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PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.3, pp 1932-1942, July-Sept 2010

Development and Validation of a Sensitive Stability Indicating Method for Quantification of Levofloxacin related substances and degradation products in pharmaceutical dosage form

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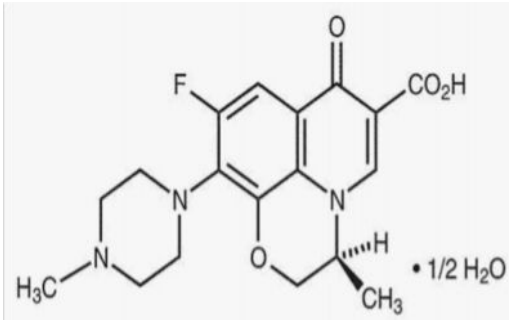
1.0 Abstract: A HPLC method was developed and validated to determine trace amounts of levofloxacin related substances & degradation products in its formulation. Separation of levofloxacin from impurity A, impurity B, impurity C and unknown degradation products was achieved on a Cosmosil C18 (250mmx4.6mm) 5µm using isocratic elution with buffer and methanol (68:32 v/v). The method is observed stability indicating by performing stressed study in various conditions such as, acid, alkali, oxidation, heat & radiation etc. The degradation pattern showed the nature that levofloxacin is highly degradable in acid & oxidation stress while much stable toward basic hydrolysis. The method was fully validated in line with pharmacopoeial and ICH guideline. In addition, solution stability, filter paper compatibility and method robustness were also evaluated to meet analytical challenges. The method was validated for accuracy from LOQ to 150% of actual standard concentration. Linearity was established including LOQ, 0.046µg/mL, 0.044µg/mL, 0.013µg/mL and 0.022µg/mL for levofloxacin, impurity-A, B and C respectively. This stability indicating related substances method can be successfully imparted for quality control purpose.
Key words: levofloxacin, HPLC, related substances, degradation, validation.

2.0 Introduction

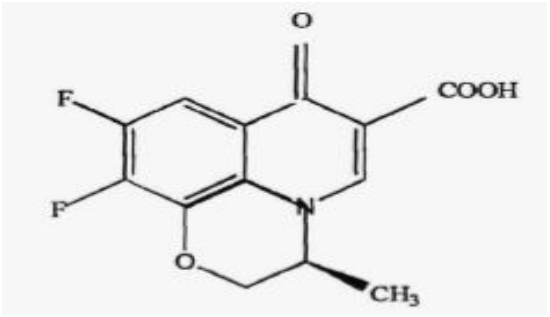
Impurity profiling of active pharmaceutical ingredients (API) in both bulk material and finalized formulations is one of the most challenging tasks of pharmaceutical analytical chemists under industrial environment [01]. The presence of unwanted or in certain cases unknown chemicals, even in small amounts, may influence not only the therapeutic efficacy but also the safety of the pharmaceutical products [03]. For these reasons, all major international pharmacopoeias have established maximum allowable limits for related compounds for both bulk and formulated APIs. As per the requirements of various regulatory authorities, the impurity profile study of drug substances and drug products has to be carried out using a suitable analytical method in the final product [01, 02].

Levofloxacin belongs to the class of fluoroquinolone (or quinolone) antiinfectives. Levofloxacin is a synthetic chemotherapeutic agent used to treat severe or life-threatening bacterial infections. Levofloxacin functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV [04]. Chemically is known as (-)-(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 hemihydrates and molecular formula is $C_{18}H_{20}FN_3O_4 \cdot 1/2H_2O$.

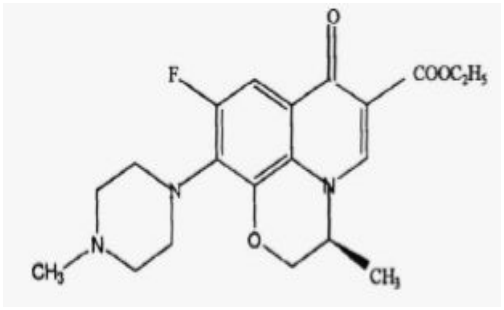
Fig. 1: Structures of Levofloxacin hemihydrates (LVF), LVF imp. A, LVF imp. B, LVF imp. C



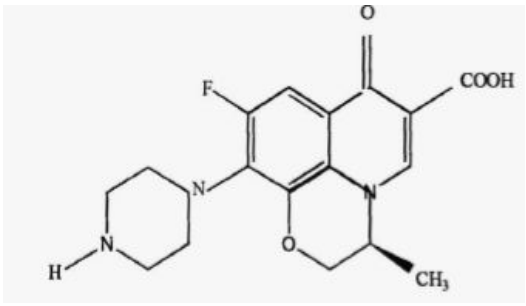
LVF



LVF Imp. A



LVF Imp. B



LVF Imp. C

As per available drug master file of levofloxacin hemihydrate the process impurities are identified as Levo acid/ 10-Fluoro Levofloxacin impurity (impurity A), Levofloxacin ethyl ester (impurity B) & Simple piperazine of Levofloxacin analog (impurity C) [05].

An extensive literature search revealed that, levofloxacin is official in Indian Pharmacopoeia [06] and contains the related substances method, but the limitation of this method is it does not consider any known impurities as well as the lowest detection can be made through this method may be 5 times higher than proposed method. Also a few methods for determination of levofloxacin, by colorimetric acid-dye complexation method [07], by UV, potentiometry & conductometry method [08], by HPLC in plasma & plasma in bone tissues [09,10], HPLC assay method with gradient elution [11] were also reported.

As a whole scenario, the previously reported work on quantification of levofloxacin is mostly on biological fluids and non-stability indicating or having less efficiency. The proposed method overcomes many difficulties of tracing out lowest determination and quantification of related substances and degradation products. Also the affirmative points are; less instrument set up time by mean of simple isocratic elution which results into a negligible noise as compare to gradient methods.

3.0 Experimental

3.1 Materials & Reagents:

All experiments were performed using 'A class' volumetric glassware, pharmaceutical grade levofloxacin hemihydrate, analytical grade sodium dihydrogen orthophosphate dihydrate (S.D.Fine Chem. A'bad, India), sodium hydroxide (Finar chem., A'bad, India), orthophosphoric acid (Spectrochem, A' bad, India), hydrochloric acid (Finar chem., A'bad, India) & hydrogen peroxide (S.D.Fine chem, A'bad, India) were used in the preparation of buffer solution and in forced degradation study of levofloxacin. Using HPLC grade triethylamine (Finar chem., A'bad, India), Methanol (Spectrochem, A' bad, India) and highly pure HPLC grade Milli Q water (Millipore, Bedford, MA, USA), mobile phase was prepared and employed for analysis. The mobile phase was filtered through 0.45µm PVDF filter (Millipore, Barcelona) and degassed under vacuum, prior to use.

3.2 Chromatography: (Instrumentation & analytical conditions)

The liquid chromatograph consisted of a Waters 2695 model with PDA isocratic pump, a Rheodyne injector with a 100µL loop and a detector (PDA 2998) operated at 294nm. The empower software used as data collector & processor.

4.0gm sodium dihydrogen phosphate and 5mL of triethylamine are mixed into 500mL Milli Q water and pH was adjusted to 6.0 by orthophosphoric acid and diluted to 1000mL. The mixture is filtered through 0.45µm filter and used as *buffer solution*. The combination of buffer solution with methanol (50:50 v/v) was used as a *diluent* (diluting solution) while preparing analytical solutions. The separation was performed using a Cosmosil C18 column (250mm×4.6 mm i.d., with a particle size of 5µm) as stationary phase and a mobile phase consisting of *buffer solution* and methanol (68:32v/v), which was filtered through 0.45µm membrane filter (Millipore, Barcelona) and degassed under vacuum, prior to use. The mobile phase was delivered at a flow rate of 1.0mL/min with maintaining the column temperature at 35ºC. The detection was achieved at 294nm by injecting 20µL of sample & standard aliquots, prepared using diluent, with the above chromatographic conditions and after partition equilibration, well shaped peaks were separated. The estimation of the impurities is done by area normalization.

3.3 Analytical solutions: (Standard and Sample solutions):

System suitability solution: Accurately weighed 20.0mg levofloxacin hemihydrate of known potency is transferred to 50mL volumetric flask, added 20mL methanol & sonicated to dissolve, 0.5ml of 80µg/mL impurity B is added and diluted up to volume with mobile phase. (400.0µg/mL Levofloxacin and 0.8 µg/mL).

Sample solution preparation: Sample tablets were triturated to get fine powder. The fine powder containing about 400mg of levofloxacin was transferred to 100mL volumetric flask, added 70mL methanol, sonicated for 15 min, diluted upto the volume with mobile phase and filtered through 0.45µm Nylon syringe filter discarding first 5mL. The sample solution contained the levofloxacin (400µg/mL)

3.4 Validation criteria:

3.4.1 Specificity:

The selectivity is defined as the capacity of an analytical method to exactly measure the concentration of analyte without interferences of impurities, products of degradation, excipients or related compounds.

***Interference from diluent, placebo and impurities*:** To check interference due to diluent, placebo & impurities, single injection of diluent and duplicate sets of placebo preparation and sample solution were injected. The spectral homogeneity of levofloxacin peak was checked by scanning in the range of 210nm-400nm into each set, retention time of all peaks & resolution between impurity B and levofloxacin was checked.

Diluent & Placebo interference: Injected duplicate injections of diluent and triplicate sets of placebo preparation (formulation components in the same quantities and conditions that in samples) and observed retention time of peaks due to diluent and common excipients. There shall not be any interference of diluent or placebo peak with levofloxacin and their known related substances.

Interference of Degradation products: (Stressed study)

Within the study of selectivity, a series of degradation studies were carried out, where the formulation samples, API and placebo were subjected to different degrees of stress, by following the ICH guidelines

The solutions of samples, placebo and the API are prepared as per optimized concentration to get the degradation up to 10%-30% at least in one condition and subjected to various stress condition as mentioned below.

Table I: Stress study conditions

Acid Stress conditions		**Alkali Stress conditions**	
Concentration of acid	5 N hydrochloric acid	Concentration of base	5 N sodium hydroxide
Time	6 hours	Time	6 hours
Temperature	60ºC	Temperature	60ºC
Oxidative Stress conditions		**Water hydrolysis conditions**	
Concentration of hydrogen peroxide	30% w/w H_2O_2	Time	6 hours
Time	60 minutes	Temperature	60ºC
Temperature	Bench top (25ºC)		
UV Radiation conditions		**Temperature Stress conditions**	
Exposure to UV Radiation	200 watt hours/m^2	Time	48 hours
		Temperature	105ºC

Injected single injection of blank, placebo preparation, test preparation for drug substance and test preparation for drug product for all conditions respectively into liquid chromatograph, chromatograms were compared and identified principal degradants by comparison of retention time of known impurities/degradants. Percentage degradation was calculated and purity of principal peak was also evaluated. To demonstrate spectral homogeneity of levofloxacin peak scanned in range of 210nm to 400nm.

3.4.2 Limit of Detection & Limit of Quantification:

The detection limit of individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value and quantification limit is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOD & LOQ concentrations of levofloxacin and impurities were determined based on standard deviation of response and slope method. Performed linearity in range of 5.0% to 25.0% (5.0%, 10.0%, 15.0%, 20.0% and 25.0%) of target concentration of impurity A, impurity B, Impurity C and levofloxacin considering limit of 0.20% (known impurities) and 0.10% (individual unknown impurity). Linearity graphs of concentration in µg/ml (X-axis) versus average area (Y-axis) were plotted. Slope of regression and residual standard deviation was calculated. LOD and LOQ concentrations of levofloxacin, impurity A, B and C were determined on the basis of equation given below.

Limit of Detection = (3.3 X σ) / S &

Limit of Quantification = (10 X σ) / S

Where, σ = Residual standard deviation of regression line, S = Slope of calibration curve.

Injected six replicate injections of these LOD & LOQ concentrations and ensured the peak is detected and responses were reproducible.

3.4.3 Precision:

The precision is supposed to be verified to show the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple analyses. Following types of precisions were studied and performed.

- *Instrumental precision (System suitability):* System suitability shall be established to prove that the suitability and reproducibility of the chromatographic system are adequate to perform the analysis. System suitability injection was injected. Tailing factor and theoretical plates are calculated. Also the resolution between levofloxacin and impurity B was determined.
- *Method Repeatability:* The method precision shows the repeatability of the results obtained by testing method. Six sets of sample preparation were prepared by spiking known amount of impurities and analyzed. Impurity A, impurity B, Impurity C, unknown individual impurity and total impurities were calculated as mentioned in test method for each set of test preparation. % RSD of Impurity A, impurity B, Impurity C, unknown individual impurity and total impurities of six sets of test preparation were calculated.
- *Intermediate precision (Ruggedness):* The purpose of this study is to demonstrate the reliability of the test results with variations. The reproducibility was conducted on two chromatographic systems by two different analysts and on two different days. %RSD of impurity A, impurity B, Impurity C, unknown individual impurity and total impurities of six sets of test preparation were calculated. Difference in result of average impurity A, impurity B, Impurity C, unknown individual impurity and total impurities between method precision and intermediate precision was determined.

3.4.4 Stability of analytical solutions:

Duplicate sets of spiked test preparation were prepared and kept on bench top (25°C±2°C) and analyzed initially (0 day), after 1 day and after 2 days by injecting single injection of each set of spiked test preparation into liquid chromatograph and chromatograms were recorded. Difference in result of impurity A, impurity B, Impurity C, unknown individual impurity and total impurities was determined at each time interval against respective initial result. Single and total impurities found well within the limit and the maximum difference observed was 0.02 & 0.03, for known & unknown impurities respectively and it was concluded that standard preparation and spiked test preparation were stable for two days on bench top (25°C±2°C).

3.4.5 Filter paper compatibility study:

The filter paper compatibility was observed for two different filters namely 0.45µm PVDF filter and 0.45µm Nylon filter. Some portions of spiked sample solution is filtered through both the filters and another portion is centrifuged and analyzed. Difference in result of impurity A, impurity B, Impurity C, unknown individual impurity and total impurities of filtered spiked test preparation against respective centrifuged spiked test preparation were compared. The %difference from centrifuged value for impurity-A, impurity-B and impurity-C were well within the acceptance criteria and results remained unchanged. The conclusion is 0.45µm Nylon filter and 0.45µm

PVDF filters both are compatible for filtration of test preparation.

3.4.6 Linearity Study:
The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Linearity was performed in range of LOQ to 150.0% (LOQ, 50.0%, 75.0%, 100.0%, 125.0% and 150.0%) of target concentration of impurity A, impurity B, Impurity C and levofloxacin considering limit of 0.20% (known impurities) and 0.10% (Limit of individual unknown impurity). In addition extended linearity was performed up to 150.0% of levofloxacin sample concentration (400µg/mL) to support calculation by area normalization. Duplicate injections of each linearity solution were injected into liquid chromatograph and chromatograms were recorded. Linearity graphs of concentration in µg/mL (X- axis) versus average area (Y- axis) were plotted. Different parameters for linearity level were calculated.

3.4.7 Accuracy (By Recovery):
Accuracy of an analytical method is closeness of test results obtained by that method to true value. Accuracy of an analytical method should be established across its range. To demonstrate accuracy of test method for impurity A, impurity B, Impurity C and unknown impurity, accuracy was performed in range of LOQ to 150.0% (LOQ, 50.0%, 100.0% and 150.0%) of target concentration of levofloxacin considering limit of 0.10% and impurity A, B, & C considering limit of 0.20%. Triplicate sets of accuracy sample preparation at each accuracy level were prepared and injected into liquid chromatograph and chromatograms were recorded.

3.4.8 Robustness:
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate changes in method parameters and provides an indication of its reliability during normal usage. The robustness of the method was demonstrated by performing the system suitability test as per the test method in normal condition and each altered condition mentioned below.

1. Changing the temperature of column (±5°c. i.e. 30°C and 40°C).
2. Changing the wavelength of detector (±2 nm i.e. 292nm and 296nm).
3. Changing the flow rate of the mobile phase (±10 % i.e. 0.8mL/min and 1.2mL/min).
4. Changing the organic solvent ratio (±5 % relative).
5. Changing the pH of aqueous phase of mobile phase (±0.2 i.e. 5.8 and 6.2)

4.0 Results & Discussions

4.1 Analytical Method development:

A strategic approach for method development was implemented to achieve the desired chromatographic parameters to develop a simple & precise one [12, 13]. The challenges optimized for method development are superiority in quantification of lowest concentrations, simple isocratic method and well resolved peaks of levofloxacin & impurity B.

In particular, a few RP-columns were compared with minor changes into mobile phase to evaluate the effect on the compound separation. To shorten the analysis time and for better resolution between trials with gradient elution were also taken, but as a result a very noisy baseline is observed. The baseline is also found unstable with variation as per slight change into pH of buffer preparation. The more precise method with less noise and high accuracy was developed by changing the ratio of buffer solution and organic solvents. As results of these experiences, using Cosmosil C18 (4.6mmx250mm), 5µm column and a mobile phase constituted from a mixture of foresaid buffer and methanol (68:32 v/v) as organic modifier was found appropriate to obtain an adequate separation of all the related impurities and degradation products of the levofloxacin. The optimal absorption wavelength for detection of the compounds was chosen especially with regard to absorption spectra of related compounds and degradation products which gave higher response at 294nm than any other. After successful development a simple, accurate and highly sensitive method is optimized with above parameters for the determination of related substances and degradation products of levofloxacin in a formulated tablet dosage form.

4.2 Method validation study:

4.2.1 Method Specificity:

The observation was made from the chromatogram of interference of diluent, placebo and impurities that No peak was found at the retention time of levofloxacin peak. All impurities were well resolved from levofloxacin peak from each other. Resolution between levofloxacin and impurity B was found more than 2.0. levofloxacin peak was spectrally homogeneous. The peak purity angle was less than peak purity threshold, which indicated that the concern peak is spectrally homogeneous. No peak due to unknown impurity was found at the retention time of impurity A, B and C peaks. All peaks were well resolved by R>1 from levofloxacin and from each other.

The degradation study showed that, the molecule is most degradable in acidic and oxidation condition, and lesser in UV radiation. In all the conditions, all the peaks due to levofloxacin, known impurities & degradation products were well separated and can be quantified separately, which showed the method stability indicating and specific.

Fig.2: Chromatogram of system suitability solution

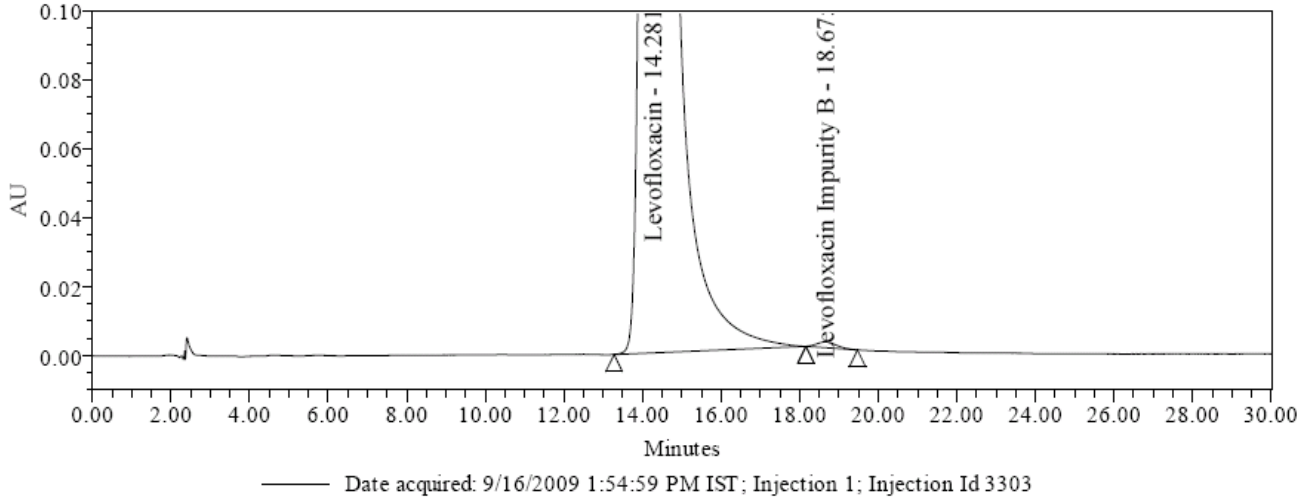


Table II: Data of forced degradation study

Drug Substances							
Peak Name	**As Such**	**Acid**	**Base**	**Oxi^n**	**Water**	**UV**	**Heat**
Impurity-A	ND	ND	ND	ND	ND	ND	0.36
Impurity-B	ND	ND	ND	ND	ND	ND	0.02
Impurity-C	0.01	0.01	ND	ND	ND	0.02	0.09
Single Unk.	0.02	34.4	0.03	8.99	0.02	0.02	0.04
Total Imp.	0.03	34.43	0.07	9.05	0.04	0.05	0.57
% LVF	100	65.6	99.9	91	100	100	99.7
Drug Product							
Condition	**As Such**	**Acid**	**Base**	**Oxi^n**	**Water**	**UV**	**Heat**
Impurity-A	ND	ND	ND	ND	ND	ND	0.55
Impurity-B	ND	ND	ND	ND	ND	ND	0.04
Impurity-C	0.02	0.01	ND	ND	ND	0.01	0.09
Single Unk.	0.02	32.84	0.15	7.33	0.03	0.03	0.12
Total Imp.	0.05	32.86	0.21	7.38	0.06	0.07	0.89
% LVF	99.9	67.1	99.8	92.6	100	99.9	99.5

Fig. 3: Chromatogram of sample solution in Acidic condition

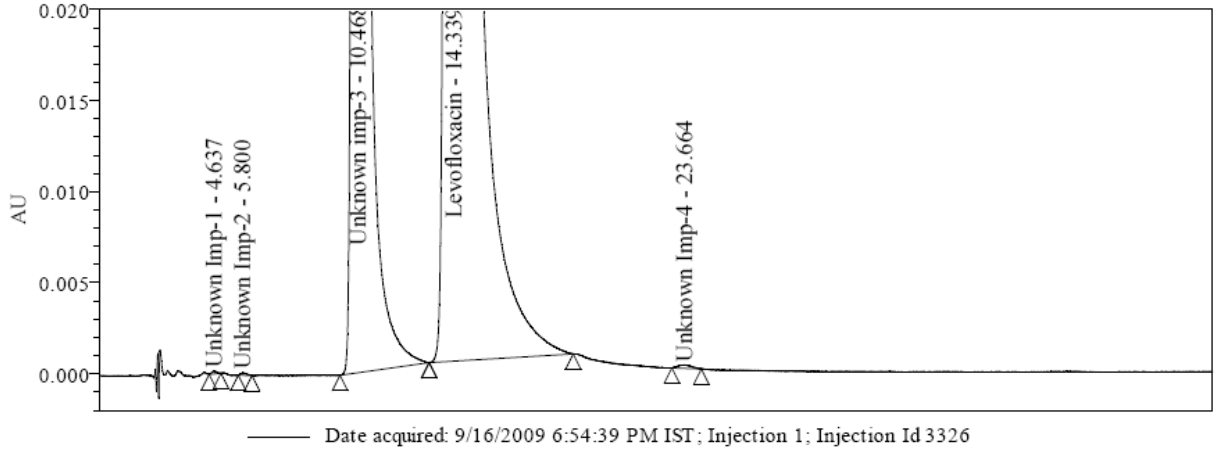


Fig. 4: Chromatogram of sample solution in Basic condition

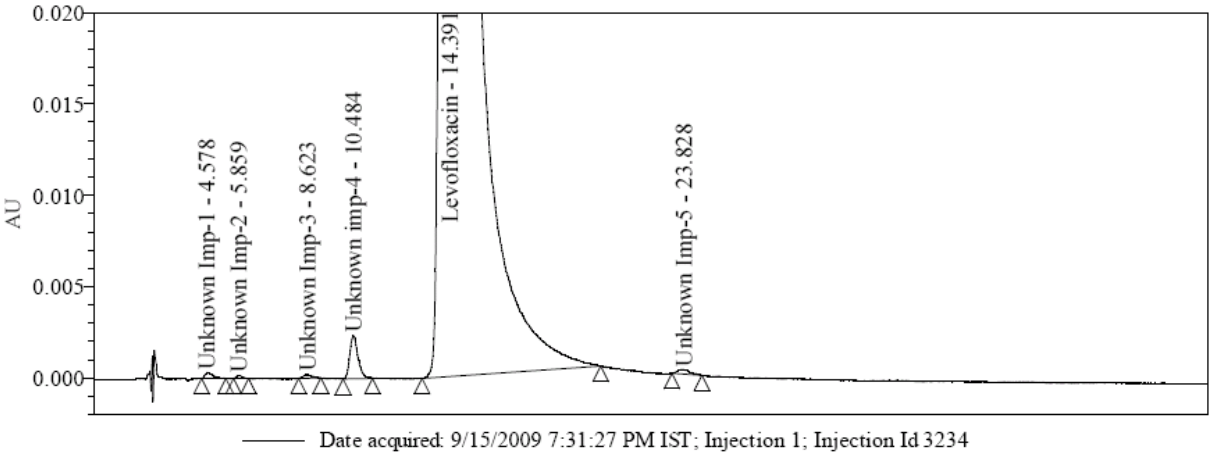
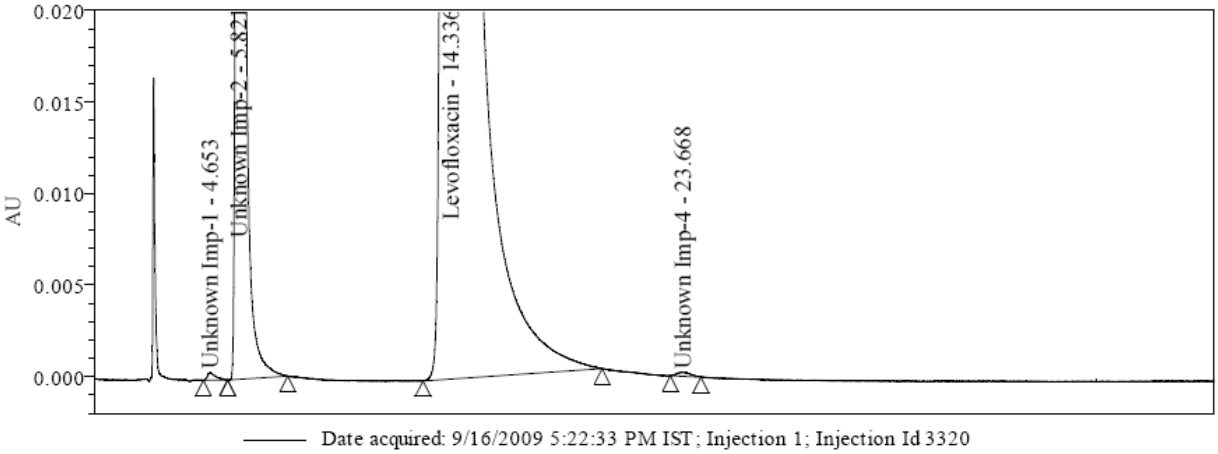


Fig. 5: Chromatogram of sample solution in Oxidation condition



4.2.2 Limit of Detection (LOD) & Limit of Quantification (LOQ):
Performed with linear regression method and calculated based on slope and residual standard deviation values. Successful lowest determination (LOD) concentrations 0.015µg/mL, 0.015µg/mL, 0.004µg/mL, 0.007µg/mL and quantification (LOQ) concentrations 0.046µg/mL, 0.044µg/mL, 0.013µg/mL and 0.022µg/mL obtained for levofloxacin, impurity A, B and C respectively. In each set of LOD, the peaks were detected successfully and for LOQ the %RSD was observed below 10%, which confirmed the acceptance.

4.2.3A System suitability Study (Instrument precision):
Chromatogram of system suitability revealed that, by analyzing this method, the desired suitability of HPLC instrument was achieved. The effectiveness of selected chromatographic system was observed by getting theoretical plates above 8300 and the tailing about 1.5 for analyte peak. Resolution between levofloxacin and impurity B found 5.2. Which showed the chromatographic system is adequate to perform the analysis.

4.2.3B Method repeatability:
For reliability and acceptance of study, %RSD for results of six sample preparation should not be more than 25.0 for impurities less than 0.05%, for impurities between 0.11-0.5, the %RSD should not be more than 15.0 and for impurities between 0.51-1.00, the %RSD should not be more than 10.0. The RSD were well within the acceptance criteria for impurity A, impurity B, Impurity C, single unknown impurity and total impurities and method is found precise for intended purpose.

4.2.3C Ruggedness of Method (Intermediate precision):
The method can be found rugged if the difference between results of normal condition and altered condition is within acceptance limit. The maximum %RSD observed for altered condition was about 10.5% which is quite less than acceptance criteria of 25.0%. The difference from method precision was about 0.0 and 0.08 which were well within acceptance range of 0.05 and 0.10. Comparison of these results complied the mentioned criteria and method found rugged for analysis.

4.2.4 Linearity:
The linearity was determined as linear regression with least square method on standard solution. Concentrations were LOQ, 50.0%, 75.0%, 100.0%, 125.0% and 150.0% of target concentration of impurity A, impurity B, Impurity C and levofloxacin considering limit of 0.20% for known impurities each and limit of 0.10% for individual unknown impurity. Extended linearity was also performed up to 150.0% of levofloxacin (400µg/mL) to establish linearity up to sample concentration. The calibration curve obtained by plotting the peak area versus the concentration for impurity A, impurity B, Impurity C and levofloxacin standard solution was linear in the mentioned concentration range. For acceptance of linearity correlation coefficient of linearity curve can not be less than 0.9800, %RSD of response factor from 50.0% to 150.0% of linearity level should not be more than 10.0 and Y-intercept bias at 100.0% linearity level should be within ± 15.0%. Comparison of these results complied the mentioned criteria that indicated that the method is linear up to the specified range of concentrations.

Table III: Data of Method Repeatability

Set number	Impurity A	Impurity B	Impurity C	Single Max. Unk. Imp. RRT=1.69	Total impurities
Set 1	0.16%	0.18%	0.18%	0.03%	0.58%
Set 2	0.16%	0.18%	0.18%	0.03%	0.58%
Set 3	0.16%	0.18%	0.18%	0.03%	0.58%
Set 4	0.16%	0.18%	0.18%	0.03%	0.58%
Set 5	0.16%	0.18%	0.18%	0.03%	0.58%
Set 6	0.16%	0.18%	0.18%	0.03%	0.58%
Average	0.16%	0.18%	0.18%	0.03%	0.58%
SD	0.00	0.00	0.00	0.00	0.00
RSD	0.0%	0.0%	0.0%	0.0%	0.0%

Table-IV: Linearity data of imp. A, B and C (LOQ to 150%)

Linearity level	Impurity A	Impurity B	Impurity C
Correlation coefficient (r) :	0.9991	0.9999	0.9992
RSD of response factor from 50.0% to 150.0% linearity level :	2.5%	0.9%	4.1%
Y-intercept :	-141.902	38.245	-1369.40
Y-intercept bias at 100.0% linearity level :	-0.8%	0.0%	-2.3%
Relative response factor	4.2	0.85	1.0

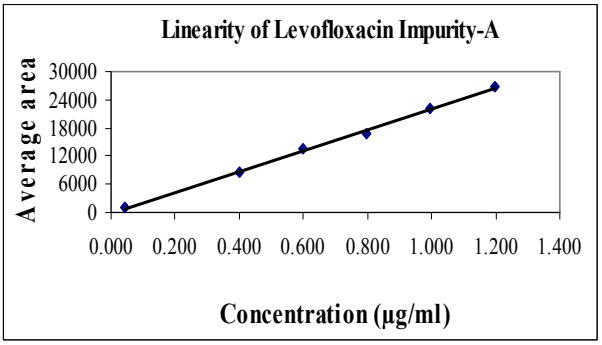
Table-V: Linearity data of Levofloxacin (LOQ to 150%)

Linearity level with respect to Levofloxacin concentration 400 µg/ml	Concentration (µg/ml)	Average area	Response factor
LOQ	0.046	6045	Not applicable
0.050% (50.0%*)	0.200	19067	95335.00
0.075% (75.0%*)	0.300	28434	94780.00
0.100% (100.0%*)	0.400	37591	93977.50
0.125% (125.0%*)	0.500	45292	90584.00
0.150% (150.0%*)	0.600	54701	91168.33
50.0%	199.836	18212184	91135.65
75.0%	299.754	26984056	90020.67
100.0%	399.672	35701253	89326.38
125.0%	499.590	47335807	94749.31
150.0%	599.508	52919004	88270.72
Correlation coefficient (r) :			0.9993
RSD of response factor from 50.0% to 150.0% linearity level :			2.7%
Y-intercept :			28413.777
Y-intercept bias at 100.0% linearity level :			0.1%

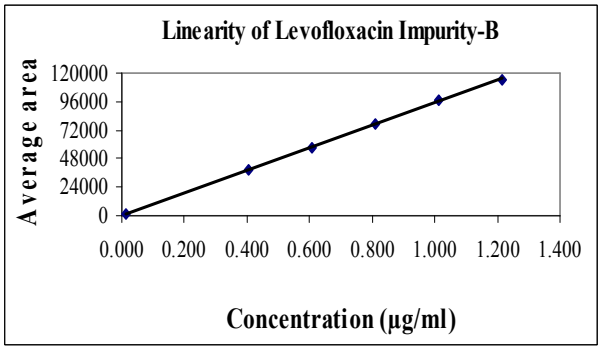
4.2.5 Accuracy (by Recovery):

The accuracy of the method was determined by measuring the drug recoveries in order to determine eventual positive or negative interferences produced by the excipients in the formulation. The results obtained for the accuracy study in the samples ranging a levofloxacin unknown impurity and known impurities concentration between LOQ to 150% which was indicated that the %recovery observed between 70.0 to 130.0 for impurity less than 0.05%, for impurities between 0.051-0.100 the observed %recovery between 75.0 – 125.0 and for impurities between 0.501-1.00 the % recovery found between 85.0-115.0. The %RSD for three sets of accuracy sample preparation at each accuracy level was found within the acceptance criteria with acceptable limit of not more than 10.0. The results showed the method accuracy for determination in said formulation.

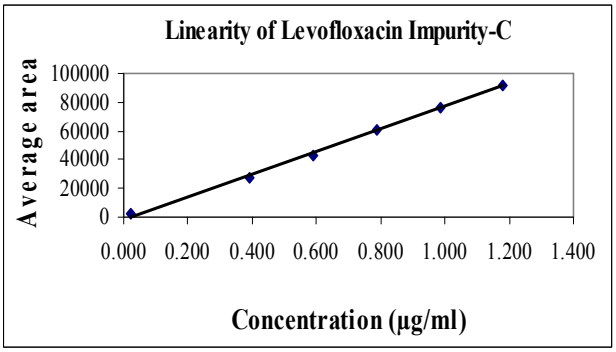
Fig. 6: Linearity curves for LVF, LVF imp. A, B & C



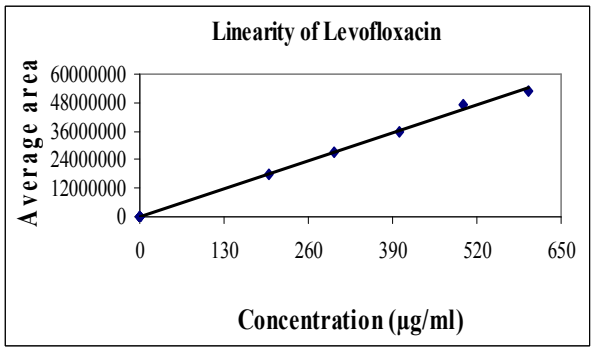
LVF Imp. A



LVF Imp. B



LVF Imp. C



LVF

Table-VI: Accuracy data of single unknown imp., imp. A, B and C (LOQ to 150%)

Accuracy level	Set No.	Recovery for Single unk. impurity		Recovery for Impurity -A		Recovery for Impurity -B		Recovery for Impurity -C	
		Average recovery (%)	RSD	Average recovery (%)	RSD	Average recovery (%)	RSD	Average recovery (%)	RSD
LOQ	Set 1 Set 2 Set 3	97.2	4.9%	124.3	4.2%	110.8	5.2%	86.0	8.1%
50.0%	Set 1 Set 2 Set 3	108.0	3.2%	111.7	2.6%	100.0	1.0%	106.7	1.1%
100.0%	Set 1 Set 2 Set 3	94.0	0.0%	109.0	2.8%	103.8	0.6%	98.3	1.1%
150.0%	Set 1 Set 2 Set 3	97.6	5.2%	107.5	2.8%	92.8	0.8%	94.3	0.4%

4.2.6 Robustness

The determination was observed with small but deliberate changes in the parameters i.e. detection wavelength, column temperature, change in organic solvent ratio etc. of analytical methodology and system suitability parameters e.g. Resolution between levofloxacin and levofloxacin imp. B, theoretical plates, tailing, %RSD were observed and found good with all the different altered conditions.

5.0 Conclusions

A sensitive, accurate and precise stability indicating RP-HPLC method was proposed for the determination of levofloxacin related substances in formulated drug product of levofloxacin and validated as per the ICH guidelines. In this method three known process impurities and other degradation unknown impurities can be identified and quantified to such a lower level. The method is found specific even after the stressed conditions and the analyte peak is free from interference from common excipients, diluent and degradation products. Method validation results have proved the method to be selective, precise, accurate, robust and stability indicating. This method can be successfully applied for the routine quality control analysis as well as stability study.

6.0 Acknowledgement

All raw data form the validation work archived at Cadila Pharmaceutical Ltd. All the validation work performed at Analytical Research Laboratory, Cadila Pharmaceutical Ltd, Dholka, Gujarat, (India). The author is thankful to M/s Cadila Pharmaceuticals LTD, Dholka, India for providing facilities and infrastructure for the study.

7.0 References

[01] ICH Guideline Q3A (R2), Impurities in New Drug Substances, October 25, 2006.
[02] ICH Guideline Q7A, Good Manufacturing Practice Guide for Active pharmaceutical Ingredients, November 2005.
[03] Van de Water, A., Xhonneux, R., Reneman, R., & Janssen, P. (1988) Eur. J Pharmacol. 156, 95-103
[04] Drlica K, Zhao X (01 Sep 1997). "DNA gyrase, topoisomerase IV, and the 4-quinolones". *Microbiol Mol Biol Rev.* **61** (3): 377–92.
[05] European drug master file (applicant's part), Dr. Reddy's laboratories ltd., 2003, 51-52.
[06] Indian Pharmacopoeia, 2007, volume 2, 1296-1297
[07] Safwan Ashour, Raghad Al-Khalil, II Farmaco, volume 60, Issue 9, September 2005, Pages 771-775
[08] G.Altiokka, Z. Atkosar, N.O.Can, Journal of Pharmaceutical and Biomedical Analysis, Volume 30, Issue3, 15 October 2002, Pages 881-885.
[09] F.A.Wong, S.J.Juzwin, S.C.Flor, Journal of Pharmaceutical and Biomedical Analysis, Volume 15, Issue 6, March 1997, Page 765-771.
[10] S.Djagarouti, E.Boselli, B. Allaouchiche, B.Ba , A.T.Nguyen, J.B.Gordien, J.M.Bernadou, M.C.Saux and D.Breilh, Journal of Chromatography B, Volume 799, Issue 1, 5 January 2004, Pages 165-172.
[11] M.Lalitha Devi, K.B.Chandrasekhar, Journal of Pharmaceutical and Biomedical Analysis, 50 (2009), 710-717.
[12] Satinder Ahuja, Henrik Rasmussen, HPLC Method Development for Pharmaceuticals, 2007
[13] L.R. Snyder (Author), J.J. Kirkland (Author), J.L. Glajch; Practical HPLC Method Development, 1997, 2nd Edition.

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