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A new HPLC method for the assay of levofloxacin and its application in drug-metal interaction studies

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Abstract

A simple reversed phase HPLC method has been successfully developed and validated for the quantitative determination of levofloxacin (LVX) in bulk material, pharmaceutical formulation and serum. Purospher STAR C18 (25 cm x 4.6 mm, 5 µ m) was used. The mobile phase MeOH: H2O (70:30, v/v) was delivered at a flow rate of 1 Ml min^{-1}. The proposed method is specific, accurate with a recovery of 100 ± 0.02. The detection limits were 2 ng with an RSD ± 0.1 (n=6).The anticipated method is applicable to routine analysis of LVX in pharmaceutical formulations and human serum samples. The method was applied to study the In vitro availability of levofloxacin in presence of various elements essential to the human body, like magnesium, calcium, chromium, copper Zinc and iron. The availability of Levofloxacin in presence of these elements was depressed up to 21% in simulated gastric juice, while up to 5% in pH 7.4 and 27% in simulated intestinal juice.

Keywords: Levofloxacin, HPLC, Metals interactions; Human serum.

Introduction

Levofloxacin is (-)-(S)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-pipe-razinyl)-7oxo-7H-pyrido [1, 2, 3-de]-1, 4-benzoxazine-6-carboxylic acid hemihydrate (structure 1), having molecular formula $C_{18}H_{20}FN_3O_4.^{½}$ H2O and molecular weight 370.38. It is a chiral fluorinated carboxy quinolone a pure (-)-(s)-enantiomer of the racemic, drug substance ofloxacin.[1-3] It is a synthetic broad spectrum antibacterial agent active against Gram-positive and Gram-negative bacteria including *Staphylococcus species*; *Streptococcus pneumoniae*[4, 5], *Streptococcus pyogenes*, *Streptococcus hemolyticus*, *Entero bacter species*, *Escherichia coli*[6], *Salmonella*, *Klebsiella*, *Serratia*, *Enterococcus*, *Proteus species* and other glucose non fermentative rods. Moreover levofloxacin has shown antibacterial activity against *Chlaymydia trachomatis*.[7] The main mechanism of action of levofloxacin is inhibition of DNA gyrase[8], it is twofold stronger than that of its l-isomer ofloxacin.[9, 10]

Already reported assay methods use determination of levofloxacin by HPLC at 294 nm using MeOH: H2O in pH 3.6 acetate buffer: THF (37:11:37:15) as mobile phase. The linear range was 6.34-12.68 µg/ml with a recovery of 99.66%.[11, 12] Bottcher *et. al*., reported the determination of drug in serum, bile soft tissue and bone, wherein the samples were prepared by protein precipitation with acid and methanol, which yielded high recoveries (for serum and bile >98% and for bone and soft tissue >90 %). The compounds were separated on a reverse phase column with an acidic mobile phase containing triethylamin and elute was monitored by fluorescence detector.

The method was linear over the concentration range of 0.1-40 µg/ml.[13] On the other hand drug concentrations in plasma, urine, and bile of renal failure were also determined by high-performance liquid chromatography. The area under the blood concentration-time curve (AUC) in each renal-failure rate was calculated and correlated.[14]

Almost all previously reported methods used expensive solvents in their mobile phase which may increase the cost of the method and time consuming. In this paper, the RPHPLC method with UV detection for determination of levofloxacin has been developed. The main purpose of our study was to develop a simple, reliable and economical method to determine LVX in a relatively short time with high linearity and low cost in bulk drug, pharmaceutical formulations and in serum. This method was further apply to determination of LVX in four different marketed formulations and also apply to in-vitro interactions of LVX with essential and trace metals along with levofloxacin as these can reduce the intestinal absorption and thus the effectiveness of the drug.[15]

Materials and Method

All chemicals used in this method were HPLC grade from Merck. Reference standard levofloxacin was a gift from Aventis Pharma (Pakistan) Limited, Karachi while dosage formulation TAVANIC (manufactured by Aventis Pharma and containing 250 mg of levofloxacin) were purchased from the market. Water used for the mobile phase and stock solution preparations was suitably purified for HPLC (double distilled and de-ionized through "GFL Water STILL" purification system and Elgacan B114 deionizer.

Chromatographic system and conditions

Development of this rapid and sensitive method used chromatographic system from Shimadzu consisted of LC 10AT VP pump, SPD 10AVP UV-Visible detector and CBM 102 communication bus (integrator). All the separation and analysis were done on Purospher STAR C18 (25 cm x 4.6 mm, 5 µ m) at the ambient temperatures. Sample was introduced in the described chromatographic system by Rheodyne injector valve with a 20 µL sample loop.

Mobile phase

Mobile phase used for the separation, and analysis was MeOH: H_2O (70:30 v/v). Mobile phase was filtered through 0.45 µm Millipore filter, mixed thoroughly & degassed by sonication for 20 minutes. The flow rate throughout the analysis was 1 mL/min and UV detection was performed at 294 nm.

Standard solution's preparations

Concentration ranges of standard solutions of levofloxacin for calibration curve were from 0.1 ppm to 0.5 ppm using MeOH: H_2O (70:30). Calibrated glassware's from Pyrex were used for the solution and mobile phase preparation. For drug-metal interaction studies, levofloxacin solutions were of 10 ppm concentration prepared in the above solvent, while the metal solutions were also of 10 ppm prepared in deionized water.

Drug-plasma solution

Blood samples were collected from healthy volunteers and immediately centrifuged at 3,000 rpm for 10 min. The supernatant obtained was stored at -200C. After thawing, serum was deproteinated by addition of acetonitrile and spiked daily with working solutions to furnish the desired concentrations of levofloxacin. Samples (20 µL) were then injected for HPLC analysis and % R.S.D and % recovery values were evaluated.

Metal interaction studies

Drug & metal standard solutions (each 10 ppm) were mixed in the ratio of 3:1 (v/v) in pH 1, 4 and in 7.4 and were allowed to react at 37°C and 40°C on a water bath for 3 hours. Resulting reaction mixture was filtered through 4.5 µm and then introduced in HPLC system by means of the Rheodyne injection system with 20 µL loop using the above-described chromatographic system. Remaining concentration of the drug after complexation with metals was calculated by means of Peak area reduction in each metal interaction.

Result and Discussion

The newly developed method for the determination of levofloxacin has been validated and holds well for the determination of drug in raw materials, dosage formulations, and even in fluids containing other medicaments. The method has also been tested for the determination of drug in presence of various metal ions, which are either already present in body fluids or may be co-administered as a result of simultaneous drug therapy in different body environment. The methods have been successful in determining the drug in concentrations as low as 2 ng, with a retention time of only 2.1 minutes. Both linearity & metal interaction studies were carried out by described chromatographic conditions. Linear relation was

observed over a concentration range of 2-10 ng with peak area (figure 1). The recovery details clearly indicate the accuracy of the method and reproducibility data shows the precision and validity of the method. Retention time slightly varies after complexation with metals.

In the presented investigation, best results were achieved using the described column specifications and solvent system (table 1). Under the same experimental conditions, duration of separation is shorter and observed peak symmetry is better. For linearity and drug metal, interaction studies the best mobile phase used was methanol water (70:30 v/v) at flow rate 1 ml /minute. After establishing the peak optimizing conditions; linearity, reproducibility, accuracy, precision, LOQ and LOD were determined. LOQ and LOD were found to be 1 & 2 ng respectively, which shows good statistical agreements.

The developed method was validated by carefully studying ICH guideline the variation of results obtained by inter operators (four operators) as well as inter & intraday variation's wherein lowest statistical result variations were observed. Similarly, the results of precision and accuracy of RP-HPLC determination are given in table 2 and 3. The important statistical values, such as standard deviation, % recoveries are given in each corresponding table. Standard deviations have very small-required values, which confirm precision of developed RP-HPLC method.

Table 1: System suitability parameters

Parameters	Selected	Difference	Retention time(tR)	Theoretical plates (N)	Tailing factor (T)	Resolution(Rs)
Flow rate	0.8	-0.2	2.3	1763	1.12	5.4
	1	0	2.1	1788	1.17	5.5
	1.2	+0.2	1.9	1788	1.16	5.6
Mobile phase	60	-10	2.3	1763	1.22	5.4
	70	0	2.1	1768	1.12	5.5
	80	+10	1.9	1738	1.17	5.6
Wavelength	290 nm	-4	1.9	1763	1.44	5.4
	294 nm	0	2.1	1787	1.12	5.5
	298 nm	+4	2.3	1786	1.154	5.6

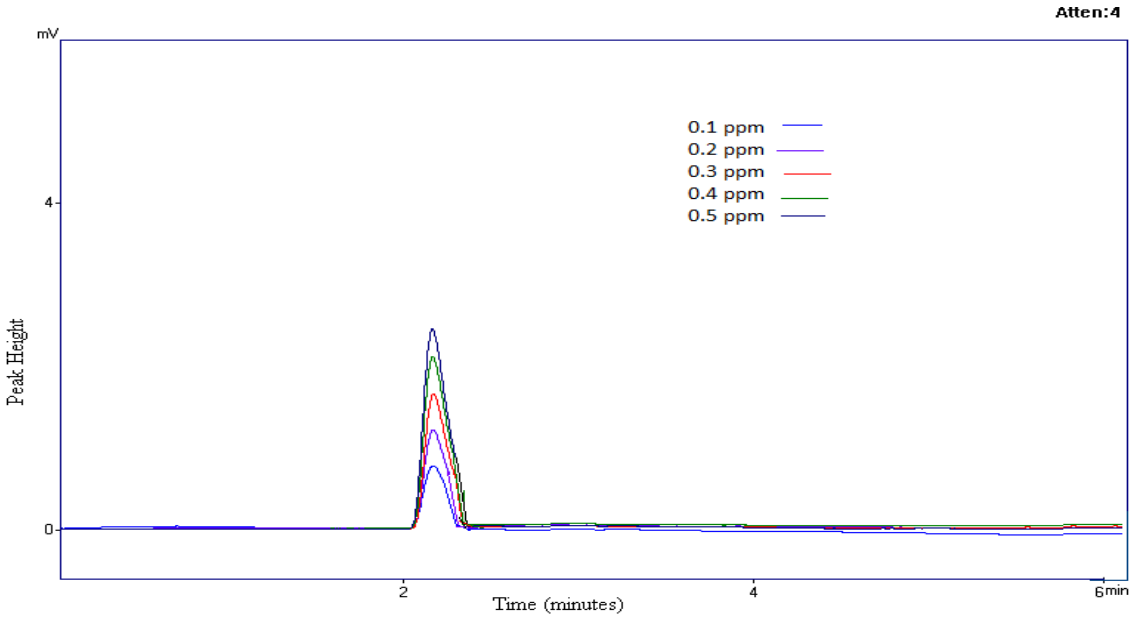

Figure 1: Linearity range of levofloxacin

Table 2: Reproducibility and accuracy of levofloxacin analysis

Drugs	Conc.* ngmL^{-1}	Conc. Found ngmL^{-1}	% Recovery	STD
Levofloxacin	2	1.991 ± 0.077	99.54	0.54
	4	4.035 ± 0.035	100.88	0.25
	6	6.097 ± 0.097	101.61	0.68
	8	8.121 ± 0.121	101.52	0.86
	10	10.00 ± 0.000	100	0

Table 3: Inter day and intraday precision of Levofloxacin

Drugs	**Conc. injected**	**in API**		**in Formulations**		**In serum**	
	ng mL^{-1}	%RSD	%Recovery	%RSD	%Recovery	%RSD	%Recovery
Levofloxacin	2	0.06	99.44	1.077	100.9	1.077	100.9
	4	0.04	100.5	1.074	101.0	1.074	101.05
	6	0.01	99.87	1.292	99.4	1.292	99.49
	8	0.09	99.02	0.602	101.1	0.602	101.18
	10	0.01	100.8	1.055	98.9	1.055	98.94

The developed method was then applied to drug metal interaction studies and their results further confer the accuracy and validity of the method. These drug metal interactions were carried out at 37°C and 40°C using simulated gastric juice, blood and intestinal pH same described experimental conditions. Representative chromatograms are presented in figures 2 & 3. The results for the metal interaction of drug and recovery of drugs after complexation are given in table 4.

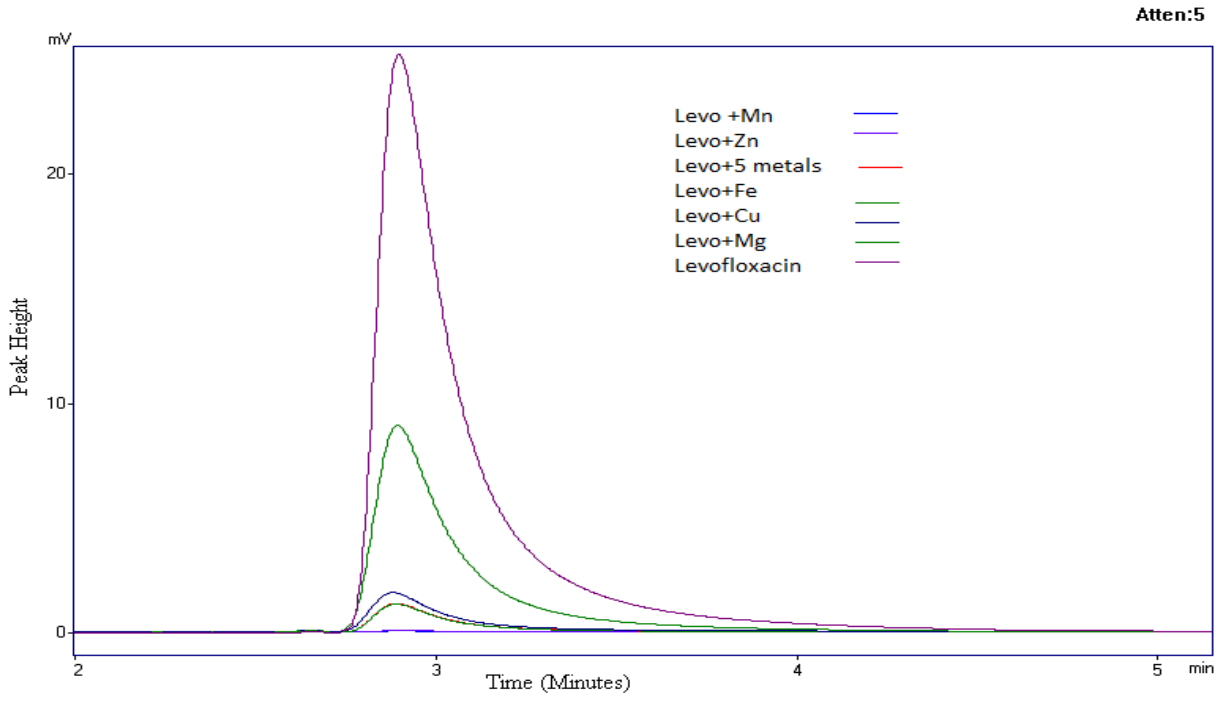

Figure 2: Levofloxacin Metal Interaction at 37°C

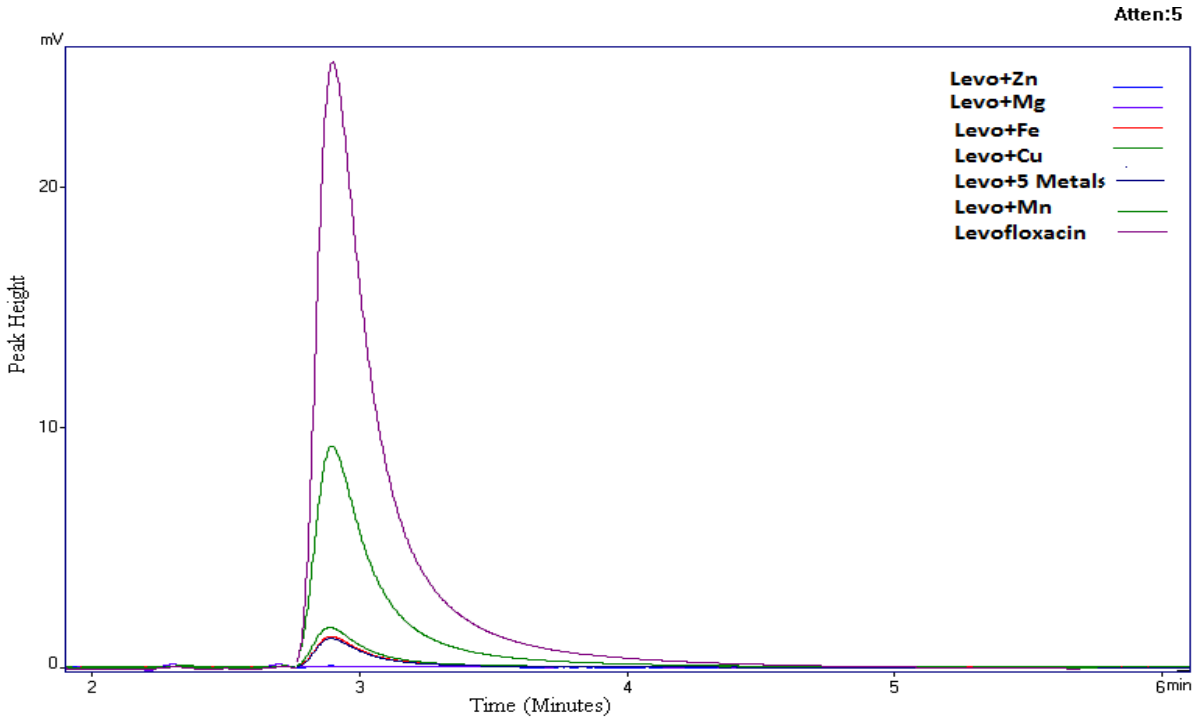

Figure 3: Levofloxacin Metal Interaction at 40°C

Table 4: % Recovery of levofloxacin in presence of essential & trace elements at 37°C and 40°C

S. No	Drug	At 40°C		At 37°C	
		Peak Area	**% Drug available**	**Peak Area**	**% Drug available**
1	Levofloxacin (Levo)	484256	100	484256	100
2	Levo + $MgCl_2$	5007	1.03	4978	1.03
3	Levo + $MnCl_2$	191820	39.61	190783	39.4
4	Levo + $FeCl_3$	35579	7.34	30364	6.27
5	Levo + $CuCl_2$	43578	8.99	44999	9.29
6	Levo + $ZnCl_2$	6186	1.27	5828	1.20
7	Levo + M*	35140	7.25	32585	6.73

M* = mixture of all the above metals

Conclusion

A rapid RP-HPLC method for the qualitative and quantitative analysis, determination and quantification of levofloxacin in raw materials, dosage formulations, and in metal interaction, products were successfully developed, with optimum retention time of 2.1 minutes. The method is able to determine levofloxacin linearly as low as 2 ng with a relative standard deviation up to 0.1 as shown in tables, while this method holds effective for LOD of 0.1 ng. Precision, reliability, rapidness, simplicity, sensitivity and economic nature of this HPLC method give it advantage over to the other reported HPLC techniques for assay of levofloxacin. The results of this HPLC method and its validation data demonstrate the satisfactory performance of the developed method in monitoring of drug administration along with essential and trace elements, in vitro studies and in different dosage formulations containing levofloxacin. There was no interference of varying parameters observed like inter-personal or inter-operator variations, inter-day and intraday variations. The obtained results are in good agreement with the declared statistical and Pharmacopoeial contents. Rapid RP-HPLC method for the qualitative and quantitative analysis, determination and quantification of levofloxacin in raw materials, dosage formulations, and in metal interaction, products were successfully developed, with optimum retention time of 2.1 minutes. The method is able to determine levofloxacin linearly as low as 2 ng with a relative standard deviation up to ± 0.1 as shown in tables, while this method holds effective for LOD of 0.1 ng. Precision, reliability, rapidness, simplicity, sensitivity and economic nature of this HPLC method give it advantage over to the other reported HPLC techniques for assay of levofloxacin. The results of this HPLC method and its

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