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DEVELOPMENT OF SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF CEFTAZIDIME WITH THE BRATTON–MARSHALL REAGENT IN PHARMACEUTICAL PREPARATION

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ABSTRACT

A simple, rapid, sensitive and accurate spectrophotometric method for the determination of ceftazidime in pharmaceutical preparation has been developed. The reaction involves a three-step process of diazotization of the ceftazidime with mixture HCl and $NaNO_2$ at 0–5 ^oC, to produce the corresponding diazonium salt, removal of residual nitrite with sulphamic acid and coupling with N-(1-naphthyl)ethylenediamine (NEDA),to give a purple colored product with λ_{max} at 575 nm and stable for five hours. Beer's law was obeyed at concentrations ranging from 1.0 to 50.0 µg/mL with correlation coefficient $R^2 = 0.997$. The molar absorptivity, Sandell's sensitivity, detection limit and quantitation limit of the method are 1.04×10^4 $L.mol^{-1}.cm^{-1}$, 0.053 $\mu g.cm^{-2}$, 0.278 $\mu g.mL^{-1}$ and 0.835 $\mu g.mL^{-1}$, respectively.Recoveries of ceftazidime from auxiliary substances were 97.4 - 104.5 %. No interference was observed from various auxiliary substances in pharmaceutical formulations. The proposed method has been applied successfully to determine ceftazidime in pharmaceutical preparation as powder for injections (Biocetum Codzidime and Kabi).

Keywords: spectrophotometry, ceftazidime, NEDA, Bratton-Marshall reagent, pharmaceutical preparation.

1. INTRODUCTION

Cephalosporins remain important agents in the treatment of many types of bacterial infections because of their broad-spectrum activity, well-characterized pharmacokinetic and pharmacodynamic properties, and proven safety and efficacy. Ceftazidime is (Z)-(7R)-7-[2-(2-aminothiazol-4-yl)-2-(1-carboxy-1 methoxyimino) acetamido]-3-(1-pyridiniomethyl)-3-cephem-4-carboxylate pentahydrate (CTZ). Ceftazidime is a third-generation cephalosporin that was introduced into clinical use in the 1980s because it demonstrated broad-spectrum activity against Gram-positive cocci and Gram-negative bacilli, including *Pseudomonas aeruginosa* and have been considered to be the drugs of choice for serious infections caused by *Klebsiella, Enterobacter, Proteus, Providencia, Serratia* and *Haemophylus* species [1, 2].

A number of analytical methods have been reported for the determination of ceftazidimein pure drug, pharmaceutical dosage forms and inbiological samples using spectrophotometric, spectrofluorimetric, high performance thin layer chromatography, liquid chromatography, electrokinetic chromatography and electrochemical methods [3 - 4]. Spectrophoto metric methods are the most convenient techniques because of their inherent simplicity, high sensitivity, low cost and wide applicability in laboratories. Determinations of ceftazidime in pharmaceutical preparation by spectrophotometric methods are based on the use of organic reagents: Folin-Ciocalteu, 3-amino phenol (AP), 1-naphthoquinolone-4-sulphonate (NQS), Neocuproin-copper(II), p-dimethyl aminobenzaldehyde,4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl), 3-methylbenzthiazolin-2-one hydrazone (MBTH), sodium nitroprusside, etc. [5 - 12].

However, the disadvantages of using these methods are that the reaction is often narrow linearity range, requiring heating or extraction, long time for the reaction to complete, use of non-aqueous systems, low stability of the colored product formed. Spectrophotometric methods based on diazotization and coupling principle are sensitive and specific. The Bratton–Marshall reagent (N-(1-Naphthyl)ethylenediamine dihydrochloride, NEDA) is a simple diamine reported as a coupling agent in spectrophotometric analysis of thiols, aromatic amines, sulfonamides, aminophenols, dinitroanilines, and chloroanilines. It is widely used for the determination of drugs and pharmaceutical containing free primary aromatic amino group.

The aim of the present work is to develop simple and accurate method for the determination of ceftazidime in pharmaceutical formulations. Based on the diazotization of CTZ and coupling with N-(1-naphthyl)ethylenediamine dihydrochloride (NEDA) reagent and applying the method to the determination of CTZ in pharmaceutical preparation.

2. EXPERIMENTAL

2.1.Chemicals and equipment

All chemicals used were of analytical grade and double distilled water was used for dilution of reagents and samples. Ceftazidime (Sigma-Aldrich, Germany) and N-(1-naphthyl) ethylenediamine dihydrochloride (NEDA) (Maya - R, China, certified to be 99 %) were used. All other chemicals and solvents used were of analytical reagent grade.

The following dosage forms containing ceftazidime were purchased from local pharmacy market and employed in the study: 1 –Kabi injections equivalent to 1000mg ceftazidime (Labesfal-Laboratorios Almiro, Portugal), 2 – Codzidime injections equivalent to 1000 mg ceftazidime (Hanlim Pharm Co., Ltd, South Korea)and 3 – Biocetum injections equivalent to 1000 mg ceftazidime (Bioton S.A, Poland).

A Biochrom Model SP-60 double beam, UV-VIS spectrophotometer (Biochrom Ltd., UK) with 1.0 cm matched quartz cells was used for absorbance measurements.

2.2. Standard solutions

A stock solution of ceftazidime (1 mg/mL) in double distilled water. The working standard solution of ceftazidime containing 100 µg/ml was prepared by dilution.

Sodium nitrite solution, 1 %. This solution was prepared by dissoslving 1 g of sodium nitrite in 100 mLdouble distilled water in a volumetric flask. Sulphamic acid solutions, 3 %. This solution was prepared by dissolving of 3 g of sulphamic acid in 100 mLdouble distilled

water. NEDA solution, 0.25 %. This solution was prepared by dissolving 0.25 g of NEDA in double distilled water in a 100 mL volumetric flask.

The content of ten vials were mixed thoroughly. An accurately weighed injection powder equivalent to 100 mg of ceftazidime was dissolved in 60 mL double distilled water and the solution were filtered through Whatman filter paper No 41. The content was transferred in 100 mL volumetric flask. The volume was made up to 100 mL with double distilled water.The working solution of the drug containing 100 µg/mL was prepared by dilution.

2.3. Procedure and calibration graph

Into a series of 10 mL volumetric flasks, volumes of CTZ working standard solution equivalent to 1.0 - 50 µg/mL were transferred. To each flask, 1.0 mL of concentrated hydrochloric acid and 2.0 mL of sodium nitrite(1 % w/v) were added and a reaction time of 5 minutes at 0 – 5°C was given for completion of the reaction. Next, 1.0 mL of sulphamic acid (3 % w/v) was added to each flask with gentle shaking and after 5 minutes, 1 mL of NEDA reagent (0.2 5% w/v) was added, and kept for 3 minutes. Finally, the volume in each flask was brought up to the 10 mL mark with double distilled water. The absorbances of violet-colored chromogen were measured at 575 nm against the reagent blank and and a calibration graphwas constructed. The colored chromogen was stable for 5 hours.

2.4. Statistical analysis

The limit of detection (LOD) and quantification (LOQ) of the method are given by $3.\frac{SD}{b}$and $10.\frac{SD}{b}$ respectively, relative standard deviation (RSD (%)= $\frac{SD}{\overline{X}}.100$; where SD is the standard deviation, b is the slope of the calibration curve equation, $\overline{X}$" is the average value of the measurement.Calculation and processing of data were done using the programs Origin Pro 8.0 and Statistica 7

3. RESULTS AND DISCUSSION

3.1. Principles of the method

It is based on diazotization of ceftazidime with nitrous acid, to form diazotized ceftazidime (1), the residual nitrite (as nitrous acid) which was undesirable due to its side reaction, such as, nitrosation of coupling agent, was removed by sulphamicacid (2), followed by its coupling with NEDA reagent to form a violet colored chromogen (3) with maximum absorption at 575 nm; it obeyed the Beer's law in the concentration range of 1.0 – 50 µg/ml. The reaction mechanism is shown in Figure 1.

The absorption spectrum shows a maximum absorption at 575 nm characteristic of the purple colored product (CTZ-NEDA). The reagent blank has negligible absorption at this wavelength (Fig. 2).

3.2. Study of the optimum reaction conditions

The various parameters affecting the colour intensity of the dye have been studied and optimum conditions are selected.

3.2.1.Choice of coupling agent

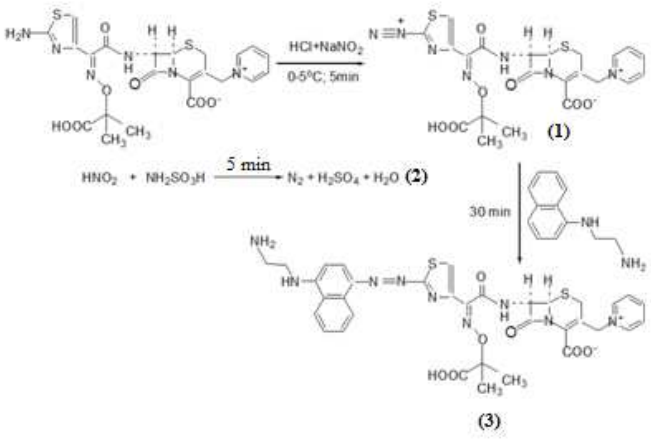

Figure 1. Proposed coupling patterns for the azo dye formation.

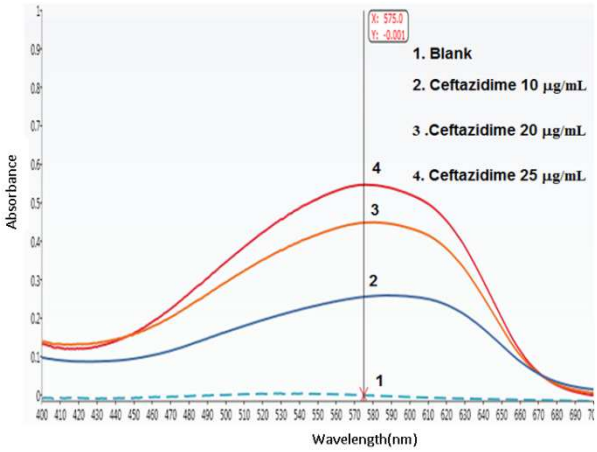

Figure 2. Absorption spectra of CTZ-NEDA and blank.

Different coupling agents are used for the reaction with diazotized ceftazidime. The results in Table 1 indicated that NEDA gave the highest intensity with a good colour contrast for coloured product.

Table 1. Selection of coupling agent.

Reagents 0.25 %	**Absorbance**	**λ_{max}(nm)**	**$\Delta\lambda^{*}$nm**
Phenol	0.122	409	52.5
8 – hydroxyl quinoline	0.129	386	107
Diphenylamine	0.165	450	54
α – napthylamine	0.260	540	65
NEDA	0.45	575	88

*Δλ= colour contrast = λmaxS - λmaxB , Where S= The dye , B=Blank

3.2.2. Effect of acids on the diazotization

The influence of acidity on the development of color was studied using different volumes (0.5 - 2.0 mL) of concentrated HCl. The maximum color intensity was observed with 1 mL of concentrated HCl and therefore 1 mL of concentrated HCl solution was used throughout the experiment (Fig. 3)

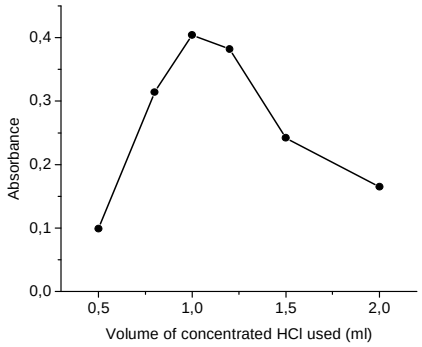

Figure 3. Effect of acid concentration.

3.2.3.Effect of nitrite amount and time

The effect of adding various amounts of sodium nitrite solution on absorbance of 30 μg. mL^{-1} CTZ was examined. The concentration of sodium nitrite was varied between 0.3 - 2.5 mL of 1 % sodium nitrite soulution with the standing time (0 - 20 min). The results showed that 2.0 mL of 1 % sodium nitrite gave maximum absorbance within 5 minutes reaction time (Table 2). Thus, 2 mL of 1 % sodium nitrite was chosen for the procedure

Table 2. Effect of nitrite amount and time on absorbance.

ml of 1% (w/v) $NaNO_2$ solution	Absorbance/minute standing time									
	0	1	2	3	4	5	7	10	15	20
0.3	0.047	0.117	0.137	0.213	0.229	0.463	0.346	0.322	0.249	0.203
0.7	0.111	0.205	0.364	0.391	0.432	0.552	0.413	0.246	0.237	0.199
1	0.132	0.288	0.359	0.433	0.467	0.404	0.357	0.318	0.259	0.145
1.5	0.162	0.216	0.311	0.384	0.531	0.582	0.489	0.313	0.273	0.123
2	0.283	0.355	0.456	0.527	0.572	0.753	0.630	0.415	0.350	0.222
2.5	0.465	0.242	0.262	0.367	0.499	0.718	0.629	0.516	0.400	0.369

3.2.4. Effect of sulphamic acid and coupling agent

The other parameters affecting the colour intensity of the solution have been studied and optimum conditions are selected. The effect of the amount of 3 % sulphamic acid solution (0.5 -

3.0 mL) for removing the excess sodium nitrite with the standing time (0 - 15 min) with occasional shaking are investigated. The results indicated that 1.0 ml of 3 % sulphamic acid solution with 5 minutes standing time was considered to be the most suitable and therefore was selected subsequently. To optimize the concentration of coupling agent, different volumes (0.5 - 3.0 mL) of 0.25 % NEDA were added to the mixture under study. It was found that 1.0 mL of NEDA solution was sufficient for maximum and stable color development. There was a decrease in absorbance at lower concentration of 0.25 % NEDA, whereas no change in absorbance was observed at higher concentration.

3.2.5. Effect of the order of addition

The effect of the order of addition on the absorbance of the product was studied under the optimum experimental conditions (Table 3). From the orders cited below, we can conclude that the reaction of nitrite with sulphamic acid is faster than the reaction of nitrite with ceftazidime in in an acidic medium and hence anabsorbance decrease is observed. Order I have been used for subsequent experiments due to the highest sensitivity.

Table 3. Effect of order of addition of reagents.

Order number	Order of addition	Absorbance
1	CTZ+ HCl + $NaNO_2$ + sunphamic acid + NEDA	0.420
2	CTZ + $NaNO_2$ + HCl+ sunphamic acid+ NEDA	0.251
3	CTZ + sunphamic acid + $NaNO_2$ + HCl + NEDA	0.007
4	CTZ + sunphamic acid + HCl + $NaNO_2$ + NEDA	0.038
5	CTZ + $NaNO_2$ + sunphamic acid + HCl + NEDA	0.011
6	CTZ + $NaNO_2$ + sunphamic acid + NEDA + HCl	0.038

3.3. Validation of the proposed method

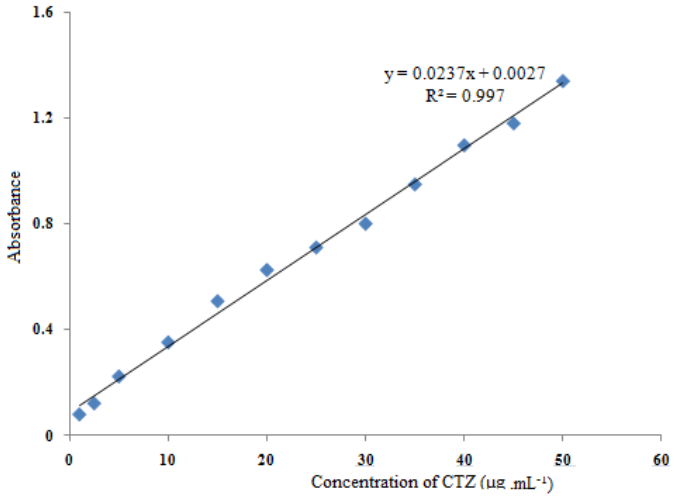

Figure 4. Calibration curve of Ceftazidime.

The calibration graph was linear in the concentration range 1.0–50.0µg.mL^{-1} of Ceftazidime, the calibration equationis A = 0.0237 C_x(µg/mL) + 0.0027. The correlation coefficient R^2 = 0.997 (Fig. 4). The molar absorptivity, Sandell's sensitivity, detection limit and quantitation limit of the method are 1.04 × 10^4 L.mol^{-1}.cm^{-1},0.053 µg.cm^{-2}, 0.278 µg.mL^{-1} and 0.835 µg.mL^{-1}, respectively.

3.4. Interference studies

The extent of interference by various auxiliary substances (sodium carbonate,magnesium stearate, glucose, lactose, glyxine and starch)which often accompany the pharmaceutical preparations was studied in a total volume of 10 mL. The interference was determined by measuring the absorbance of a solution containing 20 µg/mL of ceftazidime and 1000 µg/mL of an auxiliary substance. The tolerance limits of interfering species were established at those concentrations that do not cause more than ± 2.0 % error. It was found that these auxiliary substances do not interfere in the present method. The range of recovery is between 97.4 and 104.5 %. We consider that this variation is acceptable.

3.5.Comparison with other spectrophotometric methods

The proposed method compares favorably with other reported methods. As shown in Table 4 the proposed method is more high sensitivity than other methods, needs no heating, the product is stable for a longer time and and are free from interference with common auxiliary substances.

Table 4. Comparison of VIS spectrophotometric methods for ceftazidimedetermination.

No	Reagent	λ_{max}, nm	Range of determination (µg. mL^{-1})	Molar absorbitivity (L.mol^{-1}.cm^{-1})	Remarks	Reference
1	Folin-Ciocalteu	752	2.5-50	$3.3.10^3$	Less sensitive	[5]
2	3-amino phenol (AP)	590	24 - 168	$3.32.10^3$	Less sensitive	[6]
3	1-naphthoquinolone-4-sulphonate (NQS)	495	20-80	$4.15.10^3$	Less sensitive	[7]
4	Ammonium molybdate	716	2-70	$2.7.10^3$	Less sensitive and heating	[8]
4	Neocuproin-copper(II)	454	15-40	-	Heating	[9]
5	p-dimethyl aminobenzaldehyde (PDAB)	420	5 -35	$1.03.10^3$	Heating	[10]
6	4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl)	390	5-160	-	Tedious hydrolysis, heating and organic medium	[11]
7	N-(1-Naphthyl)ethylenediamine dihydrochloride (NEDA)	575	1 - 50	$1.04.10^4$	High sensitive and high colour stability	This paper

3.6. Application of the method

The proposed method was applied successfully for determination of the studied drugs in their pharmaceutical dosage forms (injections). The relative standard deviation values are below 2 % indicating the precision of the method. The validations of the proposed methods were further confirmed by recovery studies. The % recovery vary from 98.95 ± 0.65 to 101.32 ± 0.78. indicating high accuracy of methods (Table 5). The high % recovery value indicates non interference from excipients used in formulations.

*Table 5.*Analysis of ceftazidime in powder for injection (n = 6).

Brand name of pharmaceutical dosage form	Labeled amount (mg/vial)	Amount found by proposed methods	Recovery (%)	RSD (%)
Biocetum injections	1000	1013.20	101.32± 0.78	0.85
Codzidime injections	1000	989.50	98.95 ± 0.65	0.72
Kabi injections	1000	995.08	99.51 ± 0.49	0.54

4. CONCLUSIONS

This article reports the use of NEDA as a chromogenic reagent for the spectrophotometric determination of ceftazidime. The proposed methods are found to be simple, sensitive, selective, accurate, precise and economical and can be used in the determination of ceftazidime in powder for injection.

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