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Original Article

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Clavulanic acid and Cefpodoxime Proxetil in Combined Pharmaceutical Dosage Form

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ABSTRACT

The objective of present work was to develop and validate a simple, accurate, precise HPLC method for the estimation of clavulanic acid and cefpodoxime proxetil. The chromatographic separation was achieved on a Hypersil BDSC18column (4.6x250 mm, 5µmparticlesize). Different mobile phase systems in different proportions were tried. For HPLC method a mobile phase consisting of Acetonitrile and Methanol (75:25) produced symmetric peak shape with good resolution for both the drugs. Next, the drugs were chromatographed under different flow rates from which a flow rate of 1.0 ml/min was selected. The retention timesof clavulanic acid and cefpodoxime proxetil were found to be 2.951 min and 4.195 min, respectively.The proposed method was found to have excellent linearity in the concentration range of 20-80mg/ml with correlation coefficient r2=0.999 and 0.999 for clavulanic acid and cefpodoxime proxetil repectively.The method was validated for linearity, precision, LOD, LOQ and robustness.The proposed method optimized and validated as per ICH guidelines.

Keywords: clavulanic acid, cefpodoxime proxetil, linearity.

1. INTRODUCTION

Cefpodoxime proxetil and Clavulanic acid are antibacterial drugs. Cefexime is chemically :(6*R*, 7*R*)-7-{[2-(2-amino-1,3-thiazol-4-yl)-2 carboxymethoxyimino)acetyl]amino}-3 (methoxymethyl)-8-oxo-5-thia- 1-azabicyclo[4.2.0]oct-

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2-ene-2-carboxylic acid. It is a III generation Cephalosporin antibiotic that acts by inhibiting cell wall synthesis.It has a molecular weight of 427.455gMo/l .Cefexime is a white to light yellow crystalline powder soluble in methanol and acetonitrile. Clavulanic acid is(2*R*,5*R*,*Z*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid. Clavulanic acid is irreversible inhibitor of bacterial beta- lactamase enzymes.Clavulanic acid is solid and it is soluble in water and methanol.

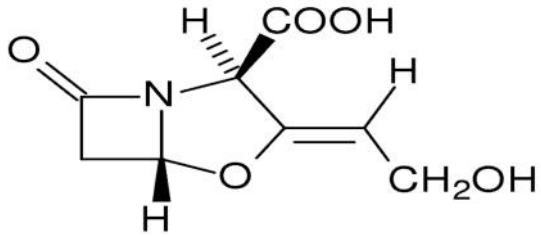

Fig 1: Clavulanic acid

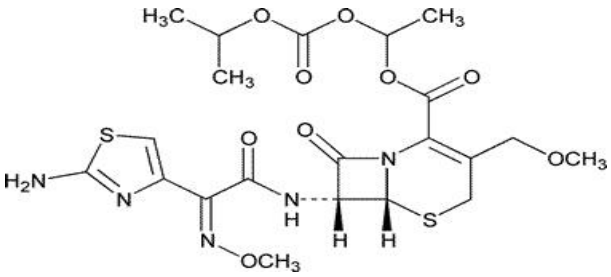

Fig 2: Cefpodoxime proxetil

Literature survey revealed that several methods were reported for cefpodoxime proxetil and clavulanic acid individually and in combinations S.Malathi,RN Dubey,R.Venkatanarayanan et al2009;Darshan shah,Smitha talaviy,Mandev patel 2012,Siddilingaiah swamy et al, Shah,2006;2010; Krzysztof, 2001; Dhoka, 2010;. Tim reyns,2006;prabhu.s, 2010; Raj k, 2010;Rathinavel,2008; ,Shah.j,2010;s.low, 1989;s.s.Zade,2013; B.thomas, 2010; Khaja, 2010; Deshpande et al., 2010; Nanda, 2009). Therefore, the main objective of this study was to attempt to develop a simple and rapid analytical method for simultaneous estimation of cefixime trihydrate and clavulanate potassium in a single dosage form and validate the proposed assay.

2. MATERIALS AND METHODS

Apparatus:The HPLC waters 2690/5 liquid chromatograph equipped with a PDA detector , the software installed was Empower, with 20µl loop, Hypersil-BDS C18 coloumn (250mmx4.6mm,5µl).The other instrument included are(SARTORIOUS) electronic balance and a sonicator (Fast clean).

Chemicals and Reagents

Active pharma ingredient of clavulanic acid and cefexime was obtained as a gift sample from Arch pharma ltd,purified water HPLC grade was prepared by triple glass distillation and filtered through a 0.45µ membrane filter.Methanol HPLC grade and Acetonitrile HPLC was run at a flow rate of 1.0ml/min, 20µl of the sample was injected in the chromatographic system.Mobile phase comprising of Acetonitrile and Methanol at the ratio (75:25).The coloumn temperature was ambient with a detection wavelength of 220.

Preparation of standard solution

Stock solutions were prepared by dissolving 10mg of clavulanic acid and 10mg of cefpodoxime proxetil in mobile phase seperatly.Aliquots of standard solution of clavulanic acid and cefpodoxime proxetil were transferred into 10ml volumetric flasks and solutions were made upto the volume to yeid concentrations of clavulanic acid and cefpodoxime proxetil.

Pharmaceutical formulation

Formulation Oratil cv,manufactured by Nacleods pharmaceutical ltd was purchased from the local pharmacy in Hyderabad.

Preparation of sample solution

For analysis of commercial formulation, 20 tablets of oratil cv of clavulanic acid 125 mg and cefpodoxime proxetil 200mg were weighed the average weight was calculated and powdered.A quantity equivalent to 125mg of clavulanic acid and 200mg of cefpodoxime proxetil was weighed and transferred to a 100ml volumetric flask which contain mobile phase and then shake it for 10mins and sonicate it for 20mins.The

solution was allowed to stand at a room temperature for 20-30mins and filterd it through a whatmann filter paper.Then suitable aliquots of formulation solution were prepared and injected intlo HPLC to obtain concentration in linearity range.

Validation of analytical method

ACCURACY:Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. Recovery studies of the drug were carried out for determining accuracy parameter. Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. It was done by mixing known quantity of standard drugs with the analyzed sample formulation and the contents were reanalyzed by the proposed method. This was carried out in 50% 100% and 150% levels.

PRECISION: The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The Precision expressed as standard deviation or relative standard deviation.

a.System precision: Standard solution prepared as per test method and injected five times.

b.Method precision: Prepare five sample preparations individually using the single as per test method and injected each solution

LINEARITY: The linearity of amalytical ,method is the ability to clicit test results that are directly proportional to the concentration of analyte in the sample within the given range.The linearity was performed by seven different concentrations,which were injected and calibration curve were plotted.The linearity of clavulanic acid and cefpodoxime proxetil was found to be in the range of 20-80µg/ml respectively The chromatograms of the resulting solutions were recorded. The plot showing linearity and range study for Clavulanic acid and cefexime is shown in figure.

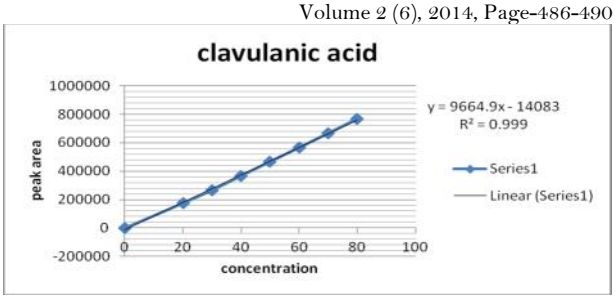

Fig 3: Plot of linearity and range study for clavulanic acid

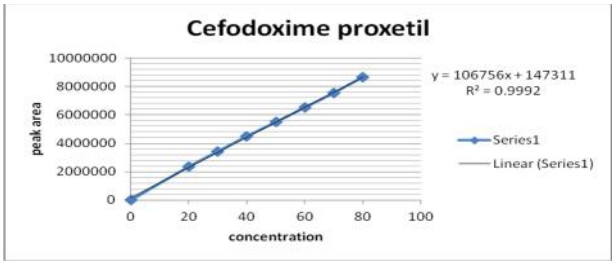

Fig 4: Plot of linearity and range study for cefexime

Ruggedness:

a) System to System variability: System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. A comparison of both the results obtained on two different HPLC systems, shows that the assay test method is rugged for System to system variables.

Robustness: The robustness of an analytical procedure are a measure of its capacity to remain unaffected by small, but deliberate changes in the method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of combination of mobile phase and flow rate. % RSD of assay was calculated.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD of an analytical procedure is the lowest concentration of an analyte in a sample which can be detected but not necessarily quantitated as an exact value where as LOQ is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

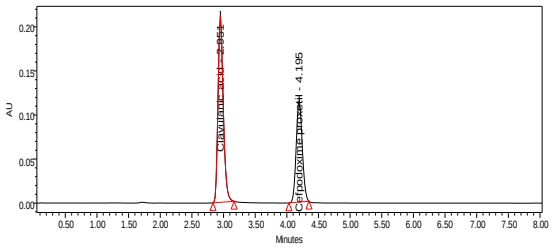

Fig 5: Optimized chromatogram

Table 1: Results of validation parameters of RP-HPLC

Sl. no	Validation Parameter	Clavulanic acid	Cefpodoxime proxetil	Acceptance Criteria
1.	Linearity (in µg)	10 – 80	20 – 80	Correlation
2.	Regression Line Equation	y = 9664.x - 14083	y = 10675x + 14731	coefficient (R^2 =0.999)
3.	R^2 Value	0.999	0.999	
4.	Precision			
	System Precision (%RSD)	1.57	0.14	RSD<2%
	Method Precision(%RSD)	1.28	1.53	
5.	LOD	0.046	0.048	
6.	LOQ	3.86	3.92	-
7.	Assay of marketed formulation	99.25%	98.52%	95-105%
8.	% Recovery	98-101	98-101	95-105%
9	Ruggedness	0.4	0.34	RSD<2%

3. RESULTS AND DISCUSSION

- The slope, intercept and correlation coefficient values were found to be 9664, 14083 and 0.999 and 10675, 14731 and 0.999 for clavulanic acid and cefpodoxime proxetil respectively.
- The LOD of clavulanic acid and cefpodoxime proxetil were found to be 0.046µg/ml and 0.048µg/ml respectively. The LOQ of clavulanic acid and cefpodoxime proxetil found to be 3.86µg/ml and 3.92µg/ml respectively.
- Precision of the developed method was studied. Low % RSD values indicate that the method is precise.

4. SUMMARY AND CONCLUSION

The proposed RP-HPLC method for the estimation of the cefpodoxime proxetil and clavulanic acid in the pharmaceutical dosage form were simple, reliable and selective providing satisfactory accuracy and precision with lower limits of detection and quantification. The recoveries achieved were good by RP-HPLC method. The methods can be recommended for routine and quality control analysis of these drugs in the pharmaceutical dosage forms. In this proposed method symmetrical peaks with good resolution were obtained.

Table 2: summury of analysis of cefpodoxime proxetil and clavulanic acid by RP-HPLC method

Drugs	Labeled amount, mg/ tablet	Estimated Amount , mg/tablet	% Label claim	% *RSD
	200	197.56		
CEF			98.52	1.73
		124.5		
CLA	125		99.25	1.13

5. ACKNOWLEDGEMENT

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