomefloxacin was supplied by Pfizer-Pharmacia (São Paulo, Brazil). The tablets were claimed to contain 400 mg (as anhydrous base) of drug and the following excipients: lactose, magnesium stearate, hydroxypropyl cellulose, carboxymethylcellulose calcium, hydroxypropylmethyl cellulose, polyethylene glycol 400. The lomefloxacin reference substance, as well as the tablets, were always kept protected from light.

Reagents and solvents

All other chemicals were of analytical grade. The water used was freshly distilled.

Instrumentation and conditions

Spectral and absorbance measurements were made with a JASCO 7800 UV-VIS and INTRAL-AB 5100 detector with 10 mm quartz cells at 280 nm. The solutions were prepared in distilled water.

Procedure

Lomefloxacin reference standard

Solutions of the lomefloxacin reference standard (200 µg.mL⁻¹) were prepared by accurately weighing 20 mg lomefloxacin reference substance into 100 mL volumetric flask. Aliquots (3 mL) of the lomefloxacin standard solution were transferred volumetrically into 100 mL flask with final concentration of 6 µg.mL⁻¹. The determination was conducted in triplicate.

Assay of lomefloxacin in tablets

To analyze the concentration of lomefloxacin tablets, twenty tablets were weighed to obtain

the average tablet weight. The tablets were ground up and 400 mg were transferred to a 1000 mL volumetric flask; 500 mL water were added and the flask was shaken for 20 minutes by mechanical shaker followed by addition of distilled water to volume (final concentration of 1.2 mg.mL⁻¹). Aliquots of 5 mL of this solution were transferred to a 100 mL volumetric flask and distilled water was added to volume to give an estimated concentration of 60 µg.mL⁻¹. After the solution was diluted 1:10 to give a final estimated concentration of 6.0 µg.mL⁻¹. This solution was prepared six times and the absorbance of each solution was determined at 280 nm. All determinations were conducted in triplicate.

Method validation

The accuracy and precision of the assay, as well linearity of the calibration curve, were determined (Fig. 2) ^{8,9}. Having established the quantitative relationships between the parameters studied, and knowing the predictive performance of their association model, a linear simple regression by the least squares method was applied. The statistical analysis was calculated by ANOVA.

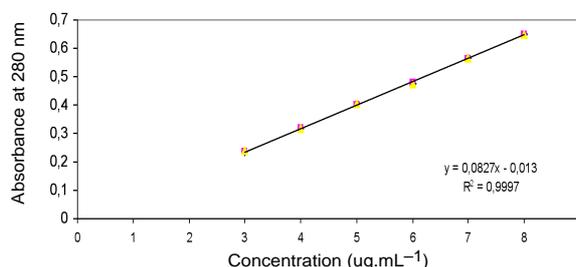


Figure 2. Calibration curve constructed for lomefloxacin from standard solutions at six concentration levels in the range 3 to 8 µg/mL.

The statistical accuracy was determined by adding known amounts of lomefloxacin reference standard to the samples at the beginning of the process. Amounts of 1.0, 2.0 e 3.0 mL of lomefloxacin standard solution (200 µg.mL⁻¹) and a aliquot of 10 of this solution (60 µg.mL⁻¹) were transferred to 100 mL volumetric flasks, respectively, R₁, R₂ and R₃ (Table 1). The percentage recovery for added lomefloxacin standard was calculated using the equation proposed by AOAC ⁸.

RESULTS AND DISCUSSION

The development of spectrophotometry methods for the determination of drugs has increased considerable in recent years because of their importance in pharmaceutical analysis. The

| | Amount of reference (200 (g.mL-1)) | | % Recovery* |
|----|------------------------------------|------------|-------------|
| | Added | Absorbance | |
| R1 | 1.00 | 0.669 | 99.25 |
| R2 | 2.00 | 0.840 | 100.80 |
| R3 | 3.00 | 1.008 | 100.60 |

Table 1. Experimental values obtained in the recovery test for lomefloxacin in dosage form. * Each value is the mean of three analysis.

calibration curve for lomefloxacin was obtained plotting the peak area versus concentration. Linearity was found to be in the range of 3.0 - 8.0 $\mu\text{g.mL}^{-1}$ with significantly high value of correlation coefficient $r^2 = 0.9997$; the representative equation was $y = 0.0827x - 0.013$ (Fig. 2). The quantitative parameters for determination of lomefloxacin in pharmaceutical dosage form are listed in Table 2.

| Parameter | Results |
|-----------------------------------|------------|
| λ_{max} (nm) | 280 |
| ϵ | 24629.76 |
| Beer's law ($\mu\text{g/mL}$) | 2.0 to 9.0 |
| Regression equation: $y = a + bx$ | |
| Intercept (a) | 0.0827 |
| Slope (b) | 0.013 |
| Correlation coefficient (r^2) | 0.9997 |
| n | 6 |

Table 2. Quantitative parameters for determination of lomefloxacin in dosage form.

The coefficient of variation (CV) on the basis of the absorbances for six triplicate measurements found to be between 0.09 and 0.80%. Lomefloxacin tablets (400 mg) were analyzed and the results obtained can be seen in Table 3. The percentage of gotten pureness was of 99.27% and the coefficient of variation of 0.45%.

The assays were validated by means of the analysis of variance, as described in official literature^{9,10}. This developed method presented no parallelism deviation and no linearity deviation ($P < 0.05$). The precision and accuracy of the assay were demonstrated. The accuracy express the agreement between the accepted value and the value found. The recoveries obtained showed that a high accuracy of the presented method (Table 1).

| | Dosage form (mg) | Experimental amount (mg) | Purity (%) |
|---|------------------|--------------------------|------------|
| 1 | 400.00 | 395.41 | 98.84 |
| 2 | 400.00 | 397.84 | 99.45 |
| 3 | 400.00 | 394.60 | 98.64 |
| 4 | 00.00 | 397.30 | 99.32 |
| 5 | 400.00 | 398.11 | 99.52 |
| 6 | 400.00 | 399.46 | 99.86 |

Table 3. Determination of lomefloxacin in dosage form. * Each value is the mean of three analyses.

CONCLUSION

The obtained and statistical parameters for determination of lomefloxacin in raw material and coated tablets demonstrate that the proposed UV spectrophotometry method by is simple, accurate, fast and precise. The method showed acceptable linearity and accuracy. The proposed method is highly sensitive; therefore it could be used easily for the routine analysis of pure drugs and their vial formulations.

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