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Analytical Method Development and Validation for the Simultaneous Estimation of Cefadroxil and Ambroxol Hydrochloride by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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Abstract
A Simple and rapid stability indicating RP-HPLC method was developed for the simultaneous determination of Cefadroxil and Ambroxol in bulk and pharmaceutical dosage form. A chromatographic separation of the two drugs was achieved with a Symmetry C_{18} (4.6 × 250 mm, 5 µm partical size, make: Xterra) analytical column using sodium acetate trihydrate buffer (adjusted to pH 4.5 by glacial acetic acid):Acetonitrile (50:50%v/v) in isocratic mode at a flow rate of 1 mL/min and column at ambient temperature. The detection was monitored at 243 nm using a PDA detector. The stressed samples were analyzed for the degradation study in acid, base, peroxide, thermal, photolytic and validated as per ICH guideline. This proposed method was found to be specific and stability indicating as no interfering peaks of degradation compounds and excipients were noticed. The described method shows excellent linearity over a range of 10-50 µg/mL of Cefadroxil and 1.2-6.0 µg/mL of Ambroxol. The correlation coefficient for Cefadroxil and Ambroxol were 0.999 and a0.999 respectively. The mean recovery values for Cefadroxil and Ambroxol were 99.9% and 100%respectively. The limit of detection for Cefadroxil and Ambroxol were 0.005 µg/mL and 0.005 µg/mL and the limit of quantification were 0.018 µg/mL and 0.017 µg/mL respectively. The retention times was observed at 2.3mins, 3.2 mins for Cefadroxil and Ambroxol respectively. The Robustness Study and percentage of assay of the formulation were found within limit as per ICH guidelines.
Key words: UV spectrophotometer, Cefadroxil, Ambroxol, High performance liquid chromatography, validation, stability indicating method, stress conditions.

1. Introduction

Analytical methods
Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available [2].

Description of the Various Analytical Methods
Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements [5]. Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [6].

Chromatography: Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to

separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate [7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC [9].

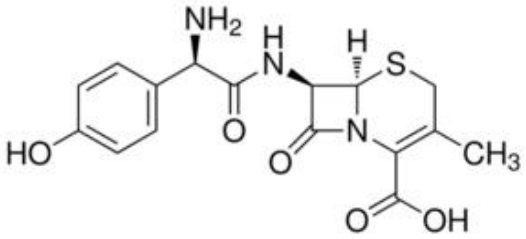

Figure 1: Cefadroxil

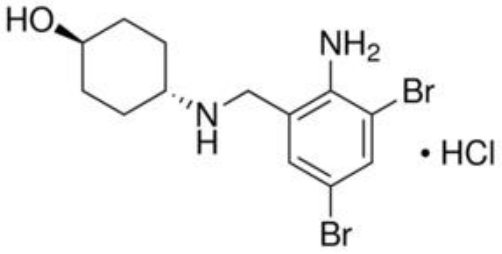

Figure 2: Ambroxol Hydrochloride

2. Materials and Methods

Apparatus

The instrument used for the study was WATERS, software: Empower, 2695 separation module, PDA detector[10].

Reagents and Materials

The solvents used were sodium acetate trihydrate buffer, Methanol, Acetonitril and Water[10].

Selection of detection wavelength:

UV spectrum of 10 µg / ml Cefadroxil and Ambroxol in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 243. At this wavelength both the drugs show good absorbance[11].

Selection of mobile phase

Initially the mobile phase tried was Methanol: Water, Phosphate buffer : Acetonitrile, Phosphate buffer : Methanol, Phosphate buffer : Methanol : Acetonitrile with varying proportions. Finally, the mobile phase was optimized with Sodium acetate trihydrate buffer (pH 4.5) : acetonitrile in proportion 50: 50 v/v respectively[12].

Optimization Chromatographic trials for Simultaneous Estimation of Cefadroxil & Ambroxol by RP- HPLC.

Optimization chromatographic conditions

Instrument used : Waters HPLC with auto sampler and PDA detector.

Column : Symmetry C_{18} (4.6 x 250mm, 5µm, Make: XTerra) or equivalent.

Preparation of buffer : 1.7 gms of sodium acetate trihydrate was dissolved in 250ml of HPLC water and pH was adjusted to 4.5 by adding glacial acetic acid dropwise.

Buffer pH	: 4.5
Mobile phase	: Acetate buffer: Acetonitrile (50:50)
Flow rate	: 1ml per min
Wavelength	: 243 nm
Temperature	: ambient.
Run time	: 6mins.

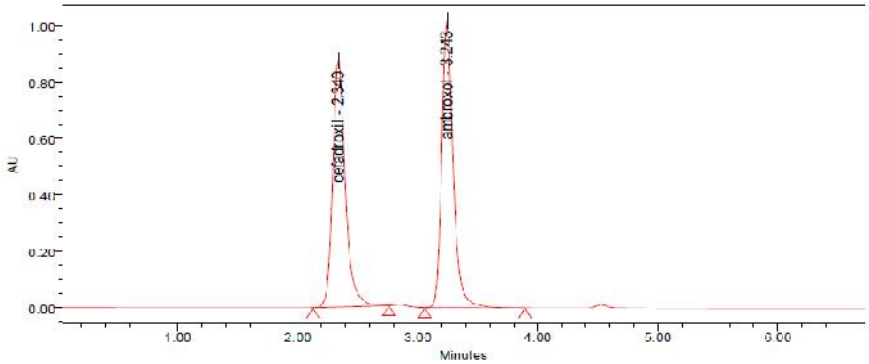

Figure 3: Optimization Chromatogram

Observation: The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one.

Procedure

Preparation of mobile phase [13].**:**

Accurately measured 500 ml (50%) of above buffer and 500 ml of Acetonitrile HPLC (50%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration

Preparation of Standard Solution:

Accurately weighed amount of 10mg Cefadroxil and 10 mg Ambroxol were taken to a 10 ml cleaned and dried volumetric flask [14]. This was then diluted with 7ml of diluent and was sonicated. The volume was made to 10 ml with the same solvent. This was marked and labelled as Stock solution [15]. Now pipette out 1 ml of Ambroxol from the above stock solution into a 100ml volumetric flask and diluted up to the mark with diluents (second dilution)[16]. Finally pipette out 0.3ml of Cefadroxil from stock solution and 3.6ml of Ambroxol from second dilution and was transferred into 10ml cleaned and dried volumetric flask and was made upto 10ml with diluents to get 30 µg/ml of Cefadroxil and 3.6 µg/ml of Ambroxol[17].

Preparation of Sample Solution:

22.4mg of Cefadroxil and Ambroxol tablet powder (equivalent to 10mg) were accurately weighed and transferred into 10 ml cleaned and dried volumetric flask. This was then diluted with 7ml of diluent and was sonicated[18]. The volume was made to10 ml with the same solvent. This was marked and labeled as Stock solution [19]. Further, pipette out an amount of 0.3 ml from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluents to get 30 µg/ml of Cefadroxil and 3.6 µg/ml of Ambroxol [20].

3. Results and Discussion

Method Validation Parameters

1. Specificity: The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak [11]. The specificity was performed by injecting blank [12].

2. Linearity: The linearity study was performed for the concentration of 10ppm to 50ppm and1.2ppm to 6.0ppm level. Each level was injected into chromatographic system [13]. The area of each level was used for calculation of correlation coefficient [14].

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range: The linearity study was performed for concentration range of 1.2 – 6.0µg and10µg-50µg of Cefadroxil and Ambroxol hydrochloride and the correlation coefficient was foundto be 0.999[15] .

4. Accuracy: Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there [16].

Standard addition method: To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration [17].

Percentage method: For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively [18].

Acceptance criteria: The mean % recovery of the Ezetimibe and Simvastatin at each level should be not less than 95.0% and not more than 105.0%.

5. Precision: The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day. Peak area and %RSD were calculated and reported [19]. The chromatograms of intra-day precision studies were shown. Inter-day precision studies, was done by injecting three (3) repeated injections for three consecutive days. Peak area and %RSD were calculated and reported [20].

Intermediate Precision (Ruggedness):

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions [21].

Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank [22].

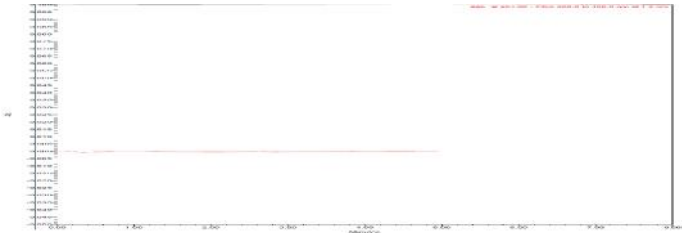

Figure 4: Chromatogram of Blank

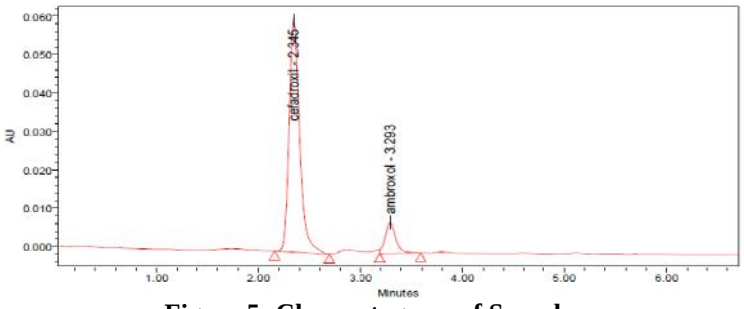

Figure 5: Chromatogram of Sample

2. Linearity: The linearity study was performed for the concentration of 10 ppm to 50ppm for Cefadroxil and 1.2ppm to 6.0ppm for Ambroxol and chromatograms are shown below[23].

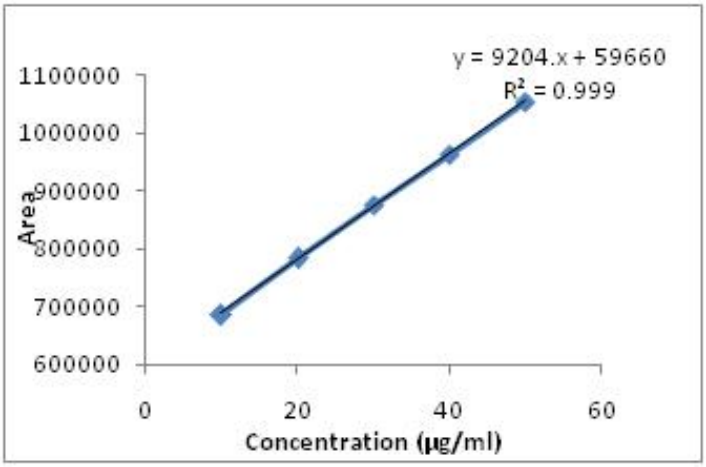

Figure 6: Calibration graph of Cefadroxil

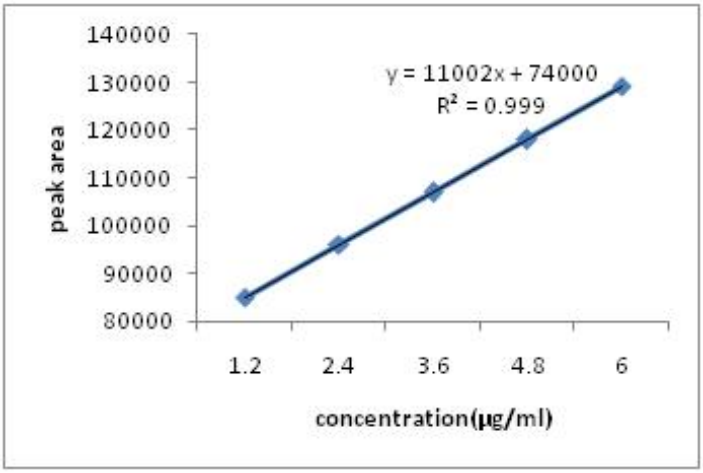

Figure 7: Calibration graph of Ambroxol

Table 1: Linearity Results

Parameters	Cefadroxil	Ambroxol
Slope (m)	9204	11002
Intercept (c)	59660	74000
Correlation coefficient (R^2)	0.999	0.999

Recovery studies: Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated [24].

Table 2: Accuracy results for Cefadroxil

%Concentration	Area	Amount present (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	444617.8	5	5	100.2	99.72
100%	885134.8	10	9.98	99.80	
150%	1319497.7	15	14.87	99.18	

Table 3: Accuracy (recovery) data for Ambroxol

%Concentration	Area	Amount present (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	50547.8	5	4.90	98.07	100
100%	104267.8	10	10.11	101	
150%	156758.1	15	15.2	101	

Table 4: Robustness: System suitability results For Cefadroxil (Flow rate)

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	SP Tailing
1	0.9	2278	1.34
2	1.0	2273	1.3
3	1.1	2034	1.30

Table 5: System suitability results for Ambroxol (Flow rate)

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	U USP Tailing
1	0.9	6685	1.22
2	1.0	6311	1.20
3	1.1	5846	1.2

Table 6: Method precision results of Cefadroxil

Injection	Area
Injection 1	885248
Injection 2	886348
Injection 3	887347
Injection 4	888565
Injection 5	890157
Average	887533.0
Standard deviation	1911.3
%RSD	0.2

Table 7: Method precision results of Ambroxol

Injection	Area
Injection 1	103601
Injection 2	103699
Injection 3	103822
Injection 4	103827
Injection 5	104197
Average	103829.2
Standard deviation	225.9
%RSD	0.2

Table 8: Ruggedness results of Cefadroxil

Injection	Area
Injection 1	886735
Injection 2	887158
Injection 3	888022
Injection 4	889259
Injection 5	889658
Average	888166.4
Standard deviation	1275.272
%RSD	0.2

Table 9: Ruggedness results of Ambroxol

Injection	Area
Injection 1	103557
Injection 2	103731
Injection 3	103854
Injection 4	104170
Injection 5	105326
Average	104127.6
Standard deviation	706.345
%RSD	0.2

Detection Limit: The LOD was performed for Cefadroxil and Ambroxol was found to be 3.05and 2.90 respectively. **Quantification Limit:** The LOQ was performed for Cefadroxil and Ambroxol was found to be 10.07 and 9.67 respectively [25].

4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Cefadroxil and Ambroxol was done by RP-HPLC. The Acetate buffer was p^H 4.5 and the mobile phase was optimized with consists of Acetonitrile : Acetate buffer mixed in the ratio of 50:50 % v/v. A C_{18} column (4.6 x 250mm, 5µm, Make: XTerra) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 243 nm. The solutions were chromatographed at a constant flow rate of 1ml/min. the linearity range of Cefadroxil and Amboxol were found to be from 10-50 µg/ml of Cefadroxil and 1.2-6.0µg/ml of Ambroxol. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 99.72-100% of Cefadroxil and Ambroxol. LOD and LOQ were found to be within limit. The stressed samples were analyzed for the degradation study in acid, base, peroxide, thermal, photolytic and validated as per ICH guideline. The results obtained on the validation parameters met ICH and USP requirements it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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