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RP-HPLC ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF CIPROFLOXACIN AND LEVOFLOXACIN IN API AND PHARMACEUTICAL FORMULATION

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Abstract:
A reliable and efficient Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Ciprofloxacin and Levofloxacin in active pharmaceutical ingredients (APIs) and pharmaceutical formulations. The chromatographic separation was performed on a Symmetry C18 column (4.6 × 150 mm, 5 µm) using a mobile phase of Methanol and Triethylamine (TEA) buffer (pH 4.2) in the ratio 40:60 v/v, at a flow rate of 1.0 ml/min. The detection was carried out at 260 nm using a Waters HPLC system equipped with an auto sampler and PDA Detector (996 model). The column temperature was maintained at 40°C, and the injection volume was 10 µl, with a run time of 6 minutes. The method was validated in accordance with ICH Q2(R1) guidelines, and parameters such as specificity, linearity, accuracy, precision, robustness, LOD, LOQ, and system suitability were assessed. The results demonstrated that the method is simple, accurate, precise, and robust, with good resolution between both analytes and minimal interference from excipients. This validated method can be effectively applied for the routine quality control and quantitative analysis of Ciprofloxacin and Levofloxacin in both bulk and finished dosage forms.

Keywords: *RP-HPLC, Ciprofloxacin, Levofloxacin, Symmetry C18 column, simultaneous estimation, validation.*

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1. INTRODUCTION:

Chromatography is a powerful analytical technique used to separate and analyze complex mixtures of substances. It involves the distribution of components between a stationary phase (which is usually a solid or a liquid) and a mobile phase (which is a liquid or a gas). The basic principle of chromatography is based on the differential interaction of the components in a mixture with the stationary phase and the mobile phase. This causes the components to move at different rates, leading to their separation.

Chromatography is widely used in various fields, including chemistry, biochemistry, pharmaceutical analysis, environmental monitoring, and food safety. There are many types of chromatography, including gas chromatography (GC), liquid chromatography (LC), and thin-layer chromatography (TLC), among others.

High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) is a sophisticated and widely used form of liquid chromatography. It is an advanced technique designed to separate, identify, and quantify compounds in a mixture. HPLC has revolutionized analytical chemistry due to its high resolution, precision, and versatility, making it a cornerstone in pharmaceutical, clinical, environmental, and food testing.

Principle of HPLC:

HPLC works on the same basic principle as chromatography, but it uses high-pressure pumps to push the mobile phase (a liquid) through a column packed with a stationary phase (typically a solid or a gel). The mixture is injected into the column, and as it moves through, the different components in the sample interact with the stationary phase to different extents. This differential interaction results in the components separating from each other as they flow through the column, with each compound exiting at a different time, known as the retention time.

The separated components are detected at the column's exit by detectors, commonly UV/Vis detectors, which measure absorbance, fluorescence detectors, or mass spectrometers, depending on the application.

Components of an HPLC System:

1. **Solvent Reservoir:** Holds the mobile phase, which is pumped through the system.
2. **Pump:** Delivers the mobile phase to the column at high pressure, enabling efficient separation.
3. **Injector:** Introduces the sample mixture into the flow of the mobile phase.
4. **Column:** Contains the stationary phase (usually silica or polymer-based particles). The column's properties (such as particle size, pore size, and chemical surface) affect the separation.
5. **Detector:** Measures the components as they elute from the column. The most common detectors include UV/Vis, fluorescence, or refractive index detectors.
6. **Data System:** Processes and displays the chromatogram (a graphical representation of the detector's response over time).

Types of HPLC:

1. **Normal-Phase HPLC (NP-HPLC):** The stationary phase is polar, and the mobile phase is non-polar. It is primarily used for separating polar compounds.
2. **Reverse-Phase HPLC (RP-HPLC):** The stationary phase is non-polar (usually C18), and the mobile phase is polar. This is the most commonly used mode for separation in pharmaceutical analysis, biochemistry, and organic chemistry.
3. **Ion-Exchange HPLC:** The stationary phase is a charged resin, and it is used for separating ionic compounds.
4. **Size-Exclusion HPLC (SEC):** The stationary phase consists of porous particles, and it is used for separating molecules based on size, often used for protein or polymer analysis.

History:

Before the invention of HPLC, scientists employed traditional liquid chromatographic methods. Liquid chromatographic methods are inefficient because of the dependence of solvent flow rate on gravity. It can take several hours, or even days, to finish a separation. It was believed that gas stage partition and the study of highly polar high atomic weight biopolymers were not feasible, even though liquid chromatography (LC) was at the time more effective. Because the solutes were thermally unstable, some organic chemists found that GC was unsuccessful. It was therefore expected that other techniques would soon propel HPLC forward. In the 1960s, building on the work of Martin and Synge in 1941, Cal Giddings, Josef Huber, and others predicted that LC could be operated in the high-efficiency mode by lowering the pressing molecule measurement well below the standard LC and GC level of 150 µm and using pressure to increase the versatile stage velocity. These expectations were the subject of much investigation and development in the 1960s and early 1970s. Early efforts were made to enhance LC particles, and the creation of the externally permeable molecule Zipax proved positive for HPLC technology. Throughout the 1970s, a lot of advancements in equipment and machinery were produced. Experts originally constructed a simple HPLC system using injectors and pumps. The reason gas amplifier pumps were ideal was that they didn't require release free seals or check valves for excellent accuracy and steady flow,

and they operated at a constant pressure. The history of HPLC is primarily the story of the development of molecular technology, even though equipment advancements played a big part. Since the introduction of permeable layer particles to boost efficacy, there has been a constant trend towards smaller molecules. However, new issues surfaced as molecule sizes decreased. It is anticipated that the disadvantage of the unnecessary pressure drop will be the challenge of uniformly pressing extremely fine materials and moving diverse liquid through the segment. Generally, each time the molecule size is fully reduced, another cycle of instrument advancement should occur to manage the pressure.[5-10]

EXPERIMENTAL METHODS:

INSTRUMENTS USED

HPLC WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detector.
pH meter Lab India
Weighing machine Sartorius
Volumetric flasks Borosil
Pipettes and Burettes Borosil
Beakers Borosil

CHEMICALS USED:

Levofloxacin Provided by Sura Pharma labs
Ciprofloxacin Provided by Sura Pharma labs
Water and Methanol for HPLC LICHROSOLV (MERCK)
Acetonitrile for HPLC Merck
Triethyl amine Sura labs

HPLC METHOD DEVELOPMENT:

TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Ciprofloxacin and Levofloxacin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.75 ml of Ciprofloxacin and 1.125 ml of Levofloxacin from the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

RESULTS AND DISCUSSION:

Optimized Chromatogram (Standard)

Mobile phase : Methanol: TEA buffer pH 4.2 (40:60)
Column : Symmetry C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 260 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 6 minutes

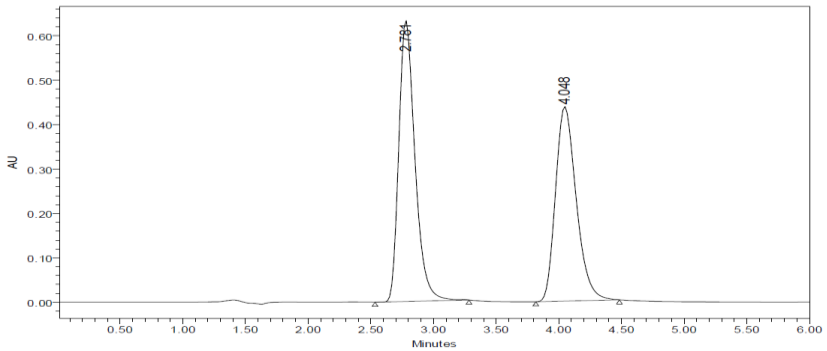

Fig-7.4: Results of Optimized Chromatogram

Table-7.4: Peak Results for Optimized Condition

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ciprofloxacin	2.781	2774027	299752		1.2	6314
2	Levofloxacin	4.048	2533532	210321	4.6	1.3	5521

Observation: From the above chromatogram it was observed that the Ciprofloxacin and Levofloxacin peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

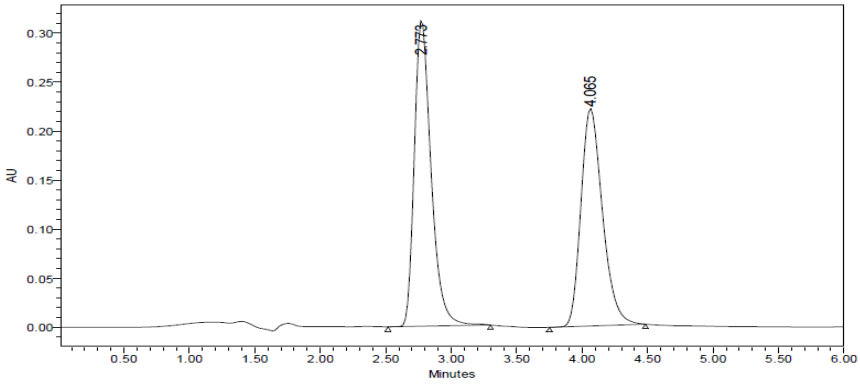

Figure-7.5: Optimized Chromatogram (Sample)

Table-7.5: Optimized Chromatogram (Sample)

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ciprofloxacin	2.773	2770123	282157		1.6	5011
2	Levofloxacin	4.065	2522041	251068	3.3	1.5	5947

Acceptance criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

METHOD VALIDATION

Blank:

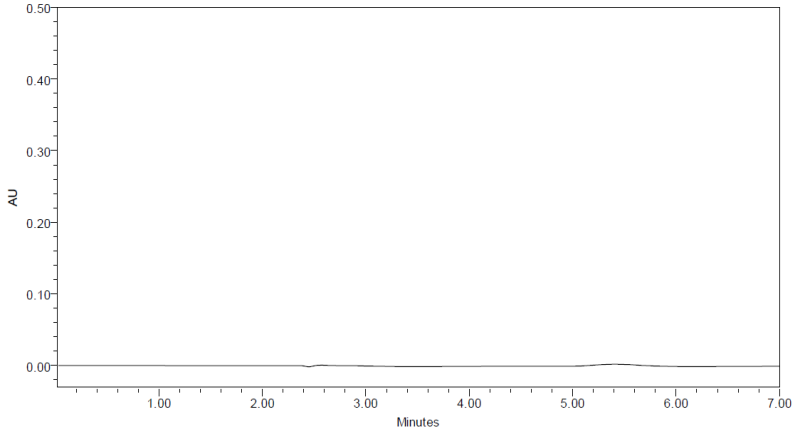

Fig 7.6: Chromatogram showing blank (mobile phase preparation)

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitates the drugs in drug product.

Assay (Standard):

Table-7.6: Peak results for assay standard of Ciprofloxacin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Tailing	USP Plate Count

1	Ciprofloxacin	2.767	2762937	357421	1.3	6344.7
2	Ciprofloxacin	2.795	2774613	388745	1.3	6344.2
3	Ciprofloxacin	2.768	2776429	364121	1.3	6344.2
Mean			2771306			
Std. Dev.			7321.9			
% RSD			0.26			

Table-7.7: Peak results for assay standard of Levofloxacin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Resolution	USP Tailing	USP Plate Count
1	Levofloxacin	4.029	2534375	210326	4.6	1.3	5937.7
2	Levofloxacin	4.067	2526189	226741	4.7	1.3	5008.8
3	Levofloxacin	4.030	2546248	231494	4.7	1.3	5990.7
Mean			2535604				
Std. Dev.			10085.82				
% RSD			0.397768				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Assay (Sample):

Table-7.8: Peak results for Assay sample

S.No.	Name	RT	Area	Height	USP	USP	USP Plate	Injection
1	Ciprofloxacin	2.764	2732203	294531		1.3	6314	1
2	Levofloxacin	4.012	2507543	216321	4.6	1.3	5954	1
3	Ciprofloxacin	2.767	2751843	286473		1.3	6369	2
4	Levofloxacin	4.016	[illegible]	216354	4.6	1.3	5944	2
5	Ciprofloxacin	2.764	2744776	312684		1.3	6329	3
6	Levofloxacin	4.013	2515628	206571	4.6	1.3	5990	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Ciprofloxacin , Levofloxacin in pharmaceutical dosage form was found to be 100. 9%, 100. 7%.

LINEARITY
CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:
Ciprofloxacin:

Concentration µg/ml	Average Peak Area
37.5	892464
75	1866364
112.5	2777423
150	3709213
187.5	4601317

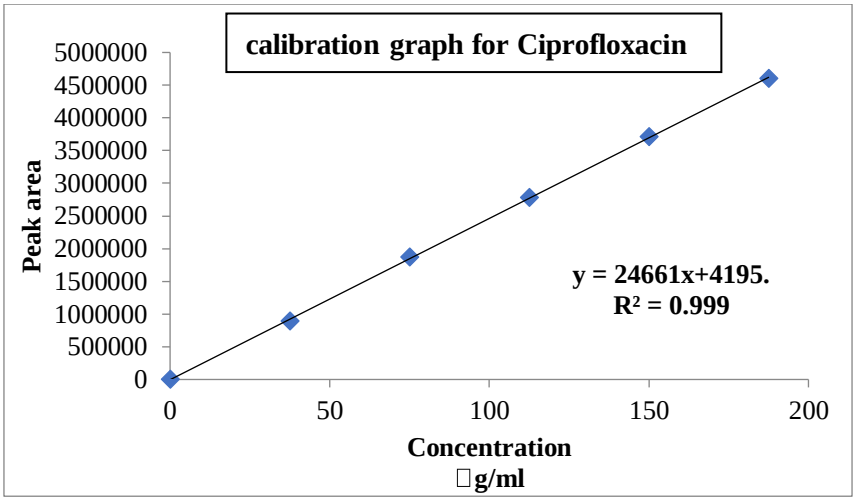

Figure 7.18: Calibration graph for Ciprofloxacin

LINEARITY PLOT:
The plot of Concentration (x) versus the Average Peak Area (y) data of DRUG is a straight line.

Y = mx + c
Slope (m) = 24661
Intercept (c) = 4195
Correlation Coefficient (r) = 0.999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.
CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 4195. These values meet the validation criteria.

Levofloxacin

Concentration µg/ml	Average Peak Area
25	920032
50	1752782
75	2521426
100	3326009
125	4217393

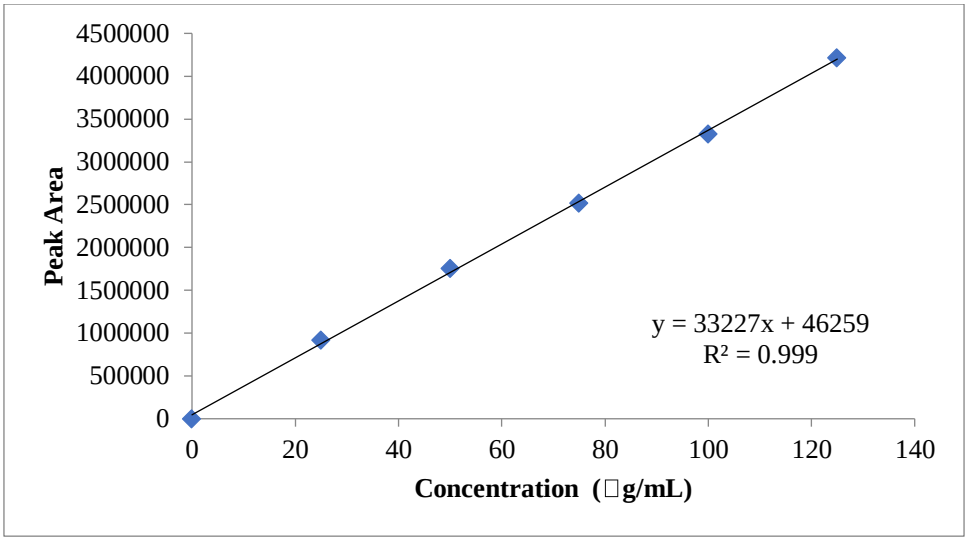

Figure 7.19: Calibration graph for Levofloxacin

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Levofloxacin is a straight line.

Y = mx + c
Slope (m) = 33227
Intercept (c) = 46259
Correlation Coefficient (r) = 0.999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 46259. These values meet the validation criteria.

PRECISION:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-:7.9 Results of repeatability for Ciprofloxacin:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ciprofloxacin	2.766	2766870	294578	6684	1.3
2	Ciprofloxacin	2.774	2771971	286541	6347	1.3
3	Ciprofloxacin	2.770	2771958	302657	6674	1.3
4	Ciprofloxacin	2.772	2780299	293412	6451	1.3
5	Ciprofloxacin	2.771	2789695	283154	6678	1.3
Mean			2776159			
Std. Dev			8969.6			
% RSD			0.32			

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table-:7.10 Results of method precision for Levofloxacin:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Levofloxacin	4.025	2534539	193240	5761	1.3	4.7
2	Levofloxacin	4.040	2539247	201647	5489	1.3	4.6

3	Levofloxacin	4.032	2544661	193472	5367	1.3	4.6
4	Levofloxacin	4.041	2548839	196475	5845	1.3	4.6
5	Levofloxacin	4.036	2558822	201394	5347	1.3	4.7
Mean			2545221				
Std. Dev			9330.0				
% RSD			0.37				

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate Precision:

Day 1:

Table-:7.11 Results of Intermediate precision for Ciprofloxacin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ciprofloxacin	2.781	2715421	294651	6647	1.3
2	Ciprofloxacin	2.780	2778540	284123	6781	1.3
3	Ciprofloxacin	2.782	2754247	274561	6984	1.3
4	Ciprofloxacin	2.780	2780545	281241	6475	1.3
5	Ciprofloxacin	2.782	2777021	286471	6647	1.3
6	Ciprofloxacin	2.774	2780254	294512	6489	1.3
Mean			2764338			
Std. Dev			25974			
% RSD			0.9			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2

Table-:7.12 Results of Intermediate precision for Levofloxacin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Levofloxacin	4.048	2506927	211541	5495	1.4	4.6
2	Levofloxacin	4.050	2504522	206141	5694	1.4	4.6
3	Levofloxacin	4.049	2541270	198641	5785	1.4	4.7
4	Levofloxacin	4.050	2507885	206741	5947	1.4	4.6
5	Levofloxacin	4.049	2504587	209487	5742	1.4	4.6
6	Levofloxacin	4.040	2504780	193481	5914	1.4	4.6
Mean			2511662				
Std. Dev			14572.01				
% RSD			0.5				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Day 2:

Table-:7.13 Results of Intermediate precision Day 2 for Ciprofloxacin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ciprofloxacin	2.764	2781856	294651	6647	1.3
2	Ciprofloxacin	2.759	2761510	284123	6781	1.3
3	Ciprofloxacin	3.015	2748811	274561	6984	1.3
4	Ciprofloxacin	2.773	2790831	281241	6475	1.3
5	Ciprofloxacin	2.765	2785112	286471	6647	1.3
6	Ciprofloxacin	2.764	2781932	294512	6489	1.3
Mean			2775009			
Std. Dev			16222.05			
% RSD			0.5			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Table-7.14: Results of Intermediate precision for Levofloxacin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Levofloxacin	4.015	2536301	211541	5495	1.4	4.6
2	Levofloxacin	4.007	2541972	206141	5694	1.4	4.6
3	Levofloxacin	4.323	2521259	198641	5785	1.4	4.7
4	Levofloxacin	4.065	2537081	206741	5947	1.4	4.6
5	Levofloxacin	4.020	2549869	209487	5742	1.4	4.6
6	Levofloxacin	4.015	2536301	193481	5914	1.4	4.6
Mean			2537131				
Std. Dev			9370.087				
% RSD			0.36				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY:

Table-:7.18 The accuracy results for Ciprofloxacin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1382603	56.25	55.05	99. 9	99.8%
100%	2777270	112.5	112.4	99. 9	
150%	41448756	225	224.6	99.6	

Table-:7.19 The accuracy results for Levofloxacin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1306990	37.5	37.5	100	99.4%

100%	2510628	75	74.8	98.6	
150%	3777999	150	149.96	99.8	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Ciprofloxacin:

=0.8µg/ml

Levofloxacin:

=0.7µg/ml

LIMIT OF QUANTITATION

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Ciprofloxacin:

= 2.4µg/ml

Levofloxacin:

= 2. 19µg/ml

Robustness

Table-: Results for Robustness

Ciprofloxacin:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2774027	2.781	6314	1.2
Less Flow rate of 0.9 mL/min	2884521	3.327	6199	1.4
More Flow rate of 1.1 mL/min	2542012	2.516	6234	1.4
Less organic phase	2888515	3.326	6298	1.4
More organic phase	2541550	2.416	6287	1.2

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Levofloxacin:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2533532	4.048	5521	1.3
Less Flow rate of 0.9 mL/min	2750214	5.319	5643	1.6
More Flow rate of 1.1 mL/min	2254107	3.649	5782	1.5
Less organic phase	2754017	5.318	5309	1.4
More organic phase	2215870	3.233	5580	1.51

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY AND CONCLUSION:

Summary:
A simple, precise, accurate, and robust Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method was successfully developed and validated for the simultaneous estimation of Ciprofloxacin and Levofloxacin in active pharmaceutical ingredients (APIs) and pharmaceutical dosage forms. The chromatographic separation was achieved using a Symmetry C18 column (4.6 × 150 mm, 5 µm) with a mobile phase consisting of Methanol and Triethylamine (TEA) buffer at pH 4.2 in the ratio of 40:60 v/v. The pH of the buffer was adjusted to 4.2, and the flow rate was maintained at 1 ml/min. Detection was carried out at a wavelength of 260 nm using a Waters HPLC system with an auto sampler and PDA Detector (996 model). The column temperature was set at 40ºC, and the injection volume was 10 µl with a total run time of 6 minutes.
The method was validated as per ICH Q2(R1) guidelines for parameters such as specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, and system suitability. The results indicated that the method provided good resolution between the two analytes with sharp, symmetrical peaks and acceptable retention times.

CONCLUSION:

The developed RP-HPLC method is highly reliable and efficient for the simultaneous estimation of Ciprofloxacin and Levofloxacin in both bulk drug and pharmaceutical dosage forms. The method met all the validation criteria as per ICH guidelines, confirming its specificity, accuracy, linearity, precision, robustness, and suitability for routine quality control analysis. The optimized chromatographic conditions enabled excellent peak separation with minimal interference from excipients or degradation products, making the method suitable for use in routine pharmaceutical analysis, stability testing, and quality control laboratories.

BIBILOGRAPHY:

1. Martin M, Guiochon G. Effects of high pressures in liquid chromatography. J. Chromatogr. Anal. 2005;(1-2)7:16-382.
2. Liu Y, Lee ML. Ultrahigh pressure liquid chromatography using elevated temperature. J.Chromatogr.2006;1104(1-2):198–2023.
3. AbidiSL. High-performance liquid chromatography of phosphatidic acids and relatedpolar lipids. J. Chromatogr.1991; 587:193-2034.
4. Hearn MT. Ion-pair chromatography on normal and reversed-phase systems. Adv. Chromatogr.1980; 18:59–100.
5. Harmita, et al. Optimation and validation of analytical method of cotrimoxazole in tablet and plasma in vitro by high-performance liquid chromatography. J Bioanal Biomed. 2012;4:26-9.
6. Nardulli P, et al. A combined HPLC and LCMS approach for evaluating drug stability in elastomeric devices: a challenge for the sustainability in pharmacoeconomics. J Pharmacovigilance. 2014;2:157.
7. Hafez HM, et al. Development of a stabilityindicating HPLC method for simultaneous determination of amlodipine besylate and atorvastatin calcium in bulk and pharmaceutical dosage form. Pharm Anal Acta. 2014;5:316.
8. Shintani H. Immobilized enzyme column combined with HPLC and column switching method for the analysis of complicated matrix such as body fluids. Pharmaceut Reg Affairs. 2014;03(5):e142. doi: 10.4172/2167-7689.1000e142.
9. Murthy TGK, Geethanjali J. Development of a validated RP-HPLC method for simultaneous estimation
10. Suresh Babu VV, et al. Validated HPLC method for determining related substances in compatibility studies and novel extended release formulation for ranolazine. J Chromatogr Separat Techniq. 2014;5:209.
11. Arayne MS, et al. Monitoring of pregabalin in pharmaceutical formulations and human serum using UV and RPHPLC techniques: application to dissolution test method. Pharm Anal Acta. 2014;5:287
12. Praveen C, et al. Method development and validation for simultaneous estimation of ethinyl estradiol and drospirenone and forced degradation behavior by HPLC in combined dosage form. Pharm Anal Acta.
13. Abdulla SA, et al. Validated HPLC method for the determination of nisoldipine. Pharm Anal Acta. 2013;S1:004.
14. Sawsan Mohammed AH, et al. Effects of blood collection tubes on determination vitamin-A by HPLC. J Chromatogr Sep Tech. 2013;4:184.
15. Subbaiah PR, Kumudhavalli MV, Saravanan C, Kumar M, Chandira RM. Method development and validation for estimation of moxifloxacin HCl in tablet dosage form by RP-HPLC method. Pharm Anal Acta. 2010;01(2):109. doi: 10.4172/2153- 2435.1000109.

16. Ahir KB, et al. Simultaneous estimation of metformin hydrochloride and repaglinide in pharmaceutical formulation by HPTLC Densitometry method. J Chromatogr Sep Tech. 2013;4:166.
17. Khodadoust S, et al. A QSRR study of liquid chromatography retention time of pesticides using linear and nonlinear chemometric models. J Chromatogr Sep Tech. 2012;3:149.
18. Vali SJ, et al. Separation and quantification of octahydro-1h-indole-2-carboxilic acid and its three isomers by HPLC using refractive index detector. J Chromatogr Sep Tech. 2012;3:136
19. Fayyad MK, et al. Effect of temperature, wavelength, ph, ion pair reagents and organic modifiers' concentration on the elution of cystatin C. stability of mobile phase. J Anal Bioanal Tech. 2010;1:103.
20. Ndorbor T, et al. Chromatographic and molecular simulation study on the chiral recognition of atracurium besylate positional isomers on cellulose tri- 3, 5-dimethylphenycarbamate (CDMPC) column and its recognition mechanism. J Chromatogr Sep Tech. 2013;4:176.
21. Hua Z, et al. Extraction and purification of anthocyanins from the fruit residues of Vaccinium uliginosum Linn. J Chromatogr Sep Tech. 2013;4:167.
22. Rogatsky E 2D or Not 2D. Column-switching techniques, multidimensional separations and.
23. Sagar KA, R Smyth MR. MultiDimensional column chromatographic method with UV detection, for the determination of propranolol at therapeutic levels in human plasma. Pharm Anal Acta. 2012;3(10):197. doi: 10.4172/2153-2435.1000197
24. Flores HE, Galston AW. Analysis of polyamines in higher plants by high performance liquid chromatography. Plant Physiol. 1982;69(3):701-6. doi: 10.1104/pp.69.3.701, PMID 16662279.
25. Hearn MTW. Ion-pair chromatography on normal and reversed-phase systems. Adv Chromatogr. 1980;18:59-100.
26. HPLC, Chemiguide; May 2, 2007. Available from: http://www.chemguide.co.uk.
27. Rao G, Goyal A. An overview on analytical method development and validation by using HPLC. ThérapiePharmaceutical and Chemical Journal. 2016;3(2):280-9.
28. Mcpolin O. An introduction to HPLC for pharmaceutical analysis. Mourne Training Service. p. 11-2.
29. Fundamentals of Analytical chemistrySkoog. West, Holler, Crouch. 2009;973.
30. Elshanawane AA, et al. Development and validation of HPLC method for simultaneous estimation ofbrimonidine tartrate and timolol maleate in bulk and pharmaceutical dosage form. J Chromatogr SeparateTechniq. 2014;5:230.
31. The European pharmacopoeia. 4th ed. Strasbourg: Council of Europe; 2002.
32. Tsai IL, Weng TI, Tseng YJ, Tan HK, Sun HJ, Kuo CH. Screening and confirmation of 62 drugs ofabuse and metabolites in urine by ultrahigh- performance liquid chromatography-quadrupole time-offlightspectrometry. J Anal Toxicol. 2013 Nov-Dec;37(9):642-51. doi: 10.1093/jat/bkt083. PMID 24084874.
33. Hearn MTW. Ion-pair chromatography on normal and reversed-phase systems. Adv Chromatogr. 1980;18:59-100
34. Siddiqui MR, AlOthman ZA, Rahman N. Analytical techniques in pharmaceutical analysis: a review. Arab J Chem. 2013.
35. Willard H, Merritt L, Dean J, Settle F. Instrumental methods of analysis. 7th ed. Stamford, CT: Wadsworth Publishing; 1998.
36. Scott RPW: Principles and Practice of Chromatography. Chrom-Ed Book Series, 2003; 1-2.
37. Chatwal GR, Anand SK : Instrumental Methods of Chemical Analysis. 5th edition 2004. 1.1-1.3, 2.566-2.2.575.
38. High performance liquid chromatography [Internet]. 2009 [Accessed 2009 Jan 20].Available from: en. wikipedia.org/wiki/file:Agilent1200HPLC.jpg. html.
39. Renlund S, Erlandsson I, Hellman U, Silberring J, Wernstedt C, Lindström L, Nyberg F: Micropurification and amino acid sequence of β-casomorphin-8 in milk from a woman with postpartum psychosis Peptides. 1993;14 :1125-1132.
40. Willard HH, Dean AJ: Instrumental Methods of Analysis. CBS Publishers and distributors, 7thedition 1986; 513-515, 580-604.
41. Connors AK. A Text Book of Pharmaceutical Analysis. A Wiley Intersciencepublication, 3rdedition 2005; 373-400.
42. Ahuja S, Ahuja S: High Pressure Liquid Chromatography. Comprehensive Analytical Chemistry. Elsevier; 2006.
43. Amesham Biosciences:Reversed Phase Chromatography. Principles and Methods ; 6-8.
44. Dorsey JG, Cooper WT: Retention mechanisms of bonded-phase liquid chromatography. Anal. Chem. 66th edition 1994; 857A-867A,
45. Tanford CW : Physical chemistry of macromolecules. 1961

46. How do I develop an HPLC method. Available from: www.sge.com
47. Snyder LR, Kirkland JJ, Glajch JL: Practical HPLC Method Development. 2nd ed. 2001
48. Sethi PD: HPLC Quantitative Analysis of Pharmaceutical Formulations. CBS Publishers & Distributors, first edition 2001.
49. Lindholm J: Development and Validation of HPLC Methods for Analytical and Preparative Purposes. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. 2004; 995
50. McCown SM, Southern D, Morrison, B.E. Solvent properties and their effects on gradient elution high performance liquid chromatography. Experimental findings for water and acetonitrile. J. Chromatogr.1986; 352: 493-509.