© June 2026 | IJIRT | Volume 13 Issue 1 | ISSN: 2349-6002

HPLC Method For Simultaneous Determination of Ambroxil and Cefadroxil in Pharmaceutical Dosage Form

Burra Lekhya[1], G Srinivasa Rao[2], Guduru Rajeswari[3], Kolavali Yalla Reddy[4]
[1]*Department of Pharmaceutical Analysis and Quality Assurance, Saastra college of Pharmaceutical Education and Research, Varigonda (v) T.P Gudur (m) SPSR Nellore Dt -524311*
[2]*Principal and Professor, Saastra college of Pharmaceutical Education and Research, Varigonda (v) T.P Gudur (m) SPSR Nellore Dt -524311*
[3]*Principal and Professor, Department of Pharmacology, Saastra College of Pharmaceutical Sciences for Women, Varigonda (v) T.P. Gudur (m) SPSR Nellore Dt -524311*
[4]*Associate Professor, Saastra College of Pharmaceutical Sciences for Women, Varigonda (v) T.P. Gudur (m) SPSR Nellore Dt -524311*

Abstract—**Background: A simple, rapid, and cost-effective Reversed-Phase High-Performance Liquid Chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of Cefpodoxime proxetil and Ambroxol HCl in combined commercial pharmaceutical dosage forms.**
Methods: Chromatographic separation was achieved on a Symmetry C18 column (150 \times 4.6mm, 5 µu maintained at ambient temperature. The optimized mobile phase consisted of a pH 3.5 phosphate buffer and acetonitrile in a ratio of 30:70 (v/v), pumped isocratically at a flow rate of 1.0\mL/min. Isopiestic UV detection was performed at a wavelength of 254nm. The method was thoroughly validated in accordance with the International Council for Harmonisation (ICH) guidelines. Results: The retention times for Cefpodoxime proxetil and Ambroxol HCl were found to be 2.162min and 3.305\min, respectively, yielding a total analysis runtime of under 5 minutes. The calibration curves demonstrated excellent linearity (R^2 = 0.999 for both analytes) over the concentration ranges of 20–100µu/g/mL for Cefpodoxime proxetil and 12–60 µu/g/mL for Ambroxol HCl. The method proved highly accurate, with mean recovery percentages of 100.0% and 100.3%, and exceptionally precise, with system precision % RSD values of 1.6% and 0.5% for Cefpodoxime proxetil and Ambroxol HCl, respectively. The limits of detection (LOD) were 0.2µu/g/mL and 1.0 \ µu g/mL, while the limits of quantitation (LOQ) were 0.5µu\g/mL and 1.2\ µu\g/mL, respectively. Robustness studies confirmed that minor operational variations did not significantly impact the method's reliability.
Conclusion: The validation metrics statistically prove that the proposed RP-HPLC method is specific, accurate, precise, and rugged. Due to its short analysis time and lack of baseline interference from common excipients, this method is highly suitable for routine batch analysis and quality control of pharmaceutical formulations containing this dual-drug combination.

Index Terms—**RP-HPLC, Cefpodoxime proxetil, Ambroxol HCl, Method Validation, Quality Control, ICH Guidelines.**

I. INTRODUCTION:

The clinical management of respiratory tract infections complicated by hyperviscous mucus requires a dual-action therapeutic approach: aggressive bacterial eradication paired with immediate mucokinetic clearance.[1] The fixed-dose combination of Cefpodoxime proxetil an advanced, broad-spectrum third-generation cephalosporin antibiotic[2] and Ambroxol hydrochloride a potent secretolytic agent represents a highly effective clinical synergy.[3] Ambroxol not only restores physiological ciliary clearance but has also been shown to enhance antibiotic concentrations directly within lung tissues. Consequently, this combination is widely manufactured and prescribed globally to accelerate patient recovery times.[4]
To uphold global pharmaceutical quality assurance standards, the availability of highly reliable, accurate, and rapid analytical methods for routine batch monitoring is critical.[5] While the literature documents various analytical protocols for these drugs ranging

from individual UV-spectrophotometric assays to basic High-Performance Liquid Chromatographic (HPLC) setups a persistent operational bottleneck remains.[6] Many established chromatographic methods are characterized by prolonged retention times, hazardous or highly complex mobile phase matrices, and significant solvent consumption. In the modern industrial landscape, these factors lead to increased cost per analysis and unacceptably low sample throughput.[7]

This study addresses these explicit limitations by developing a highly streamlined, rapid, and economically viable Isocratic Reversed-Phase HPLC (RP-HPLC) method for the simultaneous estimation of Cefpodoxime proxetil and Ambroxol HCl in commercial formulations. By utilizing a high-efficiency Symmetry C18 column and an optimized isocratic mobile phase (30:70\v/v pH 3.5 phosphate buffer to acetonitrile), we successfully compressed the analytical runtime to under 5 minutes while maintaining pristine resolution, excellent linearity ($R^2 = 0.999$), and a total absence of excipient baseline interference.[8]

The following sections detail the rigorous development, characterization, and validation of this method in accordance with current International Council for Harmonisation (ICH) guidelines. The resulting protocol provides quality control laboratories with a rugged, high-throughput, and cost-effective analytical tool perfectly suited for routine commercial production workflows.[9]

Pharmaceutical analysis serves as an indispensable pillar within the modern drug life cycle, encompassing everything from early-stage drug discovery and preclinical characterization to final commercial product release.[10] As a highly specialized discipline of analytical chemistry, pharmaceutical analysis focuses on the separation, identification, and precise quantitative determination of active pharmaceutical ingredients (APIs), their synthetic impurities, and degradation products in complex matrices.[11]

Analytical methodologies are broadly categorized into qualitative protocols, which verify chemical identity, and quantitative protocols, which numerically establish the exact concentration of an analyte.[12] Because nearly any distinct physical or chemical property of a molecular entity can serve as the foundational bedrock for an analytical assay, the field relies heavily on diverse instrumental platforms.[13] These range from classical wet chemistry titrations to sophisticated spectroscopic techniques, mass spectrometry, and advanced multi-dimensional "hyphenated" techniques (e.g., GC-MS, LC-MS).[14]

1.1. High-Performance Liquid Chromatography (HPLC) as an Industry Standard

Among the analytical platforms available to the pharmaceutical industry, High-Performance Liquid Chromatography (HPLC) is the most widely utilized tool for routine quality assurance and quality control (QA/QC).[15] Compared to classical separation methodologies, modern HPLC offers vastly superior resolution, rapid analysis turnaround times, and exceptional accuracy, precision, and sensitivity.[16]

Within the regulatory pipeline of developing a New Chemical Entity (NCE) or validating a generic formulation, robust chromatographic separation methods must be systematically tailored. These methods monitor the stability and chemical integrity of prospective drug candidates, raw excipients, and final commercial products.[17]

Chromatographic separations operate via the continuous partition of analyte molecules between a moving mobile phase and a porous stationary phase. While normal-phase chromatography utilizing a polar stationary phase and a non-polar mobile phase is highly effective for specific applications, it is inherently poorly suited for pharmaceuticals because most drug molecules are polar, leading to prolonged retention times and severe peak tailing. Consequently, Reversed-Phase HPLC (RP-HPLC), which pairs a non-polar stationary phase (such as octadecylsilane, or C18 with a polar aqueous-organic mobile phase, has become the dominant operational mode. It ensures that polar analytes elute quickly and with high peak symmetry, making it highly efficient for high-throughput industrial workflows.[18]

1.2. The Rationale for Systematic Method Development

The primary objective of HPLC method development is to achieve optimal resolution (Rs) between target analytes, synthetic process impurities, and matrix degradation products within a minimal analytical timeframe. Historically, optimization followed a manual, univariate approach manipulating one experimental variable (such as flow rate, column temperature, or mobile phase pH) at a time while

keeping others static. While valuable for understanding fundamental chromatographic interactions, this strategy is slow and resource-intensive. Modern quality control demands a more systematic approach to optimize critical parameters, such as defining specific stationary phase dimensions, ensuring proper peak symmetry (asymmetry factor, As), and strictly controlling mobile phase chemistry.[19] Crucially, variables such as buffer pH must be carefully managed during development; maintaining mobile phase parameters between pH 2.0 and 8.0 is vital to prevent the acid hydrolysis of stationary siloxane linkages or the basic dissolution of the silica core. By selecting optimal UV detection wavelengths lambda max and organic modifiers (such as acetonitrile or methanol), analysts can design rapid, rugged, and cost-effective assays that maximize sample throughput.[20]

1.3. The Imperative of Analytical Method Validation
Developing a high-performance chromatographic method is only the first step; it cannot be legally or scientifically implemented for consumer safety testing without rigorous validation. Analytical method validation provides documented, statistically sound proof that a laboratory procedure is completely suitable, reliable, and reproducible for its specific intended purpose.[21]

This requirement is strictly enforced by global regulatory bodies. For instance, the US Code of Federal Regulations (21 CFR & 211.165(e)) explicitly mandates that the accuracy, sensitivity, specificity, and reproducibility of test methods employed by a manufacturing firm must be established and thoroughly documented.[22] Beyond mere legal compliance, validation represents good science and strict quality control. It guarantees that the methods used to release commercial batches can accurately detect toxic impurities or sub-potent formulations, directly safeguarding human health.[23]

In alignment with international harmonized standards set forth by the International Council for Harmonisation (ICH) Q2(R1) guidelines, a validation protocol evaluates a specific matrix of key performance characteristics:

- Specificity:

Proving the method can unequivocally measure the target drug without baseline interference from excipients, synthesis routes, or forced degradation products.[24]

- Linearity and Range:

Demonstrating a mathematically linear response over a defined concentration window (typically spanning 80% to 120% of the target assay concentration).[25]

- Accuracy:

Verifying the closeness of agreement between the true value and the experimental value via rigorous recovery studies.[26]

- Precision:

Evaluating the scatter of data through repeatability (intraday) and intermediate precision (interday) testing.

- Detection and Quantitation Limits (LOD/LOQ):

Establishing the absolute scientific thresholds of sensitivity using signal-to-noise calculations or standard deviation regressions.

- Robustness:

Confirming the method's resilience against tiny, deliberate variations in operational parameters (e.g., small shifts in flow rate, temperature, or pH).[27]

AIM:
The main aim of the present study is to develop RP-HPLC method for the estimation of cefpodoxime proxitile and ambroxol Hcl. Validation of the developed method for routine analysis of cefpodoxime proxitile and ambroxol Hcl in tablets by quality control laboratories.To Optimize various parameters like mobile phase, λmax, column,flow rate, temperature conditions. To validate with various Parameters according to ICH guidelines.

OBJECTIVES:
The scope of this research centers on the development, optimization, and complete statistical validation of a high-throughput, isocratic RP-HPLC method. By bridging foundational chromatographic theory with strict regulatory validation frameworks, this study

outlines a rapid, simple, and economically viable analytical protocol. The developed method achieves exceptional peak resolution, excellent linearity, and a short runtime, making it perfectly tailored for routine quality control testing and batch analysis of pharmaceutical formulations.

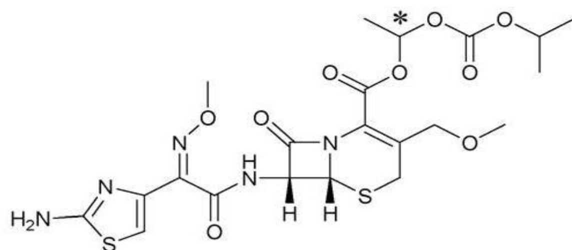
II. DRUG PROFILE:

2. 1 CEFPODOXIME PROXITILE [28]

IUPAC Name:

(6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyimino) acetamido]-3-(methoxymethyl)-8-oxo-5-thia-1- azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

2.1.2 Structural Formula:



2.1.3 Chemical formula: $C_{15}H_{17}N_5O_6S_2$

2.1.4 Molecular Mass: 427.455

2.1.5 Description: white powder

Cefpodoxime is an oral third generation cephalosporin antibiotic. It is active against most Gram positive and Gram-negative bacteria. It is commonly used to treat acute otitis media, pharyngitis, and sinusitis. Cefpodoxime proxetil is a prodrug which is absorbed and de-esterified by the intestinal mucosa to Cefpodoxime.[29]

2.1.6 Solubility:

soluble in methanol and Acetonitrile.

2.1.7 Category:

Antibacterial Agents, Cephalosporins

2.1.8 Storage:

should be stored at room temperature

2.1.9 Half-life:

2.09 to 2.84 hours

2.1.10 protein binding:

22 to 33% in serum and from 21 to 29% in plasma.

2.1.11 Indication:

For the treatment of patients with mild to moderate infecti caused by susceptible strains of the designated microorganisms.

2.1.12 Brand Names: Banan, Doxef, Vantin

2.1.13 pka values: pKa (strongest acidic): 3.22 pKa (strongest basic): 4.16

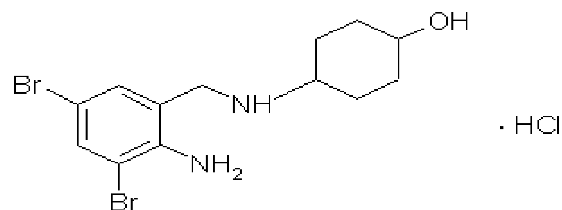
2.1.14 Mechanism of Action: Cefpodoxime is active against a wide spectrum of Gram-positive and Gram-negative bacteria. Cefpodoxime is stable in the presence of beta-lactamase enzymes. As a result, many organisms resistant to penicillins and cephalosporins, due to their production of beta-lactamase, may be susceptible to cefpodoxime. Cefpodoxime is inactivated by certain extended spectrum beta-lactamases. The bactericidal activity of cefpodoxime results from its inhibition of cell wall synthesis. [30]

2.2 AMBROXOL HCL[31]

2.2.1 IUPAC Name

trans-4- [(2-amino-3, 5 dibromobenzyl) amino] cyclohexanol hydrochloride,

2.2.2 Structural Formula:



2.2.3 Chemical formula: C13 H19 Br2 ClN2O

2.2.4 Molecular Mass: 414.6

2.2.5 Description: white powder

2.2.6

Solubility: freely soluble in methanol and Acetonitrile

2.2.7 Category: Bronchosecretolytic and Expectorant

2.2.8 Storage: should be stored at room temperature

2.2.9 Indication: ambroxol Hcl used to treat Asthma and cough.

2.2.10 Mechanism of Action:
It is a mucolytic expectorant that inhibits the release of arachidonic acid cell membrane phospholipids. Stimulates the release of surfactant by pneumocytes type II & act as a scavenger of hypochlorous and hydroxyl radicals, it blocks nitric oxide stimulated activation of guanylate cyclise.[32]

MATERIALS AND INSTRUMENTS USED
Instruments used:
Equipment: High performance liquid chromatograph equipped with Auto Sampler and PDA detector
Software: Empower 2 (WATERS)
Column: Symmetry C18 (4.6 x 150mm, 5µm, Make: Waters)
Detector: PDA detector
Elico pH meter
LABINDIA 3000 – Double beam UV-VISIBLE spectrophotometer.
Vacuum filter pump.
Digital balance.

Reagents and Standard:
- Water HPLC Grade.
- Methanol HPLC Grade
- Acetonitrile HPLC Grade
- Orthophosphoric Acid
- Potasium dihydrogen Ortho phosphate
- Ambroxol Hcl& Cefpodoxime Proxitil Working Standards
- Ambroxol Hcl& Cefpodoxime Proxitil Tablets

Preparation of Phosphate buffer:(P^H:3.5)
Weighed 7.0 grams of Potassium Di hydrogen Ortho Phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjust Ph 3.5with Orthophosphoric acid.[33]

Preparation of mobile phase:
Mix a mixture of above Buffer 250 mL (25%),750 mL of Acetonitrile HPLC (75%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.[34]

Diluent Preparation:
Use the Mobile phase as Diluent.

Preparation of the Ambroxol Hcl & Cefpodoxime Proxitil Standard & Sample Solution:

Standard Solution Preparation:
Accurately weigh and transfer 12 mg of Ambroxol Hcl and 10mg of Cefpodoxime Proxitilworking standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.3&0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:
Accurately weigh and transfer equivalent to 256.9 mg of Ambroxol Hcl and Cefpodoxime Proxitilsample into a 100mL clean dry volumetric flask add about 70mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
(Stock solution)
Further pipette 0.6ml of Ambroxol Hcland Cefpodoxime Proxitilof the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

III. METHADOLOGY

Chromatography is a technique used for the separation, Purification and identification of the compounds of mixtures by their continuous distribution, between two phases.

One is stationary phase and the other is mobile phase.
- A good method development strategy should require only as many experimental conditions as necessary to achieve the desired final result.
- Finally, method development should be as simple as possible, yet it should allow the use of sophisticated tools such as computer modeling, if these are available.

3.1. Reagents and Standard:
- Water HPLC Grade.
- Methanol HPLC Grade
- Acetonitrile HPLC Grade
- Orthophosphoric Acid
- Potasium dihydrogen Ortho phosphate
- Ambroxol Hcl& Cefpodoxime Proxitil Working Standards
- Ambroxol Hcl& Cefpodoxime Proxitil Tablets

3.2. Selection of mobile phase:

- Pure drug of Cefpodoxime proxitile and AmbroxolHcl mixed standard stock solution (10µg/mL of Cefpodoxime proxitile and 10µg/mL of AmbroxolHcl) were taken and 10µL sample was injected in to RP-HPLC system and run in different solvent systems.
- Different mobile phase compositions of pH 3.5 phospate buffer: ACN (30:70) in order to determine the best conditions for the effective separation and elution of the analytes.
- The mobile phase consisting of pH 3.5 buffer: ACN (30:70) was selected.

3.3. Preparation of Phosphate buffer:(P^H:3.5)

Weighed 7.0 grams of Potassium Di hydrogen Ortho Phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjust Ph 3.5with Orthophosphoric acid.

3.4. Preparation of mobile phase:

Mix a mixture of above Buffer 250 mL (25%),750 mL of Acetonitrile HPLC (75%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

3.5. Diluent Preparation:

Use the Mobile phase as Diluent.[35]

3.6. Selection of Flow rate:

A chromatogram was run with the optimized mobile phase, and some different flow rates of 0.8mL/min, 1mL/min, 1.2mL/min and were tried. The best retention time and separation was obtained at 1.0mL/min, so the flow rate of 1.0 mL/min has been selected.

3.7. Selection of analytical wavelength;

By appropriate dilutions of the standard stock solutions with methanol, various concentrations of Cefpodoxime proxitile and AmbroxolHcl were prepared separately and their overlain spectra was obtained using the double beam UV visible spectrophotometer in the spectrum mode between the wavelength ranges of 400 nm to 200 nm. From the overlain spectra, it was observed that Cefpodoxime proxitile and Ambroxol Hcl exhibited strong absorbance at about 254 nm (it is the coinciding maximum absorbance where the two drugs can be detected sufficiently enough for quantitative evaluation) which was selected as the analytical wavelength for further analysis.[36]

3.8. Standard Solution Preparation:

Accurately weigh and transfer 12 mg of Ambroxol Hcl and 10mg of Cefpodoxime Proxitilworking standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.3&0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

METHOD VALIDATION OF CEFPODOXIME PROXITILE AND AMBROXOL HCL IN TABLET DOSAGE FORM BY RP-HPLC RESPECTIVELY

After development of HPLC method for the estimation of the multi component dosage forms validation of the method was carried out. This section describes the procedure followed for the validation of the developed method.

System suitability studies:

System suitability studies were carried out as specified in the United States Pharmacopoeia (USP). These parameters include column efficiency, resolution, capacity factor, tailing factor and HETP were calculated in present study.

Preparation of Phosphate buffer:(P^H:3.5)

Weighed 7.0 grams of Potassium Di hydrogen Ortho Phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjust Ph 3.5with Orthophosphoric acid.

Preparation of mobile phase:

Mix a mixture of above Buffer 250 mL (25%),750 mL of Acetonitrile HPLC (75%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

Use the Mobile phase as Diluent.

Preparation of the Ambroxol Hcl& Cefpodoxime Proxitil Standard & Sample Solution:

Standard Solution Preparation:

Accurately weigh and transfer 12 mg of Ambroxol Hcl and 10mg of Cefpodoxime Proxitilworking standard

into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

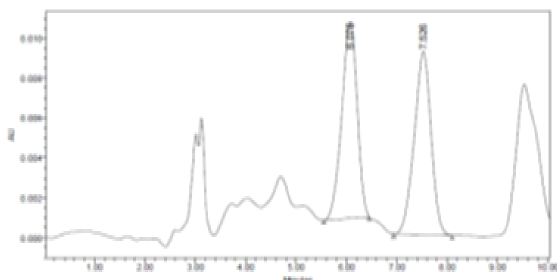
Further pipette 0.3&0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below: Accuracy, Precision, Repeatability, Intermediate Precision, Specificity, Detection Limit, Quantitation Limit, Linearity, Range. Each of these validation characteristics is defined in the attached Glossary. The table lists those validation characteristics regarded as the most important for the validation of different types of analytical procedures. This list should be considered typical for the analytical procedures cited but occasional exceptions should be dealt with on a case-by-case basis. It should be noted that robustness is not listed in the table but should be considered at an appropriate stage in the development of the analytical procedure. [37]

IV. RESULTS AND DISCUSSION

TRIAL-1: Chromatographic conditions:

- Equipment: HPLC equipped with Auto Sampler and PDA detector
- Column: Symmetry C18 (4.6 x 150mm, 5µm, Make: Waters)
- HPLC Mode: isocratic
- Mobile Phase: Methanol: Water (60:40
- Flow rate: 1.0 mL/ min
- Wavelength: 254 nm
- Injection volume : 20 µl
- Column oven: Ambient
- Run time: 10min



Chromatogram 4.1: Trial-1

Observation: peaks are not eluted clearly and some impurities was observed.

Conclusion: Method to be modified by changing Mobile phase

TRIAL-6: Chromatographic conditions:

Equipment: High performance liquid chromatography equipped with Auto Sampler and PDA detector

Column: Symmetry C18 (4.6 x 150mm, 5µm, Make: Waters)

HPLC Mode: isocratic

Mobile Phase: pH 3.5 phosphate buffer: Acetonitrile (30:70)

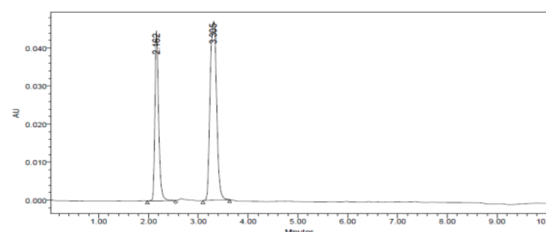
Flow rate: 1.0 mL per min

Wavelength: 254 nm

Injection volume : 20 µl

Column oven: Ambient

Run time: 10min



Chromatogram 4.2: Trial-6

Observation:

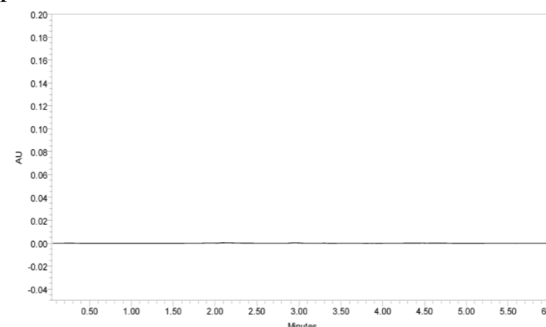
Both peaks are eluted clearly, more plate count and more resolution were observed.

Specificity:

a. Blank interference:

The prepared Blank (diluents) has been injected into HPLC as per methodology.

Acceptance criteria: Blank chromatogram should not show any peaks at the Retention times of the analyte peaks.



Chromatogram 4.3: Blank

Observation: Blank chromatogram has not shown any peaks at the Retention times of the analyte peaks.

Linearity

Preparation of stock solution:

Accurately weigh and transfer 12 mg of Ambroxol Hcl and 10mg of Cefpodoxime Proxitilworking standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I

(12ppm of Ambroxol Hcl&20ppm of Cefpodoxime Proxitil):

0.1&0.2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II

(24ppm of Ambroxol Hcl&40ppm ofCefpodoxime Proxitil):

0.2&0.4ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III

(36ppm of Ambroxol Hcl&60ppm of Cefpodoxime Proxitil):

0.3&0.6ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV

(48ppm of Ambroxol Hcl&80ppm of Cefpodoxime Proxitil):

0.4&0.8ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V

(60ppm of Ambroxol Hcl&100ppm of Cefpodoxime Proxitil)

0.5&1.0ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Procedure: Inject each level into the chromatographic system and measure the peak area.Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Linearity Results: (forAmbroxol Hcl)

Table 4.1: Linearity Results of Ambroxol Hcl

S. No	Linearity Level	Concentration	Area
1	I	12ppm	374052
2	II	24ppm	682802
3	III	36ppm	1012619
4	IV	48ppm	1324938
5	V	60ppm	1708316
Correlation Coefficient			0.999

Acceptance Criteria: Correlation coefficient should be not less than 0.999

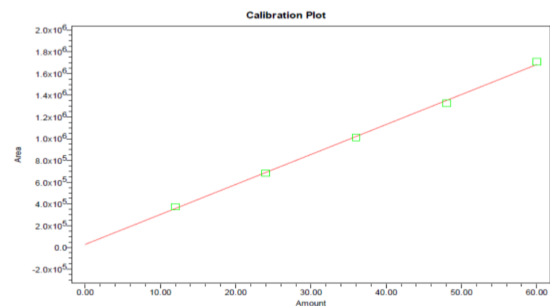
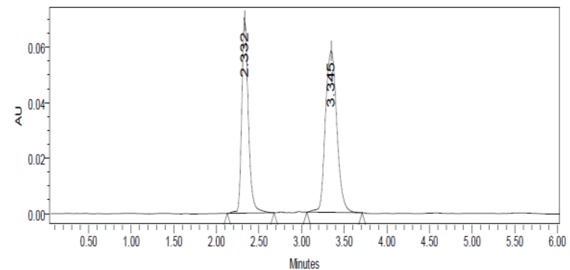


Fig 4.1: Ambroxol Hcl Calibration curve graph

Correlation Coefficient is 0.999.

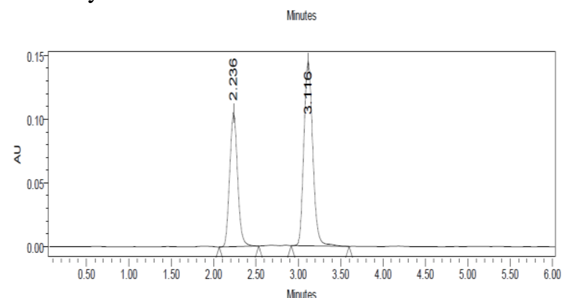
Linearity Studies Chromatograms:

Linearity level: 1



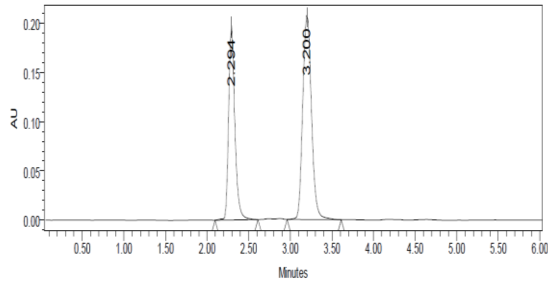
Chromatogram 4.4: Linearity level-1

Linearity level: 2



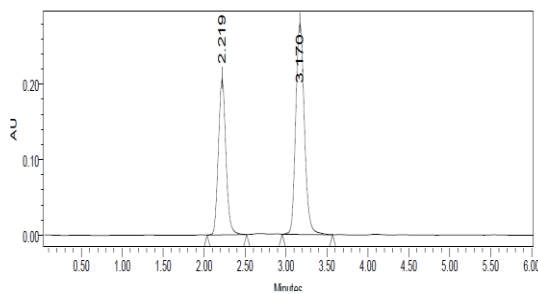
Chromatogram 4.5: Linearity level-2

Linearity level: 3



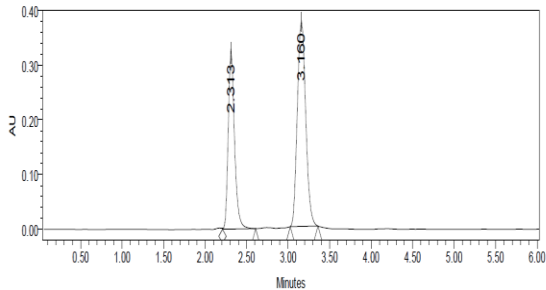
Chromatogram 4.6: Linearity level-3

Linearity level: 4



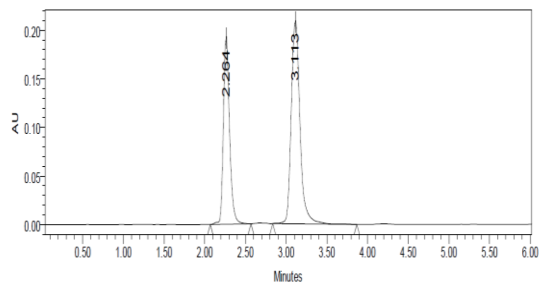
Chromatogram 4.7: Linearity level-4

Linearity level: 5



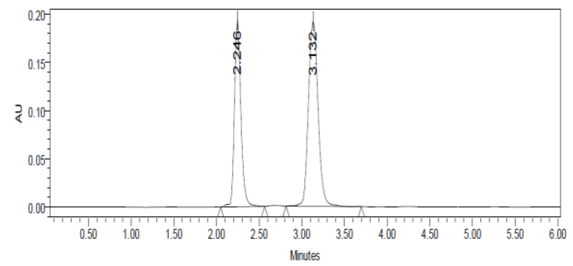
Chromatogram 4.8: Linearity level-5

Precision Studies chromatograms:
Injection-1



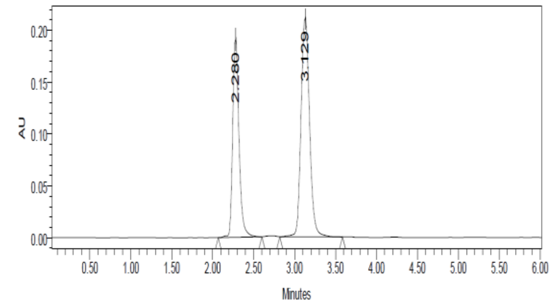
Chromatogram 4.9: Precision Studies Injection-1

Injection-2



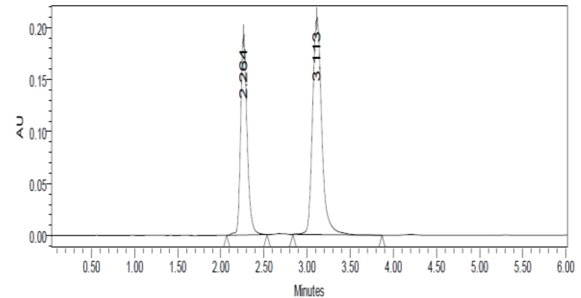
Chromatogram 4.10: Precision Studies Injection-2

Injection-3



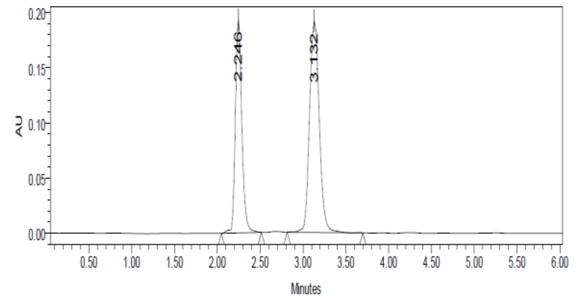
Chromatogram 4.11: Precision Studies Injection-3

Injection-4



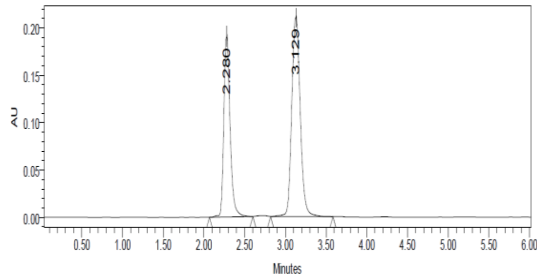
Chromatogram 4.12: Precision Studies Injection-4

Injection-5



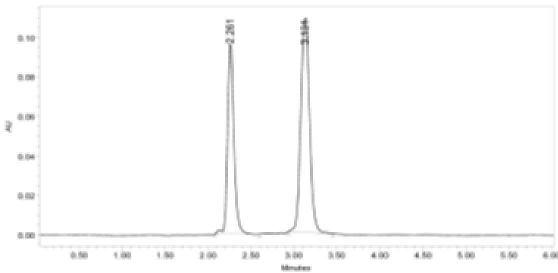
Chromatogram 4.13: Precision Studies Injection-5

Injection-6



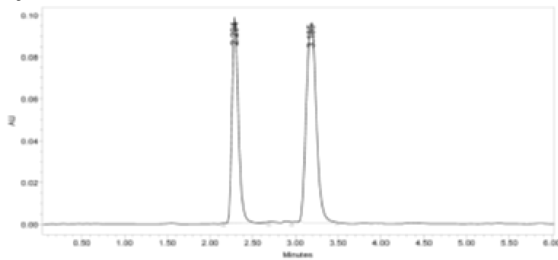
Chromatogram 4.14: Precision Studies Injection-6

Accuracy Studies chromatograms:
Accuracy study for 50%
Injection-1



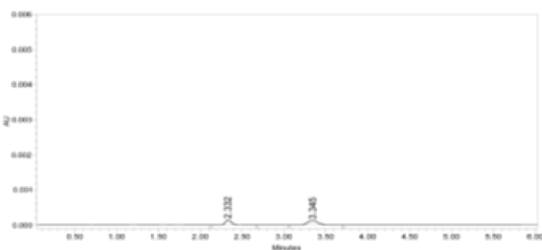
Chromatogram 4.15: Accuracy study for 50% Injection-1

Injection-2



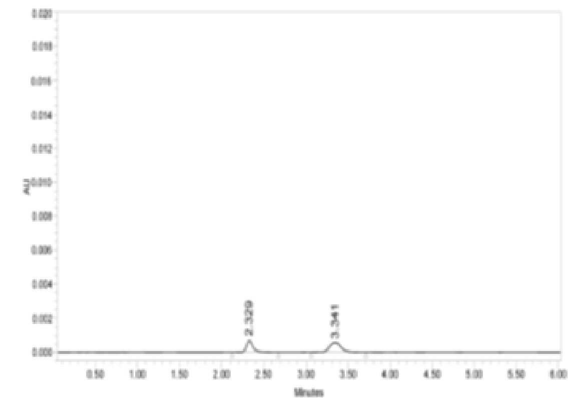
Chromatogram 4.16: Accuracy study for 50% Injection-2

Limit of detection of cepfodoxime proxitile and ambroxol Hcl chromatogram:



Chromatogram 4.17: LOD of Amb & cef

Limit of quantification of cefpodoxime proxitile and Ambroxol Hcl chromatogram:



Chromatogram 4.18: LOQ of Amb & cef

RESULT AND DISCUSSION

VALIDATION OF THE METHOD:

The suitability of the system was studied by the values obtained for Theoretical plate, Resolution and tailing factor of the chromatogram of standard drugs and presented. The selectivity of the method was revealed by the repeated injection of mobile phase and no interference was found.

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out by preparing 3 individual samples with same procedure from the formulation and injecting. The percentage recovery and percentage relative standard deviation of the percentage recovery was calculated and presented in Tables (8.6.1) & (8.6.2). From the data obtained, added of standard drugs were found to be accurate.

The precision of the method was demonstrated by system and method precision. All solutions were injected into the chromatographic system. The peak area and percentage relative standard deviation were calculated and presented in tables (8.4.1) & (8.4.2). From the data.

The standard drug solution of varying concentration ranging from 12-60µg / ml for Ambroxol Hcl and 20-100µg / ml for Cefpodoxime proxitile. The response factor, slope, intercept and correlation co-efficient were calculated. The slope, intercept, correlation co-efficient were found to be 0.999 for The Ambroxol 0.999, for Cefpodoxime proxitile calibration curves were plotted using response factor Vs concentration of standard solutions. The calibration graph shows that

linear response was obtained over the range of concentration used in the assay procedure. These data demonstrates that the method have adequate sensitivity to the analytes. The range demonstrate that the method is linear outside the limits of expected use.

The robustness of the method was studied by carrying out experiments by changing conditions discussed earlier. The response factors for these changed chromatographic parameters were almost same as that of the fixed chromatographic parameters (table8.7.1& 8.7.2) and hence developed method is said to be robust and ruggedness.

Tab 4.2: Validation parameters

Parameters	Cefpodoxime Proxitile	Ambroxol Hcl
Accuracy	% Recovery =100.0%	% Recovery =100.3
precision	% RSD =1.6%	% RSD =0.5%
Id precision	% RSD = 0.9%	% RSD = 0.7%
Linearity	R^2 =0.999	R^2 =0.999
Range	12-60	20-100
Limit of detection	0.2 µg/ml	1.0 µg/ml
Limit of quantitation	0.5 µg/ml	1.2 µg/ml

SUMMARY

Methodology consists of the general principle in HPLC instrumentation protocol for method development and the details about the proposed RP-HPLC method on the drug is included. Where the RP-HPLC method in which determination of Cefpodoxime proxitile and Ambroxol Hcl was carried out on a Symmetry C18 (4.6 x 150mm, 5µm, make: Waters) using a mobile phase consisting of pH 3.5 phospate buffer: Acetonitrile (30:70). The mobile phase was pumped at a rate of 1.0 ml/min and the detection was carried out at 254nm. The retention time of Cefpodoxime proxitile and Ambroxol Hcl was found to be 2.162 and 3.305 min respectively and linearity was in the range of 12-60µg / ml for Ambroxol Hcl and 20-100µg / ml for Cefpodoxime proxitile.

The results obtained in newer RP-HPLC method for determination of Cefpodoxime proxitile and Ambroxol Hcl are tabulated and also discussed about the developed RP-HPLC method. The proposed method is simple cost effective and gives reliable assay results with short analysis time (5min). The content of drugs in the formulation was found to be 60mg Ambroxol and 100mg Cefpodoxime proxitile.

The method was validated in terms of sensitivity, accuracy and precision and can be used for the routine determination of Cefpodoxime proxitile and Ambroxol Hcl in pharmaceutical formulations. The above method does not suffer from any interference due to common excipients. Therefore, the proposed RP-HPLC method could be successfully applied to estimate commercial pharmaceutical products containing Cefpodoxime proxitile and Ambroxol Hcl.

V. CONCLUSION

The results indicating that the proposed methods are precise, accurate, specific and simple. These methods were developed and validated according to the ICH guidelines. So, the developed methods can be easily applied for routine analysis.

It is clear from the present study that the RP-HPLC method for the determination of Acitretin is simple, accurate, specific and precise. This method was validated statistically. The results of recovery studies were in good agreement with the respective label claim of the formulation. Thus, the method is less time consuming and can be employed for routine batch analysis of Cefpodoxime proxitile and Ambroxol Hcl.

Disclosure statement

The authors declare no potential conflicts of interest.

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