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# Two Oxidative Visible Spectrophotometric Methods For The Determination Of Ceftazidime In Pharmaceutical Formulations Using N-Bromo Succinimide

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**Abstract**

Two visible spectrophotometric methods A,B have been described for the assay of Ceftazidime (CTZ) in bulk form and dosage forms based on oxidation of CTZ with an excess of oxidant N-bromosuccinimide (NBS) and the un reacted oxidant is then estimated colorimetrically by using an oxidisable dye Celestine blue (CB) in method A and Metol-Sulphanilic Acid (PMAP-SA) reagent in method B.The coloured products exhibit absorption $\lambda_{max}$ at 540 nm and 520 nm for methods A and B respectively. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges 1-5, 2-10 μg/ml, correlation co-efficients are 0.9999, 0.9999. The proposed methods are applied to commercial available formulations and the results are statistically compared with those obtained by the UV reference method and validated by recovery studies. The results are found satisfactory and reproducible.These methods offer the advantages of rapidity, simplicity and sensitivity and low cost without the need for expensive instrumentation and reagents.

**Key Words:** N-Bromo Succinimide,Oxidant,Regression Analysis

## INTRODUCTION

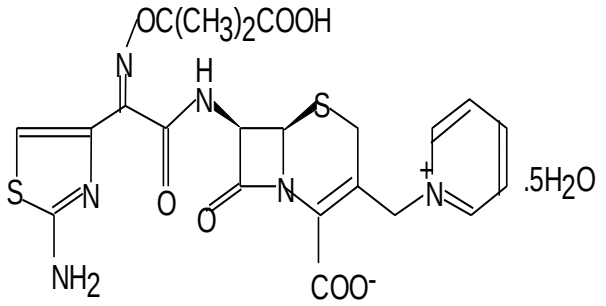



**Figure.1**.Structure of Ceftazidime

Ceftazidime (CTZ) (Fig.1) is a semisynthetic broad - spectrum, β- lactam antibiotic for vial or parenteral administration. It is pentahydrate of pyridinium,1-[[7-[[(2-amino-4thiazolyl)[(1-carboxy-1-methylethoxy)imino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-,hydroxide, innersalt,[6R-[6(alpha),7(beta)(Z)]]

[1], Methods based on HPLC [1-3] Fluorimetry [4],UV [5] and colorimetry [4,6-10] have been reported for its estimation. A few of the procedures based on colorimtry are found to be limited by relatively lack of sensitivity and selectivity.The authors have also searched for the applicability of chosen reagents NBS-CB[11-12],NBS-Metol [13-16] for the determination of the selected drug.Mohana Krishna etal [17] have developed two methods using the above reagents.

## EXPERIMENTAL

**Apparatus**: A Systronics UV-Visible spectrophotometer 117 and A Systronics 106 visible spectrophotometer with 1 cm matched quartz cells were used for the absorbance measurements . Elico LI-120 digital pH meter was used for pH measurements.All the reagents were of analytical grade and all solutions were prepared in doubled distilled water **Preparation of Standard Drug Solutions:** A 1 mg/ml stock solutions of CTZ was prepared by dissolving 100mg of the drug initially in 10ml of 0.1M NaOH and making upto 100ml with distilled water . Working standard solutions were obtained by appropriate dilution of the stock solution with distilled water 50 μg/ml for method A ,100μg/ml for method B.

**For Pharmaceutical Formulations**:

An accurately weighed portion of the Injection equivalent to 100 mg of drug was extracted with chloroform (3 x 15 ml) and filtered. The combined filtrate was evaporated to dryness and the residue was dissolved in 10 ml of 0.1N NaOH, shaken well and filtered. The filtrate was diluted to 100ml with 0.1N NaOH. Ten ml of the above solution was further diluted to 100ml with 0.1N NaOH. The absorbance of the solution was determined at $\lambda_{max}$ 254 nm. (Fig. 2). The quantity of the drug was computed from the Beer-Lambert's plot (Fig.3) of the standard drug in 0.1N NaOH.

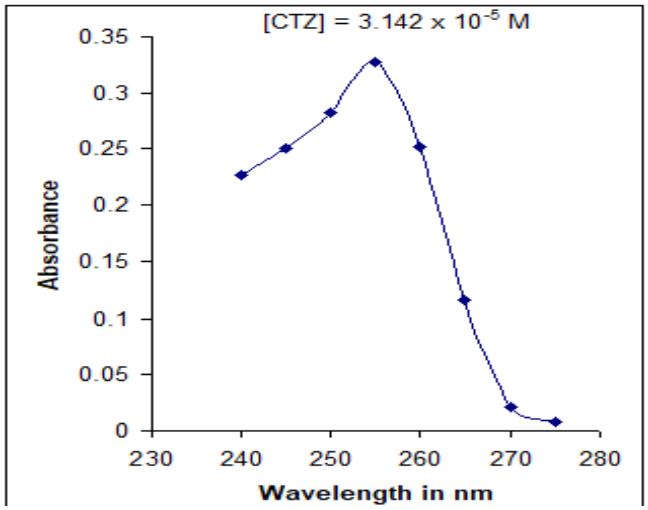



**Figure.2**.Absorption Spectra of CTZ (UV reference Method)

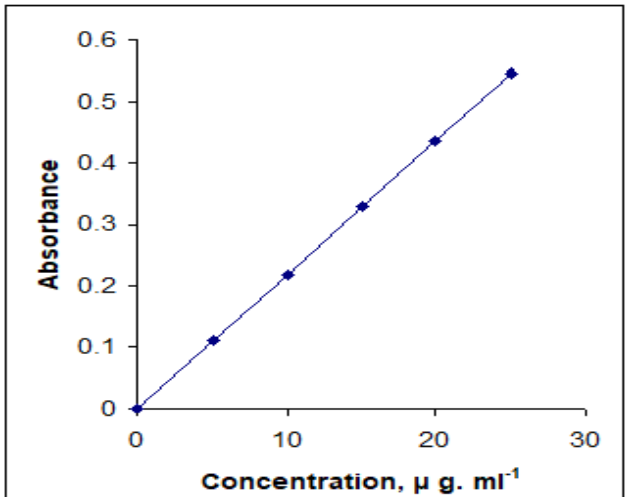



**Figure.3**.Beers plot of CTZ (UV reference Method)

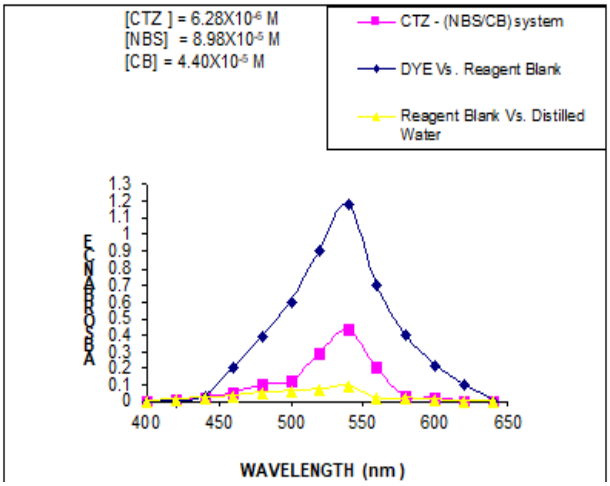
**Recommended Procedures**: Based on various parameters like Effect of acid concentrations, amount of NBS (100µg/ml) and amount of CB, Time (min) and temperature ($^0$C) required for oxidation of CTZ, Time for oxidation of dye and stability period of final color, volume of PMAP, SA solution (ml) and the following procedures were recommended

**Method -A**

Aliquots of standard CTZ solution (0.5 - 2.5 ml, 50 µg/ml) 1.5 ml of 5 M HCl and 4.0 ml of NBS solutions were delivered in to a series of 25ml volumetric flasks and the volume in each flask was brought to 20 ml with distilled water. After 10 min, 2.0 ml of CB solution was added and mixed thoroughly, and the volume was made upto the mark with distilled water. The absorbances were measured after five min at 540 nm(Fig.4). against distilled water blank. The blank (Omitting drug) and dye (omitting drug and oxidant) solution were prepared in a similar manner and their absorbance were measured against distilled water. The difference in the decrease in absorbances between test and blank (or test against a reagent blank) corresponding to the consumed NBS and in turn concentration was computed from its calibration graph (Fig.5).

**Method-B**

Aliquots of standard CTZ solution (0.5 - 2.5 ml, 100 µg/ml) were transferred in to a series of 25 ml calibrated tubes containing 0.5 ml of 5% acetic acid and 2.0 ml of NBS solutions and the volume was brought to 10 ml with distilled water. The tubes were kept aside for 15 min at room temperature. Then 1.5ml of PMAP solution was added. After 1.0 Min, 2.0ml of SA solution was added and the volume was made upto the mark with distilled water. The absorbances were measured at 520 nm ((Fig.6).) against distilled water blank after 5.0 min and before 40 min. A blank experiment was also carried out in the similar manner omitting the drug. The decrease in the absorbance, which corresponds to drug concentration, was obtained by subtracting the absorbance of the test solution from that of the blank solution. The amount of CTZ was computed from standard calibration curve (Fig.7).




**Fighure.4**.Absorption Spectra of CTZ with NBS/CB

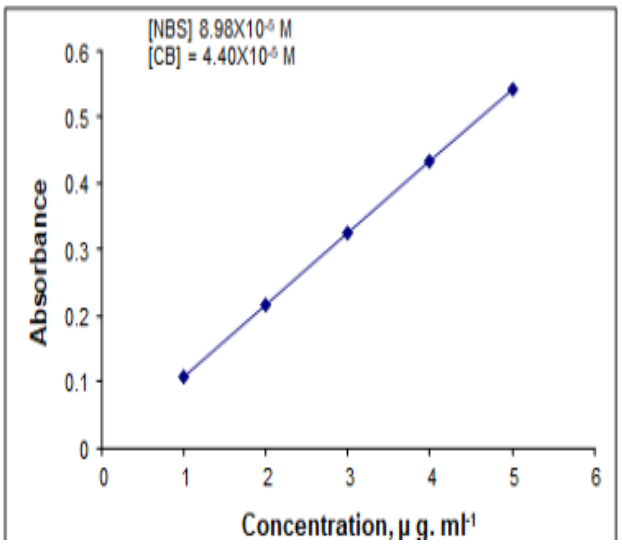



**Figure.5**.Beers plot of CTZ with NBS/CB

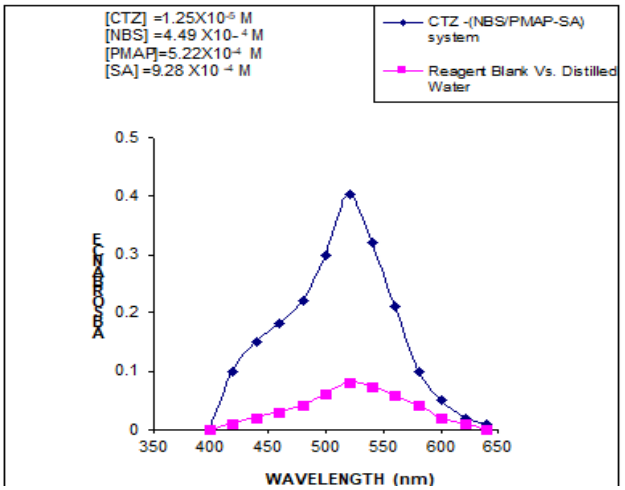



**Figure.6**.Absorption Spectra of CTZ with NBS/PMAP-SA

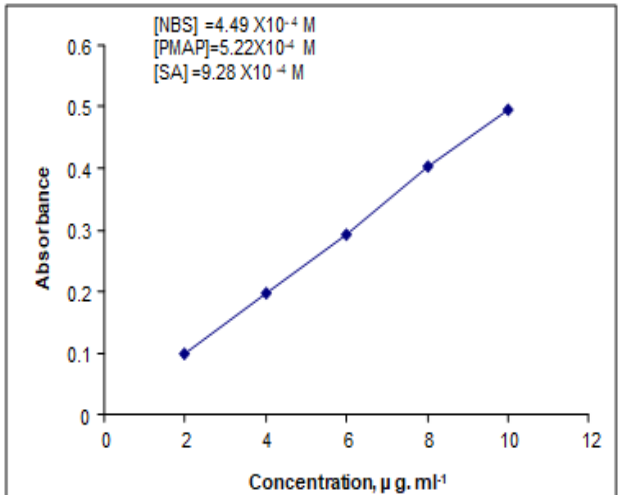



**Figure.7**.Beers plot of CTZ with NBS/PMAP-SA

**Nature of the colored species :** The structre and partial structures of CTZ earmarking the analytically useful functional groups are presented.Here, CTZ possesses four analytically useful functional moieties amino thiazole, quaternary N in substituted pyridyl, fused β-lactam dihydro thiazene ring system and carboxyl in substituted oxime. The reducing nature appears to be due to the presence of vulnerable oxidisable centers ( double bonds and hetero sulphur in amino thiazole and dihydro thiazene ) on which the methods A,B were developed.CTZ exhibits reducing property due to the presence of functional moieties ( hetero sulphur in amino thiazole and dihydro thiazene) vulnerable to oxidation selectively with oxidizing agents such as NBS under controlled experimental conditions. When treated with known excess of oxidant, CTZ undergoes oxidation, giving products of oxidation besides unreacted oxidant. It is possible to estimate the drug content colorimetrically which is equivalent to the unreacted oxidant .The unreacted oxidant can be estimated either by decrease in the intensity of dye color CB for NBS, due to disruption of chromophoric centers in the dye or color development with PMAP-SA for NBS in the proposed methods A,B respectively described in the schemes 1,2.

**Scheme-1**

The first step in methods mentioned above is the oxidation of CTZ with the oxidant.

CTZ + NBS → Oxidation products + Succinimide + un reacted NBS

The second step concerns with the estimation of the unreacted oxidant with appropriate dye or chromogenic agent.

NBS + CB → CB + Succinimide + Mixture of compounds with rupture of

(Un reacted, Conjugated system in dye (colorless)

colored ) (Reproducible but not stoichiometric

as several alternative pathways possible)

**Schem-2**

In method B, the unreacted oxidant reacts with Metol (PMAP as sulphate ) giving rise to P-N- methyl benzoquinone mono imine (PMBQMI) which in turn forms charge transfer complex with aromatic primary amine.

**RESULTS AND DISCUSSION**

The optimum conditions for the development of methods were established by varying the parameters one at a time (OVAT) and keeping the others fixed and observing the effect produced on the absorbance of the colored species.The optical characteristics and figures of merits , such as Beer's law limits, molar absorptivity and Sandell's sensitivity for the proposed methods are presented in table 1. The precision of each method was estimated by six replicate samples within the Beer's law limits and the results are incorporated in table 1.Regression analysis using the method of least squares was made to evaluate slope(b), intercept(a), correlation coefficient and the results are also summarized in table 1. commercial formulations containing CTZ were successfully analysed by the proposed method .The results obtained by the proposed UV reference methods for dosage forms were compared statistically by means of the F-and t-tests were found not to differ significantly as 95% confidence limit. As an additional check of accuracy of the proposed methods , recovery experiments were performed by adding a fixed amount of the drug to the pre analysed formulations and the results are presented in Table 2. These results indicate that the commonly used additives in the dosage forms of CTZ did not interfere in the analysis of formulations. When compared to the results by reported methods,the methods proposed were more sensitive which can be clearly observed from Beers limits, Molar absorptivity values and correlation coefficients and Sandell's sensitivity. In pharmulations also the proposed methods by the authors showed good results compared to reported methods

**Table.1**: Optical and Regression characteristics, precision and accuracy of the proposed methods for CTZ

| S.No | Parameter | Method-A | Reported Method | Method-B | Reported Method |
|---|---|---|---|---|---|
| 1 | Wave length $\lambda_{max}$ (nm) | 540 | 520 | 520 | 520 |
| 2 | Beer's law limits (µg $ml^{-1}$) | 1 - 5 | 2-10 | 2 - 10 | 4-20 |
| 3 | Detection limits (µg $ml^{-1}$) | 0.031 | ---- | 0.254 | ---- |
| 4 | Molar absorptivity (1 mole $cm^{-1}$) | 6.87x $10^4$ | 4.431x104 | 3.13 x $10^4$ | 3.227x104 |
| 5 | Sandell's sensitivity ($\mu gcm^{-2}$ / 0.001 absorbance unit) | 0.009 | 0.014 | 0.020 | 0.020 |
| 6 | Regression equation (Y = a + bC)Slope (b) | 1.41 – 4.46 | 0.0692 | 2.51 – 9.54 | 0.0632 |
| 7 | Standard deviation of slope ($S_b$) | 0.108 | 0.0692 | 0.05 | 0.0632 |
| 8 | Intercept (a) | -2.1 x $10^{-3}$ | 0.0014 | -4.0 x $10^{-3}$ | 0.0012 |
| 9 | Standard deviation of intercept ($S_a$) | 0.001133 | | 0.4247 | |
| 10 | Standard error of estimation ($S_e$) | 0.00108 | | 0.00405 | |
| 11 | Correlation coefficient ($r^2$) | 0.9999 | 0.9998 | 0.9999 | 0.9999 |
| 12 | Relative standard deviation (%)* | 1.290 | 0.4291 | 1.426 | 0.3795 |
| 13 | % Range of error(Confidence Limits) 0.05 level* | 1.354 | 0.362 | 1.497 | 0.317 |
| 14 | % Range of error(Confidence Limits)0.01 level | 2.123 | 0.531 | 2.347 | 0.469 |
| 15 | % Error in bulk samples** | -0.307 | 0.359 | -0.340 | 0.074 |

**Table:2**. Assay and recovery of CTZ in Pharmaceutical Formulations

| Sample | Amount taken (mg) | Amount found by proposed methods | | Reference Methods | Percentage recovery by proposed methods | |
|---|---|---|---|---|---|---|
| | | Method-A | Method-B | | Method-A | Method-B |
| Injection I | 250 | 247.53 ± 2.08 | 247.28 ± 2.30 | 248.06±2.30 | 99.01 ± 0.83 | 98.91 ± 0.92 |
| Injection II | 500 | 493.93 ± 6.80 | 496.72 ± 1.71 | 498.06±3.13 | 98.78 ± 1.36 | 99.34 ± 0.34 |

## CONCLUSIONS

The proposed methods exploit the various functional groups in CTZ molecule .The ingredients usually present in pharmaceutical formulations did not interfere in the color development by proposed methods . The proposed methods are simple,accurate and constitute better alternatives to the reported ones in the assay of CTZ in bulk form and pharmaceutical formulations.

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