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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFIXIME AND SATRANIDAZOLE

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ABSTRACT

An RP-HPLC method was developed and validated for the simultaneous determination of Cefixime Trihydrate and Satranidazole. The chromatographic system was equipped with Phenomenex C_{18} column and PDA detector set at 254 nm, in conjunction with a mobile phase of Phosphate Buffer, Acetonitrile and Methanol in the ratio of 50:30:20 (pH 5), at a flow rate of 1.0ml/min. The retention time of Cefixime Trihydrate and Satranidazole were found to be 2.30 ± 0.05 and 4.2 ± 0.05 minutes respectively. Linearity was observed in the concentration range of 1-10µg/ml for Cefixime Trihydrate and Satranidazole, with good linearity response of 0.998 and 0.998 respectively. Percentage recoveries obtained for Cefixime Trihydrate and Satranidazole were 98-100% and 99-102% respectively. The proposed method is precise, accurate, selective and rapid for the simultaneous determination of Cefixime Trihydrate and Satranidazole.

This method can be used to estimate either of these drugs individually when present separately in formulation or in combination.

KEYWORDS : *Cefixime Trihydrateand Satranidazole; HPLC Method.*

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INTRODUCTION

Cefixime is an oral third generation cephalosporin antibiotic. Chemically, it is (6R,7R) -7- {[2-(2-amima-1,3-thiazol-4-yl) -2- (carboxy methoxy imino) acetyl]} -3- etheynl -8- oxo-5thia-1-azabicyclo- [4.2.0]oct-2-ene-2-carboxyllic acid, clinically used in the treatment of susceptible infections including Gonorrhea, Otitis Media, Pharyngitis, Urinary Tract Infection and Lower Respiratory Tract Infection such as Bronchitis. Literature survey reveals that Cefixime can be estimated Spectrophotometric method, Colorimetric method and RP-HPLC method.

Satranidazole is Nitroimidazole derivative. Chemically, it is 1-methylsulphonyl-3-(1-methyl-5-nitro-2-imidazolyl)-2-imidazolidinone. It

is used as anti-protozoal and anti-bacterial agent in the treatment of Amoebiasis and Girardiasis.Literature survey reveals that Satranidazole can also be estimated by several methods like HPLC, HPTLC, LC-MS/MS, colorimetric and UV-Visible spectrophotometry.

Cefixime and anti protozoals like Metronidazole or Ornidazole combinations are widely available for therapy. But Cefixime is not available in combination with Satranidazole. A combination of these two drugs would be within the choice of treatment for many microbial infections. Hence in this present investigation an attempt has been made to develop a simple, accurate and precise HPLC method for the estimation of Cefixime and Satranidazole in bulk and dosage form.

MATERIALS AND METHODS

Experimental:

Reagents and materials:

Acetonitrile and Methanol of HPLC grade, Potassium dihydrogen phosphate, and Orthophosporic acid.Cefixime was obtained from Drug Testing Laboratory, Bengaluru. Satranidazole was obtained from Alkem Labs Ltd., Mumbai. The commercially available Cefixime and Satranidazole dosage form was procured from local market.

Instrumentation of HPLC:

The chromatographic separation was carried out on HPLC system (Shimadzu - LC 20 AT) with UV-Visible dual absorbance detector (PDA), Phenomenex C_{18} column (250 x 4.6mn; 5µm). The mobile phase consisting of phosphate buffer (pH 5.0 adjusted with sodium hydroxide or Orthophosporic acid), acetonitrile and methanol were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 50:30:20 v/v was pumped into the column at a flow rate of 1.0 mL/min. The detection was monitored at 254 nm. The volume of injection loop was 20 µl prior to the injection of the drug solution. The column was equilibrated for at least 30 min with the mobile phase following through the system. The column and the HPLC system were kept in ambient temperature (25º C).

Preparation of Standard Stock Solution of Cefixime

Accurately 10 mg of Cefixime was weighed into a clean and dry 10 ml volumetric flask, sonicated with sufficient volume of diluents. The volume was made up to 10 ml with diluents to get the concentration of 1000µg/ml.

Preparation of Standard Stock Solution of Satranidazole

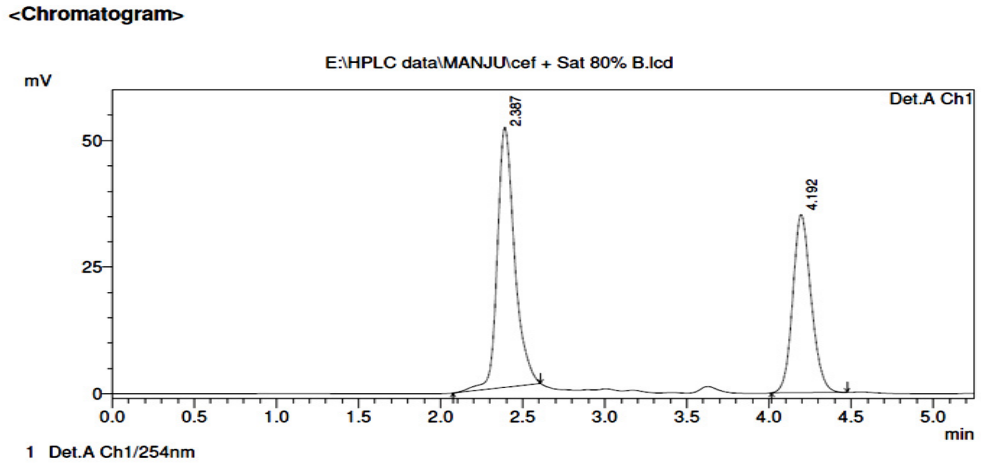
Accurately 10 mg of Satranidazole was weighed into a clean and dry 10 ml volumetric flask, sonicated with sufficient volume of mobile phase. The volume was made up to 10 ml with mobile phase to get the concentration of 1000µg/ml.

Preparation of Working Solution of Cefixime and Satranidazole (Mixture)

Pipette 0.1 ml each of the above stock solution into a clean, dry 10 ml volumetric flask and further diluted to 10 ml mobile phase to get a concentration 10 µg/ml each.

METHOD

For HPLC method, chromatographic conditions were optimized to obtain an adequate separation of eluted compounds. Initially, a series of trials were carried out with different types and ratios of solvents and buffers of different pH in mobile phase. The Retention behavior of Cefixime and Satranidazole were studied with mobile phase. The selection of best and most suitable mobile phase that provides satisfactory separation of peaks for Cefixime and Satranidazole led to solvent system of 0.05 M potassium dihydrogen phosphate (pH 5) Acetonitrile and Methanol combination in the ratio 50:30:20% v/v at 1ml/min flow rate and a wavelength of 254 nm. The retention time was found to 2.3 min for Cefixime and 4.3 min for Satranidazole respectively.



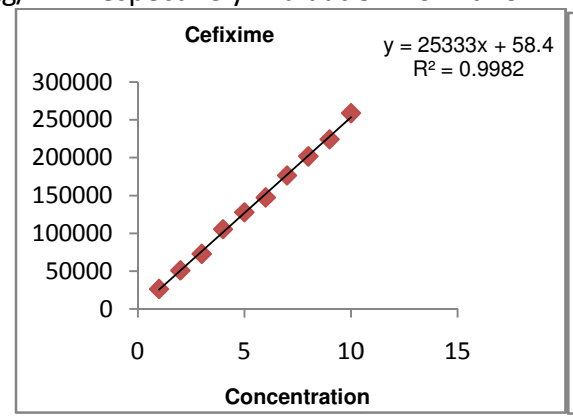
Peak#	Name	Ret. Time	Area	Tailing Factor	Resolution	Theoretical Plates
1	CEF	2.387	328453	1.38	0.00	2387.037
2	SAT	4.192	531765	1.19	8.80	6096.453

Fig 1: Chromatogram of Standard Cefixime and Satranidazole

METHOD VALIDATION

Linearity:

Several aliquots of standard solution (mixture) were taken into different 10 ml volumetric flasks and diluted up to mark with mobile phase such that final concentration of Cefixime and Satranidazole was 1-10µg/ml respectively.Evaluation of two drugs was performed and peak areas are recorded for all the peaks. The slope and intercept value for calibration curve was y=25333+58.4 (R^2=0.998) for Cefixime and y = 23378x - 2500.3 R^2 = 0.998 for Satranidazole. The results showed good correlation between peak area and concentration range indicated above regression graph are shown in Fig 2 and 3 respectively.



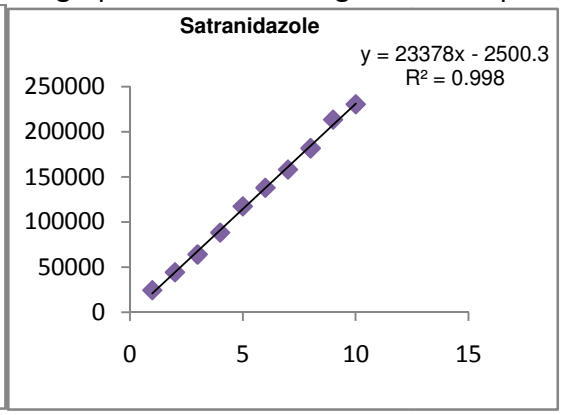


Fig 3: Linearity of CefiximeFig 3: Linearity of Satranidazole

Limit of Detection and Limit of Quantification

The limit of detection and limit of Quantification were determined individually by injecting progressively low concentrations of standard solution. The LOD is the smallest concentration that a measurable response of signal to noise ratio is of 3. The LOD for Cefixime and Satranidazole were found to be 0.6 µg/ml and 0.2µg/ml respectively. The LOQ is the smallest analyte that a measurable response of signal to noise ratio is of 10. The LOQ for Cefixime and Satranidazole were found to be 0.7 µg/ml and 0.5µg/ml respectively.

Recovery Studies:

The study of accuracy and reproducibility of the proposed method were followed by recovery studies. A fixed amount of sample mixture was taken and standard drugs were added at 80%, 100% and 120% levels. Each level was injected six

times. The contents of Cefixime and Satranidazole are shown in the Tables 1 and 2.

Level	Concentration of Cefixime Present in 10ml of sample stock solution in µg	Concentration of Cefixime present in 10ml of standard stock solution in µg	Total concentration in 10ml of mixture (µg/10ml)	Peak area	Amount found from Calibration curve	Amount of std Cefixime recovered µg/ml	% Recovery of standard
80%	5	8	13	326674	12.89	7.89	98.66
100%	5	10	15	380459	15.02	10.02	100.16
120%	5	12	17	430266	16.98	11.98	99.85

Table 1: Recovery study data of Cefixime

Level	Concentration of Satranidazole Present in 10ml of sample stock solution in µg	Concentration of Satranidazole present in 10ml of standard stock solution in µg	Total concentration in 10ml of mixture (µg/10ml)	Peak area	Amount found from Calibration curve	Amount of std Satranidazole recovered µg/ml	% Recovery of standard
80%	15	8	23	530256	22.79	7.79	97.36
100%	15	10	25	586365	25.19	10.19	101.89
120%	15	12	27	628474	26.99	11.99	99.92

Table 2: Recovery study data of Satranidazole

Ruggedness and Robustness:

The ruggedness of the method was determined by carrying out the experiment on different columns and days. Robustness is determined by making slight changes in pH of buffer, change in flow rate and mobile phase ratio. The ruggedness was determined by carrying the experiment by different analysts. It was observed that there were no marked changes in chromatograms, which indicated that the developed RP-HPLC method is robust and rugged.

Specificity:

The specificity of the method was determined by carrying out the experiment by injecting 20µl of mobile phase and working standard of Cefixime and Satranidazole separately into chromatogram to examine that the Cefixime and Satranidazole peaks are not affected by the mobile phase and the chromatogram was recorded. As no peaks were found at retention time of 2.3 min and 4.3 min, the proposed method was specific for the detection of Cefixime and Satranidazole.

RESULTS

Section	Parameter	Acceptance Criteria	Result observed for Cefixime	Result obtained For Satranidazole
1.	Linearity a. R^2 b. Range c. % curve fitting curve d. St. line eqn	 0.997 - >99.7 % Y=mx+c	 0.998 1-10 µg/ml 99.8 % y = 25333x + 58.4	 0.998 1-10 µg/ml 99.8 % y = 23378x - 2500.3
2.	LOD	S/N = 3	0.6 µg/ml	0.2 µg/ml
3.	LOQ	S/N = 10	0.7 µg/ml	0. 5µg/ml
4.	Precision of the method	Relative standard deviation within 2%	1.69%	0.86 %
5.	Precision of the system	Relative standard deviation within 2%	1.70%	1.83%
6.	Accuracy	% recovery to be found within the range of 98-102 %	98-100%	97-102%
7	Robustness	Change in pH of buffer	At 4.8 – 98.69%	99.88%
			At 5 - 100%	
			At 5.2 – 99.88%	98.89%
		Change in mobile phase (Buffer: ACN : Methanol)	(45:30:25) 99.28%	100.5%
			(50: 25: 20) 99.36%	100.52%
		Change in flow rate	0.9ml/min 94.70%	93.61%
			1.1ml/min 93.61%	103.44%
8.	Specificity	No interference at RT	No peaks were were found	No peaks found

CONCLUSION

Proposed study describes a new and simple RP-HPLC method for the simultaneous estimation of Cefixime and Satranidazole. The method validated was according to ICH guidelines, it is found to be simple, sensitive, accurate and precise and reproducible, hence can be adopted for routine quality assurance analysis. However this

study can be extended by performing the analysis on different marketed formulations.

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