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DEVELOPMENT AND VALIDATION OF NEW RP- HPLC METHOD FOR THE QUANTIFICATION OF DEGRADATION IMPURITIES IN LEVOFLOXACIN TABLET DOSAGE FORM

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ABSTRACT

A HPLC method was developed and validated to determine impurities in Levofloxacin in its formulation. Separation of Levofloxacin from unknown degradation products was achieved on Xterra RP18 (250mmx4.6mm) 5µm using gradient elution. Five impurities have been separated - Levofloxacin related compounds 1,2,3,4,5 but according to I.P, only Piperazine analogue is known impurity which is validated here. The method is observed stability indicating by performing stressed study in various conditions such as acid, alkali, oxidation, heat & radiation. The peak purity of Levofloxacin peak at every degradation sample shows that the Levofloxacin peak is homogenous and there are no co-eluting peaks indicating that the method is stability indicating and specific. The method was fully validated in line with pharmacopoeial and ICH guidelines. In addition, solution stability, filter variability, precision, linearity and method robustness were also evaluated to meet analytical challenges. The method was validated for accuracy from LOQ to 150% of actual standard concentration. This stability indicating related substances method can be successfully imparted for quality control purpose.

Key Words: Levofloxacin, Piperazine analogue, Disodium hydrogen ortho phosphate, Ammonium dihydrogen orthophosphate, gradient elution.

INTRODUCTION

Impurity profile is the description of identified and unidentified impurities present in new drug substances (Alsante KM et al., 2004). In the pharmaceutical world, an impurity is considered as any other organic material, besides the drug substance, or ingredients, arises out of synthesis or unwanted chemicals that remain with API's (ICH Q3A(R), 2000). The impurity may be developed either during formulation, or upon aging of both API's and formulated API's in medicines (ICH topicQ3A, 1995). The presence of these unwanted chemicals, even in small amount, may influence the efficacy and safety of the pharmaceutical products. Standards have been established by various organizations that attempt to define the permitted levels of various impurities in a manufactured product.

Levofloxacin is a synthetic chemotherapeutic antibiotic of the Flouroquinolone drug class (Nelson JM et al., 2007; Kawahara et al., 1998) and is used to treat severe or life-threatening bacterial infections or bacterial infections that have failed to respond to other antibiotic classes (Liu H et al., 2005; MacDougall et al., 2005). It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase iv (Drilica K et al., 1997) which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division. As per the available information of levofloxacin hemihydrate, the process impurities are identified as Levofloxacin related compounds A, B, C and D. An extensive literature survey

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revealed that Levofloxacin is official in Indian Pharmacopoeia and also a few methods for its determination by colorimetric acid dye complexation method (Safwan Ashour et al., 2005) ,by UV, potentiometry & conductometry method (Altiokka et al., 2002), by HPLC in plasma & plasma in bone tissues (Wong et al., 1997; Djagarouti et al., 2004). 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.

MATERIALS AND METHODS

Chemicals and reagents

Disodium hydrogen ortho phosphate anhydrous, ammonium dihydrogen orthophosphate, ammonia solution, acetonitrile, methanol, water.

Instrumentation

Water-alliance HPLC system (Alliance 2695) equipped with PDA detector (2996) and Empower-2 software on X terra RP18, 250×4.6mm, 5µ or equivalent column were used for the study.

Selection of wave length

A UV spectrum of Levofloxacin was recorded by scanning between 200-400nm. From this spectrum λ_{max} at 293 nm was selected for the proper study. The UV spectrum of Levofloxacin is given under figure1.

Buffer preparation

Preparation of buffer A

Weigh and transfer about 1.8g of disodium hydrogen ortho phosphate anhydrous into beaker containing 1000ml of water and dissolve completely. Filter the solution through 0.45µ nylon membrane filter.

Preparation of buffer B

Weigh and transfer 1.7g of ammonium dihydrogen orthophosphate into a beaker containing 1000ml of water. Adjust pH of the solution to 10.0 with ammonia solution.

Mobile phase preparation

Preparation of mobile phase A: use buffer A.

Preparation of mobile phase B: prepare a filtered and degassed mixture of acetonitrile and methanol in the ratio of 75:25% v/v.

Diluent preparation

Prepare a degassed mixture of water and acetonitrile in the ration of 90:10 %v/v.

Standard preparation

Weigh and transfer accurately 25mg of levofloxacin hemihydrate working standard into a 100ml volumetric flask, add about 70ml of diluent and sonicate to dissolve. Dilute to volume with diluent and mix. Transfer 1ml of this solution into a 1000ml volumetric flask and make up to the volume with diluent.

Placebo preparation

Weigh and transfer placebo powder equivalent to about 50mg of levofloxacin into a 100ml volumetric flask, add about 70ml of diluent and sonicate for 20 minutes with intermittent shaking. Dilute to volume with diluent and mix. Filter the solution through 0.45µ nylon membrane filter. Discard first few ml of the filtrate.

Sample preparation

Weigh and powder NLT 20 tablets. Accurately weigh and transfer sample powder equivalent to about 50mg of levofloxacin into a 100ml volumetric flask, add about 70ml of diluent and sonicate for 20 minutes with intermittent shaking. Dilute to volume with diluent and mix. Filter the solution through 0.45µ nylon membrane filter. . Discard first few ml of the filtrate.

Chromatographic conditions

Column	: XterraRP18, 250×4mm, 5µ or equivalent
Flow rate	: 0.8ml/min
Wavelength, λ	: 280 nm
Injection volume	: 10 µl
Column temperature	: 60˚C
Run time	: 70 mins

Procedure

Inject separately 10µl of blank (diluent), placebo solution, standard solution (6 replications) and sample solution into the chromatographic system. Record the chromatograms and measure the peak responses.

In levofloxacin molecule following impurities are present.

The impurity chromatogram is given under figure 2.

Method Validation

A. Specificity and System Suitability

Identification and RT conformation

Prepared levofloxacin hemihydrate at standard concentration level, Piperazine analogue at specification level and impurity spiked to levofloxacin hemihydrate tablets 500mg tablets at specification level and injected into the chromatographic system. The Rt of levofloxacin, piperazine analogue in individual identification solutions should be comparable with impurity spiked sample. The results were given in table 4.

Peak purity

To the levofloxacin hemihydrate tablets 500 mg sample solutions, known impurity is spiked at specification level and peak purity was observed. Purity angle should be less than purity threshold as per empower software. The results were given in table 5.

Blank and placebo solution

Prepared blank and placebo solutions of levofloxacin hemihydrate tablets 500mg as per the test method and injected into the chromatographic system. Blank and placebo of levofloxacin hemihydrate tablets 500mg should not show any interference at the Rt's of the levofloxacin and known impurity.

System Suitability

Chromatogram of system suitability revealed that, by analyzing this method, the desired suitability of HPLC instrument was achieved**.** Standard solutions as per test method were prepared and injected ten times into the chromatographic system. The theoretical plates should be not less than 2000 and the resolution between Piperazine and levofloxacin should be not less than 1.5 for the system to get desired results. The results were given in table 6.

B. Forced Degradation

Levofloxacin tablets 500mg were stressed under various conditions of acid, alkali, oxidation, heat and radiation and the solutions were prepared with respective stressed samples and each sample solution was injected into the chromatographic system. Peak should be homogenous and there should be no co-eluting peaks. Peak purity should pass. There should be no interference due to placebo at the Rt of analyte(s) peak. The results were given in tables 7 - 9.

C. Linearity

Series of solutions were prepared by using levofloxacin hemihydrate and Piperazine analogue at different concentration levels ranging from LOQ to 150% of specification and standard concentration injected and measured the peak area response of solutions at LOQ level and level 5 six times and other levels in duplicate. For the drug to pass the linearity test, Correlation coefficient should be NLT 0.99, % y- intercept should be between ±2.0, %RSD for LOQ level should be NMT 10.0 and for 150% level should be NMT 3.0. The results were given in tables 10 and 11. The chromatograms are given in figures 3 and 4.

D. Limit Of Detection (LOD) & Limit Of Quantification (LOQ)

The LOD & LOQ values of levofloxacin hemihydrate and Piperazine analogue were estimated by preparing the solutions at about predicted concentration and injected each solution into the chromatographic system and calculated the signal to noise ratio. The S/N $\geq$ 3.0 (should be between 3 to 9) for LOD and S/N $\geq$ 10.0 (should be between 10 to 13) for LOQ. The results were given in table 12 for LOD and table 13 for LOQ.

E. Precision

Precision was measured in terms of repeatability of application and measurement.

a) System precision: Prepared standard solution as per the test method and injected six times into the chromatographic system. The results were given in table 14.

b) Method precision: Prepared six sample preparations of method (A) individually using same batch of Levofloxacin hemihydrate tablets 500 mg spiked with piperazine analogue at specification level as per test method and injected into the chromatographic system. The results were given in tables 15, 16.

F. Ruggedness

The method can be found rugged if the difference between results of normal condition and altered condition are within the acceptance limit. The results are given in the tables 17 - 20.

G. Accuracy

To the sample solution of levofloxacin hemihydrate tablets 500 mg spiked with piperazine analogue at 50%, 100% and 150% of specification level in triplicate and injected into the chromatographic system. To the placebo solution of levofloxacin hemihydrate tablets 500mg spiked with piperazine analogue at LOQ level in triplicate and injected into the chromatographic system. The %recovery should be between 85.0 to 115.0%. The results were given in table 21

H. Range

Range of analytical method can be obtained from linearity, precision and accuracy data. Report the range in % with respect to sample concentration.

I. Solution Stability

a. System precision

Standard solution: Prepared standard solution as per the test method and injected six times into the chromatographic system. For standard the % assay difference for levofloxacin should be NMT 5.0. The results were given in table 22.

b. Method precision

Sample solution: Prepared sample solution spiked with piperazine analogue as per the test method and injected at different intervals. Calculated the difference of %w/w of known impurities. The difference of %w/w of known impurities should be NMT 0.05 and for total impurities should be NMT 0.2. The results were given in

table 23.

J. Robustness

The robustness of a method is evaluated by varying method parameters such as percent organic, pH, ionic strength, temperature, etc., and determining the effect (if any) on the results of the method.

a. Change in chromatographic conditions: Standard solution: Prepared standard solution as per the test method and injected six times into the chromatographic system at different variable conditions. The results were given in table 24.

Sample solution: Prepared three sample solutions spiked with piperazine analogue as per the test method and injected into the chromatographic system at different variable conditions. The results were given in table 25.

b. Filter variability*:* The difference of %w/w should be NMT 0.05 for Known impurity and 0.2 for total impurity. The results were given in table 26.

RESULTS AND DISCUSSION

Specificity and system suitability

The Rt in the individual identification solutions were comparable with the impurity spiked sample. There are no interfering peaks observed due to blank and placebo of levofloxacin hemihydrate tablets 500mg at the Rt of levofloxacin and known impurity.

Forced degradation

The peak purity of levofloxacin peak at every degradation sample shows that the levofloxacin peak is homogenous and there are no co- eluting peaks indicating that the method is stability indicating and specific.

Linearity

The response of levofloxacin and piperazine analogue was linear from LOQ to 150% of specification and standard concentration.

LOD, LOQ

The LOD for piperazine analogue is 0.0047% and for levofloxacin is 0.0027 with respect to sample concentration. The LOQ for piperazine analogue is 0.0112% and for levofloxacin is 0.00625 with respect to sample concentration.

Precision

Average theoretical plates = 12062, Average tailing factor = 1.63, Resolution = 1.74, %RSD = 0.20. Hence the test result shows that the system was precise.

Ruggedness

The maximum %RSD observed for altered condition was about 0.55 % which is quite less than the acceptance criteria of 5%. All the results were within the acceptance range and comparison of these results complied the mentioned criteria and method was found rugged for analysis.

Accuracy

The %recovery is 101% indicating that the test has an acceptable level of accuracy for the determination of levofloxacin related substances in levofloxacin hemihydrate tablet 500 mg from LOQ level to 150% of specification level.

Solution stability

The % assay difference is more than 0.5, exceeding the acceptance limit above 48 hours so it was concluded that the standard solution is stable upto 48hours on bench top. The sample solution is stable upto 48hours on bench top as the results obtained are within the limit. The difference of %w/w is 0.0 for both known impurity and total impurities.

Robustness

From the results, it is concluded that the results are not affected when the flow rate, pH and temperature are changed for the test method. The difference of %w/w is 0.03 for Known impurity and 0.2 for total impurity. From these results of filter variability, it can be concluded that the results are not affected when the samples are filtered through PVDF or NYLON 66 filter. So the test method was robust in all conditions.

Table1. Gradient program for the RS of LEV

Time (mins)	Mobile phase A%	Mobile phase B%
0.01	85	15
10	85	15
25	30	70
30	45	55
40	40	60
55	35	65
60	35	65
61	85	15
70	85	15

Table 2. Elution order of RS of Levofloxacin

Compound	RRt	RRf
Piperazine analogue	0.89	0.55
LERC-01	0.54	1.0
LERC-02	0.67	1.0
LERC-04	1.37	1.0
Levofloxacin	1.0	-

Table 3. List of Impurities present in Levofloxacin

S. No	Name of the impurity	RRt
1	Related compound-1	0.53
2	Related compound-2	0.67
3	Related compound-3	0.89
4	Related compound-4	1.37
5	Related compound-5	1.43

Table 4. Specificity results (Identification and RT conformation) of RS of Levofloxacin

Name	Rt
Levofloxacin standard	8.28
Levofloxacin sample (500 mg)	8.28

Rt of individual identification solutions:

Compound	Rt(mins)
Piperazine analogue	7.35
Levofloxacin	8.28

Rt of the impurities spiked sample:

Compound	Rt(mins)
Piperazine analogue	7.34
Levofloxacin	8.28

Table 5. Specificity results (Peak purity) of RS of Levofloxacin

Compound	Purity Angle	Purity Threshold
Piperazine analogue	2.45	2.83
Levofloxacin	5.39	78.77

Table 6. System suitability results of RS Levofloxacin

S.No	Rt(min)	Peak Area	Theoretical Plates	Tailing Factor
1	8.28	59841	29224.0	0.95
2	8.25	60993	29996.0	0.99
3	8.26	59752	30107.9	0.99
4	8.23	60418	29390.1	0.99
5	8.21	60369	29575.0	0.96
6	8.25	59093	29152.9	0.93
7	8.23	60607	29919.7	0.97
8	8.24	59743	29460.2	0.94
9	8.25	60407	31382.4	1.01
10	8.24	60003	28802.9	0.97
Mean	8.24	60123	29701.1	0.97
SD	0.01897	543.26	-	-
%RSD	0.23	0.90	-	-

Table 7. Forced degradation results (drug product) of RS of Levofloxacin

Mode Of Degradation/ Condition	Piperazine Analogue	Any Other Impurity	Total Impurities
Undegraded sample	0.019	0.082	0.219
Thermal/90˚C,48hrs	0.038	0.086	0.251
UV/ 254nm,168 hrs	0.040	0.413	1.044
Humidity/90% RH, 168hrs	0.035	0.092	0.245
Acid/1.0N HCl Reflux 2hrs	0.032	0.101	0.306
Base/ 1.0N NaOH Reflux 2 hrs	0.047	0.105	0.286
Peroxide/1.5% H_2O_2 Reflux 2hrs	0.033	2.023	2.224

Table 8. Forced degradation results (peak purity) of RS of Levofloxacin

	Mode of Degradation/ Condition	Purity Angle	Purity Threshold
Solid phase	Undegraded sample	4.090	15.440
	Thermal/90˚C, 48hrs	5.164	90.000
	UV/ 254nm, 168 hrs	4.420	90.000
	Humidity/90% RH, 168hrs	5.499	90.000
Liquid phase	Acid/1.0N HCl Reflux 2hrs	4.876	10.568
	Base/ 1.0N NaOH Reflux 2 hrs	4.475	17.758
	Peroxide/1.5% H_2O_2 Reflux 2hrs	4.18	10.24

Table 9. Forced degradation results (placebo) of RS of Levofloxacin

Mode Of Degradation/ Condition	Observation	Remarks
Undegraded sample	No interference at Rt of analyte peak	Passed
Thermal/90˚C, 48hrs	No interference at Rt of analyte peak	Passed
UV/ 254nm, 168 hrs	No interference at Rt of analyte peak	Passed
Humidity/90% RH, 168hrs	No interference at Rt of analyte peak	Passed
Acid/1.0N HCl Reflux 2hrs	No interference at Rt of analyte peak	Passed
Base/ 1.0N NaOH Reflux 2 hrs	No interference at Rt of analyte peak	Passed
Peroxide/1.5% H_2O_2 Reflux 2hrs	No interference at Rt of analyte peak	Passed

Table 10. Linearity results of RS of Levofloxacin

Level	Concentration (Ppm)	Mean Peak Area	%RSD	Statistical Analysis
LOQ	0.026	1712	4.25	r^2 =0.99971
Level 1	0.401	26662	-	Slope = 68417.56
Level 2	0.601	41811	-	Y – intercept = -126.07
Level 3	0.802	54533	-	%y intercept = -0.23
Level 4	1.002	68638	-	Residual sum of squares = 555.12
Level 5	1.202	81884	0.13	Correlation coefficient = 0.99985

Table 11. Linearity results of RS of Piperazine analogue

Level	Concentration (Ppm)	Mean Peak Area	%RSD	Statistical Analysis
LOQ	0.045	1546	2.48	r^2 =0.99955
Level 1	0.402	14169	-	Slope = 34868.95
Level 2	0.630	21951	-	Y – intercept = -87.61
Level 3	0.840	29520	-	%y intercept = -0.31
Level 4	1.050	36856	-	Residual sum of squares = 363.21
Level 5	1.260	43451	0.16	Correlation coefficient = 0.99978

Table 12. LOD results of RS of Levofloxacin

S.No	Compound	Concentration (ppm)	Conc. (in %) with Respect To Sample	S/N Ratio
1	Piperazine analogue	0.019	0.0047	3.45
2	Levofloxacin	0.011	0.0027	5.16

Table 13. LOQ results of RS of Levofloxacin

S.No	Compound	Concentration (ppm)	Conc. (in %) With Respect To Sample	S/N Ratio
1	Piperazine analogue	0.045	0.0112	11.30
2	Levofloxacin	0.025	0.0062	17.12

Table 14. System precision results of RS of Levofloxacin

S.No	Peak area	Theoretical plates	Tailing factor
01	56407	12387.05	1.59
02	56317	12243.53	1.6
03	56149	12055.93	1.63
04	56251	11892.2	1.63
05	56363	11908.67	1.65
06	56128	11884.34	1.65
Mean	56269	12062	1.63
SD	113.83	-	-
%RSD	0.20	-	-

Table 15. Method precision- A results of RS of Levofloxacin

S.No	Piperazine Analogue 500mg	Any Other Impurity 500mg	Total Impurities 500mg
01	0.016	0.082	0.205
02	0.016	0.079	0.215
03	0.016	0.081	0.212
04	0.016	0.082	0.216
05	0.015	0.081	0.207
06	0.015	0.081	0.204
Mean	0.016	0.081	0.210
SD	0.0005	0.0011	0.0052
%RSD	3.13	1.36	2.48

Table 16. Method precision- B results of RS of Levofloxacin

	Piperazine Analogue 250mg	
S.No	%w/w	%Recovery
01	0.208	104.4
02	0.210	105.4
03	0.212	107.3
04	0.209	105.2
05	0.212	106.7
06	0.201	101.3
Mean	0.209	105.1
SD	0.0041	2.12
%RSD	1.96	2.22

Table 17. Ruggedness (system precision) results of RS of Levofloxacin

S.No	Peak Area	Theoretical Plates	Tailing Factor
01	53173	9218.35	0.99
02	53341	9147.31	0.99
03	53347	9125.12	0.98
04	53374	9061.89	0.98
05	53654	8963.00	0.98
06	52774	8901.45	0.97
Mean	53277	9070	0.98
SD	291.33	-	-
%RSD	0.55	-	-

Table 18. Intermediate precision-A results of RS of Levofloxacin

S.No	Piperazine Analogue 500mg	Any Other Impurity 500mg	Total Impurities 500mg
01	0.016	0.091	0.226
02	0.016	0.089	0.224
03	0.016	0.087	0.221
04	0.015	0.089	0.222
05	0.015	0.091	0.232
06	0.015	0.088	0.225
Mean	0.016	0.089	0.225
SD	0.0005	0.0016	0.0039
%RSD	3.13	1.80	1.73

Table 19. Intermediate precision-B results of RS of Levofloxacin

	Piperazine Analogue 500mg	
S.No	%w/w	%Recovery
01	0.216	103.5
02	0.213	101.9
03	0.219	105.1
04	0.216	103.6
05	0.217	104.1
06	0.211	101.1
Mean	0.215	103.2
SD	0.0029	1.49
%RSD	1.35	1.54

Table 20. Overall analysis results of Intermediate precision of RS of Levofloxacin

Compound	Mean 500mg	SD 500mg	%RSD 500mg
Piperazine analogue	0.016	0.0005	3.13
Any other impurity	0.085	0.0045	5.29
Total impurities	0.217	0.0090	4.15
Method – B (%w/w)			
Piperazine analogue	0.212	0.0048	2.26

Compound	% Recovery	
Piperazine analogue	104.1	100.2
Set	1	11
Analyst	X	Y
Instrument ID/ HPLC	QC/LC-012	QC/LC-010
Column ID	500	503
Day	20-08-2012	22-08-2012

Table 21. Accuracy results of RS of Levofloxacin

Concentration/ Sample Id	Amount Added(ppm)	Amount Found (ppm)	%Recovery	Mean
LOQ level 1	0.0402	0.0381	94.8	96.9
LOQ level 2	0.0402	0.0392	97.5	
LOQ level 3	0.0402	0.0396	98.5	
50% level 1	0.372	0.397	106.7	106.8
50% level 2	0.372	0.392	105.4	
50% level 3	0.372	0.403	108.3	
100% level 1	0.744	0.741	99.6	100.3
100% level 2	0.744	0.752	101.1	
100% level 3	0.744	0.746	100.3	
150% level 1	1.116	1.108	99.3	100.3
150% level 2	1.116	10110	99.5	
150% level 3	1.116	1.140	102.2	
Mean % recovery				101.1

Table 22. Solution stability (standard solution) results of RS of Levofloxacin

Time Intervals	% Assay	Difference
Initial	96.3	-
6 hours	96.4	0.1
12 hours	96.1	0.2
24 hours	96.1	0.2
48 hours	95.4	0.9

Table 23. Solution stability (sample solution) results of RS of Levofloxacin

Time Intervals	Piperazine Analogue	Difference	Any Other Impurity	Difference	Total Impurities	Difference
Initial	0.020	-	0.080	-	0.200	-
6 hours	0.020	0.0	0.090	0.01	0.220	0.02
12 hours	0.020	0.0	0.080	0.0	0.210	0.01
24 hours	0.020	0.0	0.080	0.0	0.210	0.01
48 hours	0.020	0.0	0.080	0.0	0.200	0.00

Table 24. Robustness (change in chromatographic conditions-standard) results of RS of Levofloxacin

Parameter	Peak Area% RSD		Theoretical Plates	Tailing Factor	Resolution
System precision	0.20		12062	1.63	1.74
Flow variation (0.8ml/min)	0.7ml/min	0.39	16395	1.38	1.87
	0.9ml/min	0.30	9389	1.58	1.66
Temp variation (60˚C)	55˚C	0.24	13883	0.95	1.67
	65˚C	0.19	9588	0.91	1.58

Table 25. Robustness (change in chromatographic conditions-sample) results of RS of Levofloxacin

Parameter	Overall %RSD Piperazine Analogue	
Method precision	1.96	
Flow variation (0.9ml/min)	0.8ml/min	3.34
	1.0ml/min	2.15
Temp variation (38˚C)	33˚C	2.34
	43˚C	1.94

Table 26. Robustness (Filter variability) results of RS of Levofloxacin

S. No		Piperazine Analogue	Total Impurity
Nylon 66 vs. PVDF	01	0.03	0.01
	02	0.03	0.02
	03	0	0.02
Nylon 66 vs. centrifuge	01	0.03	0.02
	02	0.03	0.02
	03	0.01	0.02
The maximum difference Nylon 66 vs. PVDF		0.03	0.02
The maximum difference Nylon 66 vs. centrifuge		0.03	0.02

Figure 1. UV spectrum of Levofloxacin

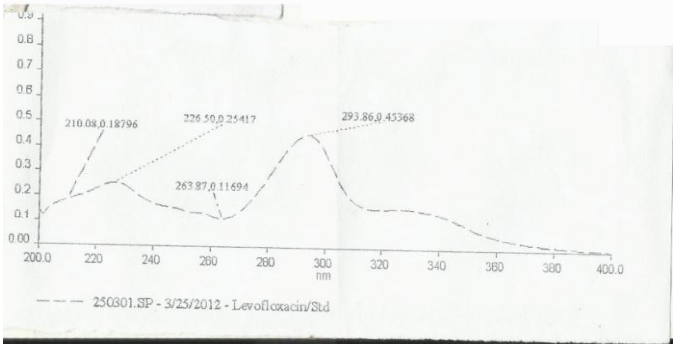


Figure 2. Levofloxacin impurities chromatogram

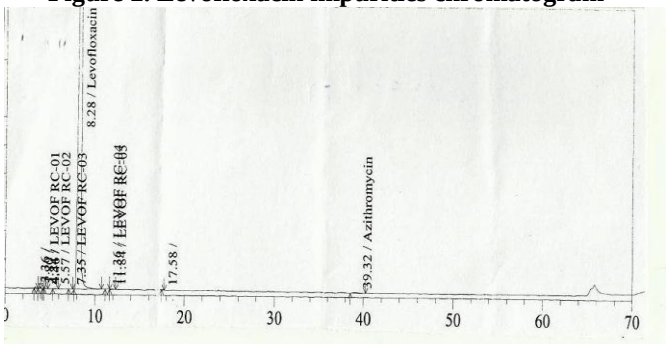


Figure 3. Linearity plot of RS of Levofloxacin

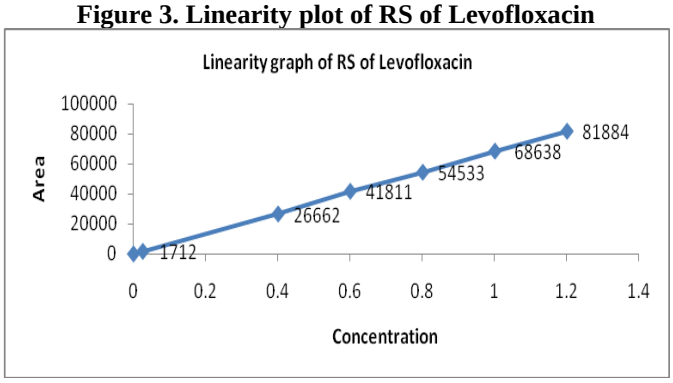
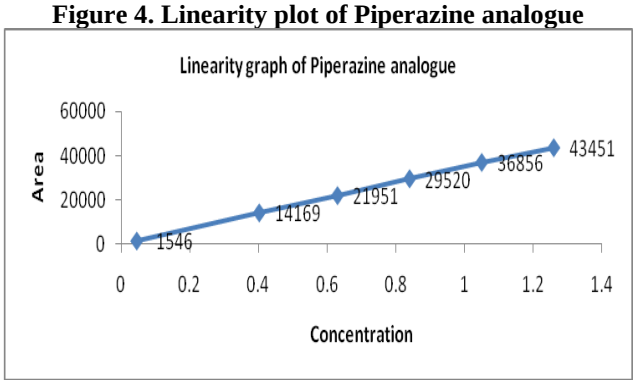


Figure 4. Linearity plot of Piperazine analogue



CONCLUSION

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 one known process impurity 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.

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