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Research Article

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF CEFIXIME AND PARACETAMOL IN BULK AND PHARMACEUTICAL DOSAGE FORM

Y.Anil kumar *, G. Indira Priyadarshini, Y.Sirisha
Department of Pharmacology, Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi road, Guntur, A. P, India
Department of Pharmaceutical Analysis, Sims College of Pharmacy, India
*Corresponding Author Email: anilkumar.yerragopu@gmail.com

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ABSTRACT

A simple, accurate precise Stability indicating RP-HPLC method for the estimation of Cefixime and Paracetamol in pure and pharmaceutical dosage form has been reported. Quantitative estimation of Cefixime and Paracetamol was done by using WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector on a BDS C18 column (250 mm x 4.6 mm, 5µ). A 10µL syringe was used for injecting the samples. Data was analyzed by using Empower 2 software. UV-VIS spectrophotometer shimadzu with special bandwidth of 2mm and 10mm and matched quartz was used for measuring absorbance for Cefixime and Paracetamol solutions. The mobile phase consists of a Buffer: Acetonitrile (60:40) And at a flow rate of 1 milliliter/minute. Cefixime and Paracetamol were eluted at approximately 7 minutes. The wavelength was found to be 260nm. A linear response was observed in the concentration ranges of 20-120µg/ml with a regression coefficient of 0.999. Forced degradation studies were performed on pure sample of Cefixime and Paracetamol using acid (0.1 Normal (N) hydrochloric acid), base (0.1 N sodium hydroxide), peroxide (30% H_2O_2) and thermal (105°C) conditions. The developed method was validated with respect to specificity, precision (% RSD about 0.4%), linearity (linearity of range about 20-120 µg/mL), robustness, LOD and LOQ values were found to be 0.255 and 0.047 respectively.

Keywords: High performance liquid chromatography, Cefixime and Paracetamol

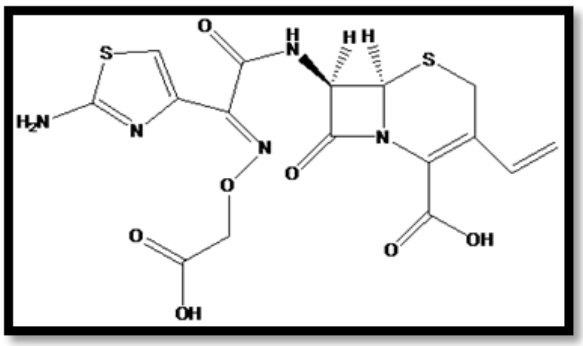
INTRODUCTION

CEFIXIME TRIHYDRATE

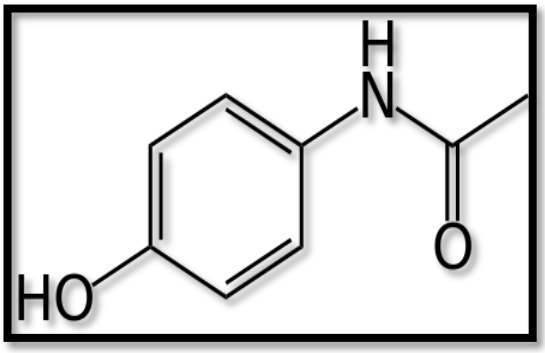
Cefixime is a semi synthetic, third generation cephalosporin antibiotic. Cefixime is a third-generation cephalosporin available in an oral formulation. In general, third-generation cephalosporins are more active against gram-negative species than the earlier generations of cephalosporins. Cefixime has enhanced antibacterial activity and increased stability against many of the beta-lactamases. It is commonly used in the treatment of otitis media, respiratory tract infections, and urinary tract infections caused by susceptible organisms. Cefixime is one of the only CDC-recommended oral antimicrobial agents to which Neisseria gonorrhea has not developed significant resistance[1].

PARACETAMOL

Paracetamol, also known as acetaminophen APAP, chemically named N-acetyl-p-aminophenol, is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). Acetaminophen is the name adopted for this pharmacologic agent in the U.S. (USAN) and Japan; paracetamol is approved in a variety of international venues (INN, AAN, BAN, etc.). Common trade names in English-speaking markets are Tylenol and Panadol. Paracetamol is classified as a mild analgesic. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics, paracetamol can also be used in the management of more severe pain such as post-surgical pain and providing palliative care in advanced cancer patients. Though paracetamol is used to treat inflammatory pain, it is not generally classified as an NSAID because it exhibits only weak anti-inflammatory activity[2].



Structure of Cefixime



Structure of paracetamol

MATERIALS AND METHODS

Cefixime and Paracetamol, Cefixime and Paracetamol tablets, HPLC grade water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydro furan, tri ethyl amine, ortho-phosphoric acid, 2N Hcl,2N NaoH,20% H_2O_2 etc.

INSTRUMENTATION

Quantitative estimation of Cefixime and Paracetamol was done by using WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector on a BDS C18 column (250 mm x 4.6 mm, 5µ). A 10µL syringe was used for injecting the samples. Data was analyzed by using Empower 2 software. UV-VIS spectrophotometer shimadzu with special bandwidth of 2mm and 10mm and matched quartz was used for measuring absorbance for Cefixime and Paracetamol solutions. Degassing of the mobile phase was done by using a shimadzu ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials[3-6].

CHROMATOGRAPHIC CONDITIONS

Chromatographic separation achieved using an analytical column, BDS (250mm 4.6mm, 5µ). Mobile phase was consisted of Buffer: Acetonitrile (60:40). The elution was achieved isocratically at a flow rate of 1 mL/min with injection volume of 10 µL. Column temperature was maintained at 30°C and chromatograph was recorded at wavelength 260nm[7].

Preparation of Sample

Preparation of standard stock solution

Accurately weighed and transferred 20mg of CEFIXIME and 25mg of PARACETAMOL working Standards into a 25ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Preparation of Sample Solution

20 tablets were weighed, powdered and then take the powder weight equivalent to 20mg of CEFIXIME and 25mg of PARACETAMOL was transferred into a 25mL volumetric flask, 15mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluent.

RESULTS AND DISCUSSION

Method development and validation

Some important parameters like pH of the mobile phase, concentration of the acid or buffer solution, etc., were tested for a good chromatographic separation. Trials showed that mobile phase with reverse phase C_{18} column gives symmetric and sharp peaks. After the optimization of chromatographic conditions, estimation of Cefixime and Paracetamol carried out by the developed RP-HPLC method. Standard solution of drug was injected separately and chromatogram of Cefixime and Paracetamol recorded in Figure 1 Now the sample solution was injected separately and chromatogram was recorded until the reproducibility of the peak areas were satisfactory.

Validation

HPLC method was validated according to the International Conference on Harmonization Guidelines (ICH Q2B, validation of analytical procedures, methodology). The method was validated for parameters such as system suitability, linearity, precision, accuracy, and robustness[8].

Linearity

Six Linear concentrations of Cefixime (20-120ppm) and Paracetamol (25ppm to 150ppm) are prepared and Injected. Regression equation of the Cefixime and Paracetamol are found to be, y = 7048.x + 332.6, y = 12126x + 336.2. And regression co-efficient was 0.999. recorded in Figure 3.

Precision

To study precision, six replicate standard solutions of Cefixime and Paracetamol were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was shown in Table 1.

Accuracy

Accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed sample solution. The standard addition method was performed at 50%, 100% and 150% level of sample solution. The resulting solutions were analyzed in triplicate at each level as per the ICH guidelines. Good recoveries were obtained for each concentration, confirming that the method was accurate and shown in Table 2.

Limit of Detection

Limit of Detection (LOD) is defined as lowest concentration of analyte that can be detected, but not necessarily quantified, by the analytical method. Limit of detection is determined by the analysis of sample with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected and it was found to be 0.255µg/ml

Limit of Quantification

Limit of quantification (LOQ) is the concentration that can be quantitated reliably with a specified level of accuracy and precision. LOQ was found to be 0.047µg/ml

Table 1: Results for Precision

Sl. No	Cefixime	Paracetamol
1	575568	1217681
2	575040	1200639
3	574412	1210933
4	566928	1205413
5	575090	1197905
6	564171	1219290
Mean	571868.2	1208644
Std. Dev.	4985.0	8832.8
%RSD	1	0.73

Table 2: Accuracy

Sample	% Level	Fixed sample concentration (µg/ml)	Amount Spiked (µg/ml)	Amount Recovered (µg/ml)	(%)Recovery	% RSD
Cefixime	50	80	40	39.92	99.8	0.44
	100	80	80	80.56	100.71	0.86
	150	80	120	119.6	99.74	0.80
Paracetamol	50	100	50	51.05	102.1	0.4
	100	100	100	100.86	100.86	0.52
	150	100	150	150.75	100.50	0.53

Table 3: Robustness data of Cefixime and Paracetamol

Sl. no	Robustness Condition	Mean Area of Cefixime	Mean Area of Paracetamol	%RSD of Cefixime	%RSD of Paracetamol	Rt	
						For cefixime	For paracetamol
	Unaltered	554078	1198527	-	-	3.82	3.13
1.	Flow rate (0.8 ml/min)	608697	1370383	0.2	0.3	4.231	3.481
2.	Flow rate (1.2 ml/min)	495573	1080098	0.0	0.2	3.463	2.852
3.	Mobile phase (62B:38A)	553061	1203391	1.6	2.0	3.779	3.102
4.	Mobile phase (58B:48A)	561146	1198837	0.5	0.7	3.830	3.174
5.	Temperature (25˚c)	600329	1342172	0.1	0.3	4.227	3.477
6.	Temperature (35˚c)	501943	1080098	0.2	0.6	3.463	2.852

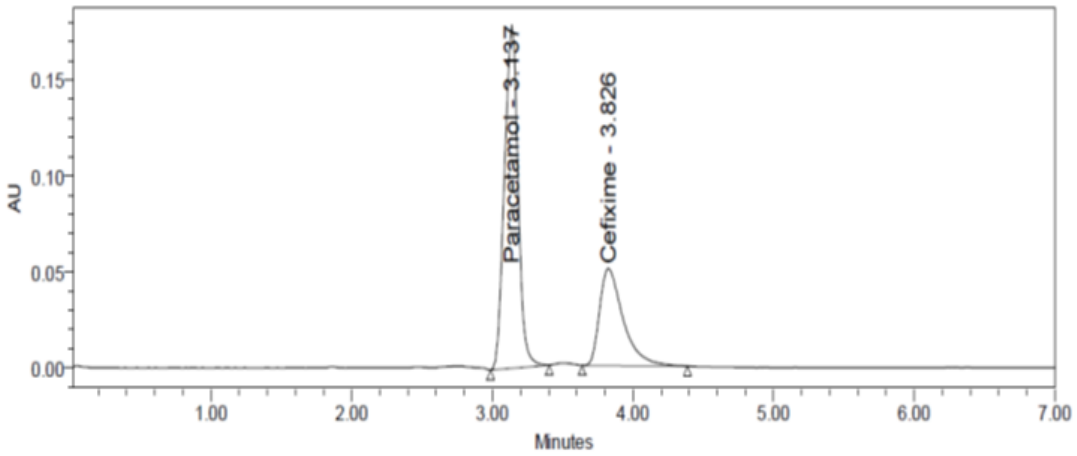


Figure 1: Chromatogram of Cefixime and Paracetamol

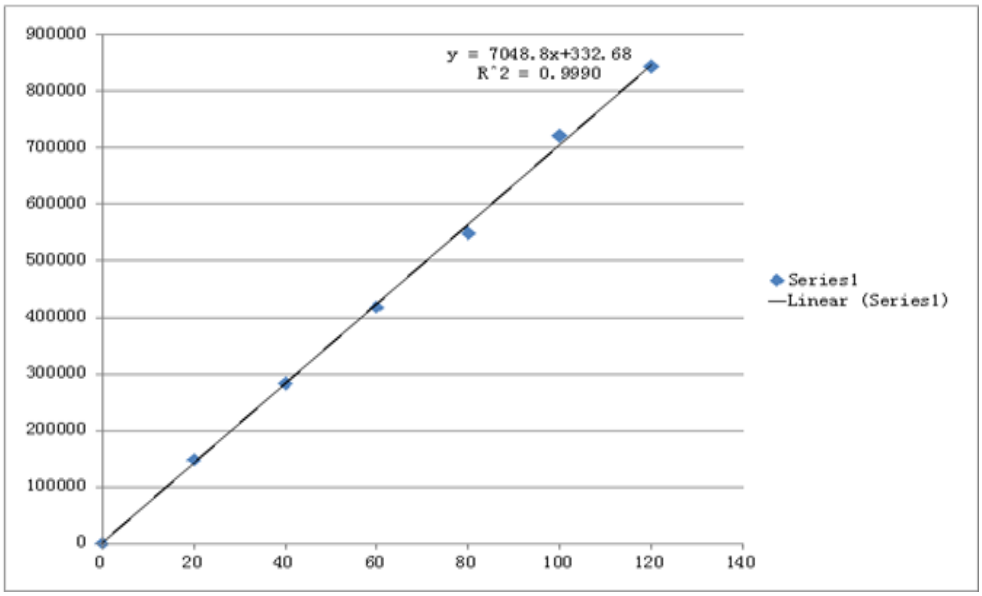


Figure 2: Calibration curve of Cefixime

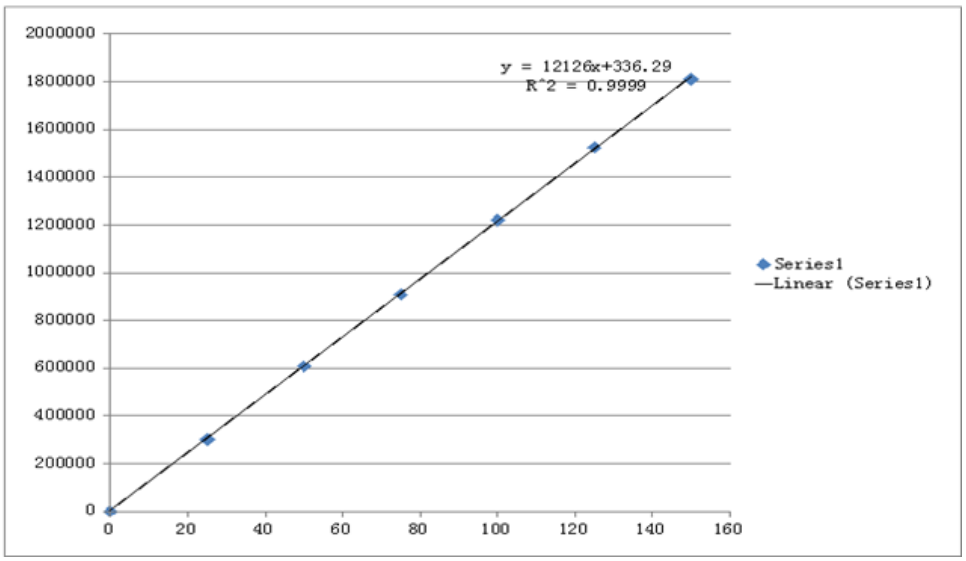


Figure 3: Calibration curve of Paracetamol

Robustness

Robustness of the developed method was demonstrated by purposely altering the experimental conditions. Robustness of method was carried out with variation of mobile phase ±0.2%, flow rate ±0.2 ml/min. It indicates that there was no effect on the results, hence the developed method is said to be more robust and shown in Table 3.

Specificity

Specificity is the ability of the analytical method to measure the analyte free from interference due to other components. Specificity was determined by comparing test results obtained from analyses of sample solution containing ingredients with that of test results those obtained from standard drug. Chromatograms for standard & samples were recorded and they represent no interference.

System Suitability

System suitability tests were carried out on freshly prepared standard stock solution of Cefixime and Paracetamol and it was calculated by determining the standard deviation of by injecting standard solutions in six replicates at frequent time interval.

CONCLUSION

The RP-LC method developed for the analysis of Cefixime and Paracetamol in their pharmaceutical preparations is simple, precise, and accurate. The method is useful for routine analysis due to short run time.

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