ORIGINAL ARTICLE

OPTIMIZATION OF LEVOFLOXACIN ANALYSIS BY RP-HPLC USING MULTIVARIATE CALIBRATION TECHNIQUE

M. SAEED ARAYNE, NAJMA SULTANA* AND FARHAN AHMED SIDDIQUI

Department of Chemistry, University of Karachi, Karachi-75270, Pakistan
Department of Pharmaceutical Chemistry, Faculty of Pharmacy
University of Karachi, Karachi-75270, Pakistan

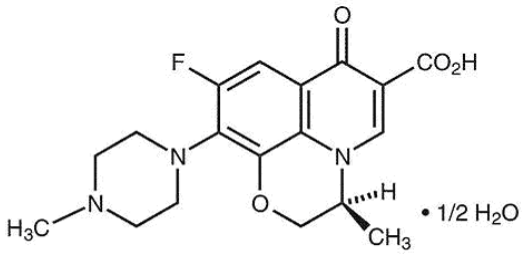
ABSTRACT

A rapid and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method for the analysis of levofloxacin from bulk materials, dosage formulations and human serum is described. This isocratic method employs, a Nucleosil, C_{18} (10µm, 25 cm x 0.46 cm) column with a mobile phase of water and acetonitrile (6:5), where in phosphoric acid was used to adjust the pH to 2.9 and propylparaben as an internal standard. Optimization of levofloxacin analysis was carried out using multivariate calibration technique and detector response was recorded at five different wave lengths. A linear response ($r > 0.9999$) was observed in the range of 40 to 10000 ng ml^{-1}. The method shows good recoveries, intra and inter-day relative standard deviations were less than 1.2 %. Validation parameters as specificity, accuracy and robustness were also determined. The method can conveniently be used for analysis of levofloxacin pharmacokinetic levels in human serum and pharmaceutical formulations.

Keywords: Levofloxacin, propylparaben, multivariate calibration, HPLC, human serum, pharmaceutical formulation.

INTRODUCTION

Levofloxacin, (–)-(*S*)-9-fluoro-2.3-dihy-dro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate (structure) (Tanaka *et al.,* 1933) is the active levo-isomer of racemic ofloxacin. It possesses wide spectrum of antibacterial activity against both Gram-positive and Gram-negative bacteria, as well as atypical pathogens such as *Mycoplasma*, *Chlamydia* and *Legionella* (Eliopoulos *et al.,* 1993). It exerts antibacterial activity via antagonism of the interaction between bacterial DNA gyrase and cell DNA (Shen *et al.,* 1993).



Structure

Levofloxacin is used for the treatment of infections of the respiratory and urinary tract, skin and soft tissues. Like other fluoroquinolones, it exerts antibacterial effect not exclusively in the blood but particularly in the inflamed tissue (Ungerstedt *et al.,* 1991, Müller, 2000).

Corresponding author: Email: arayne@gawab.com

No official (pharmacopoeia) method was found for the assay of levofloxacin in its formulations. However, many studies have been reported for the determination of levofloxacin in pharmaceuticals and biological fluids (Gong *et al.,* 2000). Numerous methods have been reported for the determination of levofloxacin in plasma, urine, bile and tissues using capillary electrokinetic chromatography (de Boer *et al.,* 2001; Hernandez 2000). Several HPLC methods have been reported by different authors for the analysis of levofloxacin (Djabarouti *et al.,* 2004; Hernandez *et al.,* 2002; Wong *et al.,* 1997; Bottcher *et al.,* 2001; Immanuel and Hemanth, 2001; Liang *et al.,* 2002; Nguyen *et al.,* 2004) by solid phase spectrofluorometry (McCourt *et al.,* 2003), with UV and fluorescence detection (Ferdig *et al.,* 2004; Ballesteros *et al.,* 2002; Carlucci 1998; Belal, 1999; Hernandez-Arteseros, 2002; Immanuel and Kumar, 2001; Cheng 2002; Epinosa-Mansilla, 2005).

Some of these methods are quite complex and lengthy, mostly because of the sample preparation, like solid phase extraction (Epinosa-Mansilla, 2005), liquid–liquid extraction (Liang *et al.,* 2002), protein precipitation combined with time consuming centrifugation steps (Nguyen *et al.,* 2004) or expensive automated sample analysis requiring column switching (Cheng *et al.,* 2002). These methods are lengthy and time consuming for routine use in clinical pharmacology, chemical and pharmaceutical laboratories.

The purpose of the present work is to develop and validate a simple liquid chromatography method with UV detection for quantitative analysis of levofloxacin in raw materials, pharmaceutical preparations and from human serum.

Multivariate HPLC-UV technique

Under optimized conditions the applied numerical method provides considerable resolving power, sensitivity, rapidity, and low cost for the quantitative analysis, quality control and routine analysis of subject compounds. The mathematical algorithm of this approach is explained as follows:

If the absorbance of an analyte (X) is measured at five wavelengths set (λ = 260, 265, 270, 275 and 280 nm), following equations can be written for each data set,

$$\begin{aligned} A_{\lambda 260} &= a * C_X + k_1 \\ A_{\lambda 265} &= b * C_X + k_2 \\ A_{\lambda 270} &= c * C_X + k_3 \\ A_{\lambda 275} &= d * C_X + k_4 \\ A_{\lambda 280} &= e * C_X + k_5 \end{aligned} \quad (1)$$

Where A_λ represent the peak area of the analyte, a, b, c, d, e are the slopes of linear regression functions of the analyte, k_1, k_2, k_3, k_4 and k_5 are the intercepts of linear regression functions at five selected wavelengths and C_X represents the concentration of analyte. The five-equation system (1) can also be rewritten as:

$$A_T = a * C_X + b * C_X + c * C_X + d * C_X + e * C_X + K_T \quad (2)$$

which can be simplified to

$$A_T = C_X (a + b + c + d + e) + K_T \quad (3)$$

Where A_T and K_T represents the sum of peak area obtained and sum of intercepts of regression equations at five-wavelength set respectively. The concentration of the X analyte in a solution of unknown concentration can be calculated by using the following equation,

$$C_x = \frac{AT - KT}{(a + b + c + d + e)} \quad (4)$$

In this case, the multivariate chromatographic calibration mood contains the use of linear regression function based on the relationship between peak area ratio and concentration mood is replaced for the prediction of unknown concentration of analyte.

EXPERIMENTAL

Materials

Levofloxacin hemihydrate was a kind gift from "Platinum Pharma Pvt. Limited (Pakistan). Dynaquin 250 mg and 500 mg tablet (Barret Hodgson Pvt. Limited) were purchased from local market. Phosphoric acid was of analytical reagent grade and purchased from Merck (Darmstadt, Germany). Double distilled water was used in all experiments. Acetonitrile was of HPLC grade and also was purchased from Merck (Darmstadt, Germany).

Equipment

The development and validation of the assay was performed on a HPLC system consisting of an LC-10 AT VP Shimadzu pump, SPD-10AV VP Shimadzu UV visible detector and a Nucleosil, C_{18} (10μm, 25 cm x 0.46 cm) column was used for separation. The chromatographic and integrated data were recorded using a CBM-102 communication Bus Module Shimadzu on a P-IV PC loaded with Class–GC software.

Chromatographic conditions

The separation was carried out under isocratic elution with mobile phase acetonitrile and water (5:6). The pH of this mobile phase was adjusted to 2.9 with phosphoric acid (85%). Mobile phase was degassed through sonication and vacuum prior to use. The flow rate of mobile phase was 1.0 ml min^{-1} and the injection volume was 10 μL. The chromatographic runs were carried out at 25°C. UV detections were performed at 260, 265, 270, 275 and 280 nm. Retention time for levofloxacin was 3.8 minutes and for internal standard (propylparaben) was 7.8 minutes.

Preparation of solutions

Levofloxacin stock solution was prepared by adding a quantity of levofloxacin hemi hydrate equivalent to 10 mg of levofloxacin to a 100 mL volumetric flask, dissolving in acetonitrile: water (1:1) and filling to the mark with the same solvent. Working standard solutions were prepared from stock solution with dilution solvent to obtain seven different concentrations within the range 40, 50, 100, 1000, 2000, 5000 and 10000 ng ml^{-1}.

Preparation of pharmaceutical formulation sample solution

Twenty tablets of each formulation were individually weighed, powdered and an amount equivalent to 100 mg of levofloxacin was diluted to 100 mL with acetonitrile: water (1:1), filtered through 0.45-μm membrane filter (Westboro, MA, USA). The first 5 ml of the filtrate were discarded. Aliquots were diluted as required.

Procedure for human serum

The availability of Levofloxacin from pooled human serum was determined by the stated chromatographic conditions. Blood samples of ten healthy volunteers were collected. Volunteers (age ranging from 22-25 years) were non-smoker, not involved in any strenuous activity and not taking any other medicaments. Multiple blood samples (10 ml) were collected in evacuated glass tubes through an indwelling cannula placed in the forearm veins

or directly from vein. The blood was then slightly shaken and centrifuged at 10,000 rpm for 10 minutes and the plasma was separated. To 1.0 ml of plasma, 10.0 ml of acetonitrile was added. The mixture was vortexed for one minute and than centrifuged for 10 minutes at 10,000 rpm. The supernatant was filtered by 0.45-micron pore size membrane filter. An aliquot serum sample was fortified with levofloxacin to achieve final concentration 40, 50, 100, 1000, 2000, 5000 and 1000 ng/ml. These solutions were stored at –20°C and analyzed regularly at interval of two days up to eight days for serum drug analysis. For analysis of each sample 10 µl of solution was injected and chromatographed.

RESULTS AND DISCUSSION

Validation of the test procedure

Reversed-phase assay was found most appropriate as compared to direct phase for initial testing. Initial method development was conducted on a Nucleosil, C_{18} (10µm, 25 cm x 0.46 cm) column for separation at room temperature. This column provides efficient and reproducible separations of non polar compounds while minimizing solvent usage. When C_8 column was used with same mobile phase, then during analysis of levofloxacin in serum peak of the later was not well resolved with serum peak. Moreover, capacity factor and theoretical plates were much less than C_{18} column, while analysis time decreased. Consequently, C_{18} column was selected for method development and remains the column utilized in validation of the method. A UV scan of levofloxacin showed a maximal absorbance at or near 275 nm. In order to optimize wavelength and minimize instrumental error, other wavelengths like 260, 265,270 and 280 were also selected (table 3).

Preliminary method development of suitable isocratic conditions to resolve levofloxacin with propylparaben on the C_{18} column was conducted with acetonitrile-water as the mobile phase. A mobile phase of acetonitrile-water (5:6 v/v) in which pH was adjusted with phosphoric acid to 2.9 was found most appropriate to provide a reproducible, baseline and resolved peak. Further more these conditions allowed for separation of levofloxacin from internal standard. The chromatographic conditions were optimized with respect to specificity, resolution and time of analysis. The specificity of the method was established through the study of resolution factor of levofloxacin peak from the internal standard peak. Peaks were identified using retention times compared with those of standards.

The objective of validation of an analytical procedure is to demonstrate that it is adequate for its intended purpose. To meet current pharmaceutical regulatory guidelines (i.e., ICH 1995, ICH 1997, USP 2000 and Eu.Ph 2005), a number of parameters were investigated in order to validate analytical method such as precision, accuracy, specificity, linearity and robustness study.

Specificity

The selectivity of assay was determined by placebo analysis. Placebo tablets were prepared using maize starch, sodium starch glycolate, lactose, magnesium stearate and hydroxypropylmethylcellulose under identical conditions as the original formulations containing levofloxacin. These placebo tablets were treated in the same manner as the normal samples, and samples were injected to study any possible interference of the excipient for the selectivity of levofloxacin separation. The chromatograms obtained from placebo with internal standard showed no interference of excipient (fig. 1). Levofloxacin reference standard along with internal standard is shown in fig. 2.

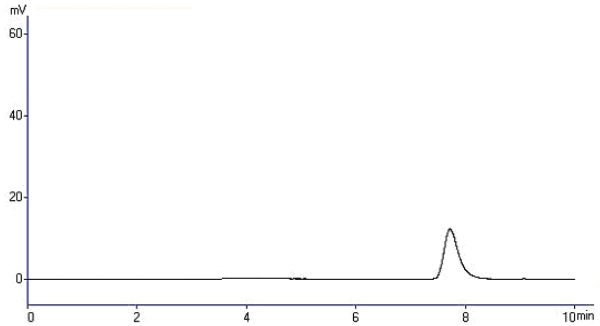


Fig. 1: Typical chromatogram of placebo with internal standard.

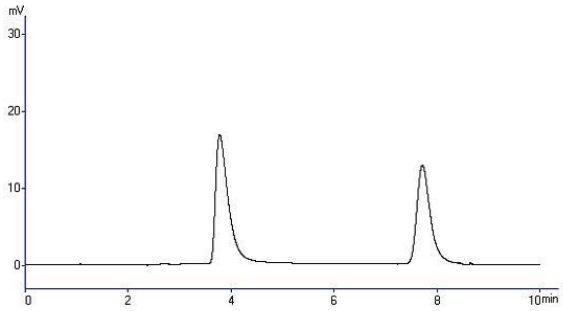


Fig. 2: Typical chromatogram of reference standard with internal standard

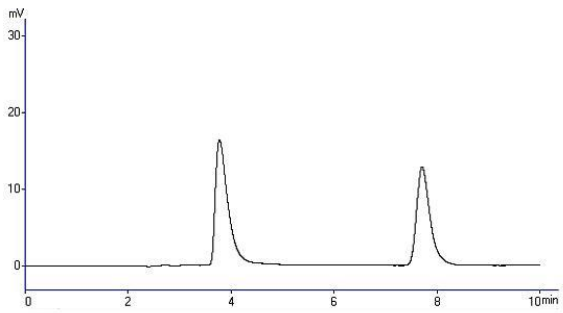


Fig. 3: Typical chromatogram of pharmaceutical formulation with internal standard.

Fig. 3 shows that the peak of levofloxacin was satisfactory enough and shows no interference from exipients. A typical chromatogram of blank serum with internal standard and levofloxacin are shown in figs. 4 and 5. Analysis of levofloxacin at different wavelengths (multivariate) is shown in fig. 6.

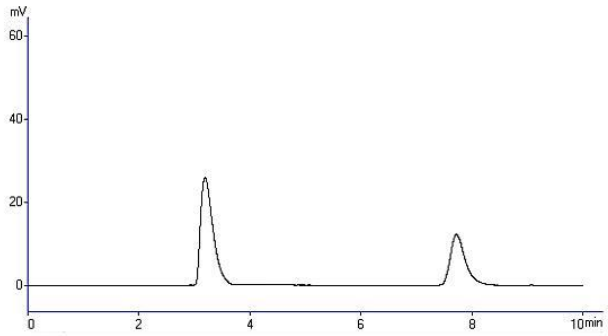


Fig. 4: Typical chromatogram of blank human serum with internal standard.

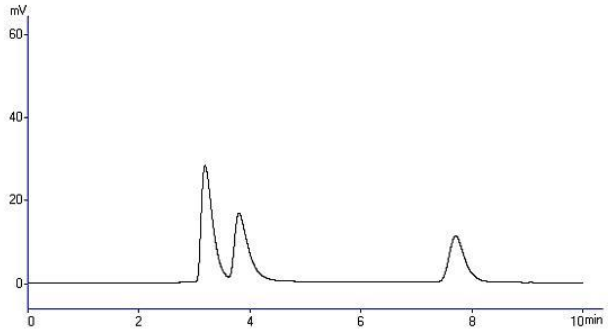


Fig. 5: Typical chromatogram of human serum with levofloxacin and internal standard.

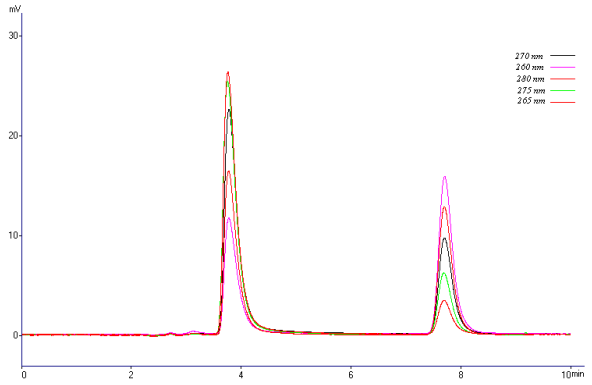


Fig. 6: Typical chromatogram of analysis of levofloxacin at different wave length (Multivariate technique).

Linearity of response

A linearity relationship was evaluated across the range of analytical procedure. The linearity was performed over the range of 40–10000 ng ml^{-1}. The regression line was calculated as A = B Cx + D, where Cx was levofloxacin concentration (ng ml^{-1}) and A was the response (peak area expressed as AUC). D is intercept and B is slope of regression line as mentioned in table 3. The calibration curve was obtained using the linear least squares regression procedure. The representative linear equation was at five different wave lengths as shown in table 3. The correlation coefficient (*r*) value is 0.9999.

Since the coefficient of correlation is not suitable as a general acceptance criterion to the linearity performance of an analytical procedure (Ermer and Ploss 2005) the relative standard error of slope was used as a parameter with respect to precision of the regression. This parameter should be comparable to the relative standard deviation obtained in precision studies in the given concentration range (Ermer and Ploss, 2005).

Limits of detection and quantitation

The limits of detection (LOD) and quantitation (LOQ) were calculated in accordance with the 3.3*s*/*m* and 10*s*/*m* criteria, respectively, where *s* is the standard deviation of the peak area (for five replicates) for the sample and *m* is the slope of the calibration curve, determined from linearity investigation (ICH 1995, ICH 1997). The LOD and LOQ were calculated and found as shown in table 3. Linear regression data (table 3) of reference drug pharmaceutical formulation and human serum shows less RSD and "r' found good and prove that method is accurate and can be used in any wavelength as mentioned in table 3.

Accuracy

Accuracy was determined by analyzing a sample of known concentration (reference standard solutions) and comparing the measured value with the true value, and using the method of standard additions.

Table 1 summarizes the accuracy and precision results, expressed as percent recovery and relative standard deviation (R.S.D) for both approaches. The method showed good recovery, % recovery of the method at different wave length and multivariate data is shown in table 1. Table 2 summarizes the % recovery of drug at different wave lengths and also using multivariate mathematical calculations.

Stability of the levofloxacin solutions

The stability of the levofloxacin solutions during time was investigated. The levofloxacin working standard solution with its concentration 100ng ml^{-1} onto the HPLC system at 0, 2, 6, 9 and 12 h after preparation was injected. The data indicates that after 12 h the chromatographic peak area of levofloxacin was not found significantly changed. It can be concluded that levofloxacin solution was stable at least for 12 h after its preparations. This solution was also analysed onto the HPLC system at 2, 4, 6 and 8 day when stored at –20^{o}C and no significant change was found when compared with freshly prepared samples.

Table 1: Intra and interday precision and accuracy of the method

	<——— Intraday ———>			<——— Interday ———>		
Drug	Drug	%Recovery	%RSD	Drug	%Recovery	%RSD
added (mg)	found (mg)	(Mean)	-	found (mg)	(Mean)	-
800 (80%)	809.33	100.87	0.433	8006.25	100.78	1
1000 (100%)	1008.66	101.17	0.302	1016.16	101.61	0.99
1200 (120%)	1222	101.83	1.044	1214.41	101.20	1.17

Table 2: % recovery of levofloxacin at different wavelengths range

Concentration	<——————————% Recovered ——————————>					
ng/ml	260	265	270	275	280	Multi.HPLC [a]
40	98.46	99.18	100.34	97.73	99.60	132.87
50	100.35	99.27	98.42	98.92	100.03	126.83
100	100.53	100.05	100.76	98.81	99.64	112.62
1000	100.56	99.96	100.42	99.48	100.50	100.40
2000	100.29	99.56	100.76	99.71	100.23	99.47
5000	100.01	99.88	99.94	99.93	99.49	98.63
10000	102.36	99.18	100.53	101.82	102.62	100.36

[a]Multivariate HPLC data

Table 3: Linear regression functions and their statistical parameters at the five-wavelength sets

Drug	λ	Regression equation	*r*	LOD (ng/ml)	LOQ (ng/ml)	%R.S.D.*
Active	260	A = 194.25 Cx -59.430	0.9999	1.118	3.728	0.939
	265	A = 199.14 Cx -1428.3	0.9999	0.898	2.992	0.758
	270	A = 193.44 Cx -802.98	0.9999	0.233	0.776	0.196
	275	A = 209.52 Cx -401.16	0.9999	1.339	4.551	1.146
	280	A = 192.32 Cx -101.68	0.9999	1.05	3.501	0.884
Tablet	260	A = 198.33 Cx -3771.2	0.9999	0.176	0.535	1.039
	265	A = 197.67 Cx -364.83	0.9999	0.106	0.319	0.636
	270	A = 194.31 Cx -1135.4	0.9999	0.078	0.237	0.459
	275	A = 212.95 Cx -4387.0	0.9999	0.178	0.539	1.128
	280	A = 196.67 Cx -4640.1	0.9999	0.077	0.232	0.446
Serum	260	A = 195.10 Cx -622.37	0.9999	0.093	0.282	0.548
	265	A = 199.41 Cx -2357.4	0.9999	0.081	0.245	0.487
	270	A = 196.27 Cx -3132.9	0.9999	0.100	0.302	0.585
	275	A = 210.11 Cx –1302.0	0.9999	0.077	0.233	0.487
	280	A = 193.97 Cx -1212.4	0.9999	0.038	0.116	0.222

* = % Relative Standard Deviation

Precision

Precision may be measured as repeatability, intermediate precision and reproducibility. Reproducibility refers to the use of analytical procedure in different laboratories. The repeatability (intra-day) and intermediate (inter-day) precision of the method were demonstrated by analyzing levofloxacin reference standard solutions of concentration 40, 50, 100, 1000, 2000, 5000, and 10000 ng ml^{-1} during 1 day and each of 4 days under the same conditions. The results obtained from these analyses are listed in table 1 as

mean recovery (%). Table 1 shows there were no significant differences between assay results either within-day or between days, implying that the precision of the proposed method was good (R.S.D less than 1.2%).

Repeatability and especially reproducibility of the proposed method was investigated by analysis of drug synthetic mixture in different laboratories. The results of repeatability and reproducibility study are listed in table 1. When results from assay of the same drugs by different laboratories were compared and variation was not found more than 1%.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters, and provides an indication of its reliability during normal usage. In order to the robustness study of the proposed method deliberate modifications in pH values of the mobile phase were made. It can be seen that in every employed condition, the chromatographic parameters are in accordance with established value (FDA, 1994). A change of $\pm$0.2 unit of pH around 2.90 (pH of mobile phase) had no considerable impact on chromatographic performance but when pH of mobile phase was changed more than $\pm$0.2 unit then chromatographic condition vanquished. When pH was increased, shape of peaks disturbed, tailing enhanced and analysis duration also increased a lot, when pH was decreased analysis time decreased and peak of levofloxacin was merged with serum peak.

Tailing (symmetry) factor (T) and number of theoretical plates (N) were calculated by formulas: $T = W/(2Wa)$ and $N = 5.545(t_R/W_{1/2})^2$, where W is the peak width at 5% height from baseline, Wa the peak front edge width at the same height and $W_{1/2}$ is the peak width at half height. According to the data of robustness test study we proposed criteria for system suitability test (tailing factors <2, numbers of theoretical plates > 4500 and repeatability (R.S.D) of five replicate must not be more than 1.5, (peak area for replicate analysis). It is used to verify that the resolution and repeatability of the system are adequate for the analysis intended.

Application of the method to pharmaceutical analysis

The two tablets were studied here, both are available in the local market Pharmaceutical formulation was presented as Dynaquin^{TM} 250 (% recovery 99.2 $\pm$ 1.23) Dynaquin^{TM} 500 (% recovery 99.6 $\pm$ 1.41) mg tablet.

CONCLUSIONS

A new reversed-phase liquid chromatographic method for the determination of levofloxacin contents in pharmaceutical formulations and also in human serum has been developed and validated which has ability to determine levofloxacin concentration up to 40 ng/ml. The developed method is very simple and results obtained confirm suitable accuracy, specificity and precision. Therefore, the developed RP-HPLC method has proved to be suitable for levofloxacin determination in routine quality control analysis as well as R&D. Samples were stable at 25°C for 8 days and no tedious clean up was required. There was no interference of excipients in the examined products, thus no additional extraction or separation procedures were required.

REFERENCES

Ballesteros O, Vilchez JL and Navalon A (2002). Determination of the antibacterial ofloxacin in human urine and serum samples by solid-phase spectrofluorimetry *J. Pharm. Biomed. Anal.,* **30**: 1103-1110.

Belal F, Al-Majed AA and Al-Obaid A.M (1999). Methods of analysis of 4-quinolone antibacterials. *Talanta.,* **50**: 765-786.

Bottcher S, von Baum H, Hoppe-Tichy T, Benz C and Sonntag HG (2001). An HPLC assay and a microbiological assay to determine levofloxacin in soft tissue, bone, bile and serum. *J. Pharm. Biomed. Anal.,* **25**: 197-203.

Carlucci G (1998), Analysis of fluoroquinolones in biological fluids by high- performance liquid chromatography *J. Chromatogr., A* **812**: 343-367.

CDER, Reviewer Guidance for Validation of Chromotographic Methods, Center of Drug Evaluation and Research – FDA (1994).

Cheng FC, Tsai TR, Chen YF, Hung LC and Tsai TH (2002). Pharmacokinetic study of levofloxacin in rat blood and bile by microdialysis and high-performance liquid chromatography. *J. Chromatogr. A.,* **961**: 131-136.

de Boer T, Mol R, de Zeeuw RA, de Jong GJ and Ensing K (2001). *Electrophoresis.,* **22**: 1413-1418.

Djabarouti S, Boselli E, Allaouchiche B, Ba B, Nguyen AT, Gordien JB, Bernadou JM, Saux MC and Breilh D (2004). Determination of levofloxacin in plasma, bronchoalveolar lavage and bone tissues by high-performance liquid chromatography with ultraviolet detection using a fully automated extraction method. *J. Chromatogr. B.,* **799**: 165-172.

Eliopoulos GM, Eliopoulos CT, Hoope DC and Wolfson JS (1993) (Editors). In: *Quinolone Antibacterial Agents*, American Society for Microbiology, Washington, pp.161-193.

Epinosa-Mansilla A, de la Pena AM, Gomez DG and Salinas F (2005). HPLC determination of enoxacin, ciprofloxacin, norfloxacin and ofloxacin with photoinduced fluorimetric (PIF) detection and multiemission scanning: application to urine and serum. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.,* **822**: 185-193.

Ermer J and Ploss HJ (2005). Validation in pharmaceutical analysis, Part II: central importance of precision to establish acceptance criteria and for verifying and improving the quality of analytical data. *J. Pharm. Biomed. Anal.*,**37**: 859-870.

European Pharmacopoeia, Directorate for the Quality of Medicines of the Council of Europe, 5th ed., Strassbourg, France, 2005.

Ferdig M, Kaleta A, Vo TDT and Buchberger W (2004). Improved capillary electrophoretic separation of nine (fluoro)quinolones with fluorescence detection for biological and environmental samples. *J. Chromatogr. A.*, **1047**: 305-311.

Gong QJ, Qiao JL, Du LM, Dong C and Jin WJ (2000). Recognition and simultaneous determination of ofloxacin enantiomers by synchronization — 1st derivative fluorescence spectroscopy. *Talanta.,* **53**(2): 359-365.

Hernandez-Arteseros JA, Barbosa J, Compano R and Prat MD (2002). Analysis of quinolone residues in edible animal product .*J. Chromatogr, A.*, **945**: 1-24.

Hernandez M, Borrull F and Calull M (2000). Determination of quinolones in plasma samples by capillary electrophoresis using solid-phase extraction. *J. Chromatogr. B.,* **742**: 255-265.

Hernandez M, Borrull F and Calull M (2002). Using nonaqueous capillary electrophoresis to analyze several quinolones in pig kidney samples. *Electrophoresis*, **23**: 506-511.

Immanuel C and Kumar AKH (2001). Simple and rapid high-performance liquid chromatography method for the determination of ofloxacin concentrations in plasma and urine. *J. Chromatogr. B.,* **760**: 91-95.

International Conference of Harmonization Q2A: Text on Validation of Analytical Procedures. US FDA Federal Register, vol. 60, March 1995, pp.11260-11266.

International Conference of Harmonization Q2B: Validation of Analytical Procedures—Methodology. US FDA Federal Register, vol. 62, May 1997, pp.27463-27467.

Liang H, Kays MB and Sowinski KM (2002). Separation of levofloxacin, ciprofloxacin, gatifloxacin, moxifloxacin, trovafloxacin and cinoxacin by high-performance liquid chromatography: application to levofloxacin determination in human plasma. *J. Chromatogr. B.,* **772**: 53-63.

McCourt J, Bordin G and Rodriguez AR (2003). Development of a capillary zone electrophoresis-electrospray ionisation tandem mass spectrometry method for the analysis of fluoroquinolone antibiotics. *J. Chromatogr. A.,* **990**: 259-269.

Müller *Adv* M. (2000) Microdialysis in clinical drug delivery studies. *Drug Deliv. Rev.,* **45**: 255.

Nguyen HA, Grellet J, Ba BB, Quentin C and Saux MC (2004). Simultaneous determination of levofloxacin, gatifloxacin and moxifloxacin in serum by liquid chromatography with column switching. *J. Chromatogr. B.,* **810**: 77-83.

Nguyen HA, Grellet J, Ba BB, Quentin C and Saux MC (2004). Simultaneous determination of levofloxacin, gatifloxacin and moxifloxacin in serum by liquid chromatography with column switching. .*J. Chromatogr. B Anal. Technol. Biomed. Life Sci.,* **810**: 77-83.

Shen LL, Hoope DC and Wolfson JS (1993). (Editors). In: *Quinolone Antibacterial Agents*, American Society for Microbiology, Washington, pp.77-95.

Tanaka M, Kurata T, Fujisawa C, Oshima Y, Aoki H, Okazaki O and Hakusui H (1993). Mechanistic study of inhibition of levofloxacin absorption by aluminum hydroxide. *Antimicrob. Agents Chemother.,* **37**: 2173.

Ungerstedt U (1991). Microdialysis - Principles and applications for studies in animals and man. *J. Intern. Med.,* **230**: 365.

United States Pharmacopoeia/National Formulary, United State Pharmacopoeial Convention (24th ed.), United States Pharmacopoeia/National Formulary, Rockville, MD (2000).

Wong FA, Juzwin SJ and Flor SC (1997). Rapid stereospecific high-performance liquid chromatographic determination of levofloxacin in human plasma and urine. *J. Pharm. Biomed. Anal.,* **15**: 765-771.

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