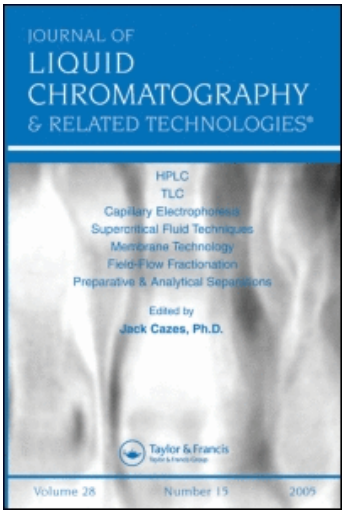

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Determination of Levofloxacin in a Pharmaceutical Injectable Formulation by Using HPLC and UV Spectrophotometric Methods

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Abstract: The objective of this study was to develop simple and rapid methods for the determination of levofloxacin (LVF) using high performance liquid chromatography and UV spectrophotometry. LVF was separated on a reversed phase Phenomenex® C18 column (150 × 4.6 mm i.d., particle size 4 μm), under isocratic elution with a mixture of water:acetonitrile:phosphoric acid 0.025 M, pH adjusted to 3.0 with triethylamine (60:20:20, v/v/v), as the mobile phase at room temperature and at a flow rate of 1.0 mL/min. The UV detector was set to 294 nm and UV-vis spectrophotometer at 292 nm. Both methods allowed the quantification of LVF and showed good linearity ($r > 0.999$) in the studied range. The relative standard deviations (RSD) were 0.66 and 1.0% for HPLC and UV spectrophotometry, respectively. The accuracy determined with HPLC was 100.68 and with UV spectrophotometry was 99.61%. The methods were validated through the parameters of linearity, accuracy, precision, specificity, and robustness. The two proposed

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methods enabled a quantitative determination of LVF in pharmaceutical injectable formulation.

Keywords: Levofloxacin, RP-HPLC, UV spectrophotometry, Validation

INTRODUCTION

The quinolones compromise a series of broad spectrum, synthetic antibacterial agents derived from nalidixic acid. They were discovered casually in 1962 and since then have been essentially used in the treatment of a wide range of infectious diseases.[1]

The fluorquinolones are quinolones with fluorine at position 6 of the naphthyridine ring. Published structure activity data showed that the fluorine atom helps to broaden their activity spectrum against both Gram-negative and Gram-positive pathogens.[1]

Levofloxacin (LVF), (s)-(−)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid, is a new quinolone antibacterial agent and the S (−) enantiomer of the racemate ofloxacin.[2,3]

Only one analytical method has been published describing the HPLC assay for levofloxacin in an injectable formulation.[2] Most of the reported methods involve a troublesome mobile phase (buffers) and difficult detection methods (fluorescence or mass detectors).[1] The objective of this study was to develop and validate rapid, economical, and sensitive analytical methods for the determination of levofloxacin in injectable preparations by using UV spectrophotometry and HPLC-UV. The results obtained from the spectrophotometer and HPLC have been statistically compared.

EXPERIMENTAL

Chemicals

Samples of levofloxacin, 100 mg bags containing 5 mg of each mL of solution, were obtained from commercial sources from the respective manufacturers and used within their shelf life period. All solvents were HPLC grade and all reagents were analytical grade. Acetonitrile was obtained from Tedia®. Phosphoric acid was obtained from Quimex® and triethylamine from Merck®. Water was purified with Milli-Q® Plus, Millipore System. All solvents and solutions were filtered through a membrane filter or filtration units (Millipore® Millex-HV filter units, 0.22 μm pore size) and degassed before use.

Instrumentation and Analytical Conditions

The HPLC method was performed on a Shimadzu® LC-10AD HPLC system, equipped with UV-vis detector model SPD-10A. Data integration was performed using Shimadzu® Class-VP software. The analytical column was a reversed phase Phenomenex® Synergi Fusion-RP (150 × 4.60 mm i.d., 4 μm particle size). All analyses were done at room temperature (24 ± 2°C) under isocratic conditions. The mobile phase consisted of a mixture of water:acetonitrile:phosphoric acid 0.025 M, pH adjusted to 3.0 with triethylamine (60:20:20, v/v/v). The flow rate was 1.0 mL/min and the volume of injection was 20 μL. The UV detection was made at 294 nm.

The spectrophotometric method was performed on a UV-vis Genesys 2 Spectronic at 292 nm and using 1.0 cm quartz cells.

Preparation of the Standard Solutions

HPLC Method

Levofloxacin reference standards (99.97%), of 20 mg, accurately weighed, were transferred to 20 mL volumetric flasks and dissolved in mobile phase (final concentration 1000 μg/mL). The resulting solution was sonicated during 10 min and diluted to obtain a final concentration of 10 μg/mL. All solutions were prepared fresh daily.

Spectrophotometric Method

Levofloxacin reference standards of 25 mg, accurately weighed, were transferred to 50 mL volumetric flasks and dissolved in distilled water (final concentration 500 μg/mL). The resulting solution was diluted to obtain a final concentration of 5 μg/mL.

Preparations of the Sample Solutions

Injectable preparations of 2.0 mL (5000 μg/mL) accurately measured were transferred into a flask to give a concentration of 100 μg/mL. The resulting solution was diluted in mobile phase to obtain a final concentration of 10 μg/mL.

Method Validation

The methods were validated according to the International Conference on Harmonisation guidelines for validation of analytical procedures.[4] Analysis of variance (ANOVA) was used to verify the validity of the methods.

Linearity

The calibration curve was obtained with five concentrations of the standard solution (5–15 μg/mL for the HPLC method, and 4–12 μg/mL for the spectrophotometric method). The solutions were prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Intra-day precision was evaluated by assaying the sample, at the same concentration and during the same day. Six sample solutions (10 μg/mL for the HPLC method and 8 μg/mL for the spectrophotometric method) were prepared and assayed. The intermediate precision was studied by comparing the assays on different days (3 days).

Accuracy

For the spectrophotometric method, the accuracy was determined by recovery of known amounts of levofloxacin reference standard added to the samples at the beginning of the process. The levofloxacin injectable preparation of 2.0 mL (5000 μg/mL), accurately measured, was tranferred to a 100 mL volumetric flask and dissolved in distilled water (final concentration 100 μg/mL). Aliquots of 3.0 mL of this solution were tranferred into 50 mL volumetric flasks containing 1.0, 2.0, and 3.0 mL of levofloxacin standard solution at 100 μg/mL, and distilled water was added to make up final volume concentrations of 8, 10, and 12 μg/mL. All solutions were prepared in triplicate and assayed. The percentage of recovery of added levofloxacin standard was calculated using the equation proposed by AOAC.[5]

For the HPLC method, the accuracy was determined by the assay of three concentrations of the sample solution (8, 10, and 12 μg/mL) in triplicate.

Specificity

The specificity was determined by the HPLC method. Standard solutions (10 μg/mL) were submitted to accelerated degradation by heat (121°C for 15 min), by the addition of 0.1 N hydrochloric acid for 17 h at 50°C, by the addition of 0.1 N sodium hydroxide for 17 h at 50°C, by the addition of 30% hydrogen peroxide for 2 h at 50°C, and by exposure to ultraviolet light ($\lambda = 254$ nm) for 24 h (10 cm) at room temperature. The objective was to verify that none of the degradation products of the analyte interferred with the quantitation of the drug.

Robustness

The robustness of the HPLC method was determined by analysis of samples under a variety of conditions by making small changes in the pH (3.0–3.4), in the percentage of acetonitrile (2%) in the mobile phase, in the flow rate (0.8–1.2 mL/min), in the temperature of the column (25–30°C), and by changing the wavelength (292–296 nm).

LOQ and LOD

Limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified, and the limit of quantitation (LOQ) was defined as the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy.

RESULTS AND DISCUSSION

The development of the HPLC method for the determination of drugs has received considerable attention in recent years because of its importance in analysis of quality control of pharmaceutical formulations. A reversed-phase HPLC method was proposed as a suitable method for the estimation of levofloxacin in a pharmaceutical dosage form. The chromatographic conditions were adjusted in order to provide a good assay performance. Mobile phase selection was based on peak parameters, run time, ease of preparation, and cost. Figure 1 shows a typical chromatogram obtained from the analysis of a sample solution of levofloxacin using the proposed method. As shown in

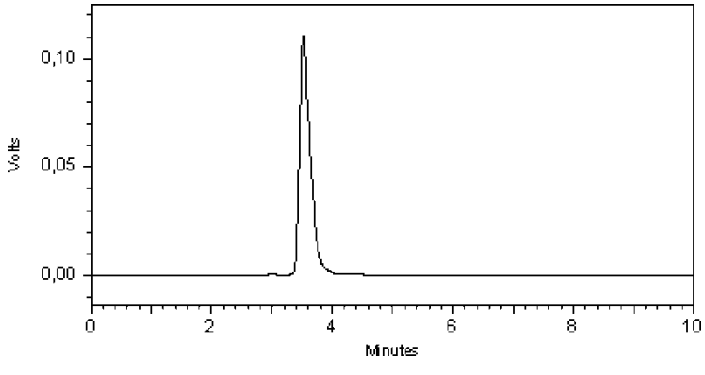

Figure 1. Chromatogram of levofloxacin 10 μg/mL. Phenomenex® C18 column (150 × 4.6 mm i.d, 4 μm). Water:acetonitrile:phosphoric acid 0.025 M, (pH 3.0) (60:20:20, v/v/v) as mobile phase. Flow rate 1.0 mL/min. 294 nm.

this figure, levofloxacin formed a symmetrical peak, well separated from the solvent front. The retention time observed (3.52 min) allowed a rapid determination of the drug.

The limit of detection and limit of quantitation were obtained using the slope and standard deviation of the intercept from three curves and determined by the linear regression line. The LOD and LOQ obtained were 0.1 and 0.3 $\mu g\ mL^{-1}$, respectively. These values were also used in an experimental assay confirming the calculation.

The proposed UV method allowed a rapid and economical quantitation of levofloxacin without any time consuming sample preparation. Moreover, the spectrophotometric methods involved relatively simple instrumentation. The λ_{max} was found to be 292 nm after running the absorption spectra of levofloxacin in an aqueous solution. This wavelength was used for all measurements.

The calibration curves for levofloxacin were constructed by plotting concentration versus peak area and showed good linearity in the 5–15 $\mu g/mL$ range. The representative linear equation was $y = 101830x + 113675$, with a highly significant correlation coefficient ($r = 0.9999$) for the method. For the spectrophotometric method, the calibration curves showed good linearity in the 4–12 mg/mL range. The representative linear equation was $y = 0.0796x + 0.0126$ ($r = 0.9998$).

Accuracy and precision of the proposed method were assessed by performing triplicate analyses of the standard solutions. Three different concentrations diluted in the mobile phase, were prepared in the linear range of the calibration curve and analyzed to determine intra-day and inter-day variability and accuracy. The inter and intra-day precision were calculated as the RSD%. The results and the mean values were shown in Table 1, demonstrating good precision and accuracy.

Table 1. Intra-day and inter-day accuracy and precision data of RP-HPLC and UV methods for levofloxacin

Theoretical concentration ($\mu g\ mL^{-1}$)	Intra-day[a]		Inter-day[a]	
	Accuracy (%)	Precision (RSD%)	Accuracy (%)	Precision (RSD%)
HPLC				
8	100.34	0.18	101.21	0.53
10	101.47	0.22	101.90	0.72
12	100.25	0.84	101.48	1.19
UV				
8	100.00	1.22	101.61	0.95
10	99.50	0.47	100.91	0.43
12	99.33	0.65	101.08	0.17

[a]Mean of five determinations for each concentration.

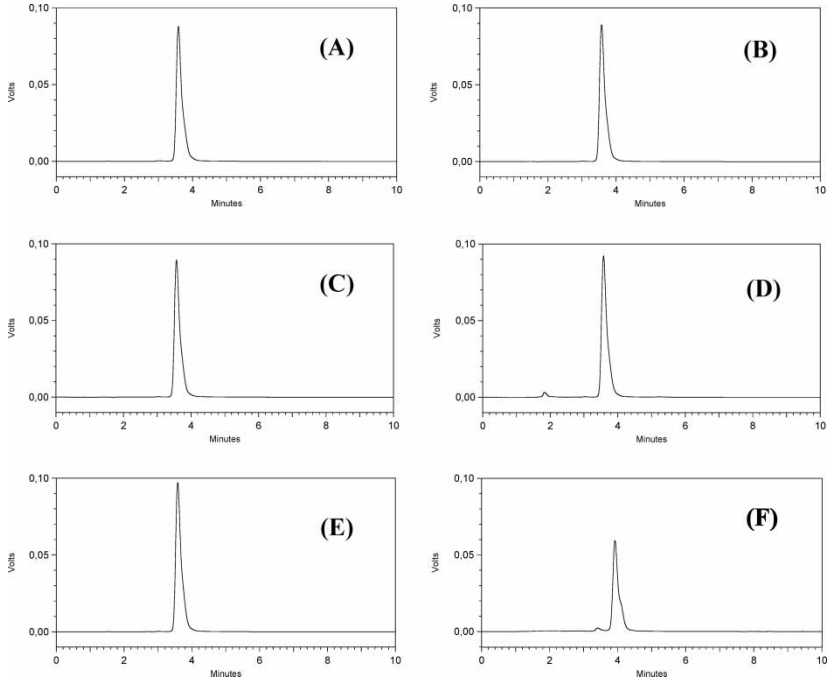

Figure 2. HPLC representative chromatograms of levofloxacin at standard conditions (A), submitted to accelerated degradation by HCl 0.1 N (B), NaOH 0.1 N (C), H_2O_2 30% (D), by heat (E) and submitted to photolysis by ultraviolet light for 24 h (F).

No degradation product of levofloxacin was observed after acid, base, peroxide, and temperature stress. After ultraviolet light exposure, a decrease in the peak area of the levofloxacin standard substance was observed, confirming the results investigated by Yoshida et al. for the photodegradation of levofloxacin in an aqueous solution (Figure 2).[6]

Table 2. Results of the determination of levofloxacin in injectable solution by HPLC and UV methods

Method	Sample (μg)	Experimental amount[a] (μg)	(%)	(RSD%)
HPLC	10	10.03	100.34	0.66
		10.14	101.47	
		10.02	100.25	
UV	10	10.22	102.19	1.0
		10.09	100.99	
		10.02	100.20	

[a]Mean of five determinations for each concentration.

Table 3. Comparison of the two methods for the determination of levofloxacin in injectable formulation, ANOVA test

Amount labelled (100%)	HPLC (%)	UV spectrophotometric (%)
Amount found[a]	100.68	101.12
RSD	0.67	0.99

[a]Average of six experiments. ANOVA, ($p < 0.01$); $F_{calc} = 2.27$; $F_{tab} = 5.18$.

When chromatographic conditions were intentionally altered, no significant effect was observed in the chromatogram, confirming the robustness of the method.

The intra-day precision obtained by the proposed methods showed a RSD of 0.66 and 1.0% for HPLC and UV spectrophotometry, respectively. Inter-day variability was calculated and showed a RSD of 0.81 and 0.51% for HPLC and UV spectrophotometry, respectively. The accuracy of the HPLC method was 100.68% and the accuracy of the UV spectrophotometric method was 99.61%, confirming the accuracy of the proposed methods. The results are expressed in Table 2.

The proposed analytical methods were compared using statistical analysis. ANOVA was applied and did not reveal a significant difference between the experimental values obtained by the two methods. The calculated F-value ($F_{calc} = 2.27$) was found to be less than the tabled F-value ($F_{tab} = 5.18$) at a 1% significance level (Table 3).

CONCLUSION

The proposed methods enable a quantitative determination of levofloxacin in pharmaceutical injectable preparations. The preparation of samples is easy and efficient. All calibration curves were found to be linear with correlation coefficientes above 0.999. Analytical results of the samples were in accordance with those of the standard solutions at the same concentrations. Both methods are fast, precise, accurate, and efficient, and can be used in routine analyses in quality control laboratories.

REFERENCES

1. Santoro, M.I.R.M.; Kassab, N.M.; Singh, A.K.; Kedor-Hackmam, E.R.M.J. Quantitative determination of gatifloxacin, levofloxacin, lomefloxacin and pefloxacin fluoroquinolonic antibiotics in pharmaceutical preparations by high performance liquid chromatography. J. Pharm. Biomed. Anal. **2006**, *40* (1), 179–184.
2. Böttcher, S.; Baum, H.V.; Hoppe-Tichy, T.; Benz, C.; Sonntag, H.-G. An HPLC assay and a microbiological to determine levofloxacin in soft tissue, bone, bile and serum. J. Pharm. Biomed. Anal. **2001**, *25*, 197–203.

3. Altiokka, G.; Atkosar, Z.; Can, N.O. The determination of levofloxacin by flow injection analysis using UV detection, potenciometry, and conductometry in pharmaceutical preprarations. J. Pharm. Biomed. Anal. **2002**, *30*, 881–885.
4. International Conference on Harmonisation (ICH) of Technical Requirements for the Registration of Pharmaceutical for Human Use, Validation of Analytical Procedures: Methodology (ICH-Q2B), November, 1996, 1–8.
5. *Official Methods of Analytical Chemists of AOAC*, 15th Edn.; AOAC: 1990; XVII.
6. Yoshida, Y.; Sato, E.; Moroi, R. Photodegradation products of levofloxacin in aqueous solution. Arzneimittelforschung. **1993**, *43* (5), 601–606.

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