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SPECTROPHOTOMETRIC DETERMINATION SOME CEPHALOSPORINS CONTAINING AMINO GROUP USING 1, 2-NAPTHAQUINONE-4-SULFONIC ACID SODIUM IN PHARMACEUTICAL DOSAGE FORM

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Abstract

A new, simple and sensitive spectrophotometric method for the determination of some cephalosporins drugs has been developed. The method is based on the condensation of ceftazidime and cefepime with 1, 2-napthaquinone-4- sulfonic acid sodium (NQS) in alkaline media to yield orange colored products respectively. Ceftazidime and cefepime showed maximum absorbance at 495 nm and 475 nm with linearity was observed in the concentration range of 20-80 µg mL^{-1} and 20-140 µg mL^{-1} respectively. The relative standard deviations of 1.64% for ceftazidime and 0.46 % for cefepime were obtained. The recoveries of ceftazidime and cefepime injections are in the range 95.0-97.0±0.009, 98.50-107.5±0.0026 respectively. The proposed method is simple, rapid, precise and convenient for the assay of ceftazidime and cefepime in commercial injection preparations.

Keywords: Cephalosporins drugs, Condensation, 1, 2- Napthaquinone 4- sulfonic acid sodium, Spectrophotometry, Pharmaceutical formulation.

Introduction

Ceftazidime is chemically (Z)-(7R)-7-[2-(2-aminothiazol-4-yl)-2-(1-carboxy-1- ethoxyimino) acetamido]-3-(1-pyridiniomethyl)-3-cephem-4-carboxylate pentahydrate [1, 2]. It is a third generation cephalosporin antibiotic characterized by a broad antibacterial spectrum. Several analytical procedures are available in the literature for the analysis of ceftazidime, *via* high performance liquid chromatography [3-6], charge transfer complexation [7], spectrophotometric methods [8-13] and kinetic spectrophotometric method [14]. Ceftazidime in

combination with pyridine [15], vancomycin [16], salbactum [17, 18] and cefepime by HPLC [19] method were developed.

Cefepime chemically (2-aminothiazol-4-yl)-2(Z)-(methoxyiminoacetamido)-3-(methyl-1-pyrrolidino) methyl-ceph3-em4-carboxylic acid, is a new, injectable, fourth-generation, β-lactamase-resistant parenteral cephalosporin with a broad spectrum of activity against many Gram-positive and Gram negative bacteria [20]. It differs from the third-generation cephalosporins in the quaternary N-methylpyrrolidine ring bonded to C-3 of the cephem nucleus. Cefepime is suitable for treatment of severe infections such as bacterial meningitis, although data on the penetration of cefepime into human cerebrospinal fluid and its use in the treatment of bacterial meningitis are scarce [21]. Several methods have been reported for determination of cefepime in pharmaceutical dosage form. Spectrophotometric [22], Second derivative spectroscopic method [23] has been used for determination of cefepime in pharmaceutical preparations. Determination of cefepime in individual [24, 25] and combined dosage forms [26- 28]. Polarographic techniques [29] are available for the quantitation of cefepime in biological fluids.

1, 2-naphthoquinone-4-sulphonic sulphonate (NQS) has been used as a chromogenic reagent for the spectrophotometric determination of many pharmaceutical amines [30, 31]. However, the reaction between NQS with ceftazidime and cefepime has not been investigated so far. The present study describes the evaluation of NQS as a chromogenic reagent in the development of simple and rapid spectrophotometric method for the determination of ceftazidime and cefepime in its pharmaceutical dosage forms.

Experimental

Apparatus

A Shimadzu UV-visible spectrophotometer model 1800 functioning with UV-probe software and having 1 cm matched quartz cell was used for the absorbance measurements. Systonics electronic balance was used for weighing the samples.

Reagents

All employed chemicals were of analytical grade and high-purified water was used throughout the study.

Ceftazidime and cefepime pure samples were obtained as a gift samples from Strides Arcolab Limited, Bangalore, India.

1, 2-Naphthoquinone-4-sulphonate (NQS) 0.5 %(w/v)

0.5 g of NQS was accurately weighed transferred into a 100 ml calibrated flask, dissolved in 10ml distilled water, and make up the volume up to the mark with distilled water to obtain a solution of 0.5% (w/v). The solution was freshly prepared and protected from light during the use.

10 x 10^{-3}N (0.01N) Sodium hydroxide solution

0.2 g of sodium hydroxide is accurately weighed and transferred into a 500.0ml volumetric flask and made up to the mark with distilled water.

Standard solutions

Ceftazidime and cefepime stock solutions (1.0 mg/ml) were prepared separately by dissolving in distilled water. Working solutions of the drug were prepared by dilution of the stock solution. The injection forms of ceftazidime and cefepime which are used in the determination was Betazidim, Nepecef® respectively with a labelled amount of 1 g and manufactured by Strides Arcolab Limited, Bangalore, India.

Selection of Analytical Wavelengths for ceftazidime and cefepime

Two volumetric flasks of volume 10.0ml are taken and to each volumetric flask 1.0 ml of 0.5% NQS solution and 1.0 ml of 0.01% sodium hydroxide were added and then 0.3 ml of ceftazidime is added to one flask and 1.2 ml of cefepime is added to get 30µg mL^{-1} and 120µg mL^{-1} respectively. Then after the development of the orange colour solutions were made up to the volume with water. The absorption spectra of the colour complex were determined against blank solution. The maximum absorption point of each drug after the reaction with NQS was determined.

Optimization Studies

Effect of NQS Concentration

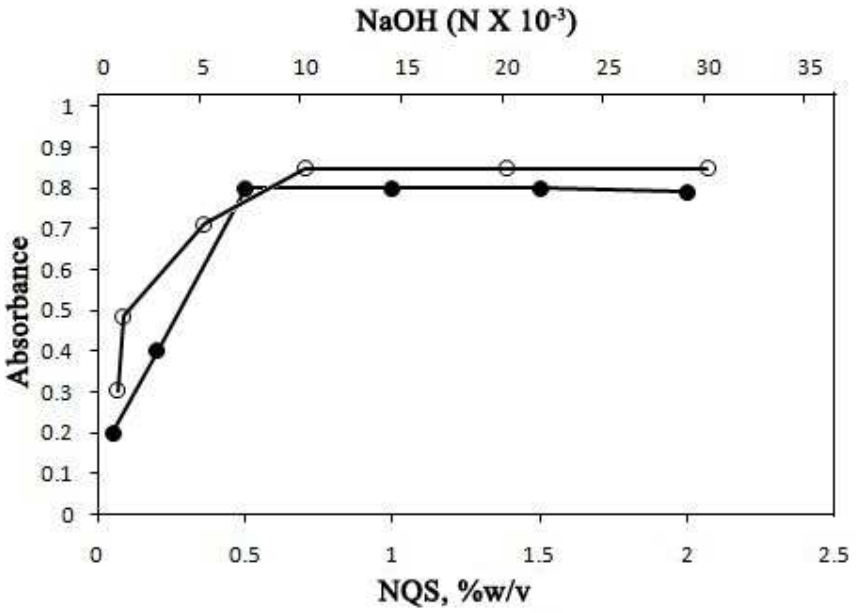
The effect of varying the concentration of NQS was carried out using reagent concentrations of 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5% in 10 x 10^{-3}N NaOH. After mixing 1ml of each reagent concentration with the drug solutions of ceftazidime and cefepime and made up to 10.0 ml with water, the absorbance readings of the complex

formed were measured at 495nm and 475nm on the UV-visible spectrophotometer. Maximum absorbance is seen when 0.5% w/v solution of NQS was used. Further experiments are carried out using 0.5% w/v solution of NQS reagent (Figure 1).

Effect of alkalinity:

To generate the nucleophiles from ceftazidime and cefepime that is, to activate the nucleophilic substitution reaction alkaline medium was necessary. Different inorganic bases were tested: sodium hydroxide, disodium hydrogen phosphate, and sodium bicarbonate, all prepared as aqueous solution of a concentration range of 0.5 – 30 x 10^{-3} N. Best results were obtained in case of sodium hydroxide where with other bases either precipitation of white colloid occurred upon diluting the reaction solution with organic solvent, high blank readings, non reproducible results, and/or weak sensitivity were observed. Studies for optimization of sodium hydroxide concentration revealed that the optimum concentration was 10 x 10^{-3}N (Figure 1).

Figure-1: Effect of Alkalinity and Concentration of NQS on the reaction of ceftazidime (80µg/ml)/cefepime (140µg/ml).

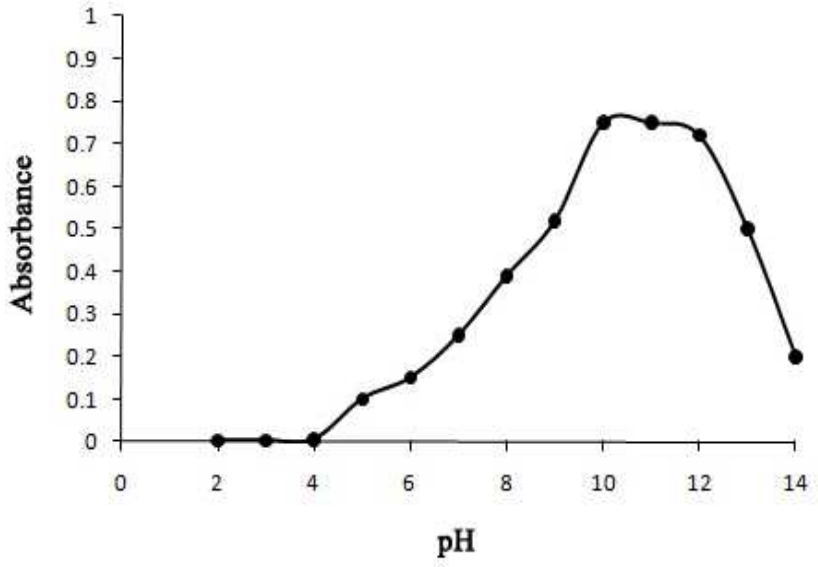


Effect of pH

In a separate series of experiments, the influence of pH on the absorbance of ceftazidime/cefepime-NQS product was investigated. The results revealed that the absorbances at pH < 6 were close to 0, indicating that under acidity, ceftazidime/cefepime have difficulty to react with NQS (Figure 2). At pH > 6, the absorbance

increased rapidly with the increase in the pH, as the amino group of ceftazidime and cefepime turns into the free-NH_2, facilitating the nucleophilic substitution reaction. The maximum absorption values were attained in the range of pH at 10 – 11.5. So in order to obtain 11.5 pH 10 x 10^{-3}N NaOH was used. At pH > 11.5, the absorbance of solution obviously decreased. This was attributed probably to the increase in the amount of hydroxide ion that holds back the condensation reaction between ceftazidime/cefepime and NQS. In order to keep the high sensibility for determination of ceftazidime and cefepime, the experiment was carried out at pH 11.5.

Figure-2: Effect of pH on the reaction of ceftazidime (80µg/ml) and cefepime (140µg/ml) with NQS.



Preparation of calibration curve

Standard solutions of ceftazidime and cefepime in water, having final concentrations in the range of 20-80 µg mL^{-1} and 20-140 µg mL^{-1}, were transferred into a series of 10 ml volumetric flasks, to these solutions 1 ml of 10 x 10^{-3}Nsodium hydroxide is added, 1 ml of 0.5% NQS is added. The mixture was then gently shaken until the appearance of orange colour. The contents were diluted up to 10 ml with distilled water. The absorbance of each solution was measured at 495nm and 475nm respectively against the reagent blank prepared in the same

manner, without the analyte and the calibration curve and absorption spectra are represented in the (Figure 3 and 4) respectively.

Figure-3: Calibration graphs of ceftazidime and cefepime, conc. (ceftazidime) = 20-80 µg mL^{-1}; conc. (cefepime) = 20-140 µg mL^{-1}

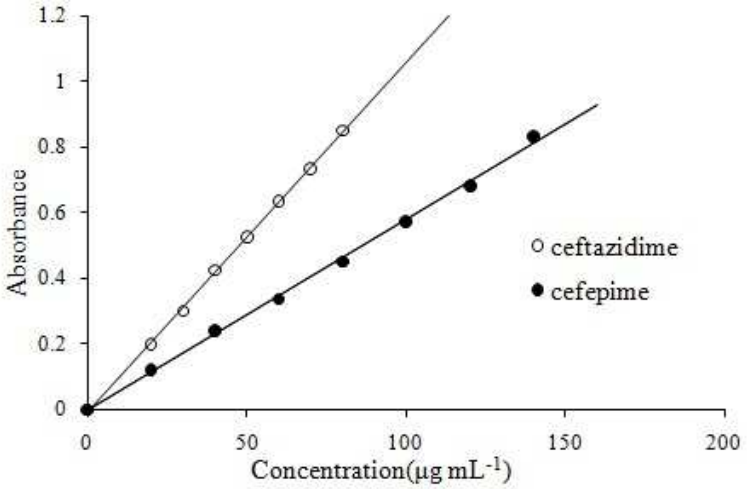
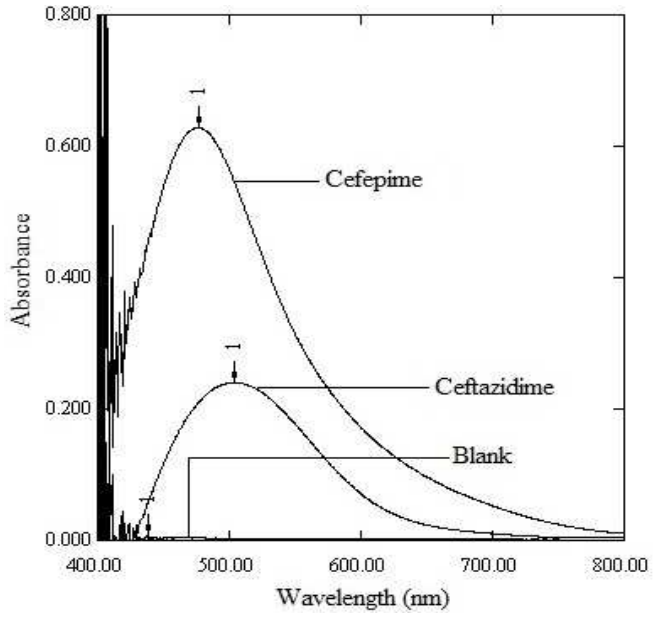


Figure-4: Absorption spectra of blank Vs blank, blank Vs ceftazidime (30 µg mL^{-1}) and cefepime (120 µg mL^{-1})



General procedure:

Several standard solutions of ceftazidime and cefepime were taken in individual standard flasks. To each standard flask, 1 ml of 10 x 10^{-3}N sodium hydroxide and 1 ml of 0.5% NQS was added. The mixtures were

then shaken until the appearance of orange colour. The absorbance was measured at λ max at 495 nm and 475 nm for Ceftazidime and cefepime respectively against a blank similarly prepared by omitting the drug solution with water. The concentration of ceftazidime and cefepime in each standard flask was obtained by interpolating the corresponding absorbance value from Beer's plot of standard ceftazidime and cefepime solutions.

Analysis of commercial pharmaceutical preparations

Injections

An appropriate amount of ceftazidime and cefepime were dissolved in water for injection so as to prepare 1.0 mg/ml solution. An aliquot of this solution was diluted with water to obtain concentrations of 40 µg mL^{-1} and 80 µg mL^{-1} respectively. To that solution 1 ml of 10 x 10^{-3}N sodium hydroxide is added, 1 ml of 0.5% NQS is added. The mixture was then gently shaken until the appearance of orange colour. The contents were diluted up to 10 ml with distilled water.

Quantification

The limits of the Beer's law, the molar absorptivity and the Sandell's sensitivity values were evaluated. Regression analyses of the Beer's law plots at their respective λmax values revealed a good correlation. Graphs of absorbance versus concentration showed zero intercept, and are described by the regression equation, $Y = bX + c$ (where Y is the absorbance of a 1 cm layer, b is the slope, c is the intercept and X is the concentration of the drug in µg/ml) obtained by the least-squares method. The results are summarized in Table 1.

Table-1: Optical Characteristics and Statistical Data for the Regression Equation of the Proposed Method

Parameter	Values	
	Ceftazidime	**Cefepime**
λ_{max}/ nm	495 nm	475 nm
Beer's law limits (µg mL^{-1})	20-80	20-140
Molar absorptivity (L /moL/cm)	0.415 x 10^4	0.2884 x 10^4
Correlation coefficient (R)	0.9994	0.998

Sandell's sensitivity(ng cm^{-2})	0.131	0.166
Regression equation (y)	y = 0.0107x – 0.0079	y = 0.005x + 0.003
Slope, *b*	0.0107	0.005
Intercept, *c*	0.0079	0.003
Relative standard deviation%	1.64	0.46
Repeatability (SD)	0.65	0 .82
Limit of detection (µg mL^{-1})	4.842	2.560
Limit of quantification (µg mL^{-1})	14.52	7.681

$Y = bX + c$, where X is the concentration of drug in µg mL^{-1}; Average of six determinations

Validation

Six measuring flasks containing varying volumes of ceftazidime and cefepime stock solution, (0.2-0.8 and 0.2-1.8 ml) with respective concentrations, (20-80 µg mL^{-1} and 20-180 µg mL^{-1}) were prepared. 1.0 ml of 0.5% w/v NQS was added to each of these tubes. Then 1.0 ml of 0.05N sodium hydroxide was also added. Then the volume is made up to the volume. The absorbance readings of each of the mixtures of both the drugs were then recorded at 495 and 475nm respectively. These processes were repeated three times and on each occasion fresh stock solutions of ceftazidime and cefepime were prepared and used. The average absorbance reading was obtained from the determinations, and used to generate the calibration curves. Linear regression analysis was used to calculate the slope, intercept and coefficient of determination (R^2) of each calibration curve.

Accuracy (% Recovery)

Accuracy of an analysis is determined by systemic error involved. It is defined as closeness of agreement between the actual (true) value and analytical value and obtained by applying test method for a number of times. Accuracy may often be expressed as % recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. The recovery experiments were carried by standard addition method by spiking previously analysed injection samples (ceftazidime 20µg mL^{-1} and cefepime 20µg

mL^{-1}) with three different concentrations of standards (ceftazidime 20, 40, 60 µg mL^{-1} and cefepime 20, 40, 60 µg mL^{-1}). The results are given in the Table 2.

Table-2: Results of recovery study by standard addition method for ceftazidime and cefepime.

Drug	Standard Drug Solution (µg mL^{-1})	Sample Drug Solution (µg mL^{-1})	Absorbance at respective λ_{max}	Amount of Drug Found from std.graph (µg)	Recovery of sample (µg)	% Recovery
Ceftazidime	20	20	0.415	39.0	19.0	95.0%
	40	20	0.629	59.5	19.5	97.5%
	60	20	0.831	79.0	19.0	95.0%
Cefepime	20	20	0.238	39.7	19.7	98.5%
	40	20	0.350	41.5	21.5	107.5%
	60	20	0.451	80.1	20.1	100.5%

Limit of Detection

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. Limits of detection can be calculated using following equation as per ICH guidelines.

LOD = 3.3 x N/S

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Limit of Quantification

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and

accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guidelines.

LOQ = 10 x N/S

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Precision

The precision is measure of either the degree of reproducibility or repeatability of analytical method. It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, relative standard deviation or coefficient of variance of a series of measurements. The precision values are represented in table 3.

Table-3: Evaluation of precision.

Drug	S.no	Drug Present in pharmaceutical dosage form (g)	Amount found (mg)	%Purity	% RSD	SEM
Ceftazidime	1	1.0	0.976	97.60	1.64	0.0090
	2		0.956	95.60		
	3		0.945 *t*=0.189 *F*=2.074	94.50		
Cefepime	1	1.0	0.976	97.60	0.46	0.0026
	2		0.985	98.50		
	3		0.980 *t*=(-)1.072 *F*=1.3819	98.0		

RSD relative standard deviation:

SEM. Standard error of mean

Inter-day and intra-day analysis

It express within laboratory variations as on different days analysis or experiment within the laboratory. Variation of results within same day is called intra- day precision and variation of results amongst days called inter- day precision. The intra-day precision was determined for standard solutions of ceftazidime (20.0-80.0 µg/ml) and cefepime (20.0- 140.0 µg/ml) for three times on the same day by using the same stock solutions and the average readings are reported in table 4. The inter-day precision was determined for standard solutions of ceftazidime and cefepime for two days.

Table-4: Intra-day and Inter-day analysis.

Sample	Drug Present in pharmaceutical dosage form (g)	% Purity±SD
Ceftazidime injection (Betazidim)		
Intraday analysis	1.0	97.60±0.015
Interday analysis	1.0	100.53±0.65
Cefepime injection (Nepecef®)		
Intraday analysis	1.0	98.50±0.0045
Interday analysis	1.0	99.58±0 .82

Robustness and ruggedness

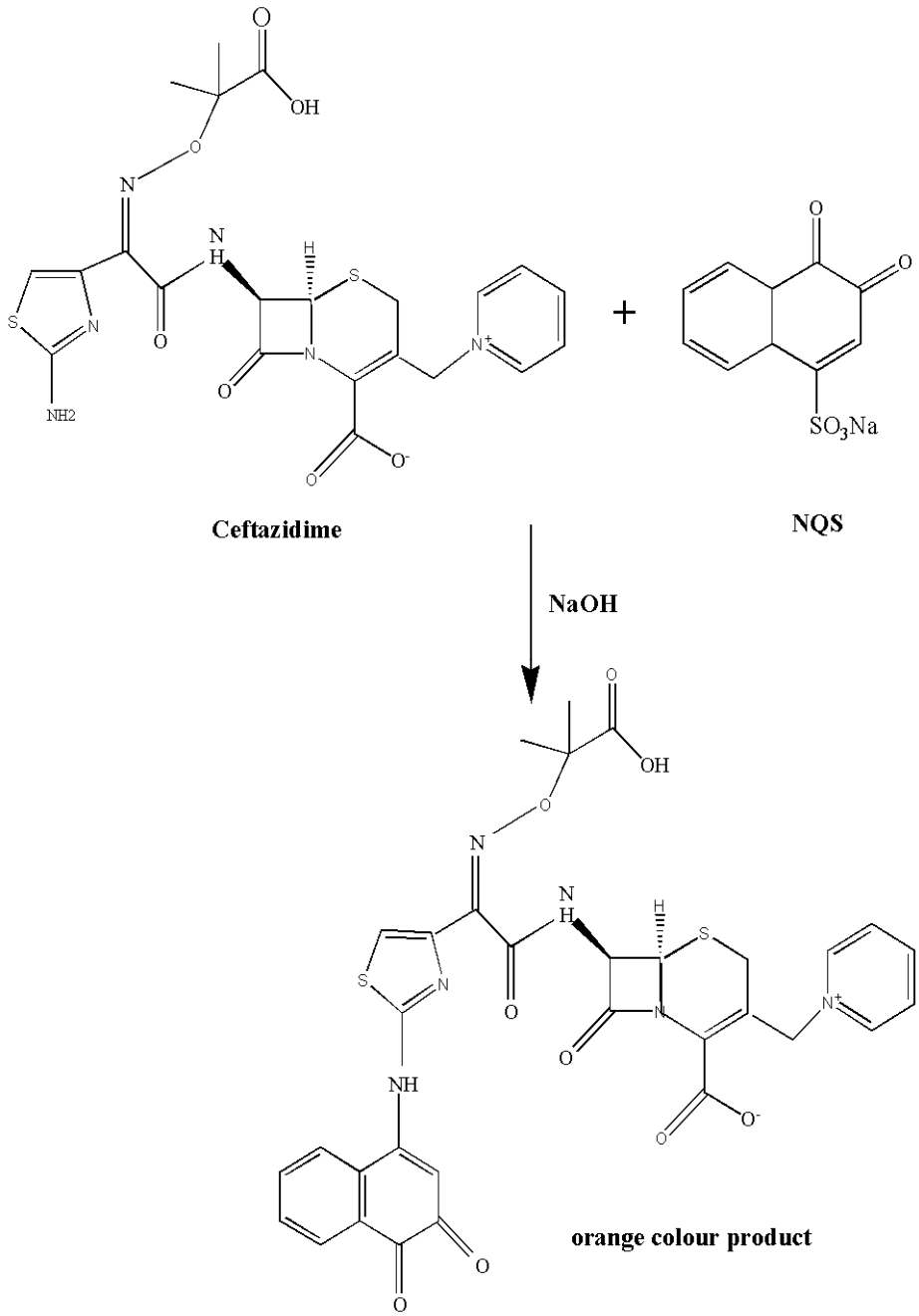
Robustness was examined by evaluating the influence of a small variation of the method variables including the concentration of analytical reagent and the pH of the sodium hydroxide solution. The effect of change in the concentration of NQS and sodium hydroxide was studied. This was an indication of the reliability of the proposed method during its routine application for the investigated drugs. The robustness was tested by applying the proposed method of analysis for both the drugs using the same operational conditions.

RESULT AND DISCUSSION

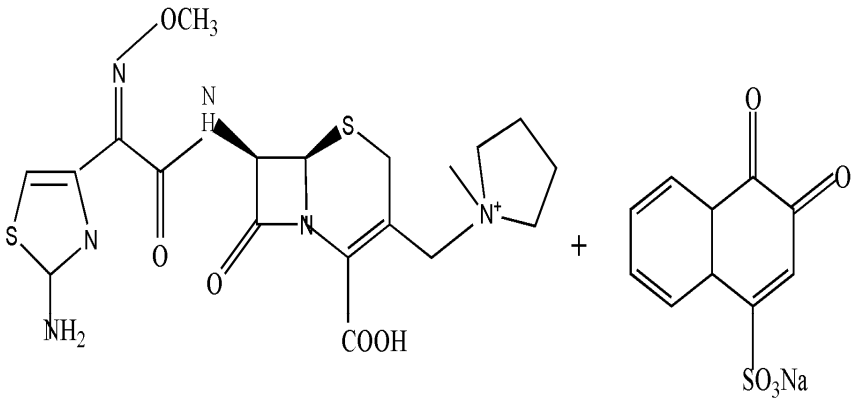
Derivatisation of cephalosporins was attempted in the present study for the development of spectrophotometric method for its determination. The present method is based on the reaction between the NQS and

cephalosporin molecules. The NQS reagent reacts with cephalosporins at the free NH_2 group represented in scheme 1 & 2. The reagent blank has negligible absorbance in the range used for detection of the cephalosporins. Beer's law is obeyed in the range of 20-80 µg mL^{-1} for ceftazidime and 20-140 µg mL^{-1} for cefepime.

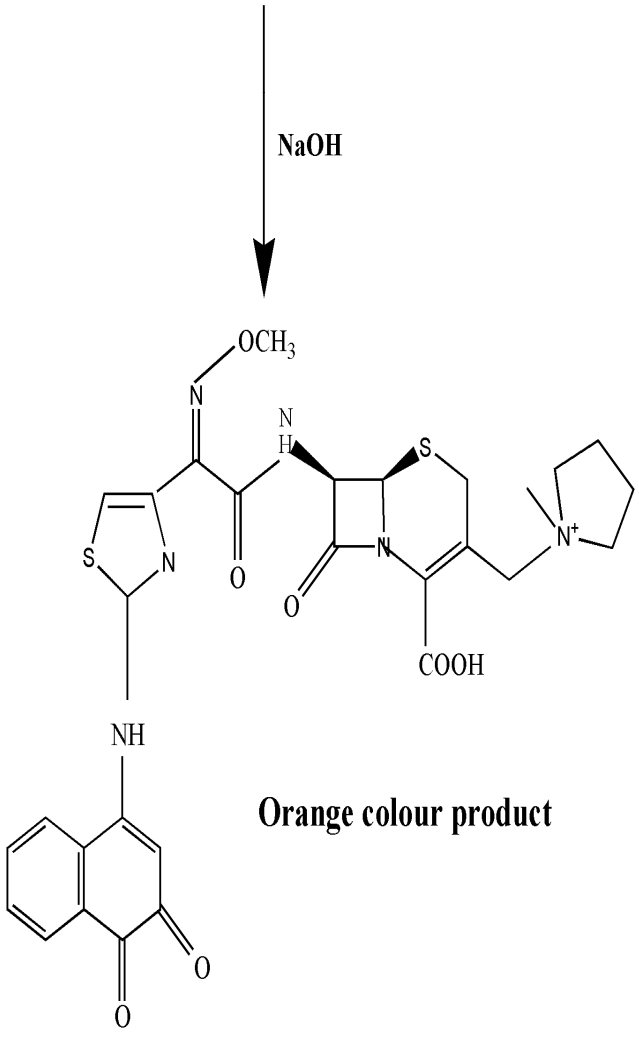
Scheme-1: Mechanism of reaction of ceftazidime with NQS and formation orange colored product.



Scheme-2: Mechanism of reaction of cefepime with NQS and formation orange colored product.



Cefepime NQS



Derivatisation using NQS has attracted considerable attention for quantitative analysis of many pharmaceutically active compounds. In the present investigation, NQS forms a coloured complex with ceftazidime and cefepime in alkaline conditions and their absorbances were measured at 495nm and 475nm respectively. Because of the presence of amine as chromophoric group in ceftazidime and cefepime molecules, derivatization of these compounds was attempted in the present study for the development of spectrophotometric methods for its determination. NQS has been used as chromogenic and fluorogenic reagent for primary and secondary amines, however, its reaction with ceftazidime and cefepime has not been investigated yet. Therefore, the present study was devoted to explore NQS as a derivitising reagent in the development of spectrophotometric method for the determination of ceftazidime and cefepime in pharmaceutical dosage forms.

Optimisation of the spectrophotometric conditions was intended to take into account the various goals of method development. Analytical conditions were optimised via a number of preliminary experiments. The effect of NQS concentration was studied and found that 0.5% gave good absorbance values so ffurther experiments were carried out using 0.5 % NQS. To generate the nucleophiles from ceftazidime and cefepime activate the nucleophilic substitution reactions, alkaline medium was necessary. Different inorganic bases were tested: sodium hydroxide, disodium hydrogen phosphate, and sodium bicarbonate, all prepared as aqueous solution of a concentration range of 0. 01 - 0.05 N. Best results were obtained in case of sodium hydroxide in the concentration of 1.0ml of 0.01 N solution.

Stability of the Chromogen:

Under the optimum conditions, the reaction between ceftazidime / cefepime and NQS was completed within 2 minutes at room temperature. The effect of time on the stability of the chromogen was studied by following the absorption intensity of the reaction solution (after dilution) at different time intervals. It was found that the absorbance of the chromogen remained stable for longer period of time of about 4.0 hours. This increased the convenience of the method as well as made it applicable for large number of samples.

The proposed method is compared with the reference methods [14, 24] and the corresponding values are given in the table 5.

Table-5: Statistical comparison of proposed method with reported reference method.

Parameter	Ceftazidime	Reference method for ceftazidime	Cefepime	Reference method for Cefepime
λ_{max}	495nm	610nm	475nm	256nm
Beer's Range (µg mL^{-1})	20-80	5.0- 15.0	20-140	0.3- 200
Intercept	0.0079	2.044	0.9983	0.0066±0.00009
Slope	0.0107	1.046	0.0058	0.0399±0.0002
Correlation Coefficient	0.9994	0.9995	0.9982	0.9998
Limit of Detection (µg mL^{-1})	4.842	0.233	2.560	0.08
Limit of Quantification(µg mL^{-1})	14.52	0.699	7.681	0.24
% Recovery	95.0 - 97.0±0.009	99.18± 0.8714 – 100.16±0.8680	98.50 – 107.5±0.0026	98.5±0.2 – 101.9±0.3

CONCLUSION

The reagents utilized in the proposed methods are cheap, readily available and the procedures do not

involve any critical reaction conditions or tedious sample preparation. Moreover, the methods are free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control was well established by the assay of cephalosporins (ceftazidime and cefepime) in pure form and in pharmaceutical preparations.

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