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Method Development and Validation of Cefixime and Paracetamol in Pharmaceutical Dosage Form by Using RP-HPLC

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ABSTRACT

The Developed RP- HPLC method allows rapid and precise determinations of Cefixime and Paracetamol. The scope of the present work is to expand and optimization of the chromatographic conditions and to develop RP-HPLC method. A series of mobile phases and columns were tried, among the various mobile phases, Buffer: Acetonitrile (65:35A) (PH-3.5) as an ideal mobile phase, since it gave a good resolution and peak shapes with perfect optimization. The flow rate was optimized at 1 ml/min. The Linearity and correlation coefficient of Cefixime and Paracetamol was found to be 0.999, and 0.999 respectively. Precision was performed and % RSD for Cefixime and Paracetamol were found to be 1.17% and 1.32% respectively. Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount recovered and % Recovery was found to be 100%. Limit of detection was calculated by standard deviation method Cefixime and Paracetamol and LOD for Cefixime and Paracetamol were found to be 0.03and 0.02 respectively. Limit of Quantification was calculated by standard deviation method Cefixime and Paracetamol and LOQ for Cefixime and Paracetamol were found to be 0.08 and 0.06 respectively. Small deliberate changes in method like flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. The average % Assay was calculated and found to be 100.46% and 99.84% for Cefixime and Paracetamol respectively. Hence, the chromatographic method developed for Cefixime and paracetamol is said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in Industries, approved testing laboratories, Bio-pharmaceutics and Bio-equivalence studies and in clinical pharmacokinetic studies.

Keywords: Cefixime, Paracetamol, Optimization, RP-HPLC.

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INTRODUCTION

Cefixime is a semi synthetic, third generation cephalosporin antibiotic. Cefixime has enhanced antibacterial activity and increased stability against many of the beta-lactamases. The antibacterial activity of the drug results from inhibition of mucopeptide synthesis in the bacterial cell wall. Paracetamol also known as acetaminophen, chemically named N-acetyl-p-aminophenol, is a widely used analgesic and antipyretic. Several methods have been developed using various chromatographic studies and the scope of the present work is to expand and optimization of the chromatographic conditions, to develop RP-HPLC method. A series of mobile phases were tried, among the various mobile phases Buffer: Acetonitrile (65:35A) (PH-3.5) as an ideal mobile phase, since it gave a good resolution and peak shapes with perfect optimization. These drugs are evaluated for Linearity, Precision, Accuracy, LOD, LOQ, Specificity, % Assay etc.

High Performance Liquid Chromatography

High Performance Liquid Chromatography[3-5] (HPLC) is the fastest growing analytical technique for the analysis of drugs. Chromatographic separation in HPLC is the result of specific interaction between sample molecules with both the stationary and liquid mobile phases. HPLC has been rapidly developed with the introduction of new pumping methods, more reliable columns and wide range of detectors. HPLC is also being automated which involve automated sampling, separation, detection, recording, calculation and printing of results.

HPLC offers a wide choice of chromatographic separation methodologies from normal to reverse phase and whole range of mobile phases using isocratic or gradient elution techniques. Various detectors available for HPLC are electrochemical detectors, refractive index detectors, fluorescence detectors, radiochemical detectors, mass-sensitive detectors and Ultra-violet (UV) detectors[6-10].

To develop a new HPLC method for any drug, knowledge of its molecular weight, polarity, ionic character, pK_a values, wavelength of absorption, purity of compound and the solubility should be known. Method development involves considerable effort and time. The most commonly applied method is reversed phase and reverse coupled with ion-pairing. These two techniques probably account for more than 85% of the applications for a typical pharmaceutical compound. The typical pharmaceutical compounds are considered to be an active pharmaceutical ingredient of molecular weight of less than 1,000 Daltons. Depending on the number of active compounds to be resolved or separated, the more complex is the separation, the more gradient elution will be advantageous over isocratic mode. Optimization[11-15] can be started only after reasonable chromatogram has been obtained.

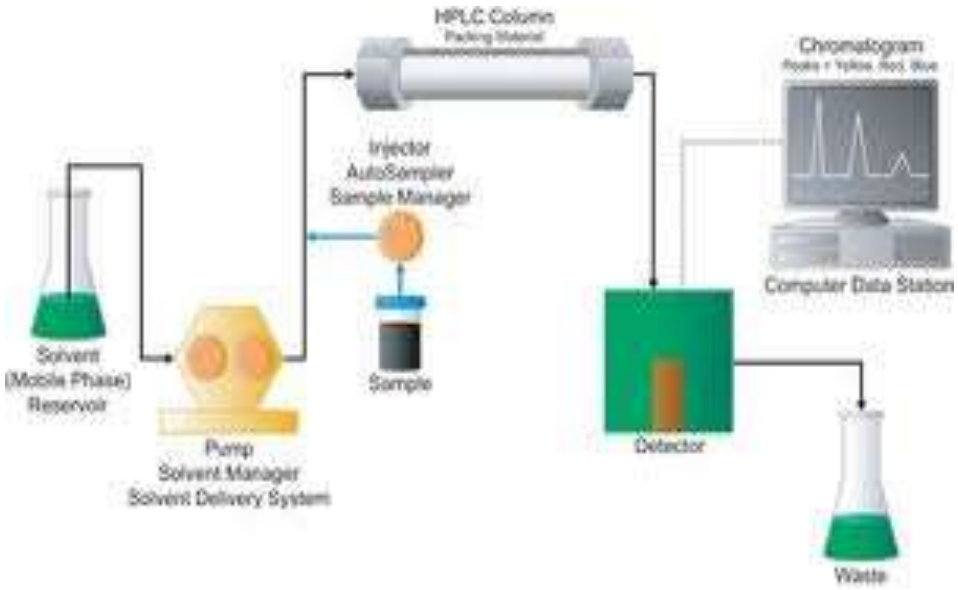

Figure 1: Schematic Diagram of HPLC Instrument

The main objective was to develop a sensitive, simple, rapid, reliable and accurate analytical method for the simultaneous estimation of Cefixime and Paracetamol in formulations and validation of developed method by using RP-HPLC technique.

MATERIALS AND METHOD

Materials:

Cefixime and Paracetamol, Combination Cefixime and Paracetamol tablets, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid etc.

Instrument:

HPLC instrument used was of *Shimadzu* HPLC System with Auto Injector and PDA Detector. Software used is LC Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Cefixime and Paracetamol solutions.

Methods:

Preparation of buffer:Buffer: (0.01N Ammonium buffer)

0.77 gm of ammonium acetate was dissolved in 700ml of HPLC grade water; it was sonicated to degas for 20min and made up to 1000ml with the same HPLC grade water. pH was adjusted to 3.5 with dilute glacial acetic acid.

Standard Preparation: (80ppm Cefixime &100ppm Paracetamol)

Accurately Weighed and transferred 20mg & 25mg of CEFIXIME & PARACETAMOL working Standards 25 ml clean dry volumetric flasks, add 3/4th volume of diluent, sonicated for 30 minutes

and make up to the final volume with diluents. From the above stock solution of cefixime, 1 ml was pipette out in to a 10ml Volumetric flask and then make up to the final volume with diluent, and 1 ml of paracetamol solution was pipette out in to a 10ml Volumetric flask and then make up to the final volume with diluents.

Sample Preparation:

1 tablet was transferred into a 250 mL volumetric flask, 200 mL of diluent added and sonicate for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1 ml was pipette out into a 10 ml volumetric flask and made upto 10ml with diluent.

Linearity:

Linearity solutions are prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml from the Stock solutions Cefixime and Paracetamol are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 20ppm, 40ppm, 60ppm, 80ppm, 100ppm, 120ppm of Cefixime and 25ppm, 50ppm, 75ppm, 100ppm, 125ppm, 150ppm of Paracetamol.

ACCURACY:

Standard Preparation : (80ppm Cefixime &100ppm Paracetamol)

Accurately Weighed and transferred 20mg & 25mg of CEFIXIME & PARACETAMOL working Standards 25 ml clean dry volumetric flasks, add 3/4thvol of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution of cefixime, 1 ml was pipette out in to a 10ml Volumetric flask and then make up to the final volume with diluent, and 1 ml of paracetamol solution was pipette out in to a 10ml Volumetric flask and then make up to the final volume with diluent.

Preparation of 50% Spiked Solution:

625mg of drug was taken into a 250ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labeled as Accuracy 50% Sample stock solution. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents.

Preparation of 100% Spiked Solution:

1250 mg of drug was taken into a 250ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labeled as Accuracy 100% Sample stock solution. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents.

Preparation of 150% Spiked Solution:

1875mg of drug was taken into a 250ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labeled as Accuracy 150% Sample stock solution. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents.

METHOD DEVELOPMENT:

Many trials were done by changing columns and Mobile phases and were reported .Among them the optimized method was discussed below. the chromatogram was shown in figure 2.

Optimized Method:

Drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within the limits.

Column used : STD ODS 250 x 4.6 mm, 5µ.

Buffer used : Ammonium acetate

Mobile phase : Buffer: Acetonitrile (65:35A) (PH-3.5)

Flow rate : 1ml/min

Diluent : Firstly dissolved in Water and Acetonitrile (50:50) and then made up with mobile phase.

Wavelength : 212

Temperature : 30°C

Injection Volume : 10µl

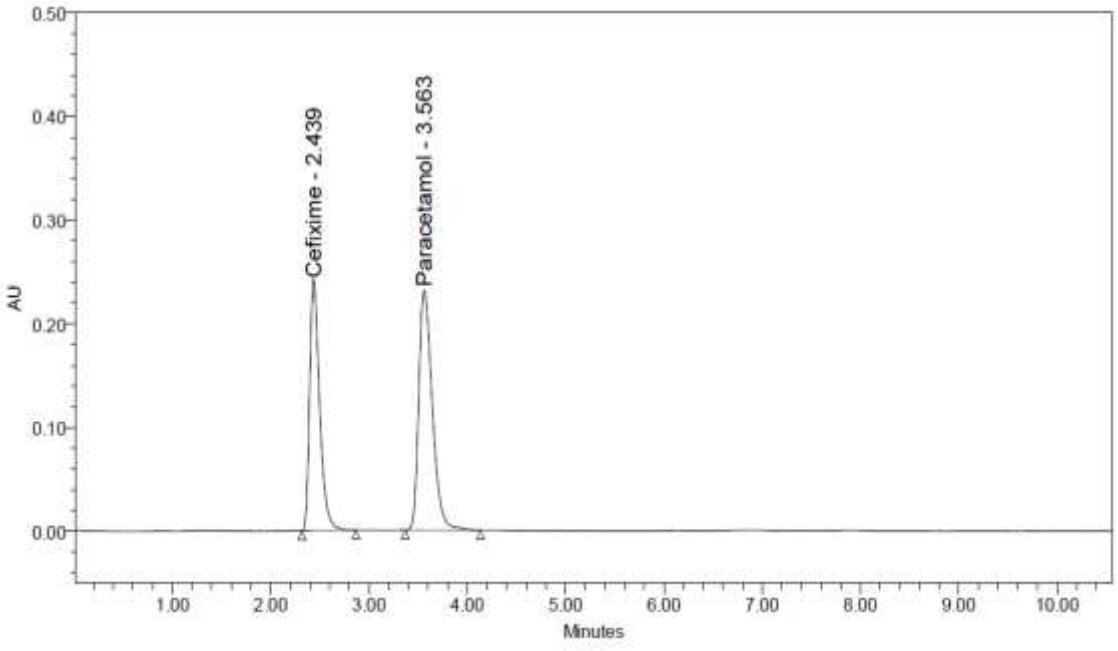

Figure 2: Optimized chromatogram of Cefixime and Paracetamol

Observation: Peak shape and retention time is good

RESULTS AND DISCUSSION

System suitability:

All the system suitability parameters are within range and satisfactory as per ICH guidelines. The results are shown in table 1.

Table: 1. System suitability studies of Cefixime and Paracetamol method

Property	Cefixime	Paracetamol
Retention time (tR)	2.439 min	3.563 min
Theoretical plates (N)	2801 ± 63.48	3735 ± 63.48
Tailing factor (T)	1.50 ± 0.117	1.45± 0.117

Linearity:

Six Linear concentrations of Cefixime(20-120ppm) and Paracetamol (25-150ppm) are prepared and Injected. Regression equation of the Cefixime and Paracetamol are found to be, y = 22107.x + 779.4, and y = 28770x + 337.5 and regression coefficient value was 0.999.linearity graphs are shown in figure. 3 and 4.

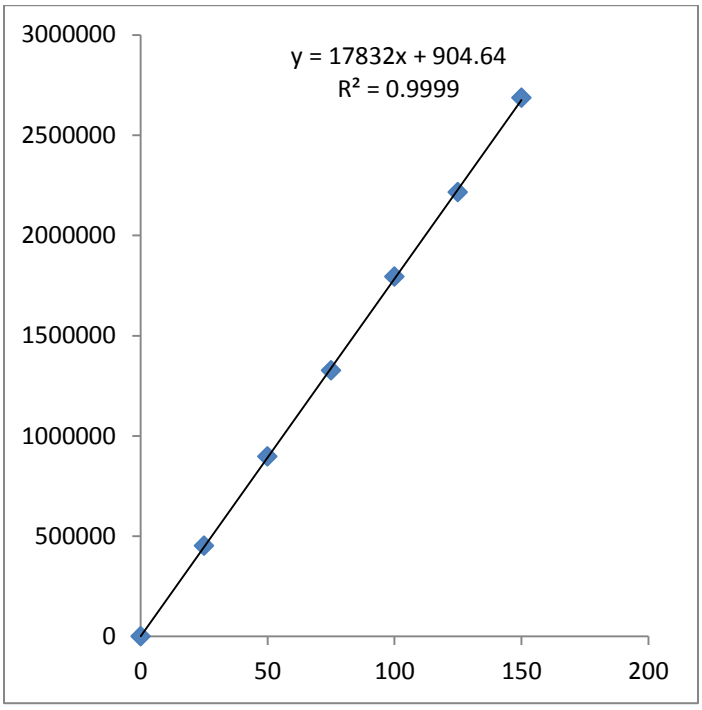

Figure 3: Calibration curve of Cefixime

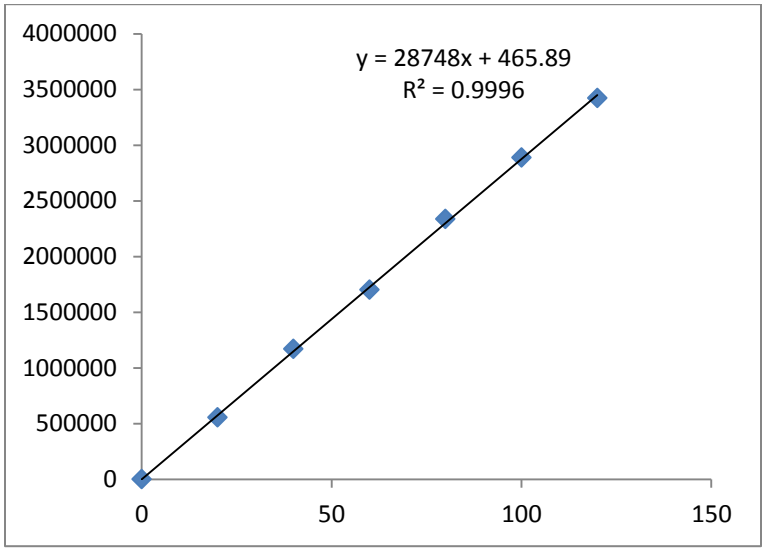

Figure 4: Calibration curve of Paracetamol

Precision:

Intraday precision (Repeatability): Intraday Precision was performed and % RSD for Cefixime and Paracetamol were found to be 1.17% and 1.32% respectively. The chromatogram was shown in figure 5.

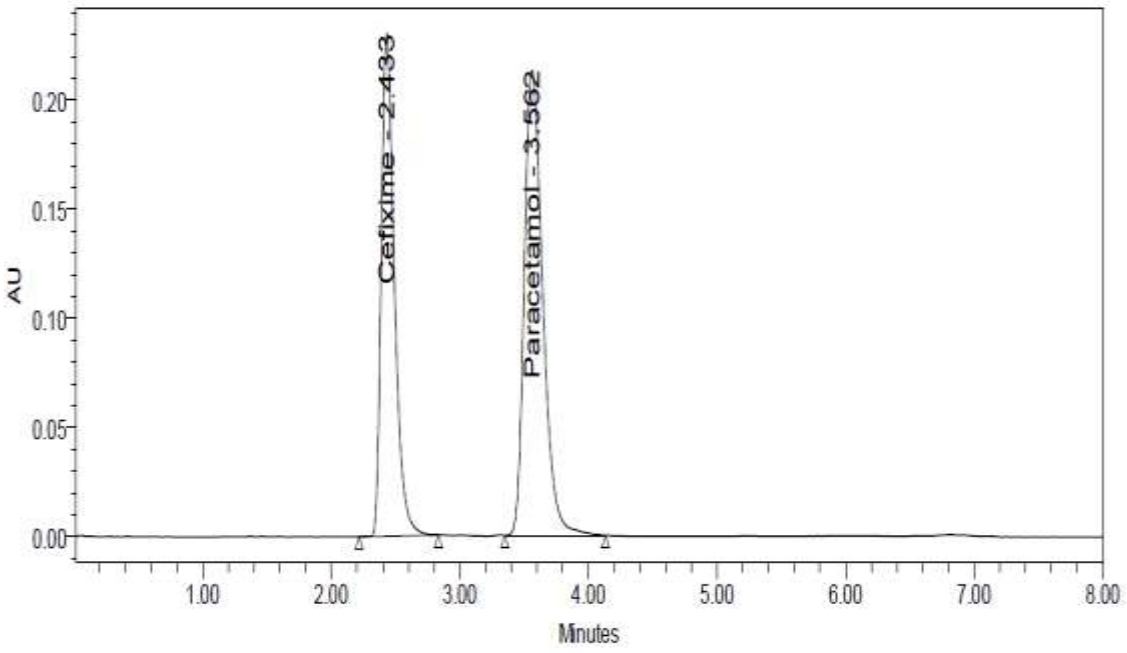

Figure 5: Inter Day precision Chromatogram of Cefixime and Paracetamol

Accuracy:

Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table .2.

Table 2: Table of Accuracy

Sample	Concentration (%)(µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Cefixime	40	40.38	100.95	0.38
	80	80.63	100.79	0.70
	120	120.42	100.35	1.37
Paracetamol	50	50.77	101.55	0.57
	100	99.47	99.47	1.49
	150	152.14	101.43	0.59

LOD:

Limit of detection was calculated by standard deviation method Cefixime and Paracetamol and LOD for Cefixime and Paracetamol were found to be 0.03 and 0.02 respectively**.** The chromatogram was shown in figure 6**.**

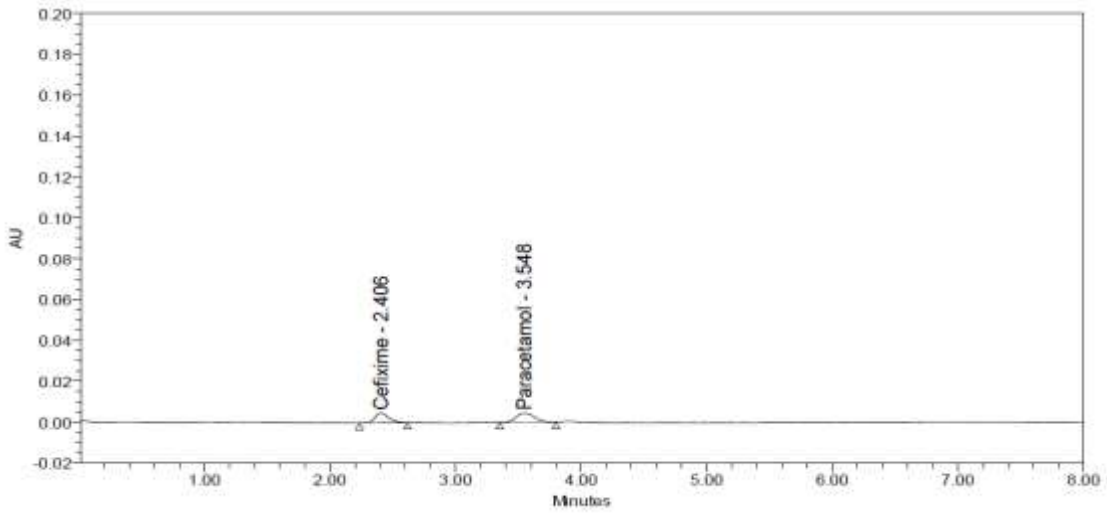

Figure 6: LOD Chromatogram of Cefixime and Paracetamol

LOQ:

Limit of Quantification was calculated by standard deviation method Cefixime and Paracetamol and LOQ for Cefixime and Paracetamol were found to be 0.08 and 0.06 respectively. The chromatogram was shown in figure 7.

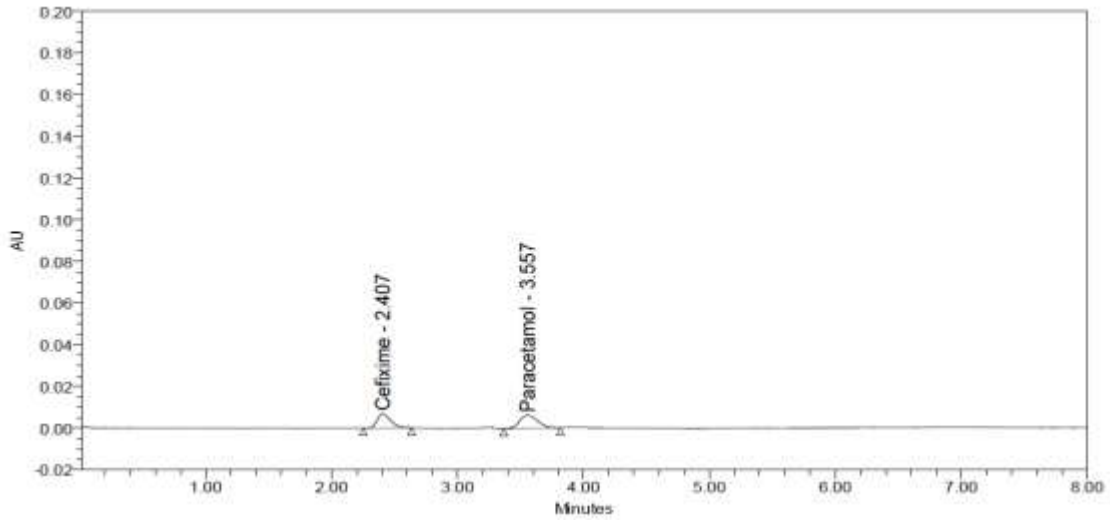

Figure 7: LOQ Chromatogram of Cefixime and Paracetamol

Robustness:

Small deliberate changes in method like flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. The results are shown in table.3.

Table: 3. Robustness data of Cefixime and Paracetamol

S.No	Robustness condition	Cefixime %RSD	Paracetamol %RSD
1	Flow minus	0.2	0.3
2	Flow Plus	0.16	0.89
3	Mobile phase minus	0.1	0.3
4	Mobile phase Plus	1.4	0.5
5	Temperature minus	0.1	0.8
6	Temperature Plus	0.12	1.17

Assay:

Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 100.46% and 99.84% for Cefixime and Paracetamol respectively. The chromatogram was shown in figure.8.

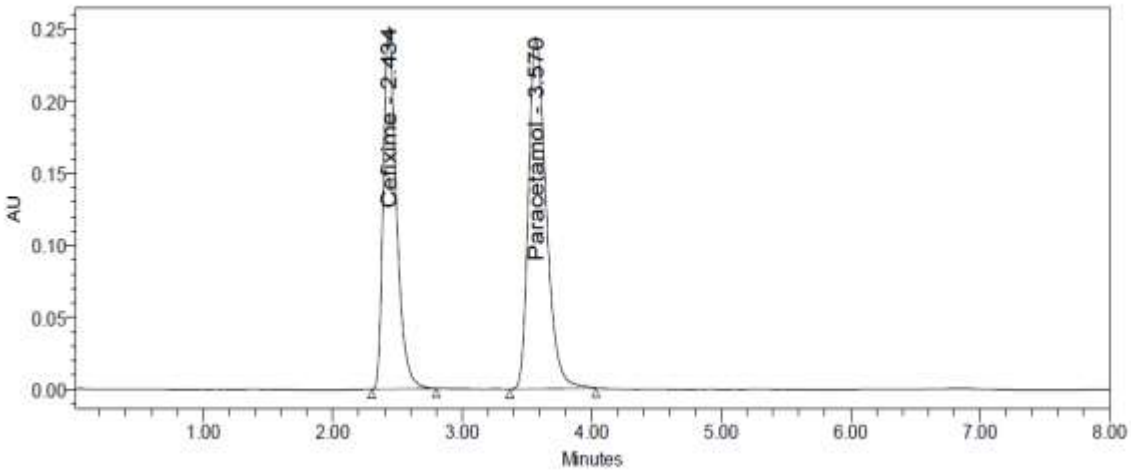

Figure 8: Assay of Tablet

Table-4: Summary of all the parameters

Parameters	Cefixime	Paracetamol
Calibration range (mcg / ml)	20-120 ppm	25-150 ppm
Optimized wavelength	212nm	212nm
Retention time	2.434min	3.570 min
Regression equation (Y*)	y = 22107.x + 779.4	y = 28770.5x + 337.5
Correlation coefficient(r2)	0.999	0.999
Precision (% RSD*)	1.14	0.71
% Assay	100.46%	99.84%
Limit of Detection (mcg / ml)	0.03ppm	0.02ppm
Limit of Quantization (mcg / ml)	0.08ppm	0.06ppm

CONCLUSION:

Hence, the chromatographic method developed for cefixime and paracetamol is said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

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