Research Article

SIMULTANEOUS RP-HPLC DETERMINATION OF CEFPODOXIME PROXETIL AND OFLOXACIN

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ABSTRACT

A rapid and simple high Reverse-phase performance liquid chromatography method was developed and validated for simultaneous determination of Cefpodoxime Proxetil and Ofloxacin from pharmaceutical preparation. The separation was achieved on A kromosil (technokroma) 100 C-8 (3.5 μ m, 15x0.46) performed using Acetonitrile and phosphate buffer as mobile phase the pH of buffer was adjusted to 4.8 using Ortho-phosphoric acid, the ratio was 58:42 (v/v) eluents were monitored at 275 nm. The detector response was linear in the range of 100 to 300 μ g, for both Cefpodoxime Proxetil and Ofloxacin. The percentage assay Cefpodoxime Proxetil and Ofloxacin was found between 99.76 and 99.31% respectively. The described method has the advantage of being rapid and easy hence it can be applied for routine quality control analysis.

Keywords: Cefpodoxime Proxetil, Ofloxacin, RP-HPLC, Pharmaceutical formulation.

INTRODUCTION

Cefpodoxime Proxetil (CEFPO) has the molecular formula $C_{15}H_{17}N_5O_6S_2$ and molecular weight 427.455 g/mol. Its chemical name is (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.^{1,2} Few methods such as spectrophotometric method^{3,4,5}, HPLC^{6,7} method have been reported for individual Cefpodoxime Proxetil or in combination with other drugs in formulation

Ofloxacin has the molecular formula $C_{18}H_{20}FN_3O_4$ and a molecular weight 361.67 g/mol. 7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1,2,3,4-tetrahydro-1,8-naphthyridine-3-carboxylic acid. It is used for the treatment of gonorrhea

and is an alternative treatment to ciprofloxacin for anthrax. Few methods such as spectrophotometric method^{4,5}, HPLC method⁸⁻¹¹ and other^{12,13} have been reported for individual Ofloxacin or in combination with other drugs in formulation

Cefpodoxime Proxetil 200 mg and Ofloxacin 200 mg in combination are available in market by brand name Zedocof-O which is anti biotics.

A literature survey reveals that there are no analytical methods reported for the simultaneous determination of these drugs in combined dosage form. The present study reports a HPLC method for simultaneous determination of Cefpodoxime Proxetil and Ofloxacin in combined tablet dosage form.

Cefpodoxime Proxetil

Ofloxacin

Figure 1. Structures of Cefpodoxime Proxetil and Ofloxacin

MATERIALS AND METHODS

Cefpodoxime Proxetil and Ofloxacin working standards were obtained as a gift sample from Emcure Pharmaceutical Ltd. Pune, India with purity of 98.99 and 99.69% respectively. The formulation of the tablet with combination of Cefpodoxime Proxetil 200 mg and Ofloxacin 200 mg is available in market by brand name Zedocof-O. All the reagents used were of HPLC grade, and analytical grade. All dilutions were performed in standard volumetric flask. Triple distilled water was prepared in the laboratory.

Instrumentation and chromatographic conditions

Chromatography was performed on Water 996 high performance liquid chromatography equipped, PDA detector, auto sampler with windows based Empowerpro and Thermo UV/ Double beam. The stationary phase is A kromosil (technokroma) 100 C-8 (3.5 μ m 15x0.46) using Acetonitrile : Phosphate Buffer pH 4.8 (58:42v/v) mobile phase. The chromatographic conditions had previously been optimized to achieve the best resolution and peak shape. Detection

was performed at 275 nm having flow rate of 1.2 mL/min. The typical chromatogram is shown in Figure 2.

Stock solution of Cefpodoxime Proxetil (Solution 1)

50 mg of Cefpodoxime Proxetil was accurately weighed, transferred into 50 ml of volumetric flask and dissolved in the minimum amount of Acetonitrile and diluted up to the mark with acetonitrile. (1000 µg/ml)

Stock solution of Ofloxacin (Solution 2)

50mg of Ofloxacin was accurately weighed and transferred into 50 ml of volumetric flask. It was dissolved in the minimum amount of Acetonitrile and then diluted up to the mark with Acetonitrile. (1000 µg/ml)

Figure 2. Chromatogram showing separation by RP-HPLC (1) Ofloxacin, (2) Cefpodoxime Proxetil

Preparation of working standard solution

Working standard has been prepared by Pipetting out 2ml from stock solution of both Cefpodoxime Proxetil and Ofloxacin mixed in volumetric flasks. These mixtures were then diluted up to the mark with Acetonitrile which gives concentration 200 µg/ml of both Cefpodoxime Proxetil and Ofloxacin.

Preparation of sample solution (Solution 3)

Twenty tablets were weighed accurately and average weight of one tablet was calculated. The tablets were crushed to furnish a homogeneous powder and a quantity equivalent to one tablet (867 mg) was weighed and dissolved in a minimum quantity of acetonitrile. This was transferred to flask and diluted up to 867ml with Acetonitrile and filtration by using a Whatman filter paper 41. The filter paper was washed with Acetonitril and take filtrate and make concentration 200 µg/ml.

Preparation of Phosphate Buffer pH 4.8 solution (Solution 4)

Accurately weighed quantity 1.36 gm of potassium dihydrate has been dissolve in sufficient quantity of 1% triethylamune in100 ml volumetric flask and diluted up to the mark and pH has been adjusted to 4.8 by ortho phosphoric acid.

VALIDATION PROCEDURE

Linearity

Different concentrations of Cefpodoxime Proxetil (100.00 to 300.00 µg) and Ofloxacin (100.00 to 300.00 µg) were prepared from stock solution of respective API in Acetonitrile. And mix the solution of both drugs. 20 µL of each solution was injected and the detector response for the different concentration was measured. The drug peak area was calculated for each concentration level and a graph was plotted of drug concentration against the peak area. The plot was linear in the range mentioned above. This experiment was performed thrice and the mean peak area response was used for calculations. The data was analyzed by linear regression least square fitting. The statistical data obtained is given in Table 1.

System suitability

The coefficient of variation for peak area and retention time value for all the drugs was less than 2.00% for three replicate measurements of the same sample.

Robustness of the method

In the present work effect of flow rate, effect of organic phase & effect of pH was studied for the robustness of the method. The analysis was performed by using flow rate of ± 0.1 ml/minute, organic phase ± 10 % and pH by ± 0.2 units. The results are summarized in Table 2, 3 and 4 which shows that method is not affected by deliberate change in the flow rate, organic phase and pH.

Peak asymmetry and peak tailing

The result shows that within the concentration ranges mentioned above there was an excellent correlation between peak area ratio and concentration of the each drug. The results are given in Table 5.

Assay (from pharmaceutical preparation)

20 µL of working standard solution of Cefpodoxime Proxetil and Ofloxacin and sample solution were injected and chromatograms were developed and evaluated. The procedure was repeated three times, individually weighing the tablet powder (average weight of one tablet) each time. The responses from the standard and sample were used to calculate the amounts of the drug in the tablet. Results obtained are shown in Table 6.

Recovery

The accuracy of method was determined by recovery experiments. The recovery studies were carried out using standard addition method at 50, 100 and 150 % level; known amount of standards was added to reanalyzed sample and subjected them to the proposed HPLC method. Percentage recovery was calculated from the amount found and actual amount added result. The mean recovery is within acceptable limits which indicate that the method is accurate. That shows in Table 7.

Table 1. Linear study data data

Drugs	Slope	Intercept	Correlation Coefficient (R ²)
Cefpodoxime Proxetil	21078	8332	0.997
Ofloxacin	48950.0	12391	0.998

Table 2. Effect of flow rate

System Suitability parameter		Observations			Limits
		As Such	-0.2 units	+0.2 units	
The % RSD of peak area response for five replicate injections	Ofloxacin	0.83	0.90	0.21	NMT 2.0
	Cefpodoxime Proxetil	0.13	0.07	0.20	
Theoretical plates	Ofloxacin	2486	2416	2401	NLT 1000
	Cefpodoxime Proxetil	3039	3118	2714	
Tailing factor	Ofloxacin	1.57	1.64	1.59	NMT 2.0
	Cefpodoxime Proxetil	1.07	1.17	1.10	

Table 3. Effect of organic phase variation

System Suitability parameter		Observations			Limits
		As Such	- 10%	+ 10%	
The % RSD of peak area response for five replicate injections	Ofloxacin	0.83	0.90	0.21	NMT 2.0
	Cefpodoxime Proxetil	0.13	0.07	0.20	
Theoretical plates	Ofloxacin	2486	2416	2401	NLT 1000
	Cefpodoxime Proxetil	3039	3118	2714	
Tailing factor	Ofloxacin	1.57	1.64	1.59	NMT 2.0
	Cefpodoxime Proxetil	1.07	1.17	1.10	

Table 4. Effect of pH variation

System Suitability parameter		Observations			Limits
		As Such	-2units	+ 2units	
The % RSD of peak area response for five replicate injections	Ofloxacin	0.11	0.64	0.38	NMT 2.0
	Cefpodoxime Proxetil	0.68	0.60	0.43	
Theoretical plates	Ofloxacin	2486	2040	2465	NLT 1000
	Cefpodoxime Proxetil	3039	3206	3103	
Tailing factor	Ofloxacin	1.57	1.66	1.39	NMT 2.0
	Cefpodoxime Proxetil	1.07	1.17	1.07	

Table 5. Asymmetry factor and peak tailing factor Components

Parameters	Ofloxacin	Cefpodoxime Proxetil
Theoretical plates	2417	2950
Asymmetry factor	1.40	1.23
Tailing factor	1.41	1.15

Table 6. Results of HPLC assay studies (n=3)

Drugs	Wight of std mg	Wight of sample mg	% LC	SD	% RSD
Cefpodoxime Proxetil	1000	4832.5	99.32	0.257	0.259
Ofloxacin	1000	4832.3	98.69	0.088	0.089

Table 7. Recovery data

Drug	% Recovery	%RSD
Cefpodoxime Proxetil	100.89	0.45
Ofloxacin	100.61	0.26

Figure 3. Chromatogram of variation in flow rate (A)1.3 ml/min, (B)1.2ml/min &(C)1.1ml/min

Figure 4. Chromatogram of variation in organic phase of mobile phase (A)+10% (B)such as & (C)-10%

Figure 5. Chromatograms of variation in pH of mobile phase (A)+0.2 units (B) as such (C) 0.2 units

RESULTS AND DISCUSSION

Use of Acetonitrile and Phosphate buffer pH4.8 (58:42v/v) resulted in good separation of the drugs with no interfering peak at the retention time of the Cefpodoxime Proxetil And Ofloxacin 1.28 minute and 2.98 minute at 275 nm respectively as shown in Figure 2.

Figure 2 shows typical chromatogram obtained from Cefpodoxime Proxetil and Ofloxacin. Regression analysis for the calibration data for all the drugs showed that the dependent variable (peak area) and independent variable (concentration) were represented by the equation $y=21078x + 8332$ for Cefpodoxime Proxetil, $y=48950x+12391$ for Ofloxacin.

The correlation coefficient obtained was 0.997 and 0.998 for Cefpodoxime Proxetil and Ofloxacin respectively. It shows the good linear relationship between the concentration range 100 to 300 µg/ml for both drugs Cefpodoxime Proxetil and Ofloxacin.

The % RSD for peak area and retention time value for all the drugs was less than 2% for three replicate measurements of the same sample. This showed that the method and the system are suitable for determination of Cefpodoxime Proxetil and Ofloxacin.

The assay of Cefpodoxime Proxetil and Ofloxacin was found to be 99.32 and 98.69% respectively. The recovery of Cefpodoxime Proxetil and Ofloxacin was found to be 100.89 and 100.61 % respectively from recovery studies which indicates high accuracy of the method.

CONCLUSION

As the proposed method is highly accurate, selective and precise hence can be used for a routine quality control analysis and quantitative simultaneous determination of Cefpodoxime Proxetil and Ofloxacin in pharmaceutical preparations. The method is also fast and requires approximately 10 minute for analysis.

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