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METHOD DEVELOPMENT AND VALIDATION OF ANTIBACTERIAL DRUGS BY USING RP-HPLC METHOD IN SOLID DOSAGE FORM

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ABSTRACT

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 230nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was WATERS RP-C_{18} (250mm×4.6mm, 5µm) produce good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Different ratios of mobile phase were studied, mobile phase with ratio of 70:30v/v orthophosphoric acid Buffer: Methanol was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonocation time was fixed to be 15min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 6min because analyte gave peak around 3.09 and 4.55 for Levofloxacin and Cefixime respectively and also to reduce the total run time. This system produced symmetric peak shape, good resolution and reasonable retention times of Cefixime and Levofloxacin. The mean recoveries were found to be 100 and 100 respectively for Levofloxacin and Cefixime respectively was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 0.006 and 0.002 respectively for Cefixime and Levofloxacin. Linearity study was, correlation coefficient and curve fitting was found to be 0.99 and 0.99 for Cefixime and Levofloxacin respectively. The analytical method was found linearity over the range of 50-150ppm of the target concentration. The average retention time for Cefixime and Levofloxacin were found to be 4.56 and 3.09 respectively. The analytical passed robustness test. Relative standard deviation was well satisfactory.

Keywords: RP HPLC**,** Levofloxacin, Cefixime, Estimations, Validation parameters

INTRODUCTION [1-5]

High-performance liquid chromatography (HPLC) is the fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids. High performance liquid Chromatography (HPLC) is the term used to describe liquid chromatography in which the liquid mobile phase is forced through the column at high speed as a result, the analysis time is reduced by 1-2 orders of the magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possibly increasing the column efficiency substantially.

High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. It also allows using a very much smaller particle size for the column packing material which gives a much greater surface area

for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture. The other major improvement over column chromatography concerns the detection methods which can be used. These methods are highly automated and extremely sensitive.

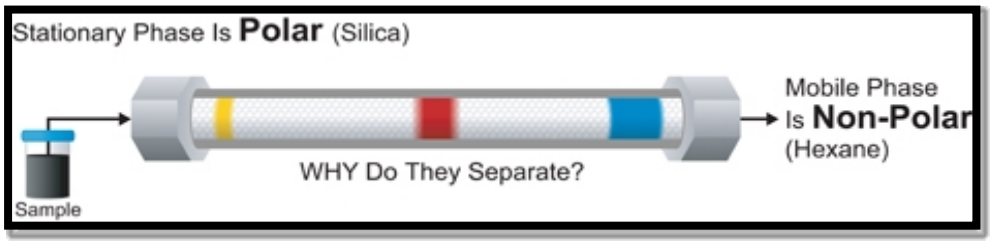

Fig 1: Normal phase chromatography

Instrumentation of HPLC

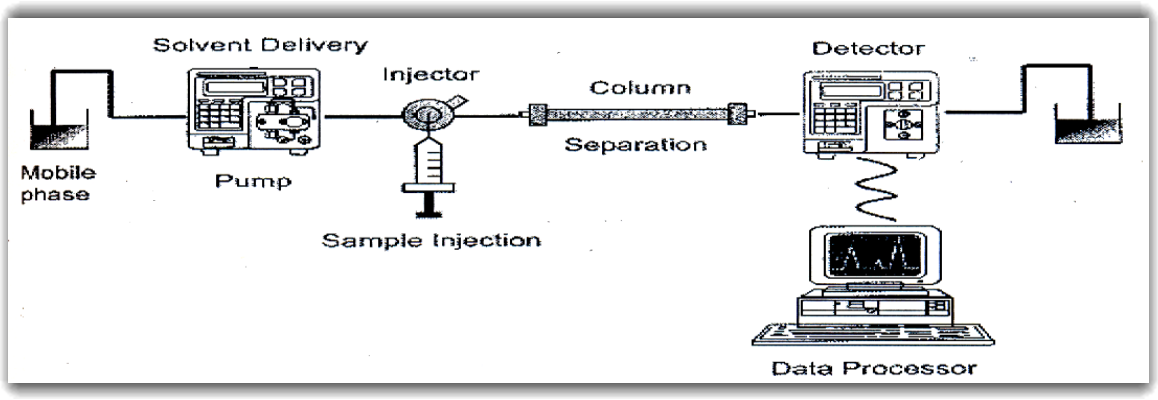

Fig 2: Block diagram of HPLC

METHOD VALIDATION: Typical performance characteristics of LC methods are specificity, selectivity, precision, linearity, robustness, recovery, range, limit of quantification, limit of detection and ruggedness. Validation should refer to an "analytical system" (system precision) rather than an "analytical method" (method precision), the analytical system comprising a defined method protocol, a defined concentration range for the analyte, and a specified type of test material.

DRUG PROFILE: LEVOFLOXACIN

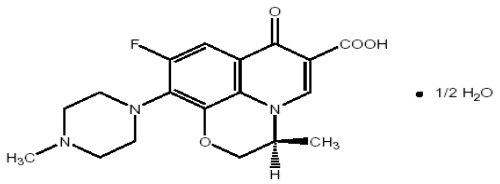

Fig 3: Molecular Structure of Levofloxacin

Molecular formula: $\underline{C}_{18}\underline{H}_{20}\underline{F}\underline{N}_3\underline{O}_4$
Solubility: Soluble in water. Freely soluble in glacial acetic acid and Chloroform, sparingly soluble in Methanol, slightly soluble in ethanol, practically insoluble in ether.
Category: Anti-Bacterial Agents, Quinolones, Nucleic Acid Synthesis inhibitors, Anti-Infective Agents, Urinary.

CEFIXIME

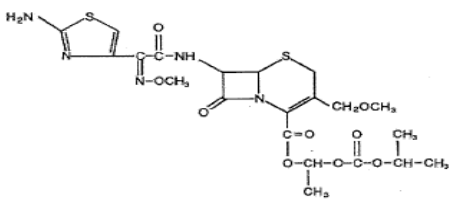

Fig 4: Molecular Structure of Cefpodoxime Proxetil

Solubility **:** slightly soluble in water, soluble in methanol and sparingly soluble in ethanol.
Category **:** Anti Bacterials, Cephalosporins

MATERIAL AND METHODS

SAMPLE: A gift sample is collected from Lara Drugs Private Limited

APPARATUS USED

HPLC : Equipped with a photodiode array detector capable of operating in the range of 190 nm to 400 nm (Waters)
pH meter : Range from 0-14 (Sartorius)
Analytical Balance : Accurate to 0.1 µg (Sartorius)
Graduated Cylinder : 10ml, 50 ml, 100ml (Borsil, Rankem)
Volumetric Flasks : 100 ml, 50 ml, 10 ml, 5 ml (Borsil, Rankem)
Volumetric Pipettes : 10 ml, 5 ml, 3 ml, 2 ml, 1 ml (Borsil, Rankem)
Graduated Pipettes : 10 ml, 5 ml, 2 ml, 1 ml (Borsil, Rankem)
Solvent filtration unit : Millipore (Rankem)
Syringe Filters : PVDF filters (Zodiac Life Sciences, India)
Sonicator : (Sonorex)

STANDARDS USED: Levofloxacin

CHEMICALS USED

Orthophosphoric Acid (OPA) : (Merck or GR grade)
Water : (HPLC grade or Milli-Q or TKA grade)
Methanol : (HPLC grade)
Software used : Empower-2

LIST OF INSTRUMENTS

HPLC : Water
Ultra Sonicator : Ultra Clean
Electronic Balance: Sartorius
pH meter : Sartorious

METHOD DEVELOPMENT: The following studies were conducted for this purpose.
Detection wavelength: The UV spectrum of Cefpodoxime Proxetil and Levofloxacin was recorded by scanning between 210- 400 nm. From this spectrum λ_{max} at 230 nm was selected for the proper study.
Choice of stationary phase: ACE C-18 columns, WATERS C-18 column.
Selection of the mobile phase**:** A mixture of buffer and methanol in the ratio of 70:30v/v was proved to be the most suitable of all combinations since the chromatographic peaks obtained were better defined and resolved and almost free from tailing.
Flow rate: It was found from the experiments that 1.0 ml/min flow rate was ideal for the successful elution of the analyte.
Selection of Chromatographic method: Based on the P_{ka} of the candidate drug the solubility, and as the drug is non-polar the chromatographic technique was selected for initial separations from the knowledge of properties of the compound.

RESULTS AND DISCUSSION

System suitability:The % Relative standard deviation of five replicate injections for Cefixime and Levofloxacin was found to be 1.0 and 0.3. The %Relative standard deviation of five replicate injections for Cefixime and Levofloxacin standard were found to be within limits.
The tailing factor for Cefixime and Levofloxacin peaks was found to be 1.269 and 1.1964. The tailing factor for Cefixime and Levofloxacin peaks was found to be within limits. The theoretical plates for Cefixime and Levofloxacin were found to be 8134 and 10362 respectively. The resolution was found to be 8.809.

Table 1: Results from system suitability studies of Cefixime and Levofloxacin

S.NO	Injection Number	Peak area for Cefixime	Peak area for Levofloxacin	Acceptance criteria
1	01	3820597	4648890	The % RSD of peak areas of Cefixime and Levofloxacin should not be more than 2.0
2	02	3862227	4681298	
3	03	3764240	4691774	
4	04	3829312	4675353	
5	05	3778733	4680978	
Mean		3811022	4675659	
%RSD		1.0	0.3	

Precision

System precision: To study the system precision, six replicate mixed standard solutions of Cefixime and Levofloxacin were injected the %RSD was calculated and it was found to be 0.05 and 0.02 respectively for Cefixime and Levofloxacin, which are well within the acceptable criteria of not more than 2.0.

Table 2: System precision of Cefixime and Levofloxacin

Injection number	Area of Cefixime	Area of Levofloxacin	Acceptance criteria
1	3818769	4678289	The % RSD of peak areas of Cefixime and Levofloxacin should
2	3812585	4677549	
3	3812077	4676508	

4	3812886	4675202	not be more than 2.0
5	3817130	4677862	
6	3811304	4678822	
Mean	3814125	4677372	
% RSD	0.08	0.02	

Method precision: The Relative standard deviation of individual % assay of Cefixime and Levofloxacin from six sample preparations should be not more than 2.0%. The Relative standard deviation of individual % assay of Cefixime and Levofloxacin were found to be 0.05 and 0.02 respectively. The method is considered "PRECISE" if the assay results of Cefixime and Levofloxacin should be not less than 95.0% and not more than 105.0%. It is observed from the data tabulated above, that the % RSD of the peak responses as peak area was found to be within acceptance criteria indicating an acceptance level of precision for Repeatability precision studies

Table 3: Method Precision for Cefixime and Levofloxacin

Sample number	% Assay of Levofloxacin	% Assay of Cefixime
1	100.03	100.04
2	100.04	100.06
3	100.03	100.05
4	100.05	100.03
5	100.06	100.03
6	100.04	100.04
Mean	100.036	100.035
% RSD	0.03	0.08

Accuracy: The results were found within acceptance criteria. Hence the method is accurate throughout the selected range.

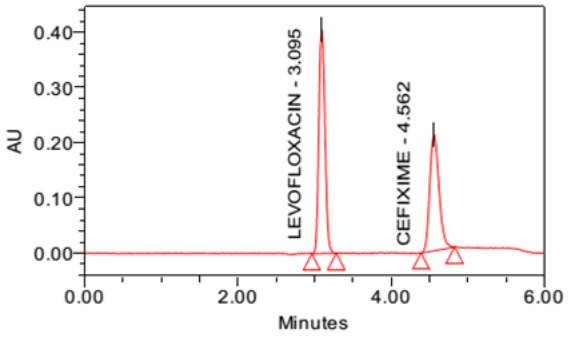

Fig 5.1: Chromatogram of Accuracy at 50%

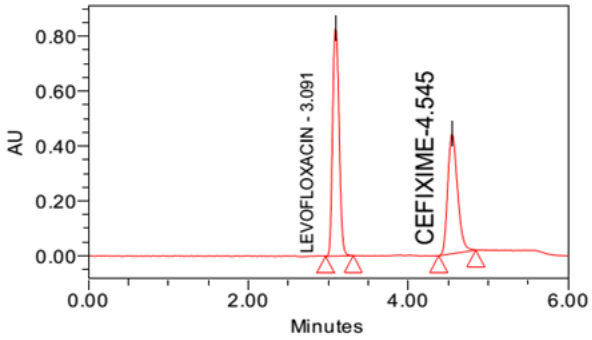

Fig 5.2: Chromatogram of Accuracy at 100%

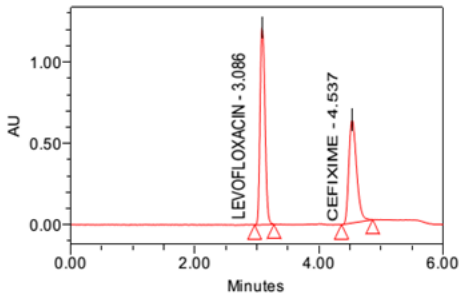

Fig 5.3: Chromatogram of Accuracy at 150%.

LINEARITY OF DETECTOR RESPONSE: The *Correlation coefficient* for Levofloxacin and Cefixime was found to be 0.99 and 0.99 respectively, which indicates that the peak responses are linear. This concluded that the method was linear throughout the range selected.

Table 4: Results for Linearity of Detector Response

% Level	Mean peak area	Concentration (ppm)
Levofloxacin		
50	2333535	250
75	3505494	375.00
100	4673695	500.00

125	5841370	625
150	7015486	750
Cefixime		
50	1906610	200
75	2859061	300
100	3810769	400
125	4763750	500
150	5716217	600

SPECIFICITY: On the basis of these chromatograms we can say that there is no interference of blank and placebo at the retention time of Cefixime and Levofloxacin.

Table 5: specificity study

Name of the solution	Retention time (min)
Blank	No peaks
Levofloxacin	3.8
Cefixime	5.6

Effect of variation in mobile phase flow rate

Table 6: Results of Effect of variation in mobile phase Flow Rate

S. No	System suitability parameters	Flow 0.8ml/min	Flow 1.0ml/min	Flow 1.2ml/min
Cefixime				
1	Tailing Factor for standard preparation	1.24	1.28	1.29
2	Theoretical Plates for Standard solution	7448	8801	7274
3	The Resolution between Cefixime and Levofloxacin	8.326	9.160	8.163
Levofloxacin				
1	Tailing Factor for standard preparation	1.09	1.20	1.22
2	Theoretical Plates for Standard solution	8403	11627	7582

Effect of variation in temperature

Table 7: Results of Effect of variation in Temperature

S. no	System suitability parameters	20°C	25°C	30°C
Cefixime				
1	Tailing Factor for standard preparation	1.29	1.28	1.22
2	Theoretical Plates for Levofloxacin Standard solution	7274	8801	7899
3	The Resolution between Cefixime and Levofloxacin peaks in solution	8.16	9.16	8.54
Levofloxacin				
S. no	**System suitability parameters**	**20°C**	**25°C**	**30°C**
1	Tailing Factor for standard preparation	1.22	1.20	1.15
2	Theoretical Plates for Levofloxacin Standard solution	7582	11627	9234

From the above results obtained, it was found that the robustness parameters were within the limit at all variable conditions.

Limit Of Detection And Limit Of Quantification

From the above results obtained, it was found that the LOD and LOQ parameters were within the limit at all variable conditions.

Table 8: LOD and LOQ

Parameter	Measured value (µg/ml)	
	Cefixime	Levofloxacin
Limit of detection	0.006	0.002
Limit of quantification	0.02	0.008

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 230nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was WATERS RP-C_{18} (250mm×4.6mm, 5µm) produce good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Different ratios of mobile phase were studied, mobile phase with ratio of 70:30v/v orthophosphoric acid Buffer: Methanol was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonocation time was fixed

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The mean recoveries were found to be 100 and 100 respectively for Levofloxacin and Cefixime respectively were linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 0.006 and 0.002 respectively for Cefixime and Levofloxacin. Linearity study was, correlation coefficient and curve fitting was found to be 0.99 and 0.99 for Cefixime and Levofloxacin respectively. The analytical method was found linearity over the range of 50-150ppm of the target concentration. The average retention time for Cefixime and Levofloxacin were found to be 4.56 and 3.09 respectively. The analytical passed robustness test. Relative standard deviation was well satisfactory.

From the overall results obtained by RP-HPLC method for the estimation of Cefixime and Levofloxacin in a tablet dosage form it was found that the method was precise and simple and it was estimated by using a simple method without using the advanced instruments like GC, LC-MS, UPLC etc. Finally, it was concluded that the developed method was more accurate, precise, specific and robust with ±2ºC in temperature and ±0.2ml/min in flow rate.

CONCLUSION

From the reported literature review, there were few methods established for the determination of Cefixime and Levofloxacin in individual and in combination with other drugs. In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and low cost RP-HPLC method. It is successfully applied for the determination of Cefixime and Levofloxacin in pharmaceutical preparations.

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