Development and validation of RP-HPLC method for simultaneous determination of cefpodoxime proxetil and H_2 receptor antagonists in pharmaceutical dosage forms

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Abstract: A new method on RP-HPLC is devised and validated, as per ICH guidelines, for the synchronous estimation of cefpodoxime proxetil and H_2-receptor antagonits that are Cimetidine, Famotidine and Ranitidine. The method is simple, accurate, expeditious, reproducible, robust and precise. Chromatography was done on a C_{18} (250 x 4.6mm) column with methanol: water as mobile phae in the ratio of 70:30 (v/v), pumped at a flow rate of 1ml/min and pH was maintained using 85% ortho-phosphoric acid at 3. The λ max 240 nm was preferred for UV detection. A good linear relationship was attained, over the concentration ranges of 20-70 µg/ml and 5-30µg/ml, for cefpodoxime proxetil and H_2 blockers respectively, with a correlation coefficient of R= 0.9987 to 0.9992. The method was validated and found precised (i.e. intra day and interday analysis) with RSD <2%. LOD and LOQ observations were under 0.4806 to 2.6069µg/ml which proved the method to be sensitive. The method provided satisfactory results of robustness and reproducibility, when validated and applied successfully for analysis of dosage forms.

Keywords: Cefpodoxime Proxetil, H_2 receptor blockers, RP-HPLC

INTRODUCTION

Cefpodoxime proxetil, an orally given cephalosporin, is actually an inactive ester of an antibiotic cefpodoxime (Saathoff *et al*., 1992). It is considered as a broad spectrum semi-synthetic antibiotic (Frampton *et al*., 1992). Cefpodoxime proxetil works at penicillin-binding proteins (PBPs) and inhibits synthesis of bacterial cell wall by effecting peptidoglycan transpeptidation (Pahwar *et al*., 2015). Cefpodoxime is immensely active against *H. influenza* and *M. catarrhalis* strains making β-lactamase, and is claimed to have like effectiveness as of cefixime (Chugh and Agarwal, 2003). The drug has proved its efficiency in control of Urinary tract and Respiratory infections. It is also used skin infections, acute otitis media, pharyngitis, tonsillitis and sexually transmitted diseases. H_2 receptor antagonist are used in duodenal ulcers, in benign gastric ulcer, gastroesophageal reflux disease (GERD), hypersensitivity conditions (e.g., Zollinger Ellison Syndrome). Famotidine differs from Cimetidine and Ranitidine, due to presence of a guanyithiazole ring, it possess neither the imidazole ring of the former nor the furan ring of the latter. Famotidine has been reported to be 2 to 160 fold more potent than cimetidine and 3-20 fold more potent than ranitidine (Smith *et al*., 1985). An HPTLC method was developed and validated for the estimation of cefpodoxime proxetil in Pharmceutical dosage form by Darji *et al* in 2007. Linearity of the Caliberation curve was found between 100 to 700ng/spot. The mobile phase used was chloroform: methanol: Toluene (4:2:4v/v/v). The detection of spot was carried out at 289.0 nm (Darji *et al*., 2007). In pharmaceutical dosage forms simultaneous determination of moxifloxacin and H_2 receptor blockers, by RP-HPLC, was performed. The method showed accuracy and was rapid with acceptable linearity (Sultana *et al*., 2011). Novakovic developed a new HPTLC method for Ranitidine Hydrochloride and Famotidine where silica plates were used for separation. Mobile phases were toluene-methanol-diethylamine (9:1:1, v/v) for ranitidine and USP 23 mobile phase for famotidine. Quantification was done at UV absorption maxima 320 nm for ranitidine hydrochloride and 276 nm for famotidine. Linearity was found in ranges from 30 to 230 ng and 80 to 580 ng for ranitidine hydrochloride and famotidine respectively (Novakovic, 1999). In another work three spectrophotometric methods were devised for investigation of H_2 receptor antagonists cimetidine, ranitidine hydrochloride and famotidine (Kahdiga *et al*., 2002). Many instrumental HPLC and Spectrophotometric methods have been developed and validated for the estimation of cefpodoxime proxetil alone and

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simultaneous determination of cefpodoxime proxetil with other commonly used drugs (Dubala *et al*., 2013; Kakumanu *et al*., 2006; Malathi *et al*., 2009). Simultaneous estimation of ceftriaxone sodium and H_2 blockers in pharmaceutical dosage forms and human serum was done by RP-HPLC, the technique was accurate, rapid, and precise and showed linear results (Shah *et al*., 2013). The present research aims at developing a simple, easy and robust method for determination of two important drugs i.e cefpodoxime proxetil and H_2 receptor antagonists simultaneously, as no such method have been found to be reported in literature.

MATERIALS AND METHODS

Cefpodoxime proxetil standard was given as a gift by Pharmevo (Pvt) Ltd., Cimetidine was presented by Bosch Pharmaceuticals (Pvt) Ltd, Famotidine and Ranitidine were a gift from GSK (Pvt) Ltd. Tablets were used in the below mentioned strengths Cefpodoxime proxetil 100 mg/tab. Cimetidine 200mg/tab, Famotidine 40 mg/tab, Ranitidine 150 mg/tab. Analytical grade reagents were utilized. Methanol HPLC grade and 85% ortho-phosphoric acid (Merck), distilled water was used for the mobile phase. Reagents were passed through double distillation and filteration.

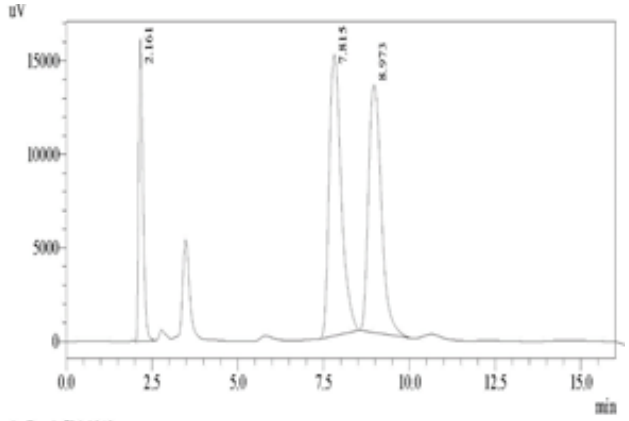


Fig. 1: Chromatogram of cefpodoxime + cimetidine

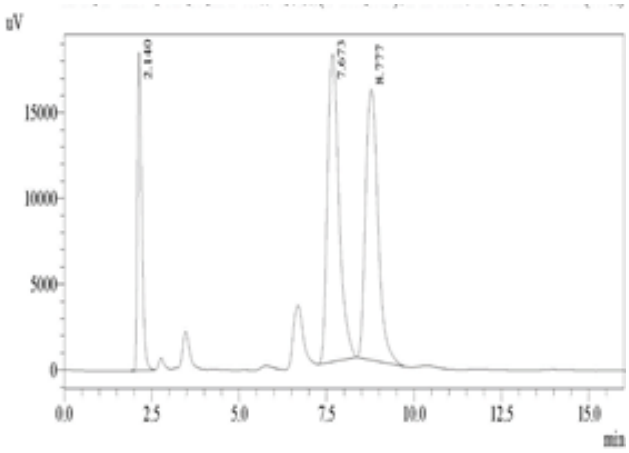


Fig. 2: Chromatogram of cefpodoxime + Famotidine

Chromatographic conditions

The chromatographic conditions are summarized in table1.

Preparation of stock solution and working solution

Standard solution i.e. Stock solution of cefpodoxime proxetil and H_2 receptor blockers were obtained by dissolving 50mg Cefpodoxime proxetil and 5mg H_2 receptor blockers Cimetidine, Famotidine and Ranitidine in 100ml of diluent to prepare a final concentrations of 500µg/ml and 50µg/ml of cefpodoxime proxetil and H_2 receptor antagonists respectively. The stock solutions were diluted, with the diluent, to give 30 to 70µg/ml and 2 to 10µg/ml concentrations of cefpodoxime proxetil and H_2 receptor blockers respectively. These are used as working solutions for calibration curves.

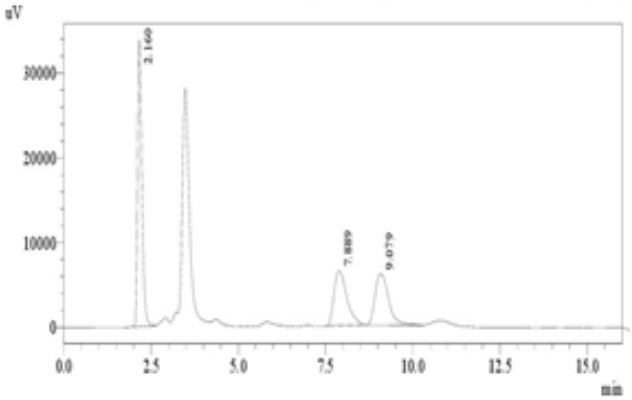


Fig. 3: Chromatogram of cefpodoxime + ranitidine

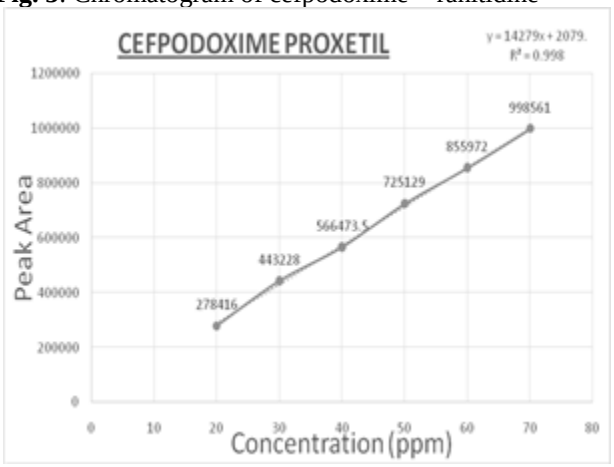


Fig. 4: Linearity graph of Ceprodoxime Proxetil

Assay of the dosage formulation

Weighed 20 tablets of all drugs under consideration and then powdered them individually. From the weighed powder the amount of drug (according to the label claim) was taken in 100ml volumetric flask, which was dissolved in 60ml of diluent and then volume was made up to 100 ml with same diluent. Finally sonicated all four solutions. Final dilutions were prepared using the similar diluent to obtain specific concentration and all dilutions were filtered separately through 0.45µm filter.

Table 1: The Chromatographic conditions

Method	Reversed phase high performance liquid Chromatography
Chromatographic System used	Two Shimadzu HPLC systems, with automatic injectors, were used to perform Chromatographic studies. One equipted with LC-10 AT VP pump, SPD-10 A UV/VIS detector and the other HPLC system was attached with LC-20AT and SPD-20A UV/VIS detector
Column	CLC-ODS, C18 (4.6 x 250 mm) Shim-Pack RP column was used for the detection.
Column temperature	Room temperature
Mobile phase	Methanol: Water (60:40) pH 3.0 was maintained with 85% phosphoric acid. The mobile phase initially filtered through 0.45µm filter and after that degassed using an ultrasonic bath before its injection into the HPLC system.
Flow rate	1ml/min at room temperature.
Diluent	Methanol: Water in the ratio 80:20.
Injection	10µl sample (in triplicate using autosampler)
Detection	240nm wavelength was selected for recording of chromatogram.

Table 2: Regression characteristics of Cefpodoxime Proxetil and H_2 receptor antagonists

Analytes	Conc. Range (µg/ml)	R^2	Intercept	Slope
CP	20-70	0.9989	2079.7	14279
Cimetidine	5-30	0.9992	2399.6	9204.7
Famotidine	5-30	0.9987	5446.9	10456
Ranitidine	5-30	0.9989	10694	19382

Table 3: Sensitivity (µg/ml) of the proposed method

Analyte	LOD	LOQ	Regression Equation
Cefpodoxime proxetil	0.4806	1.4564	Y=14279x + 2079.7
Cimetidine	0.8602	2.6069	Y=9204.7x +2399.6
Famotidine	1.719	5.2	Y=10456x + 5446.9
Ranitidine	1.8207	5.517	Y=19328x + 10694

Table 4: Accuracy of Cefpodoxime Proxetil, Cimetidine, Famotidine & Rnitidine

Analyte	Spiked Conc. (µg/ml)	Mean Conc. Recovered (µg/ml)	% Recovery
Cefpodoxime proxetil	40	39.673	99.1825
	50	50.826	101.652
	60	59.246	98.7433
Cimetidine	12	12.108	100.9
	15	15.402	102.68
	18	18.264	101.46
Famotidine	12	12.083	100.691
	15	14.899	99.326
	18	18.456	102.533
Ranitidine	12	11.793	98.275
	15	15.182	101.213
	18	17.963	99.794

Method validation

System suitability testing

HPLC system was injected with the mobile phase on daily basis. Six injections of the standard were injected consecutively to monitor system suitability and Peak area was measured.

Range & linearity

Linearity was examined, at six concentrations, for all drugs under investigation that are cefpodoxime proxetil and Histamine receptor antagonists. Linearity plot, slope, intercept and the correlation coefficient were determined. Linearity curves were produced by plotting peak areas against concentrations.

Specificity

The specificity and selectivity of the method were found out.

Accuracy (Recovery)

Accuracy of the method was questioned and then justified by individually evaluating solutions of Cefpodoxime

Proxetil and all three H_2 antagonists (Cimetidine, Famotidine and Ranitidine) at three different concentrations i.e., 80%, 100% and 120% by spiking known amount of the drugs. Each concentration was injected in triplets and the mean was taken for the calculation of percent recovery.

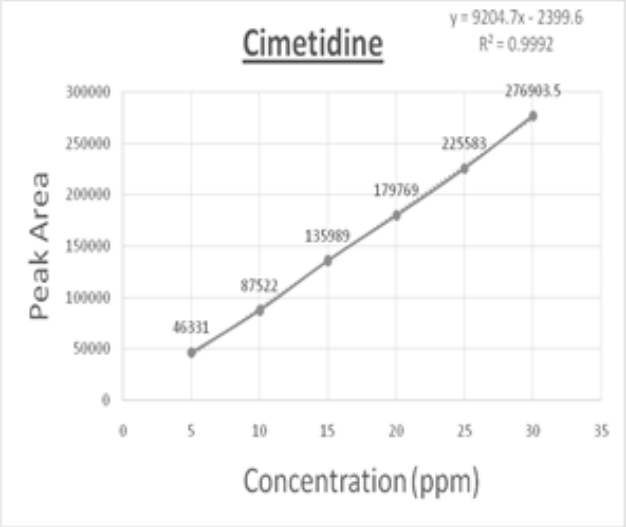


Fig. 5: Linearity graph of Cimetidine

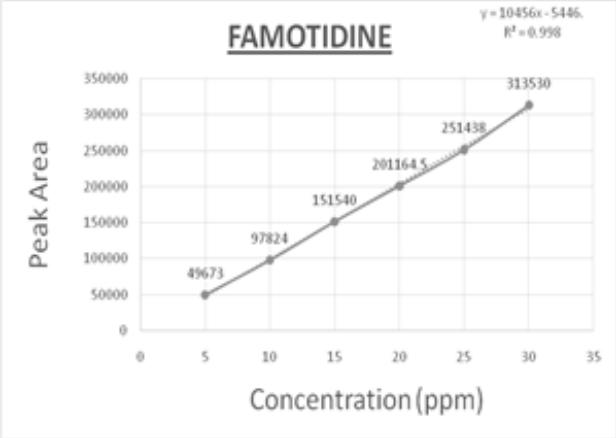


Fig. 6: Linearity graph of Famotidine

Precision

The precision of the newly developed method was investigated by intra-day (repeatability) and inter-day (intermediate precision) analysis. The method proved precised when six times each concentration (in two days) was injected and % RSD was determined.

Limit of detection & limit of quantitation

The *limit of detection* (LOD) is usually defined as the lowest quantity or concentration of a component that can be reliably detected with a given analytical method. Limit of quantification (LOQ) is that concentration which exhibit numerical result with least chances of errors. Calibration curve of standard was drawn and LOD and LOQ were calculated by standard formulas: LOD = (3.3 ×σ)/m LOQ = (10×σ)/m. In the above formulas σ is regarded as standard deviation of y-intercept of regression line where as m is considered as slope of regression line.

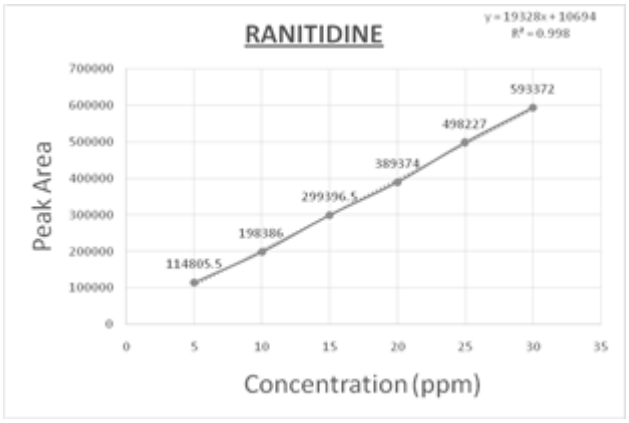


Fig. 7: Linearity graph of Ranitidine.

Ruggedness

The method when performed in two different labs (First was RIPS, Depart. of Pharm. Chemistry, the other research lab was at Depart. of Chemistry Uni. of Karachi) didn't give any significant deviations from acceptable values. Two different instruments were employed for the purpose that is LC 10 and LC 20.

Robustness

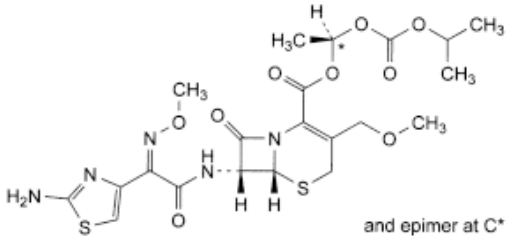
Robustness was estimated by insignificant alterations in ratio or pH levels of mobile phase (Methanol: Water), flow rate and wavelength. pH was changed ±0.2%, change in flow rate ±0.2% and in wavelength ±5% from optimal condition were adopted and it was noted that the % R.S.D did not surpass over 2%. Five recurrent samples were injected with slight variations in the above said parameters. Fluctuation in retention time ±0.2% was observed that was assessed as insignificant.

RESULTS

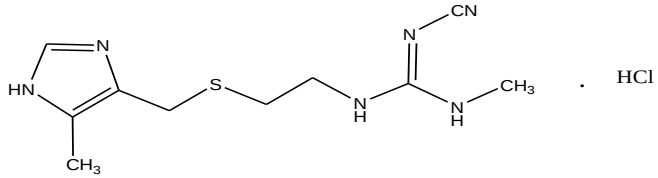
The investigational results of validation, were stated by SPSS INC. Software. System Suitability parameter includes peaks symmetry and others such as column's plates. All the values obtained for system suitability were within range. The retention times for cefpodoxime proxetil-cimetidine were 2.161, 7.815, 8.973 minutes as indicated in fig. 1. For cefpodoxime proxetil-famotidine 2.140, 7.673, 8.777 minutes were recorded (fig. 2) and considering cefpodoxime proxetil-ranitidine the retention times were 2.160, 7.889, 9.079 minutes (fig. 3). Linearity was recorded in ranges 20 -70 µg/ml and 5-30 µg/ml for Cefpodoxime proxetil and H_2 receptor blockers respectively (fig. 4-7). The concentration of drugs in parts per million and peak areas were investigated for least square linear regression. A linear regression line was observed with R^2>0.998 where R is correlation coefficient

Table 5: Precision of Cefpodoxime Proxetil and H_2 receptor antagonist

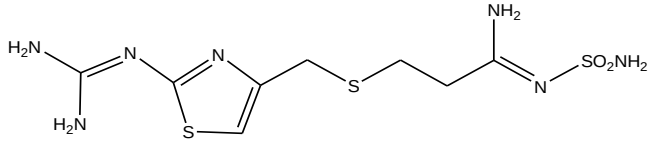
Analytes	Conc. (µg/ml)	RSD (%)		t-stat	P (T<t) two-tail
		Day 1	Day 2		
Cefpodoxime proxetil	40	0.543	1.338	-1.953	0.190
	50	0.459	1.384		
	60	0.547	0.538		
Cimetidine	12	1.540	1.939	0.533	0.647
	15	0.179	0.111		
	18	1.750	0.772		
Famotidine	12	0.959	0.151	0.198	0.862
	15	0.441	1.574		
	18	0.789	0.091		
Ranitidine	12	0.819	0.230	0.400	0.728
	15	0.632	1.575		
	18	1.438	0.348		



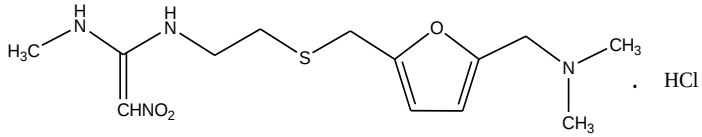
Cefprodoxime proxitel



Cimetidine HCl



Famotidine



Ranitidine HCl

(table 2). The table 3 presents the values of LOD and LOQ. All the ideal chromatograms were developed and showed good resolution of cefpodoxime proxetil, parent analyte from all H_2 antagonists which clearly illustrated the specificity of this new method in raw materials. Specificity of the system showed it is free of obstructions of the excipients utilized in pharmaceutical preparations. The method proved to be of high accuracy, by exhibiting high % recoveries ranges from 98.275-102.68% as shown in the table (table 4). Samples injection were repeated five times. Results of Precision showed relative S.D in range of 0.091-1.939% approving remarkable precision (table 5).

DISCUSSION

Various methods have been reported in literature for determination of cefpodoxime in bulk and pharmaceutical formulations (Patle *et al*, 2014; Mathew *et al*., 2013; Acharya and Patel, 2013). In a method reported by Mathew *et al* linearity was in the concentration range of 5-100µg /ml which confirms the results, of linearity, of present study i.e 20-70µg /ml (fig. 4) (Mathew *et al*., 2013). In the present study LOD and LOQ were found 0.4806, µg /ml 1.4564µg /ml for cefpodoxime proxetil which is greater than LOD 0.0726µg /ml and LOQ 0.220 µg /ml reported in literature by Acharya and Patel

(Acharya and Patel, 2013). In 2011 a dual wavelength spectrophotometric method was determined for simultaneous determination of ofloxacin and Cefodoxime Proxetil in tablet dosage form (Sanket and Satish, 2011).

CONCLUSION

The suggested method turned out to be an easy, rapid and cost-effective analytical evaluation for assaying cefpodoxime proxetil and H_2 receptor antagonist in bulk samples and, as expected, in pharmaceutical dosage forms. Furthermore, the method met all validation requirements such as linearity, accuracy, sensitivity, robustness, precision requested by international guidelines.

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