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TO VALIDATE STABILITY INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS DETERMINATION OF ASSAY OF LEVOFLOXACIN AND CEFPODOXIME DRUGS IN THE PHARMACEUTICALS TABLET FORMULATIONS USING LAMOTRIGINE AS A COMMON INTERNAL STANDARD.

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ABSTRACT

Levofloxacin is a synthetic broad-spectrum antibacterial agent administered orally and intravenously. Cefpodoxime proxetil is an orally administered, extended spectrum, semi-synthetic antibiotic of the cephalosporin class. Validation of stability indicating Simple, Specific, Precise, Accurate, Linear, Rugged, Robust High Performance Liquid Chromatographic method of analysis for simultaneous determination of assay of Levofloxacin and Cefpodoxime drugs in the pharmaceuticals Tablet formulations using Lamotrigine as a common internal standard was performed. The assay was accomplished using a mixture of 0.1% Trifluoroacetic acid in Water and methanol in the volume ratio of 35:65 v/v as mobile phase on a Zorbax SB- C18, 150 mm x 4.6mm, 3.5µ as chromatographic column at a flow rate 0.700 mLmin-1 and with a uv detector at a wavelength 278 nm. The temperature of auto injector and column oven was 10^0C and 30^0C receptively. The Injection volume kept as 30 µL. linearity of the analytical method was evoluted at concentration range of 7.0041 µg/ml to 350.0350 µg/ml for Levofloxacin and 5.6048 µg/ml to 280.1050 µg/ml for Cefpodoxime respectively with Correlation coefficient (r) value more than 0.9999.The LOD and LOQ was 0.9135µg/ml and 2.7681µg/ml for Levofloxacin and 1.2387µg/ml and 3.7536µg/ml for Cefpodoxime respectively. The retention time found to be 5.01 min for Levofloxacin, 7.28 min for Cefpodoxime and 10.39 min for internal standard. Specificity, Method Precision, System Precision, Ruggedness, Robustness, Recovery, Stability of analytical solution, Filter paper selection study, Stress testing(Force Degradation) at various conditions were performed as per the ICH (Q2) recommendations. All the results were found with in acceptance criteria.

KEYOWRDS: Levofloxacin, Cefpodoxime Hydrochloride, Lamotrigine Hydrochloride, High Performance Liquid Chromatographic, Force degradation studies, Assay.

INTRODUCTION

Levofloxacin is a synthetic broad-spectrum antibacterial agent given orally and intravenously. Chemically, levofloxacin is a chiral fluorinated carboxyquinolone, i.e. (-)-(S)-enantiomer of the racemic drug substance ofloxacin. The chemical name is (-)-(S)-9fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4benzoxazine-6-carboxylic acid hemihydrate. The molecular formula for Levofloxacin hemihydrate is C18H20FN3O4 • ½ H2O. The molecular weight of Levofloxacin hemihydrate is 370.38 and that of free Levofloxacin is 361.37. Levofloxacin is light yellowish-white to yellow-white crystal or crystalline powder and soluble in organic solvents such as methanol. The pKa of Levofloxacin is 5.45.[1-4]

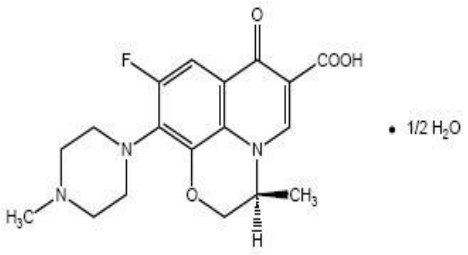

Figure 1: Chemical structure of Levofloxacin Hemihydrate.

Cefpodoxime proxetil is an orally administered, extended spectrum, semi-synthetic antibiotic of the cephalosporin class. The chemical name is (RS)-1(isopropoxycarbonyloxy) ethyl (+)-(6R,7R)-7-[2-(2-

amino-4- thiazolyl)-2-{(Z)methoxyimino}acetamido]-3-methoxymethyl-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene- 2-carboxylate. The molecular formula is $C_{21}H_{27}N_5O_9S_2$. The molecular weight of Cefpodoxime proxetil is 557.6. The molecular weight of Cefpodoxime sodium is 449.43 and that of free Cefpodoxime is 427.45. Cefpodoxime proxetil is white to slightly brownish white powder and soluble in organic solvents such as ethanol, methanol,acetonitrile. The pKa of Cefpodoxime is 3.20.[1-4]

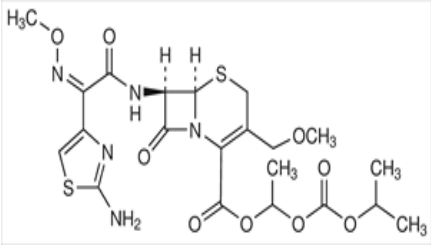

Figure 2: Chemical structure of proxetil.

Lamotrigine is an anticonvulsant drug used in the treatment of epilepsy and bipolar disorder. For epilepsy it is used to treat partial seizures, primary and secondary tonic-seizures associated with Lennox-Gastaut syndrome. Lamotrigine also acts as a mood stabilizer. It is the first medication since lithium granted Food and Drug Administration the maintenance treatment of bipolar type I. chemically unrelated to other anticonvulsants, lamotrigine has relatively few side-effects and does not require blood monitoring. lamotrigine works is unknown. The molecular formula is C9H7N5Cl2. The molecular weight of Lamotrigine is 256.09. Lamotrigine a white to pale cream-colored powder and has a pKa of 5.7. Lamotrigine is very slightly soluble in water and soluble in organic solvents such as methanol.[1-4]

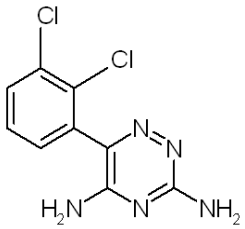

Figure 3: Chemical structure of Lamotrigine.

While Reviewing Literature for analytical method of analysis it was observed that many methods have been reported for determination of Levofloxacin and Cefpodoxime in combination and individually[5-25] but none of the reported HPLC methods have not been validated using internal standard to compensate any processing related and method related variability. Most of the published method is not performed stability-indicating studies (Acid, Alkali, Peroxide, Thermal, Photolytic, Humidity degradation,) which is mandatory as per the ICH(Q2) recommendations.

The main objective of the work is to develop and validate stability indicating HPLC method of analysis which is Simple, Specific, Precise, Accurate, Linear, Rugged, Robust etc. for simultaneous determination of assay of Levofloxacin and Cefpodoxime drugs in the pharmaceutical Tablet formulations using Lamotrigine as an common internal standard.

MATERIAL AND METHODS

Instrumentation

Shimadzu Prominence HPLC system equipped with dual pump, SIL-HTc auto-sampler with cooler, column oven, variable wavelength UV detector and a data acquisition system (Lab Solution Software) were used for the simultaneous determination of assay of Levofloxacin and Cefpodoxime drugs in the pharmaceutical Tablet formulations using Lamotrigine as a common internal standard.

Reagents and Materials

The reagents used during analysis include Methanol [HPLC Grade], Water [Milli-Q /HPLC Grade], Trifluoroacetic acid (TFA), Levofloxacin standard; Cefpodoxime Hydrochloride standard and Lamotrigine Hydrochloride were used obtained as a gift samples from Wockhardt Pharmaceutical limited. Fixed dose combination tablets containing 200 mg Cefpodoxime proxetil and 250 mg Levofloxacin of Hetero Drugs. Ltd. was purchased from Local medical, Aurangabad (Maharashtra). Trifluoroacetic acid (TFA), Rinsing solution, Mobile Phase was prepared by dissolving required volume and quantity of reagents and chemicals.

Analytical solutions

Stock solutions having concentrations approximately, 100.1 µg/mL of Levofloxacin in methanol, 800.3 µg/mL of Cefpodoxime in methanol and 2085.0 µg/mL of Lamotrigine in methanol were prepaed and solutions were filtered through 0.45µm nylon membrane filter with discarding first 2 mL of the filtrate before use. The solution of Lamotrigine was used as internal standard dilution solution during various experiments performed in an analytical method validation and assay calculations of pharmaceutical formulation.

Standard solutions having concentrations approximately, 120.00 µg/ml of Levofloxacin, 96.00 µg/mL of Cefpodoxime and 50.00 µg/mL of Lamotrigine were prepared in mobile phase and use as a reference solution for related activities and system suitability. Filter the solution through 0.45µm nylon membrane filter with discarding first 2 mL of the filtrate before use.

Sample solution having concentrations 120.00 µg/ml of Levofloxacin, 96.00 µg/mL of Cefpodoxime and 50.00 µg/mL of Lamotrigine was prepared in mobile phase by dissolving a quantity of powder equivalent to Strength of 250 mg of Levofloxacin and 200 mg Cefpodoxime and use as a sample solution for related activities. Filter the

solution through 0.45µm nylon membrane filter with discarding first 2 mL of the filtrate before use.

RESULT AND DISCUSSION

Method development

Primarily, several trials under optimization of analytical method was performed using different mobile phases composition, different ratios of organic to buffer, different organic solvents, different buffer with different pH, different stationary phases, different internal standards and variable chromatographic settings in an effort to achieve the finest peak resolution and separation between Levofloxacin, Cefpodoxime and internal standard as depicted in Figure 4.

A summarized chromatographic condition was as follows:

Methanol and 0.1% Trifluoroacetic acid in Water (35:65v/v)

Rinsing Solution: Methanol:	Mill-Q water (35:65v/v)
Chromatographic Column:	Zorbax SB- C18, 150 mm x 4.6mm, 3.5µ
Wavelength:	278 nm
Column Oven Temperature:	30 ^{0}C
Sample cooler Temperature:	10 ^{0}C
Flow rate:	0.700 ml per minute
Injection Volume:	30 µl
Run Time:	15 minute
Retention Time (minute):	Levofloxacin-5.01 Cefpodoxime-7.28 Lamotrigine-10.39

Analytical method validation

The Analytical method was optimized and validated in accordance with the current ICH guidelines and recommendations by means of a vision to accomplish Simple, Specific, Precise, Accurate, Linear, Rugged, Robust method.[26-30]

Specificity

For the evaluation of specificity; Blank solution, placebo solutions, sample solution, standard solution in triplicate were injected into HPLC system. No interference was observed from blank solution and placebo at the retention time of chromatographic peak of Levofloxacin, Cefpodoxime and internal standard. Peak purity was passes (purity angle was less than purity threshold) for Levofloxacin and Cefpodoxime and % assay difference with respect to method precision was found 0.10% for Levofloxacin and 0.30% for Cefpodoxime.

The typical chromatograms of various samples under optimized HPLC conditions was depicted in Figure 4.

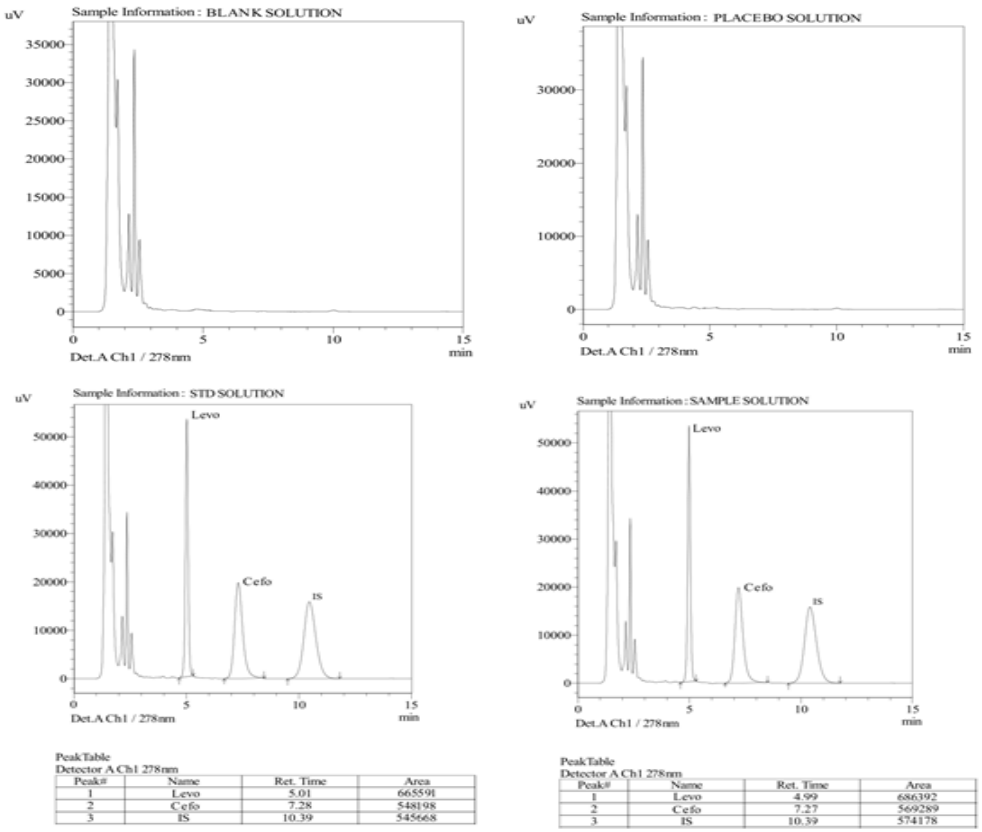

Figure 4: Typical chromatograms of Blank solution, Placebo solution, Sample solution & standard Solution.

System Precision

Six replicates injections of standard solution was injected in to the HPLC system and the chromatograms and area ratio of Levofloxacin to the Lamotrigine and Cefpodoxime to the Lamotrigine are recorded. % RSD for area ratio of Levofloxacin to the Lamotrigine and Cefpodoxime to the Lamotrigine of six replicate injections of standard solution was found 0.71% and 0.57% respectively implies that system is précises as tabulated in table no1.

Table No.1.

Injection No.	Area ratio (Levofloxacin to Lamotrigine)	Area ratio (Cefpodoxime to Lamotrigine)
1	1.2198	1.0046
2	1.2074	1.0055
3	1.1990	0.9942
4	1.2147	1.0005
5	1.2038	1.0017
6	1.2196	1.0113
Mean	**1.21072**	**1.00297**
Standard Deviation	**0.008642**	**0.005711**
% R.S.D.	**0.71**	**0.57**

Method Precision

For the evaluation of Method precision of the analytical method, six samples from homogenous mixture of single batch were prepared as per the test procedure of methodology and analyzed on HPLC system .%RSD for % assay of Levofloxacin and Cefpodoxime of six samples found was 0.78% and 0.30% as tabulated in Table no.2.

Table No.2.

Sample No.	% Assay of Levofloxacin	% Assay of Cefpodoxime
1	99.7	99.8
2	101.2	100.1
3	99.6	99.4
4	99.3	100.2
5	99.7	99.6
6	98.9	99.8
Mean	**99.7**	**99.8**
Standard Deviation	**0.78**	**0.30**
% R.S.D.	**0.78**	**0.30**

Method Ruggedness

The ruggedness was evaluated through analysis of six samples from a homogenous mixture of single batch by different analyst by using different column, different system and different day. % RSD for % assay of ruggedness found was 0.53% for Levofloxacin and 0.35% for Cefpodoxime and Overall % RSD found was 0.68% for Levofloxacin and 0.40% for Cefpodoxime as tabulated in Table no.3.

Table No.3.

Sr. No.	Levofloxacin		Cefpodoxime	
	% Assay of Levofloxacin	% Assay of Levofloxacin	% Assay of Cefpodoxime	% Assay of Cefpodoxime
	Method precision	Ruggedness	Method precision	Ruggedness
1	99.7	99.6	99.8	99.4
2	101.2	99.5	100.1	98.9
3	99.6	99.8	99.4	99.1
4	99.3	98.3	100.2	99.2
5	99.7	99.4	99.6	99.5
6	98.9	99.2	99.8	99.9
Mean	**99.7**	**99.3**	**99.8**	**99.3**
Standard Deviation	**0.78**	**0.53**	**0.30**	**0.35**
% R.S.D.	**0.78**	**0.53**	**0.30**	**0.35**
Overall Mean	**99.5**		**99.6**	
Overall S.D.	**0.68**		**0.40**	
Overall R.S.D.	**0.68**		**0.40**	

Accuracy (Recovery)

Accuracy of the analytical method was evaluated at a known concentration of Levofloxacin and Cefpodoxime at about 50%, 100% and 150% of test concentration of sample solution and 50% (1X Blend) and 150% (3x Blend) was calculated. % accuracy at individual level and overall average of % Recovery at all level for both Levofloxacin and Cefpodoxime was found 99% to 100% as tabulated in table no.4.

Table No.4.

Spike level in %	Levofloxacin				Cefpodoxime			
	% Recovery	Mean	SD	% RSD	% Recovery	Mean	SD	% RSD
50% (Assay)	99.9	**99.7**	**0.21**	**0.21**	99.4	**99.2**	**0.26**	**0.26**
	99.5				99.3			
	99.6				98.9			
100% (Assay)	99.5	**99.4**	**0.21**	**0.21**	99.8	**99.5**	**0.26**	**0.26**
	99.6				99.4			
	99.2				99.3			
150% (Assay)	99.9	**99.8**	**0.15**	**0.15**	100.1	**99.8**	**0.25**	**0.25**
	99.6				99.8			
	99.8				99.6			
50% (1X Blend)	99.1	**99.4**	**0.36**	**0.36**	99.0	**98.9**	**0.40**	**0.40**
	99.8				99.3			
	99.3				99.5			
150% (3X Blend)	99.9	**99.9**	**0.06**	**0.06**	100.0	**99.9**	**0.10**	**0.10**
	100.0				99.8			
	99.9				99.9			
Overall Mean	**99.6**				**99.5**			
Overall SD	**0.28**				**0.45**			
Overall % RSD	**0.28**				**0.45**			

Linearity

For the evolution of the linearity of the analytical method, a mixture of standard solution of Levofloxacin and Cefpodoxime in a concentration range of 7.0041 µg/ml to 380.0350 µg/ml for Levofloxacin and 5.6048 µg/ml to 280.1050 µg/ml for Cefpodoxime respectively were prepared as per the test procedure of methodology and analyzed on the HPLC system.

Correlation coefficient (r) value for Levofloxacin and Cefpodoxime using a regression equation with a 1/ (concentration^2) of weighting factor was calculated.

Correlation coefficient (r) value was found 1.0000 for Levofloxacin and 0.9999 for Cefpodoxime. Lower limit of Detection (LOD) and Lower limit of Quantification (LOQ) was calculated using following formulas.

Limit of detection (LOD) =3.3 X S.D. of Y intercept / Slope of the calibration curve.

Limit of Quantification (LOQ) =10 X S.D. of Y intercept / Slope of the calibration curve.

The LOD and LOQ were 0.9135µg/ml and 2.7681µg/ml for Levofloxacin.

The LOD and LOQ were 1.2387µg/ml and 3.7536µg/ml for Cefpodoxime.

The linearity plot was depicted in Figure 5 for Levofloxacin and Cefpodoxime.

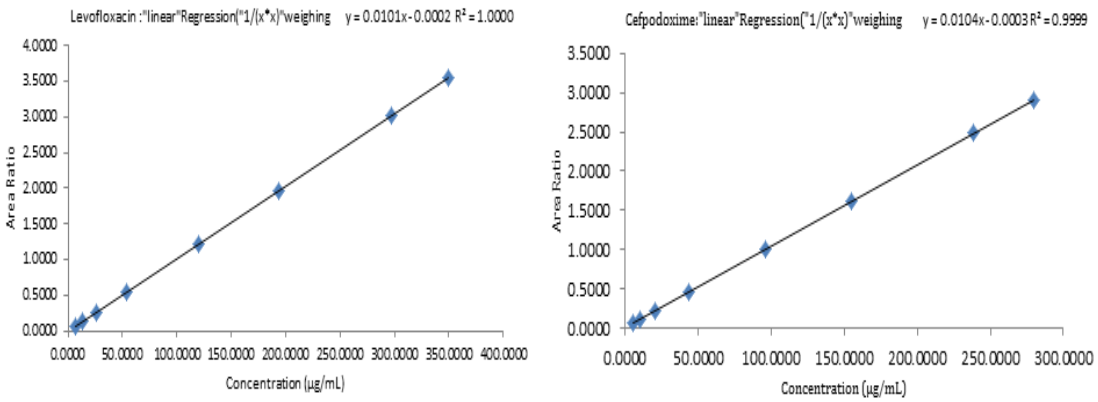

Figure 5: Linearity plot for Levofloxacin and Cefpodoxime.

Results was tabulated in table no.4.

Table No.5

Sample no.	Levofloxacin		Cefpodoxime	
	Concentration in µg/mL	Area Ratio	Concentration in µg/mL	Area Ratio
1	7.0041	0.0699	5.6048	0.0577
2	12.9706	0.1274	10.3793	0.1057
3	25.9412	0.2572	20.7586	0.2122
4	54.0441	0.5400	43.2471	0.4516
5	120.0979	1.2120	96.1047	1.0032
6	193.3944	1.957	154.7580	1.6123
7	297.5298	3.0108	238.0893	2.4815
8	350.0350	3.5366	280.1050	2.9084
Slope	**0.0101**		**0.0104**	
Intercept	**-0.0038**		**-0.0003**	
CC (r)	**1.0000**		**0.9999**	

The results of the linearity confirmed that an excellent correlation was exists between area ratio and concentration of both drugs within the concentration range.

Stability in analytical solution

For the evolution of stability in analytical solution; standard solution and sample solution was prepared freshly injected on the HPLC system at initially and different time intervals up to 48 hours and 54 hours respectively and the results of standard solution and sample solution were recorded. Absolute % difference and similarity factor were calculated.

For sample solution; absolute % difference between the initial result and results obtained at different time intervals was found 0.40% for Levofloxacin and 0.20% for Cefpodoxime.

For standard solution; similarity factor between the initial result and results obtained at different time intervals was found 99.6 for Levofloxacin and 99.6 for Cefpodoxime.

The sample solution is stable up to 48 hours and standard solution is stable up to 54 hours y on bench top at room temperature.

Filter paper study

Filter paper study was performed to measure the analysis impact of filter paper used during various experiments of analytical method validation. For the evolution of the filter paper study of the analytical method, standard solution was prepared as per test procedure of methodology and distributed the standard solution in two different portions. One portion centrifuged at 4000 rpm for 5 minutes and second portion was filter through 0.45-µm nylon membrane filter with discarding first 2mL of the filtrate and all the samples were analyzed on HPLC system.

Similarity factor between as such standard solution and filtered standard solution was found 100.3 for Levofloxacin and 100.7 for Cefpodoxime.

% absolute difference between average % assay of centrifuged sample solution and filtered sample solution was found 0.40% for Levofloxacin and 0.20% for Cefpodoxime.

Form the results it was concluded that the 0.45-µm nylon membrane filter with discarding first 2mL of the filtrate is suitable for the determination of the Assay Levofloxacin and Cefpodoxime in tablet formulation.

Forced degradation study

Forced degradation study was performed by treating sample tablet of 200MG/250MG strength containing 200 mg Cefpodoxime and 250 mg Levofloxacin under acidic, basic, peroxide, thermal, photolytic and humidity conditions but somewhat degradation of the Levofloxacin observed under peroxide stress condition and slightly acidic degradation detected for Cefpodoxime as tabulated in table no.6**.**

Table No.6 Results of Force degradation.

Degradation Condition	% Degradation	
	Levofloxacin	Cefpodoxime
Acid Treated	6.3	6.6
Alkali Treated	5.0	4.2
Peroxide Treated	5.2	6.3
Thermal Treated	11.3	0.1
Photolytic Treated	10.6	0.3
Humidity Treated	0.2	0.4

Method robustness

Robustness of the analytical method was evaluated by accomplishment of analysis under marginally changed in the chromatographic method of analysis such as change in detection wavelength, change in flow rate, change in composition of the mobile phase and change in column oven temperature, and the assay results were compared with the assay result of method precision i.e. with

finalized chromatographic conditions. The analytical method used is robust for change in flow rate, change in column oven temperature, and change in wavelength and change organic component of mobile phase.

The result was tabulated in table no.7 and in table no.8.

Table No.7 for Levofloxacin.

Sr. No	Method precision	Minus Flow	Plus Flow	Minus Temp	Plus Temp	Minus Organic comp. (MeOH)	Plus Organic comp. (MeOH)	Minus Wavelength	Plus Wavelength
1	99.7	99.4	99.9	99.8	99.5	99.8	99.7	99.6	99.7
2	101.2	99.7	99.5	99.7	99.8	99.6	99.5	99.4	99.3
3	99.6	99.5	99.8	99.6	99.6	99.8	99.6	99.4	99.4
4	99.3								
5	99.7								
6	98.9								
Overall mean		**99.7**	**99.7**	**99.7**	**99.7**	**99.7**	**99.7**	**99.6**	**99.6**
Overall SD		**0.63**	**0.63**	**0.62**	**0.62**	**0.62**	**0.62**	**0.63**	**0.64**
Overall %RSD		**0.63**	**0.63**	**0.62**	**0.62**	**0.62**	**0.62**	**0.63**	**0.64**

Table No.8 for Cefpodoxime

Sr. No	Methodprecision	Minus Flow	Plus Flow	Minus Temp	Plus Temp	Minus Organic comp. (MeOH)	Plus Organic comp. (MeOH)	Minus Wavelength	Plus Wavelength
1	99.8	99.4	99.5	99.9	99.7	99.4	99.5	99.2	99.5
2	100.1	99.6	99.4	99.6	99.9	99.8	99.6	99.4	99.4
3	99.4	99.5	99.7	99.5	99.7	99.5	99.7	99.3	99.2
4	100.2								
5	99.6								
6	99.8								
Overall mean		99.7	99.7	99.8	99.8	99.7	99.7	99.6	99.7
Overall SD		0.29	0.29	0.27	0.24	0.29	0.27	0.35	0.34
Overall % RSD		0.29	0.29	0.27	0.24	0.29	0.27	0.35	0.34

Range

From the analytical procedure data of precision, accuracy and linearity, the range of the analytical method used for simultaneous determination of assay of Levofloxacin and Cefpodoxime drugs in the pharmaceutical Tablet formulations using Lamotrigine as a common internal standard was tabulated in table no.7.

Table No.9

Name of Analyte (s)	Concentration (µg/mL)
Levofloxacin	7.0041 µg/ml to 350.0350 µg/ml
Cefpodoxime	5.6048 µg/ml to 280.1050 µg/ml

Analysis of Marketed Products

The potency test of marketed tablet products were performed after the complete validation of the method for simultaneous determination of assay of Levofloxacin and Cefpodoxime drugs in the pharmaceutical Tablet formulations using Lamotrigine as a common internal standard were performed by the proposed validated method.

The potency of tested brands was found to be within the limit of 98.00-102.00%.The results are tabulated in table no.10.

Table No. 10

Sr.No.	Brand name code	Cefpodoxime Label Claimed (mg)	Cefpodoxime Amount found (mg)	Cefpodoxime Potency (%)	Levofloxacin Label Claimed (mg)	Levofloxacin Amount found (mg)	Levofloxacin Potency (%)
1	Cefo and Levo **A**	200	203	101.50	250	250	100.00
2	Cefo and Levo **B**	200	201	100.50	250	251	100.40
3	Cefo and Levo **C**	200	202	101.00	250	252	100.80

CONCLUSIONS

This is the first reported High Performance Liquid Chromatographic method developed used for simultaneous determination of assay of Levofloxacin and Cefpodoxime drugs in the pharmaceuticals Tablet formulations using Lamotrigine as a common internal standard was stability indicating as recommended by ICH guidelines and validated for Specificity, System precision, Method precision, Ruggedness, Robustness, Accuracy etc. The present analytical method has a

widespread linear concentration range augmenting its applicability to different strength of Levofloxacin and Cefpodoxime tablet formulations. The chromatographic method may also be applied for simultaneous estimation of analytes in plasma, serum, urine after using appropriate sample extraction technique. Thus the method is Simpler, Accurate and Economical as compare to the previous methods.

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