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DEVELOPMENT AND VALIDATION OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR DETERMINATION OF CEFIXIME TRIHYDRATE AND ITS DEGRADED PRODUCTS FORMED UNDER STRESS CONDITION OF UV LIGHT

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ABSTRACT

A simple, accurate, precise and sensitive reverse phase high performance liquid chromatography (RP-HPLC) method for the determination of cefixime trihydrate and its degraded products have been developed and validated. Drug was resolved on a C18 column (waters spherisorb 25 cm × 4.6 mm, 5µm), utilizing mobile phase of sodium dihydrogen phosphate monohydrate (0.1M aqueous) pH adjusted to 2.5 with diluted orthophospharic acid (10 % aqueous) and methanol in a ratio of 3:1 respectively. Mobile phase was delivered at the flow rate of 1.0 ml/min. Ultra violet detection was carried out at 254 nm. Separation was completed within 9.75 minutes. Calibration curve was linear with correlation coefficient (r^2) = 0.9996 over a concentration range 10-50µg/ml. Recovery was between 98.84, 100.25 percentage. Method was found to be reproducible with relative standard deviation (RSD) for intra and interday precision of < 1.0 over the said concentration range. The method was successfully applied to the determination of the decomposed products of cefixime trihydrate, it can be very useful and an alternate to performing the stability studies.

INTRODUCTION: Cefixime is a semi synthetic, aminothiozolyl, broad spectrum third generation cephalosporin, active against gram positive and gram negative aerobic bacteria. Its Pharmacokinetic profile has been extensively studied in healthy volunteers as well as in patients.

Besides, its use in urinary tract infection, respiratory tract infection it has been documented efficacious in the treatment of gonorrhea [1] (Plourde, P.J. *et al*, 1992). It is used also to treat many different types of bacterial infections such as bronchitis, tonsillitis, ear infections, and skin infections. Cefixime is available in oral formulation.

Like ceftriaxone and cefotaxime, cefixime has enhanced antibacterial activity and increased stability against many of the β-lactamases [2] (Martindale, 2009).

Very few methods for analysis of cefixime trihydrate are reported in the literature. Those reported include high performance liquid chromatography [3, 4] (Madhura V. Dhoka *et al*., 2010; Dhoka MV *et al*., 2010).

The aim of the present work was to develop a relatively simple, sensitive, validated, reliable, and inexpensive HPLC method for the determination of cefixime trihydrate and its degraded products.

MATERIALS AND METHODS:

Chemicals and Reagents: All chemicals and regents used were of a HPLC grade .Cefixime trihydrate was kindly supplied from AUROBINNDO PHARMA LTD – INDIA .Sodium dihydrogen phosphate monohydrate was obtained from MERCK laboratory, Germany. Methanol HPLC grade was obtained from Scharlau chemie S.A, Spain .Orthophospharic acid 85 % were obtained from BDH laboratory, England.

Equipment:

1. HPLC was performed using a PERKIN ELMER HPLC system 200 consisting of LC binary pump series 200,Diode Array Detector 235C,Link (Interface) series 600,Software Turbo chrome and turbo scan program, and Desk Jet exi for windows 660
2. Column used was C18, Waters Spherisorb®5.0µm ODS2 4.6 mm x 250mm.
3. Sartorius model cp224s balance
4. Mi 180 Bench pH meter, MARTINI instruments.

Preparation of 0.1 M sodium dihydrogen phosphate monohydrate solution: Solution was prepared by weighing 13.67 g of sodium dihydrogen phosphate monohydrate and dissolving into 900 ml with distilled water and adjusted to pH 2.5 with diluted orthophosphoric acid and diluted up to 1000 ml with distilled water. The mobile phase was prepared by mix a solution of sodium dihydrogen phosphate monohydrate and Methanol with a ratio of 3:1 respectively and degassed.

Preparation of Standard Stock Solutions: Stock standard solution having concentration of 0.1 mg/ml was prepared by dissolving pure drug of cefixime trihydrate in distilled water. The calibration curve was prepared by diluted stock solution to get concentrations of 10, 20, 30, 40, and 50µg/ml of cefixime trihydrate.

Dilution for Accuracy and Recovery Studies: To study the accuracy of the method and to check the interference from excipients, recovery studies were carried out by addition of standard drug solution to sample at 3 different levels (80%, 100%, and 120 %) of the test concentration. The first recovery study was conducted on the excipients mixture (placebo) prepared by adding accurately weighed amounts of cefixime trihydrate to the excipient mixture and calculating the percentage recovery in each case.

Dilution for Precision Studies: Precision of the method was checked by repeatability of intraday and interday assay. The intra- assay precision (repeatability) and accuracy were studied by analyzing repeatedly six replicate concentration of 100% of cefixime trihydrate on one laboratory on the same day ,three different concentration levels (10, 20 and 30µg/ml). reading of a e and 3 replicate The interday precision (reproducibility) and accuracy were studied by analyzing the different concentration levels (10, 20, 30 and 40 µg/ml) of cefixime trihydrate (3x) over three days and results were expressed as a Relative standard deviation percentage (RSD%)

Robustness Studies: Robustness of the method was determined by small, changes in flow rate, mobile phase ratio and wave length of detection. Flow rate was changed to 1±0.5 ml/min. The mobile phase ratio was changed to ±5% for both components. Wave length of detection was changed to 254±2 nm.

Determination Limit of Detection (LOD) and Limit of Quantitation (LOQ): Limit of detection can be calculated by using the following formula:

$$LOD = \frac{3.3\ \sigma}{S}$$

Limit of quantitation can be calculated base on standard deviation of the response and the slope.

$$LOQ = \frac{10\ \sigma}{S}$$

Where σ = Standard deviation of the response; S = Slope of the calibration curve

System Suitability Testing: System suitability testing is used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. Parameters such as theoretical plates; tailing factors; resolution and reproducibility (% RSD for retention time and for area of six replicates) are determined and compared against the specifications.

RESULTS AND DISCUSSION: Various mobile phases were used containing acetonitrile, methanol, sodium and potassium phosphate buffers. Literature revealed that, tetra butyl ammonium hydroxide solution mix with acetontrile with a ratio of 3:1 or 2:1 respectively were preferred as it is used as mobile phase used for resolved cefixime trihydrate.

The use of 0.1 M sodium dihydrogen phosphate monohydrate pH 2.5 mixes with methanol with a ratio of 3:1 respectively as mobile phase revealed good resolution and peak shape for cefixime trihydrate. On the other hand UV absorption spectra of cefixime in the range 200-400 nm showed maximum absorbance at 254 nm (**Figure 1**). Therefore, the above mentioned parameter was selected as detection wavelength. Flow rate of 1.0 ml/min provided run time of about 9.75 minutes and was used as flow rate.

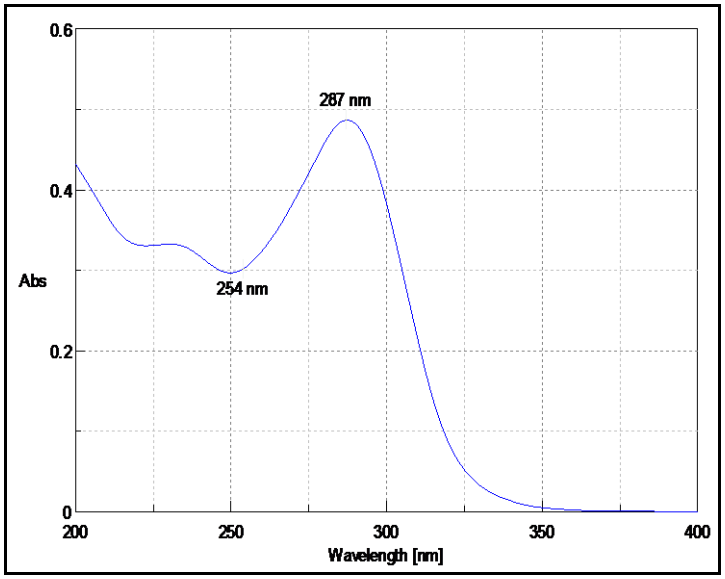

FIGURE 1: UV SPECTRUM GRAPH OF CEFIXIME TRIHYDRATE REFERENCE STANDARD

C18 is common stationary phase suitable for many pharmaceuticals and was found to be suitable in this case (**Table 1**).

TABLE 1: CONDITION USED FOR CHROMATOGRAPHY ANALYSIS

Parameter	Condition used for analysis
Mobile phase	Sodium dihydrogen phosphate monohydrate (0.1 M aqueous) pH2.5 with diluted orthophosphate: Methanol (3:1)
Flow rate	1.0 ml/min
Detection Wavelength	254 nm
Sample injector	50µl loop
Column	C18 waters (4.6 x 250 mm,5µm)

With above selected method parameters, system suitability testing provided good resolution and reproducibility and was adequate for analysis to be performed for the resolution of cefixime trihydrate as shown in **table 2 and figure 2** and its degraded product is shown in **figure 3**. The results of system suitability and system precision were shown in **table 2**.

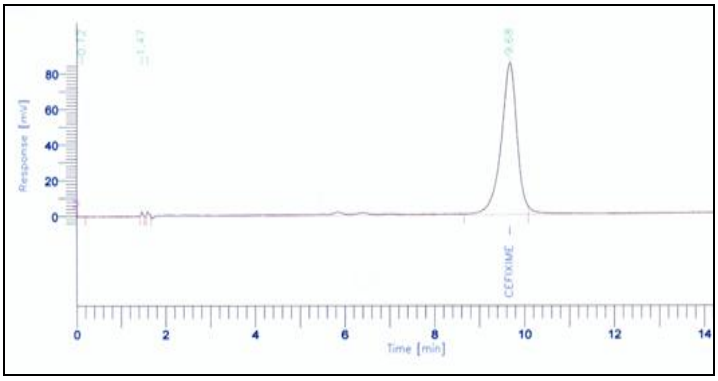

FIGURE 2: TEST HPLC CHROMATOGRAM FOR THE ANALYSIS OF CEFIXIME TRIHYDRATE REFERENCE STANDARD BY DEVELOPED VALIDATED METHOD

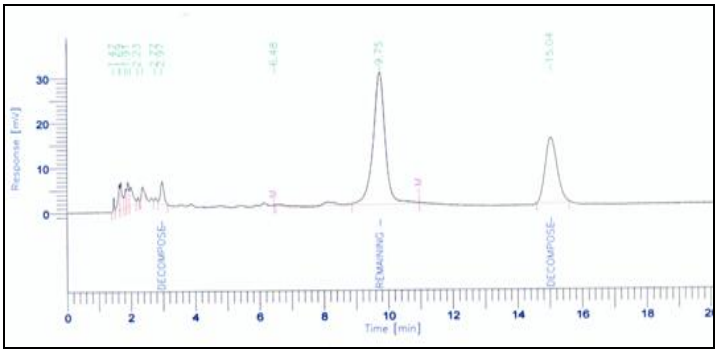

FIGURE 3: TEST HPLC CHROMATOGRAM FOR THE ANALYSIS OF CEFIXIME TRIHYDRATE ALIQUOTS UV DECOMPOSED BY DEVELOPED VALIDATED METHOD TO ILLUSTRATE RESOLUTION STUDY

TABLE 2: SYSTEM SUITABILITY TESTING

No. of injection	Retention time (min)	Mean Peak height	% RSD	No. of Plates	Resolution	Asymmetry 5 %
6	9.75	129958.19	0.71	4925	0.000	1.05

The linear relationship was observed when plotted peak height versus concentration over the range of 10-50µg/ml. The linearity was expressed as correlation coefficient, which was 0.9996. Correlation coefficient, Y-Intercept, slope of regression line is shown in **table 3 and figure 4**.

TABLE 3: STANDARD CALIBRATION CURVE FOR THE ANALYSIS OF CEFIXIME TRIHYDRATE BY HPLC (r = 0.9996)

Concentration of cefixime trihydrate µg/ml	Peak Height
10	7772.59
20	15869.13
30	23704.02
40	32119.42
50	41224.84

LOD = 0.67 µg/ml; LOQ =2.03 µg/ml

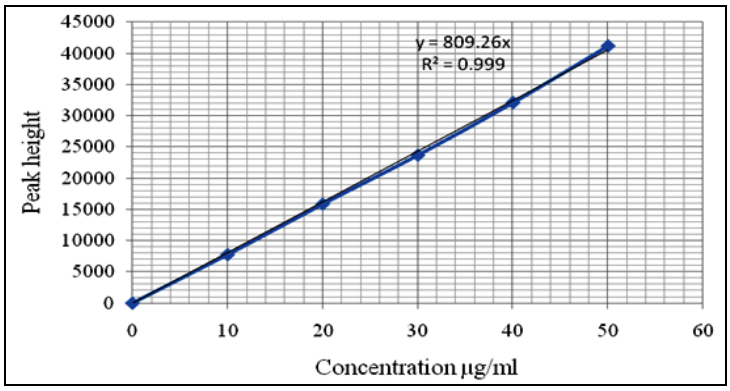

FIGURE 4: CALIBRATION CURVE FOR REGRESSED PEAK HEIGHT VALUES VERSUS CEFIXIME TRIHYDRATE CONCENTRATION

The method was validated for various parameters as per ICH guidelines'. The results of method validation are shown in **table 4**.

TABLE 4: RESULTS OF VALIDATION PARAMETERS

Parameters	Results
Linearity (R^2)	0.999
Y-Intercept	40475
Slope of regression line	809.26
%RSD (Indicates precision)	0.71 %
Mean % Recovery	99.99 %
Limit of Detection (LOD)	0.61 µg/ml
Limit of Quantitation(LOQ)	2.03 µg/ml
Range	10-50 µg/ml

As per ICH guidelines, for assay procedure of active substance or finished product, range should be 80-120% of the test concentration. Results of recovery studies ranged from 98.84-100.25 are shown in **table 5**, indicating very good reproducibility of these methods. The excipients used in the mixture did not interfere in the analysis.

TABLE 5: RESULTS FOR RECOVERY STUDY

Run	% Amount addition	% Amount Found	Recovery %	RSD
1	80	80.94	98.84	
2	80	79.96	100.05	0.70
3	80	79.97	100.04	
1	100	100.10	99.90	
2	100	99.75	100.25	0.60
3	100	98.93	101.08	
1	120	119.99	100.01	
2	120	119.94	100.05	0.18
3	120	120.34	99.72	
Accuracy = Mean over all Recovery			% 99.99	

Precision was carried out as per ICH guidelines, it was determined at one concentration with six replicate for intra-precision assay (**Table 6**) and three concentration levels with 3 replicates at each level (**Table 7**). For all three concentration levels % RSD obtained is less than 1.0%. The results obtained of precision are RSD% values indicated that the proposed methods were accurate and precise.

TABLE 6: RESULTS FOR METHOD INTRADAY – ASSAY PRECISION (REPEATABILITY)

PRECISION	
RUN	Height
1	131493.90
2	130213.81
3	128773.77
4	129562.36
5	129451.59
6	130253.73
Mean	129958.19
Deviation	930.30
RSD	0.71%

TABLE 7: RESULTS FOR METHOD INTERDAY–ASSAY PRECISION (REPRODUCIBILITY)

Conc .in µg/ml	Mean height	SD	%RSD
10	7772.59	164.61	0.77
20	15869.13	6.245	0.06
30	23421.225	282.795	0.63

Robustness studies were carried out after change of flow rate, wavelength of detection and mobile phase composition. It was observed that the small changes in this parameters, did not lead to changes of retention times of peak of interest.

CONCLUSION: The method described enables the quantification of cefixime trihydrate and its degraded products. The validation data demonstrate good precision and accuracy, which prove the reliability of proposed method. Hence, this HPLC method can be used routinely for quantitative estimation and apply as stability indication method.

It can be concluded that the proposed methods are fully validated. They were found to be simple, sensitive, accurate, precise, reproducible, relatively inexpensive, and they give an acceptable recovery of the analyte. The method was specific to drug and also selective to degradation products.

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