RESEARCH ARTICLE

# Analytical Method Development and Validation of Cefixime Trihydrate in Bulk and Dosage Form by UV-Visible Spectroscopy

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## Abstract

**Objective:** This work aimed to validate a simple UV-Visible spectrophotometric method for estimating Cefixime trihydrate in bulk and to produce an accurate, precise, repeatable, and cost-effective method.

**Material and Methods:** The pH 7.4 Phosphate buffer was utilized as the solvent throughout the experiment. The drug's absorption maxima (max) was discovered to be at 288 nm. Beer's law was found to be obeyed in the range of 10-45 µg/mL during the quantitative analysis of the substance at 288 nm. In the doses tested, the approach was found to be linear, with the line equation y = 0.035x – 0.002 and a correlation coefficient of 0.999.

**Results and Discussion:** Cefixime Trihydrate recovery values varied from 99.656 percent to 101.825 percent. Six duplicates of the experiment had a relative standard deviation of less than 2%. The interday precision range was 0.52-1.02%, and the intraday precision range was 0.57-0.995 percent relative standard deviation (RSD percent). The detection and quantification limits were 0.914 and 3.142 µg/mL, respectively. The method's robustness and ruggedness had a percent relative standard deviation of 0.532-0.827 percent.

**Conclusion:** As a result, the proposed procedure was precise, accurate, and economical. This method could be used to determine the quantity of medicine in bulk.

**Keywords:** UV-Vis Spectrophotometer, Method Validation, Accuracy, Precision.

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## Introduction

Cefixime is a third generation cephalosporin antibiotic which is semisynthetic and derived from marine fungus *Cephalosporium acremonium* with antibacterial properties. It is a broad spectrum antibiotic but more specific and effective against gram-negative organisms than gram positives in comparison to second generation cephalosporin.[1,2] The creation and validation of analytical methods are critical in the discovery, development, and manufacturing of pharmaceuticals.[3]

Every year, a greater number of medications are added to the market. These medications could be brand-new or a partial structural alteration of an existing medicament. There is frequently a time lag between the release of a medicine to the market and its inclusion in pharmacopeias. This is due to the potential for uncertainty in the continued and expanded use of these treatments, reports of new toxicities (leading in their withdrawal from the market), the development of patient resistance, and the introduction of superior drugs by competitors. Standards and analytical procedures for these substances may not be available in pharmacopoeia under certain circumstances. As a result, newer analytical procedures for such medications must be developed.

Cefixime is not a USP drug; since it is not included in the monograph, yet is in the market and in high demand because of its spectrum, it is imperative to develop an analytical method that conforms to the validation parameters.[4] UV-Visible Spectrophotometer is an easy-to-use and available instrument; in this project the objective was to develop a simple, accurate, easy to reproduce method by UV spectrophotometry.[5-7]

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**Conflict of interest:** None

## Materials and Methods

Cefixime Trihydrate was collected from Beximco pharmaceutical Ltd Bangladesh. Disodium hydrogen phosphate and sodium di-hydrogen phosphate were purchased from Sigma Aldrich, Germany, and Scharlab S.L., Spain.

## Method Development

### Preparation of Buffer Solution

One liter phosphate buffer of pH 7.4 was prepared by mixing 405 mL 0.2 M disodium hydrogen phosphate and 95 mL 0.2 M sodium dihydrogen phosphate. The two solutions were prepared by weighing 17.799 gm and 3.122 gm reagents, respectively, and adjusting the volume up to 1 liter.[8]

### Preparation of Stock Solution

10 mg of Cefixime were weighed and taken in a 100 mL volumetric flask, then some buffer was added and shaken well to dissolve and placed in a vortex to dissolve fully. Then the volume was adjusted up to 100 mL to make the concentration 100 µg/mL.[9]

### Determination of λ max

Solutions containing 10 µg/mL of Cefixime Trihydrate were scanned in the range of 200-800 nm to determine the wavelength of maximum absorbance for the medicines after suitable dilution of standard solutions with distilled water. Cefixime Trihydrate had the highest absorption at 288 nm.

### Preparation of Standard Solution

A wide range of solutions were prepared by calculating the amount of the stock solution and the amount of the buffer solution. 1-70 µg/mL concentration range solutions were prepared for the determination of validation parameters and other studies. 1, 2.5, 5, 10, 15, 20, 25, 35, 45, 50, 60, 70 mL stock solution was taken in 100 mL volumetric flasks and diluted with buffer up to the mark.

### Preparation of Assay Solution

A 400 mg drug from the capsule was weighed, then taken in a 100 mL volumetric flask and volume was adjusted up to 100 mL. Then desired concentrations were prepared from this solution by dilution.

### Quantitative Analysis of the Drug

A regression equation was obtained after constructing a calibration curve by graphing absorbance versus concentration. The quantitative analysis of the drug in a sample was estimated using the standard curve equation by taking absorbance of the test solution at 288 nm and multiply it with dilution factor and total volume of solution.

## Method Validation

The proposed method was tested for linearity, precision, accuracy, specificity, robustness, LoD, LoQ, and assay accuracy.[10]

### Linearity

Standard stock solutions, 100 µg/mL were further diluted with the buffer to obtain 10, 15, 20, 25, 30 µg/mL solutions. All the absorbances were measured at 288 nm. The calibration curves were constructed by plotting absorbance versus concentration and calculating the regression equations.[11]

### Specificity

Various aliquots ranging from 2-20 µg/mL were produced from the stock solution (100 µg/mL). To identify the wavelength of maximum absorbance for the medications, the solutions were scanned in a UV-Visible spectrophotometer in the range 200-400 nm.[6]

### Precision

System precision refers to the variability and repeatability of a procedure. Three repeat analyses of the same working solution were used to determine it. Intraday and interday fluctuation studies [12] were used to determine the method's precision. The method's intraday precision was assessed by preparing samples from the same batch in nine measurements on the same day, each with three replicates of concentrations (15, 20, 25 µg/mL). The precision of the method is shown by the percent RSD of the test findings. The interday precision was obtained by testing samples three times per day for three days in a row.

### Accuracy

Percent recovery studies determined the accuracy of the study at three levels by the standard addition method. Capsule filling powder for twenty capsules was weighed, and the average weight was calculated. The segregated powders were crushed to obtain a fine powder. Then 80, 100, and 120% of the stock solution was prepared by spiking the drug. The solution was then filtered through a Whatman filter paper (No. 41).

### Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The results from the linearity investigations were used to calculate LoD and LoQ. The linearity plot's slope was determined. The standard deviation (SD) responses were computed for each of the ten replicate assays of the same concentration (10 µg/mL). On the basis of the standard deviation and slope of the regression equation, the LoD and LoQ were calculated from these values.

### Robustness

The experiment was carried out by two analysts at two distinct temperatures, e.g. 20°C and 30°C, to determine robustness. All of the absorbances was collected, and the assay was repeated six times, with the result represented in percent RSD.

### Assay

Twenty capsules were disassembled, and the weight of content was measured. The contents were grinded to make fine powders. 10 mg of the fine powder was dissolved in 70 mL of buffer solution in a 100 mL volumetric flask with the help of a vortex mixer and then diluted to 100 mL with the same buffer solution. The First 30-40 mL of solution was discarded after filtration. The aliquot of the filtrate was further diluted to yield Cefixime Trihydrate at a concentration of 10 µg/mL, and then the percent drug potency was calculated based on the standard curve equation.

## Result and Discussion

The method described in this paper is a simple and accurate approach for analyzing Cefixime Trihydrate. Within the concentration range of 10-45 µg/mL (Table 1), the substance follows Beer's law with an $R^2$ value of 0.9997

(Figure 2). According to ICH guidelines, the $R^2$ value should be greater or equal to 0.999.[13] So, the $R^2$ of this research meets the required limit. In this study, every concentration was prepared three times (triplicate), and absorbance of each one was measured and the average one was used for other concentrations.[13] Every measure was taken and performed following the ICH Q2 (R1) instructions, mainly for the development and validation of analytical method. The validation parameters are described there and followed diligently.

Table 1 shows the preparation of the serial dilution solution from the stock solution (which was initially 100 µg/mL) and their absorbance measurements. This table also helps for the linearity study and the standard curve preparation. According to ICH Q2 (R1) version, the standard curve should be prepared with at least six concentrations, including zero concentration. Here seven concentrations had been used without zero concentration to keep pace with ICH guidelines. The standard curve was prepared with the concentrations of 10, 15, 20, 25, 30, 35, 45 µg/mL and show an equation of:

$$y = 0.035x - 0.002$$

Table 2 helps to determine the linearity of the data. From the Absorbance/Concentration vs Log of Concentration graph, it can be noticed that the linearity range is in between (10-45) µg/mL. A wide range of solutions between (1-70) µg/ml was prepared from the stock solution of 100 µg/mL and their responses were taken with the help of a UV-Visible Spectrophotometer.[11]

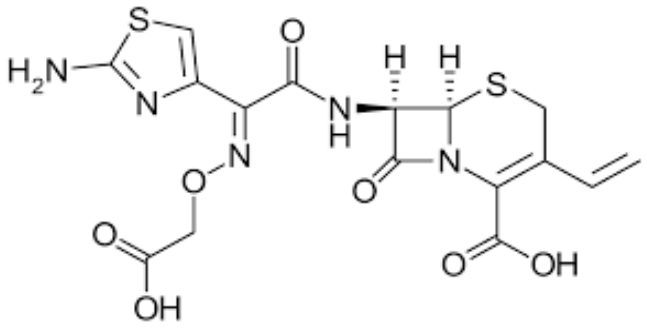

**Figure 1:** Chemical structure of Cefixime Trihydrate.[10]

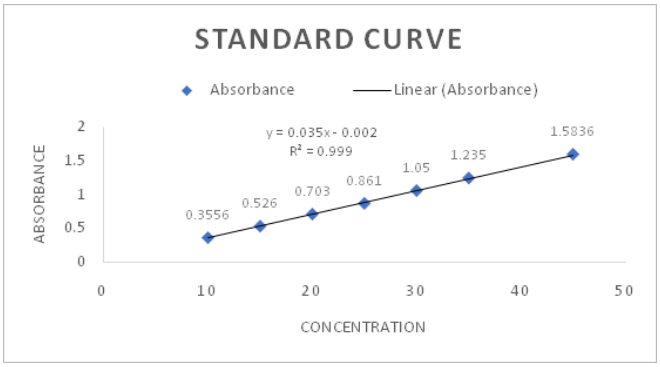


**Figure 2:** Calibration curve of Cefixime Trihydrate

Figure 4 gives information about the specificity. Cefixime Trihydrate was mixed with a variety of excipients, and absorbance of the mixture was taken to ensure that no other ingredients of the mixture interfered with the maximum wavelength of Cefixime Trihydrate. It can be seen that at the maximum wavelength 288 nm, only Cefixime trihydrite gives the desired peak. No other excipients interfere with the λmax.

The accuracy of the method was measured by preparing a known concentration (24, 30, 36 µg/mL) in a triplicate manner and then their absorbances were taken and concentration was calculated with the help of the standard curve (which has been mentioned earlier). After that, percent recovery and relative standard deviation were calculated. ICH limit for percent recovery is 100 ± 10% and relative standard deviation for ≤ 2%. Table 2 gives the information about the accuracy of the method which is in the range of (99.02-101.78) % and % RSD in the range of (0.0092-1.012). Both results obey ICH limit.[14]

Precision is a measure of closeness of the values to one another. This is measured by relative standard deviation. For this test, three concentrations were prepared (15, 20, and 25) µg/mL triplicate, and their absorbance was taken. Then the average of the values, standard deviation, and relative standard deviation were calculated. Both the interday and intraday precision were calculated by taking a reading on the same day at different times and on different days.[11]

Intraday and interday precision results are given in Tables 3 and 4, respectively. According to the ICH guideline, the %RSD is within the range of (0.14-1.06), which is below 2%.[13]

Two analysts prepared six solutions of the same concentration in two different labs, and their absorbance was measured at two different temperatures. And then, relative standard deviation was calculated for the Ruggedness and Robustness. The ruggedness and robustness data are shown in Table 5. %RSD is in the range of (0.532-0.827), which is below 2% and obeys ICH guidelines.

**Table 1:** Linearity range table

| *Concentration (µg/ml)* | *log Concentration* | *Response (Abs/Conc)* |
|---|---|---|
| 1 | 0 | 0.034 |
| 2.5 | 0.3975 | 0.0176 |
| 5 | 0.6989 | 0.03666 |
| 10 | 1 | 0.0351 |
| 15 | 1.178 | 0.0351 |
| 20 | 1.301 | 0.0351 |
| 25 | 1.3979 | 0.0344 |
| 35 | 1.544 | 0.0352 |
| 45 | 1.6532 | 0.03519 |
| 50 | 1.6989 | 0.0332 |
| 60 | 1.78 | 0.031 |
| 70 | 1.85 | 0.0367 |

Twenty capsules were opened, and powder was weighed, and the average weight was calculated. The required amount for 400 mg API was weighted and taken in a 100 mL volumetric flask and mixed with buffer, and after that, volume was adjusted. A 10 µg/mL solution was prepared from the stock solution in a triplicate manner, and absorbance was taken, and concentration was calculated with the help of a standard curve prepared with the serial dilution solutions of the drug.

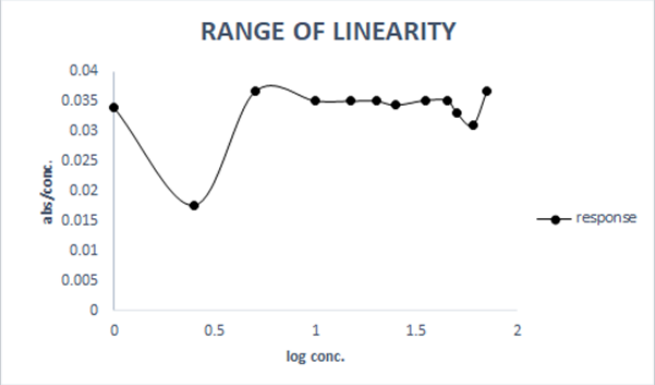


**Figure 3:** Absorbance/Concentration vs. log Concentration curve

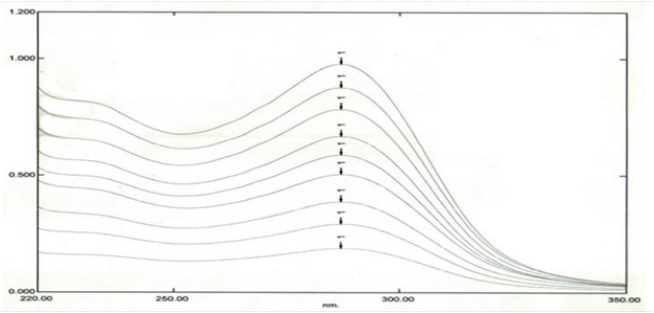

**Figure 4:** Specificity of Cefixime

**Table 2:** Determination of accuracy of Cefixime Trihydrate by UV-Visible Spectrophotometer

| *% Recovery* | *Formulation concentration (µg/mL)* | *Drug found (µg/mL)* | *% Recovery* | *Avg. Recovery* | *% RSD* |
|---|---|---|---|---|---|
| 80 | 24 | 24.428 | 101.785 | | |
| 80 | 24 | 24.426 | 101.777 | 101.78% | 0.0092 |
| 80 | 24 | 24.424 | 101.768 | | |
| 100 | 30 | 29.9 | 99.67 | | |
| 100 | 30 | 30.38 | 101.28 | 100.84% | 1.012 |
| 100 | 30 | 30.46 | 101.56 | | |
| 120 | 36 | 35.59 | 98.86 | | |
| 120 | 36 | 35.61 | 98.916 | 99.02% | 0.28 |
| 120 | 36 | 35.75 | 99.31 | | |

**Table 3:** Intraday Precision

| *Concentration (µg/mL)* | *Abs 0 hour* | *Avg* | *Abs 4 hour* | *Avg* | *Abs 8 hour* | *Avg* | *SD* | *% RSD* |
|---|---|---|---|---|---|---|---|---|
| 15 | 0.524 | 0.526 | 0.53 | 0.524 | 0.522 | 0.5236 | 0.0014 | 0.268 |
| | 0.53 | | 0.519 | | 0.525 | | | |
| | 0.526 | | 0.523 | | 0.524 | | | |
| 20 | 0.7 | 0.703 | 0.712 | 0.704 | 0.704 | 0.702 | 0.001 | 0.14 |
| | 0.697 | | 0.72 | | 0.705 | | | |
| | 0.711 | | 0.68 | | 0.69 | | | |
| 25 | 0.853 | 0.861 | 0.855 | 0.855 | 0.87 | 0.873 | 0.009 | 1.06 |
| | 0.861 | | 0.858 | | 0.869 | | | |
| | 0.87 | | 0.853 | | 0.88 | | | |

**Table 4:** Interday precision

| Concentration (µg/mL) | Abs Day 1 | Avg | Abs Day 2 | Avg | Abs Day 3 | Avg | SD | % RSD |
|---|---|---|---|---|---|---|---|---|
| 15 | 0.511 | 0.514 | 0.516 | 0.516 | 0.521 | 0.51 | 0.003 | 0.6 |
| | 0.515 | | 0.52 | | 0.505 | | | |
| | 0.516 | | 0.512 | | 0.503 | | | |
| 20 | 0.699 | 0.702 | 0.697 | 0.699 | 0.69 | 0.698 | 0.0022 | 0.319 |
| | 0.695 | | 0.706 | | 0.698 | | | |
| | 0.713 | | 0.694 | | 0.706 | | | |
| 25 | 0.866 | 0.861 | 0.865 | 0.858 | 0.863 | 0.859 | 0.0015 | 0.18 |
| | 0.859 | | 0.855 | | 0.859 | | | |
| | 0.858 | | 0.854 | | 0.857 | | | |

**Table 5:** Ruggedness and Robustness

| | *% Assay (20°C)* | *% Assay (30°C)* | | *% Assay (20°C)* | *% Assay (30°C)* |
|---|---|---|---|---|---|
| Analyst 1 | 0.633 | 0.639 | Analyst 2 | 0.619 | 0.622 |
| | 0.646 | 0.636 | | 0.629 | 0.629 |
| | 0.644 | 0.638 | | 0.63 | 0.628 |
| | 0.641 | 0.639 | | 0.628 | 0.625 |
| | 0.640 | 0.635 | | 0.635 | 0.626 |
| | 0.642 | 0.634 | | 0.628 | 0.62 |
| Mean | 0.641 | 0.637 | | 0.628 | 0.625 |
| %RSD | 0.697% | 0.532% | | 0.827% | 0.554% |

**Table 6:** Assay of Cefixime Trihydrate Capsule

| *Concentration (µg/mL)* | *Absorbance* | *%Assay* | *Mean%* | *%RSD* |
|---|---|---|---|---|
| 10 | 0.479 | 107.39 | 107.39 | 0.242 |
| 10 | 0.480 | 107.65 | | |
| 10 | 0.478 | 107.13 | | |

**Table 7:** Summary of the study

| *Parameter* | *Result* |
|---|---|
| Range | 10-45 µg/ml |
| Linearity | r≥ 0.999 |
| Accuracy | 100.52 ± 1.25 |
| Precision | 0.428 ± 0.5 |
| LOD | 0.914 µg/ml |
| LOQ | 3.142 µg/ml |
| Ruggedness | 0.65 ± 0.17 |
| Assay | 107.39% |

After that, the percent assay and relative standard deviation were calculated. According to USP, the assay result is 107.39%, which is within the limit of (100 ± 10) %.[12]

## Conclusion

This analytical method was rather simple, accurate, precise, and sensitive. This method is developed for determining the quantity of Cefixime Trihydrate in bulk drugs. All the pharmaceutical companies of Bangladesh are manufacturing the generic version of the drug. Most of the raw materials are imported from abroad. So, they must develop a method for the bulk drug analysis which is also validated. Moreover, Cefixime is a very popular antibiotic in Bangladesh because of its broad spectrum. The validation method ensures that it is a doable method for the quantization of the drug in bulk. So this endeavor can be of use to many parties. The experiments were performed by obeying laboratory ethics. No animals or humans were hurt throughout the process. Cefixime Trihydrate is available in both tablet and capsule form. There is abundant research on the tablet form, but the

popular dosage form for Cefixime is a capsule in Bangladesh. In this research, all the measures were taken according to ICH and USP and followed through with caution. All the instruments that were used in this experiment were handled with care, such that no damage was done. So, the research performed was rational.

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