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# Selective and validated spectrophotometric methods for the determination of ceftazidime in pharmaceutical formulations

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## ABSTRACT

Two simple and sensitive validated spectrophotometric methods have been described for the assay of ceftazidime in drug formulations. Method A is based on the oxidation of the drug with ferric ion followed by complex formation reaction with 1,10-phenanthroline (1,10-phen) to form orange red colored chromogen exhibiting $\lambda_{max}$ at 510nm. Method B is based on the formation of colored Schiff's base obtained when ceftazidime in acidic conditions reacted with anisaldehyde (ANLD) in ethanol to form yellow colored chromogen exhibiting $\lambda_{max}$ at 383nm. The products are stable for more than 10 and 2 h respectively. Common excipients used as additives in pharmaceutical preparations do not interfere in the proposed methods. Both the methods are highly reproducible and have been applied to a wide variety of pharmaceutical preparations and the results compare favorably with those of official method. 

## KEYWORDS

Ceftazidime;
1,10-Phen;
ANLD;
Spectrophotometry;
Dosage form.

## INTRODUCTION

Ceftazidime is chemically known as (Z)-(7R)-7-[2-(2-aminothiazol-4-yl)]-2-(1-carboxy-1-methoxy imino) acetamido]-3-(1-pyridiniomethyl)-3-cephem-4-carboxylate pentahydrate[1,2]. Ceftazidime is a new β-lactamase resistant third generation cephalosporin, which is noted for its antipsuedomonal activity. It is active against some strains of *Pseudomonas. aeruginosa* that are resistant to cefoperazone and ceftriaxone. Ceftazidime is also highly effective against β-lactamase producing strains of the *Enterobacteriaceae* family. It is generally less active than cefotaxime against gram-positive bacteria and *B.fragilis*. The noteworthy structural features of ceftazidime is that the presence of methylpropionioxaminoacyl group at its 2-position which confers β-lactamase resistance and possibly increased permeability through the porin channels of the cell envelope and the other pyridinium group at its 3-position which confers zwitter ionic properties to the molecule. It has been used effectively for the treatment of meningitis caused by *H.influenzae* and *N.meningitides*. Several analytical procedures are available in the literature for the analysis of ceftazidime, via high performance liquid chromatographic[3-8], charge transfer complex[9], official methods[10,11] and spectrophotometric methods[12-18]. Most of the spectrophotometric methods reported earlier suffer from the disadvantages like narrow range of determination, requires heating or extraction, long time for the reaction to complete, use of non-aqueous systems, stability of the colored product formed, etc.

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The simplicity of the present methods is that the reagents used in both the methods are easily available and the chemistry of the reagents is already well established. The reactions involved with these reagents are simple, rapid and sensitive in the ranges of determinations compared with other established methods. Further, spectrophotometric methods involve simple instrumentation which is cost effective compared with other instrumental techniques, which ordinary laboratories cannot afford to have. The present methods involve the formation of highly colored species which are stable for 10 and 2 h respectively which makes it easier for their determination. The idea of the present work is to produce simple, sensitive and rapid spectrophotometric determination of ceftazidime and the methods are free from interference when excipients are present. In continuation of our research work on drug analysis[19-23], we hereby report two sensitive spectrophotometric methods for the determination of ceftazidime in either pure form or in pharmaceutical preparations. The methods are based on oxidation of drug with ferric ion followed by complex formation reaction with 1,10-phenanthroline and the condensation reaction between the drug and anisaldehyde (ANLD) in ethanol.

## EXPERIMENTAL

### Apparatus

An ELICO Model SL-164 double beam UV-visible spectrophotometer with 1.0 cm matched quartz cells were used for absorbance measurements.

### Reagents and materials

Drug(Gift sample from Alkem Laboratories Ltd, Daman), 1,10-phen(Merck), $FeCl_3$ (A.R), o-phosphoric acid(A.R), anisaldehyde(A.R) and concentrated hydrochloric acid (A.R) were used. All other chemicals and solvents used were of analytical reagent grade. De-ionized water was used to prepare all solutions and in all experiments. Commercial dosage forms were purchased from local sources.

### Solutions

About (~100mg) of ceftazidime(pure or formulation) was accurately weighed and dissolved in 20ml of absolute alcohol. Concentrated hydrochloric acid (1.0ml) was added and the final volume made upto 100ml with absolute alcohol. The final concentration was brought upto 100µg/ml with absolute alcohol. However, for method(B), standard and sample solutions were prepared in deionised water without adding concentrated hydrochloric acid. A 0.03M alcoholic solution of 1,10-phenanthroline and 2.0% v/v alcoholic solution of anisaldehyde were freshly prepared. About 0.03 M $FeCl_3$ solution, 0.02M o-phosphoric acid and concentrated hydrochloric acid were used.

### Procedure

In method A, aliquots of the working standard solution (0.2-1.0ml) of ceftazidime (1.0ml =100µg) were transferred into a series of 10ml volumetric flasks. To each, 0.2ml of 0.03M $FeCl_3$ and 0.5ml of 0.03M 1,10-phenanthroline solution were added, heated on water bath for 10 min and then cooled to room temperature. To each flask 2.0ml of 0.02 M o-phosphoric acid solution was added and final volume made upto 10ml with de-ionized water. The solutions were mixed thoroughly and the absorbance was measured at 510nm against reagent blank and calibration graph was constructed.

In method B, aliquots of the working standard solution(1.0-5.0ml) of ceftazidime (1.0ml=500µg) were transferred into 10ml volumetric flasks. To each, 1.0ml alcoholic solution of anisaldehyde 2.0% v/v was added and heated at 60-70$^0$C for 30min. After cooling, the volume was brought upto mark with absolute alcohol and the absorbance of the yellow colored species was measured at 383nm against reagent blank. The colored species was stable for more than 2 h. The amount of ceftazidime was computed from its calibration curve.

### Procedure for the assay of the drug in commercial samples

**Injection :** Ten to twenty vials depending on content per vials were weighed and mixed thoroughly. An amount of the powder equivalent to 100mg of active component was weighed into a 100ml volumetric flask. About 60ml of de-ionized water added and shaken thoroughly for about 20min, the volume was increased to the mark with de-ionized water shaken and filtered using filter paper. For spectrophotometric determination, the filtrate was diluted sequentially to get 100µg/ml for drug. A suitable portion was then used for analysis.

## RESULTS AND DISCUSSION

In the method A, the ferric ion was reduced by the drug to ferrous ion followed by complex formation reaction with 1,10-phenanthroline to form a ferrion. In the method B, the presence of amino group in ceftazidime, enabled the use of its condensation reaction with anisaldehyde (ANLD) forming a yellow colored chromogen and the absorbances were measured at 510 and 383nm, respectively.

### Spectral characteristics

The absorbance spectra of the orange red colored product (CFZM+1,10-phen) with $\lambda_{max}$ at 510nm and of the yellow colored product (CFZM+ANLD) with $\lambda_{max}$ at 383nm are shown in figure 1 and 2, respectively. The above mentioned reagent blanks have practically negligible absorption in both the systems.

### Optimum reagent concentration

For the method A, 0.03M $FeCl_3$ in the range of 0.2-1.0ml, 0.2-1.0ml of 0.03M 1,10-phenanthroline and 1.0-5.0ml of 0.02M o-phosphoric acid solution were necessary for the development of maximum color intensity.

For the method B, 2.0% alcoholic solution of anisaldehyde in range of 0.5-2.5ml was necessary for the development of maximum color intensity.

Hence, required volumes of all the reagent solutions were used as mentioned in the recommended procedure.

### Quantification

Beer's law was obeyed over the CFZM concentration range of 2-10μg/ml, 50-250 μg/ml for method A and B respectively. The proposed procedures are validated by determining various optical parameters which are listed in TABLE 1. The linearity, slope and the intercepts have been calculated using the regression equation y=ax + b, where 'y' represents optical density, 'x' the concentration of drug in μg/ml and 'a' and 'b' represent slope and intercept, respectively. Precision and accuracy of the proposed methods were tested by carrying out the determination of eight replicates of pure and commercial samples of the drug, whose concentration lie within Beer's law range. The values of standard deviation (SD), relative standard deviation (RSD) and range of error at 95% confidence level were calculated.

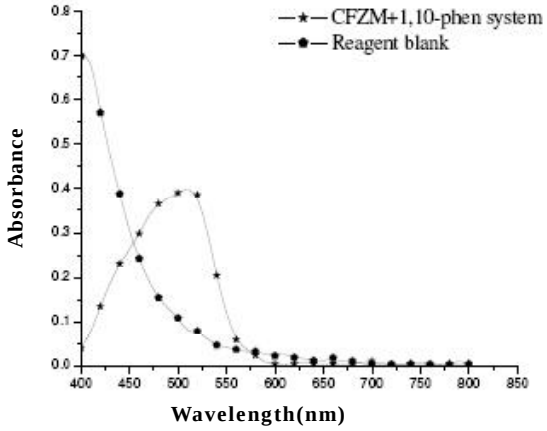


**Figure 1: Absorption spectra of ceftazidime drug. Initial concentration of ceftazidime is 4μg/ml**

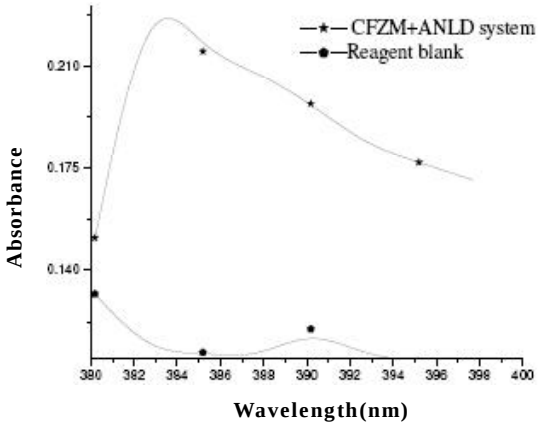


**Figure 2: Absorption spectra of ceftazidime drug. Initial concentration of ceftazidime is 200μg/ml**

**TABLE 1: Parameters for the spectrophotometric determination of ceftazidime**

| Parameter/characteristics | Method A | Method B |
|---|---|---|
| Color | Orange red | Yellow |
| $\lambda_{max}$/nm | 510 | 383 |
| Stability/h | 10 | 2 |
| Beer's law range (C) (μg/ml) | 2 -10 | 50-250 |
| Limit of detection/(μg/ml) | 0.091 | 0.82 |
| Limit of quantification/(μg/ml) | 0.30 | 2.71 |
| Molar absorptivity/(l $mol^{-1}cm^{-1}$) | $6.36\times10^4$ | $0.61\times10^3$ |
| Sandell's sensitivity/(μg $cm^{-2}$ ) | 0.010 | 0.12 |
| Regression equation(Y)[a] | | |
| Slope (a) | $2.48\times10^{-3}$ | $1.06\times10^{-3}$ |
| Intercept (b) | $9.99\times10^{-2}$ | $3.36\times10^{-2}$ |
| Correlation co-efficient (r) | 0.9999 | 0.9979 |
| R.S.D. (%)[b] | 0.30 | 1.39 |
| % Range of error's[b] | ± 0.0014 | ±0.0022 |

[a]y =ax + b, where x is the concentration in μg/ml; [b]Eight replicates

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There was no change in $\lambda_{max}$ when least concentration of the analytes was determined, for the calculation of LOD. LOQ was found to be three times that of LOD which is in accordance with thumb's rule. The experiment for the proposed methods were conducted by the second analyte on different days and the results produced justified the ruggedness of the proposed methods. The two methods have been applied to various pharmaceutical formulations and recovery studies have been made. The optical characteristics and precision data for both the methods suggested are presented in (TABLE 1).

## Reaction sequence

The method A is based on the reduction of ferric ion by the drug to ferrous ion followed by complex formation reaction of ferrous ions with 1,10-phenanthroline to form orange red colored species. The method B, describes the condensation reaction of drug with anisaldehyde to form Schiff's base to yield a yellow colored chromogen.

## Stability

The colored products formed in the proposed method A and B were found to be stable for 10 and 2 h respectively at room temperature. In method A the reaction of colored complex formation was slow at room temperature ($25^0$C) and required longer time for completion. Hence, efforts were made to accelerate by carrying out the reaction at higher temperatures. It was observed that the maximum color intensity was obtained by heating the reaction mixture at $80^0$C on a water bath for 10min. The absorbance's remained constant at room temperature for more than 10 h. In method B, the color was found to be stable for more than 2 1827h at room temperature.

## Interference

A detailed study on the interference of various concomitant substances on the determination of the drugs was made. For method A, 4µg/ml CFZM was selected to check the interference. For method B, 50µg/ml CFZM was used to study the interference. Before adding the reagents, a known amount of the interfering substance was added and the reaction was carried out for both the methods. The extents of interference by various excipients that often accompany the pharmaceutical formulations are tabulated in TABLE 2. It was found that both the methods gave excellent results for the determination of pure CFZM in presence of excipients which do not interfere in both the methods. The results are given in TABLE 2. An error of 2.0 % in the absorbance reading was considered tolerable.

**TABLE 2: Determination of ceftazidime in the presence of excipient and other substances**

| Material | Amount (mg) | % Recovery of drug±RSD[a] | |
|---|---|---|---|
| | | Method A | Method B |
| Magnesium stearate | 30 | 99.80±0.13 | 99.79±0.20 |
| Lactose | 30 | 99.73±0.16 | 99.24±0.56 |
| Dextrose | 30 | 99.57±0.12 | 99.84±0.66 |
| Starch | 30 | 99.81±0.13 | 99.96±0.29 |
| Gum acacia | 30 | 99.66±0.49 | 99.25±0.89 |
| Talc | 30 | 99.56±0.29 | 99.42±0.27 |
| Carboxy methyl cellulose | 30 | 99.74±0.19 | 99.10±0.10 |
| Sodium alginate | 30 | 99.66±0.31 | 99.28±0.73 |

**Method A, *4µg/ml of drug taken. Method B, * 50µg/ml of drug taken; [a]Average of five determinations**

## Application

The applicability of the proposed methods was examined for pharmaceutical preparations. The results of analysis of drug in injection are given in TABLE 3. The same batch vials were also analyzed by official method[11]. A statistical analysis of the results using t- and F-values compared with the official method[11]. Mean values were obtained in student t- and F- test at 95% confidence limits for seven degree of freedom[24]. The results showed no significant differences with regard to the accuracy and precision. Vials containing 250, 500 and 1000mg of drug samples labeled as 1, 2 and 3 respectively, were analyzed by the proposed methods

**TABLE 3: Results of an assay of drug in pharmaceutical formulations**

| Preparations[a] | Label claim mg/vial | Found[b](recovery±SD) | | | Students t-value[c] | | F-value[d] | |
|---|---|---|---|---|---|---|---|---|
| | | Method A | Method B | Reference method | A | B | A | B |
| Tazid (1) | 250 | 99.80±0.43 | 99.22±0.55 | 99.85± 0.63 | 0.18 | 2.17 | 2.14 | 1.31 |
| Tizime (2) | 500 | 99.52±0.38 | 99.48±0.62 | 99.98± 0.38 | 2.10 | 1.94 | 1.59 | 2.66 |
| Cefazid (3) | 1000 | 99.74±0.39 | 99.55±0..76 | 99.84± 0.59 | 0.41 | 0.85 | 2.13 | 1.66 |

**(a) Marketed by:1,Alkem;2,Lupin and 3,Biochem; (b) Mean value of eight determinations; (c) Tabulated value at 95% confidence level is 2.365 d. Tabulated value at 95% confidence level is 3.79**

**TABLE 4: Results of recovery studies by the standard-addition technique**

| | Method A | | | | Method B | | | |
|---|---|---|---|---|---|---|---|---|
| Formulations[a] | Amount of drug in formulation/mg | Amount of drug added/mg | Total found/mg | % Recovery of pure drug[b] | Amount of drug in formulation/mg | Amount of drug added/mg | Total found/mg | % Recovery of pure drug[b] |
| | 5.94 | 3 | 8.92 | 99.33 | 3.04 | 4 | 7.01 | 99.25 |
| 1 | 5.94 | 6 | 11.91 | 99.50 | 3.04 | 8 | 11.0 | 99.50 |
| | 5.94 | 9 | 14.93 | 99.88 | 3.04 | 12 | 15.08 | 100.33 |
| | 3.98 | 3 | 6.99 | 100.33 | 3.04 | 5 | 8.02 | 99.60 |
| 2 | 3.98 | 6 | 9.96 | 99.66 | 3.04 | 10 | 13.06 | 100.20 |
| | 3.98 | 9 | 12.97 | 99.88 | 3.04 | 15 | 18.01 | 99.80 |
| | 4.02 | 3 | 7.01 | 99.66 | 3.04 | 6 | 9.03 | 99.83 |
| 3 | 4.02 | 6 | 9.99 | 99.50 | 3.04 | 9 | 12.0 | 99.55 |
| | 4.02 | 9 | 13.03 | 100.11 | 3.04 | 12 | 15.06 | 100.16 |

[a]Branded by: 1, Tazid (250mg); 2, Tizime (500mg) and 3, Cefazid (1000mg); [b]Average of three determinations

and the accuracy was assessed by the standard addition method in which variable amounts of pure drug was added to the previously analyzed portion of pharmaceutical formulations. Results are recorded in TABLE 4 and confirmed that the proposed methods are not liable to interference by fillers usually formulated with standard drugs.

## CONCLUSION

From an analytical point of view, it is concluded that the described procedure allows the determination of CFZM in pure and pharmaceutical dosage forms. The results are reproducible. Unlike gas chromatographic and HPLC, the spectrophotometer is simple and inexpensive. Its importance lies in the chemical reaction upon which the procedure is based, rather than upon the sophistication of the instrument. The aspect of spectrophotometric analysis is of major interest in analytical pharmacy, since it offers a distinct possibility in the assay of a particular component in complex dosage formulations. The reagents utilized in the proposed method are cheaper and readily available. The proposed methods for the determination of CFZM are simple, rapid and sensitive. The methods do not require extraction or tedious sample preparation. The statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the methods. These merits, in addition to the use of simple reagent, suggest the utility of the proposed method for routine quality control and in clinical laboratories.

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