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NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF LEVOFLOXACIN IN TABLET DOSAGE FORM

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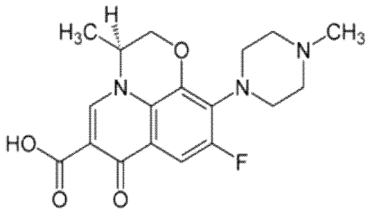
ABSTRACT

A simple and accurate RP-HPLC method has been developed for the estimation of Levofloxacin hemihydrate in bulk and pharmaceutical dosage forms using ACE C_{18} Column 250 x 4.6mm; 5µm particle size in isocratic mode, with mobile phase comprising of buffer (p^H 5.0) and Acetonitrile in the ratio of 80:20 v/v. The low rate was 1.0 ml/min and detection was carried out by UV detector at 294nm. The retention time for Levofloxacin hemihydrate was found to be 4.930 min. The proposed method has permitted the quantification of Levofloxacin hemihydrate over linearity in the range of 30-90µg/ml and its percentage recovery was found to be 98-102%. The intra day and inter day precision were found 0.38% and 0.38% and 1.43%.

Key words: Levofloxacin hemihydrate, HPLC, Isocratic, Acetonitrile.

INTRODUCTION:



Levofloxacin is a Antibacterial agent, its Chemical Name is (*S*)-7-fluoro-6-(4-methylpiperazin-1-yl) -10-oxo-4 thia1-azatricyclo[7.3.1.0] trideca-5(13),6,8,11 tetraene-11 carboxylic acid, And its Molecular Formula is $C_{18}H_{20}FN_3O_4$. Molecular Weight of this Levofloxacin is 361.368 g/mol. And its physical state is a light yellowish-white to yellow-white crystal. The data demonstrate that from pH 0.6 to 5.8, the solubility of levofloxacin is essentially constant (approximately 100 mg/ mL). Levofloxacin is considered soluble to freely soluble in this pH range, as defined by USP nomenclature.

Mechanism of Action:

Levofloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division.

EXPERIMENTAL:

Reagents & Chemicals

Pure standard of Levofloxacin hemihydrate (96.8%) was obtained as gift sample from Hetero Drugs Pvt. Ltd, Hyderabad along with certificate of analysis (COA). HPLC grade Acetonitrile, HPLC grade water, Potassium di hydrogen phosphate, Triethylamine ortho phosphoric acid, Hydrochloric acid and Methanol. All the glass ware employed in the work cleaned with hot water followed acetic anhydrate then acetone and dried in hot air oven when ever required. Working environment was maintained in between 18-25°C.

HPLC apparatus and chromatographic conditions:

The HPLC system (Waters Alliance with 2695 [separation module & (PDA) detector] UV Visible-spectrophotometer 1700 series Shimadzu, p^H meter advanced instruments, Mettler electronic balance and Sonicator Sandelin- sonorex super Rx-106.

Isocratic elution of mobile phase (80:20 v/v of buffer p^H 5.0 and Acetonitrile) with flow rate of 1.0 ml/min was performed on ACE C_{18} Column 250 x 4.6mm; 5µm. the contents of mobile phase were 80:20 v/v Buffer p^H 5.0 and acetonitrile. They were filtered through 0.45µm membrane filter and degassed by sonication before use. The flow

rate of mobile phase was optimized to 1.0 ml/min. The run time was set at 15 min and column temperature was maintained at ambient. The volume of injection was 10µl. The eluent was detected at 294nm.

Procedure:

Preparation of mobile phase:

Buffer p^H 5.0 and Acetonitrile in the ratio of 80:20 v/v were employed as a mobile phase and Buffer solution was prepared as directed by the procedure of United States of Pharmacopoeia.

Preparation of stock solution of Levofloxacin hemihydrate:

A stock solution was prepared by dissolving 60mg of Standard Levofloxacin hemihydrate in to a 100 ml of volumetric flask, added 60 ml of diluent and sonicated to dissolved. Diluted to volume with diluent and mixed.

Transfer 5.0 ml of above solution into a 50 ml of Volumetric flask, dilute to volume with diluent and mixed.

Construction of Linearity:

The Linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample.

Procedure:

A series of solutions were prepared using Levofloxacin API at concentration level 25-150 of standard concentration and each solution was analyzed.

The calibration curve for Levofloxacin was constructed by plotting the mean peak area (Y-axis) against the Concetration (X-axis). It was found to be linear in the concentration range 30-90 µg/ml with good correlation in between concentration and mean peak area. The values were shown in Table 1.

Preparation of sample solution:

Weighed and powdered not less than 10 tablets. Accurately weighed and transfer tablets powder equivalent to about 1000mg of Levofloxacin in to a 500 ml of volumetric flask, added about 300 ml of diluent and sonicated for not less than 30 min with occasional shaking (maintain the sonicator bath temperature between 20-25ºC). Diluted to volume with diluent and mixed. Filtered a portion of the solution through 0.45µm membrane filter and discard the first few ml of the filtrate.

Transferred 3.0 ml of the above solution into a 100 ml of a volumetric flask, diluted to a volume with diluent and mixed.

METHOD VALIDATION:

LINEARITY:

The linearity for the detection of Levofloxacin was 30-90µg/ml with (R^2=1; y = 51901x + 39446). Standard was transferred to a volumetric flask and made up to a sufficient volume with mobile phase to get desired concentration of 10µg/ml. The prepared dilution was injected into the column to obtain chromatogram. From that peak area, the drug content in the tablets was quantified.

PRECISION:

Precision is a degree of closeness of results for homogeneous done under replicates.

Method precision (Repeatability):

For Chromatographic conditions, Preparation of Mobile phase, diluent and standard preparation refer quantitation. Establish the repeatability by injecting six individual sample solutions prepared as follows.

Preparation of sample solution

Weigh and powder not less than 10 tablets. Accurately weigh and transfer tablets equivalent to about 1000 mg of Levofloxacin in to a 500 ml of volumetric flask and add about 300 ml of diluent and sonicated for not less than 30 min. with occasional shaking (Maintain the sonicator bath temperature between 20- 25 ^{0}C). Dilute to volume with diluent and mix. Filter a portion of in the solution through 0.45 µm membrane filter and discard first few ml of the filtrate.

Transferred 3.0 ml of the above solution into a 100 ml volumetric flask, diluted to volume with diluent and mixed.

ACCURACY & RANGE:

It is measure of exactness of the method. An assay is accurate if the mean result from the assay is the same as the true value.

Procedure:

Solutions were prepared in duplicate at levels 100% of test concentration using Levofloxacin drug substance and Levofloxacin Tablets placebo as per test method and injected each solution into HPLC as per test methodology.

SYSTEM SUITABILITY:

Preparation of sample solution:

Weigh and powder not less than 10 tablets. Accurately weigh and transfer tablets equivalent to about 1000 mg of Levofloxacin in to a 500 ml of volumetric flask and add about 300 ml of diluent and sonicate for not less than 30 min. with occasional shaking (Maintain the sonicator bath temperature between 20- 25 ^{0}C). Dilute to volume with diluent and mix. Filter a portion of in the solution through 0.45 µm membrane filter and discard first few ml of the filtrate.

Transfer 3.0 ml of the above solution into a 100 ml volumetric flask, dilute to volume with diluent and mix.

Procedure:

Equilibrate the column for not less than 30min with mobile phase at a flow rate of 1.0 ml/min. Separately inject 10 µl of Diluent as blank, standard solution (5 injections) and sample solution in to the chromatographic system. Record the chromatograms and measure the peak responses.

SPECIFICITY:

It is the ability to measure the analyte concentration in the presence of components, which may be expected to present which includes impurities, degradants, matrix etc. It generally refers to a method that produces a response for a single analyte only.

Procedure:

For Chromatographic conditions, Preparation of Mobile phase, Diluent preparation and Standard preparation. Specificity shall be established by demonstrating that the procedure is unaffected by the presence of interference at the retention time of the Levofloxacin with respect to Diluent, all known impurities, placebo solution and degradants.

Preparation of Placebo solution:

Weigh and powder not less than 10 tablets. Accurately weigh and transfer tablets equivalent to about 1000 mg of Levofloxacin placebo in to a 500 ml of volumetric flask and add about 300 ml of diluent and sonicate for not less than 30 min. with occasional shaking (Maintain the sonicator bath temperature between 20- 25 ^{0}C). Dilute to volume with diluent and mix. Filter a portion of in the solution through 0.45 µm membrane filter and discard first few ml of the filtrate.

Transfer 3.0 ml of the above solution into a 100 ml volumetric flask, dilute to volume with diluent and mix.

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.

A) LINEARITY:

Typical Chromatograms for Linearity were shown in fig 1. The calibration curve for Levofloxacin was shown in fig. 2.

Fig.1

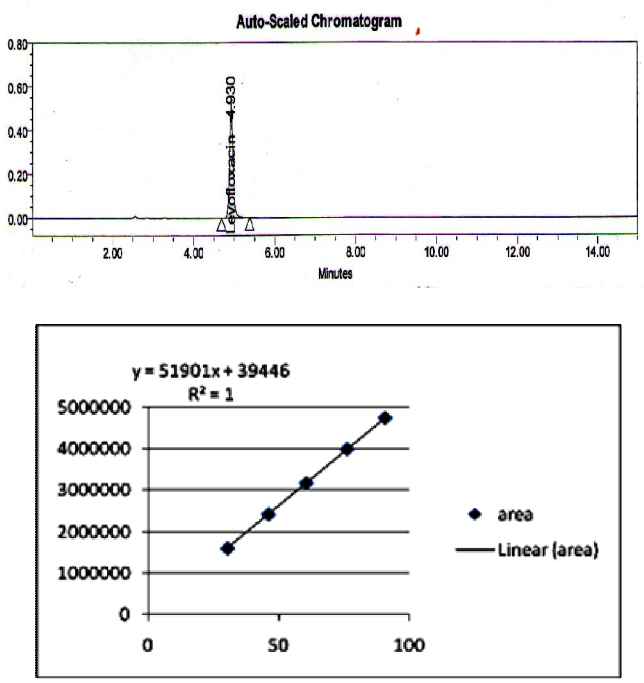


Fig: 2. Calibration curve of Levofloxacin at 294 nm

Table 1: Linearity results for Levofloxacin

Conc. (mcg / ml)	**30.192**	**45.892**	**60.384**	**76.084**	**90.576**
Avg. Area**	1604557	2421716	3176262	3989043	4738377
Correlation	1.00000				

** Average of five determinations.

Table 2: Calibration parameters of Levofloxacin

Parameter	Results
Slope	51901.25
Y-Intercept	39446.31
Correlation co-efficient	1.00000

Acceptance criteria:

Correlation Coefficient should not be less than 0.998.

PRECISION:

The chromatogram for Precision was shown in fig.3

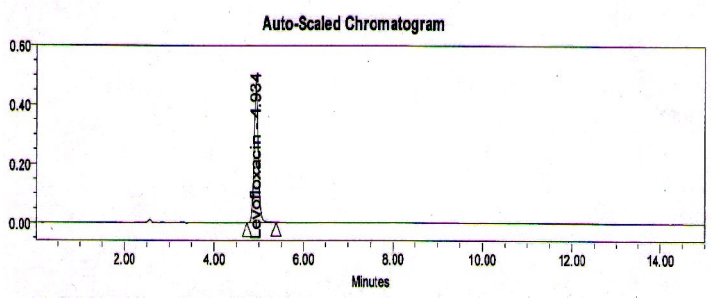


Table 3

Sr. No.	Concentration (mcg / ml)	Intraday precision (%)	Interday precision (%)
1	60	100.1	101.7
2	60	100.4	100.2
3	60	100.4	100.2
4	60	100.2	99.6
5	60	101.1	101.4
6	60	100.8	97.7
Mean		**100.5**	**100.1**
Std.Dev		**0.379**	**1.433**
%RSD.		**0.38**	**1.43**

Acceptance criteria:

% RSD of Assay results does not exceed 2.0%.

C) ACCURACY AND RANGE:

The chromatogram for Accuracy & Range was shown in fig 4.

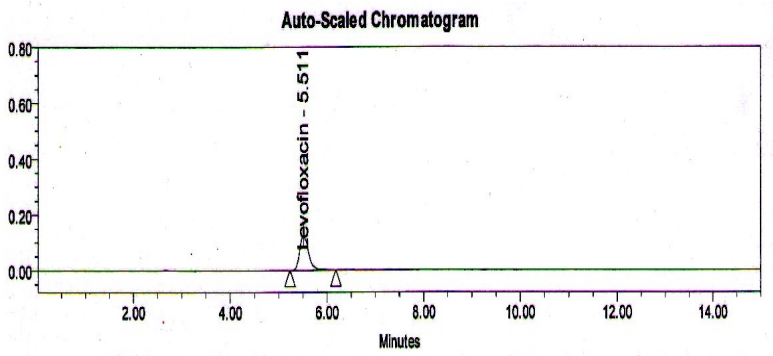


Table 4 : Accuracy results for LEVOFLOXACIN

Sample No.	Spike Level	Amount (mcg / ml) added	Amount (mcg / ml) found	% Recovery	Mean % Recovery
1	50 %	0.041	0.0410	99.1	**99.0**
	50 %	0.041	0.0409	99.0	
	50 %	0.041	0.0408	98.9	
2	100 %	0.081	0.0803	97.1	98.6
	100 %	0.081	0.0807	100.7	
	100 %	0.081	0.0810	98.1	
3	150 %	0.123	0.12281	98.1	98.9
	150 %	0.123	0.12261	99.0	
	150 %	0.123	0.12258	98.8	

Table 5

Recovery Level	% Mean Recovery
Recovery level -50%	99.0
Recovery level -100%	98.6
Recovery level-150%	98.9
Mean Recovery	**98.8**
SD	**0.19**
% RSD	**0.19**

Acceptance criteria:

- Recovery should be in the range of 98.0% to 102.0%

- % RSD should not be more than 2%

D) SYSTEM SUITABILITY:

The chromatogram for system suitability was shown in fig.5

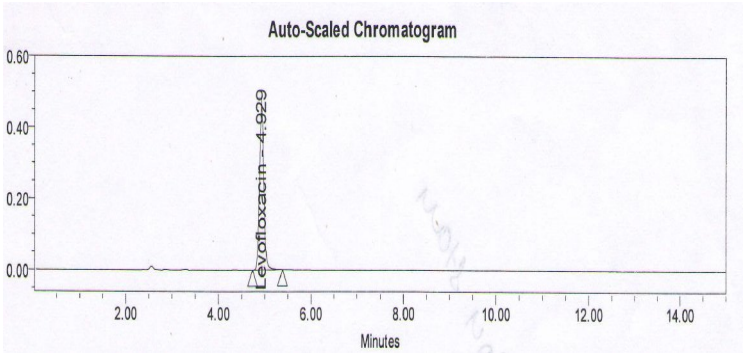


Table 6:

	Sample name	Inj	RT(min)	Area	USP Tailing	USP Plate count
1	Standard	1	4.934	3049611	1.11	13617
2	Standard	2	4.929	3069317	1.10	13334
3	Standard	3	4.931	3068311	1.10	13531
4	Standard	4	4.930	3044206	1.10	13500
5	Standard	5	4.927	3042898	1.10	13134

Table 7 : System suitability studies of Levofloxacin by RP-HPLC method

Property	Values
%RSD	0.425
Theoretical plates (N)	13423
Tailing factor (T)	1.10

Acceptance criteria:

The % is RSD for peak areas obtained from five replicate injections of standard solutions is not more than 2.0.

E) SPECIFICITY:

The chromatogram for specificity was shown in fig.6

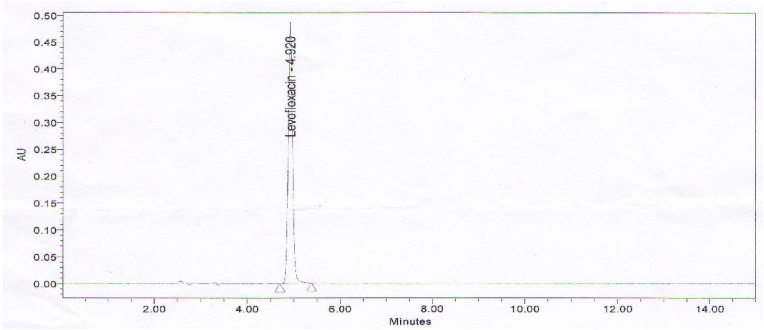


Table 7

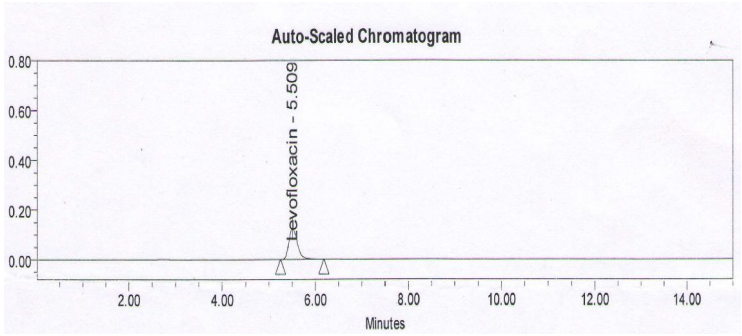
	Interferences
Diluent	No interference
Mobile phase	No interference
Blank solution	No interference
Placebo solution	No interference
Peak purity of Levofloxacin	0.99

Acceptance Criteria:

- Diluent should not show any interference at the retention time corresponding to the peak of Levofloxacin.
- Mobile phase should not show any interference at the retention time corresponding to the peak of Levofloxacin.

F) ROBUSTNESS:

The chromatogram for Robustness was shown in fig. 7



(A). Chromatographic Condition: Change in flow rate

Table 8

SI. No	Change in flow rate	R.T
01	0.8 ml / min	5.509
02	1.2 ml / min	4.927

Table 9: Robustness results for Levofloxacin: (Flow rate 0.8)

RT	Peak Area	USP Plate Count	USP Tailing
5.509	1588770	13617	1.11

Table 10 : Robustness results for Levofloxacin: (Flow rate 1.2)

RT	Peak Area	USP Plate Count	USP Tailing
4.927	3042898	13134	1.10

CONCLUSION:

The results of the study reveal that the proposed RP-HPLC method for the estimation of Levofloxacin hemihydrate is simple and accurate in tablet dosage forms. Hence, the developed chromatographic method for Levofloxacin is said to be rapid, simple, precise, accurate, and cost effective that can be effectively applied for the routine analysis in research institution, quality control department in industries, approved testing laboratories, biopharmaceutical studies, and in clinical pharmacokinetic studies.

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