Original Research Article

ULTRA SENSITIVE VISIBLE SPECTROSCOPIC METHODS FOR CEFPODOXIME PROXETIL USING NQS AND PDAC REAGENTS

ABSTRACT

Very sensitive and accurate visible spectrophotometric methods were developed and validated for the quantification of Cefpodoxime Proxetil in API and marketed formulation. Method A is based on the measurement of absorbance of reddish orange coloured chromogen at 454.9nm, formed by the condensation reaction of the primary amino group of Cefpodoxime Proxetil with NQS reagent. Method B is based on the measurement of absorbance of light yellowish green coloured chromogen at 401.3nm which is based on the formulation of Schiff's base in acidic medium between Cefpodoxime Proxetil and PDAC reagent. Beer-lambert's law is obeyed in the concentration range of 0.1-1 µg/mL for method A and for method B it was 1-10 µg/mL. With NQS reagent, the regression equation was y=1.083x+0.014 and the correlation coefficient value (R^2) was 0.999. For Schiff's base reaction the regression equation was y=0.107x+0.0068 and the correlation coefficient value (R^2) was 0.9991. The values of LOD and LOQ were found to be 0.009 µg/mL and 0.027 µg/mL respectively for the condensation reaction with NQS reagent. For Schiff's base reaction, LOD and LOQ values were 0.154 µg/mL and 0.467 µg/mL respectively. The recovery studies were performed by the standard addition method. The %RSD values for the intraday and interday precision studies were found to be less than 2. The developed method is simple, sensitive, specific and can be successfully employed in routine analysis of Cefpodoxime Proxetil in pharmaceutical dosage forms.

Keywords: Cefpodoxime Proxetil, 1, 2-Napthoquinone 4-Sulphonate (NQS), P-Dimethylamino Cinnamaldehyde (PDAC).

INTRODUCTION

Cefpodoxime Proxetil (CFP) is chemically 1-(Isopropoxy carbonyloxy) ethyl (6R,7R)- 7-[2-(2-amino-4-thiazolyl) - (z)-2-(methoxyimino) acetamido]-3-methoxymethyl-3-cephem-4-carboxylate **[1],** is a semi-synthetic third-generation cephalosporin anti-biotic with a structure given in **Figure 1**. It is used for infections of the respiratory tract, urinary tract, skin and soft

tissues. It has greater activity against *Staphylococcus aureus* **[2].** Cefpodoxime Proxetil is a prodrug that is de-esterified in vivo to its active metabolite, cefpodoxime, to exhibit antibiotic activity **[3]**. It is active against most Gram-positive and Gram-negative organisms. It is commonly used in the treatment of a variety of infections of skin, respiratory tract, urinary tract, and systemic infections and also to treat acute otitis media, pharyngitis, and sinusitis **[4].** The drug is available for use as a prodrug-cefpodoxime Proxetil which is absorbed readily from the gut. It reaches adequate levels exceeding the minimum inhibitory concentration (MIC) in most of the body fluids. It is excreted by kidneys, unchanged. Also, dose needs adjustment in compromised renal function. It is a bactericidal agent like rest of the cephalosporins. After de-esterification by the intestinal esterases, the drug acts by inhibiting the bacterial cell wall synthesis. The molecular weight of the active molecule is 557.6, which allows its free passage through the porins present in the bacterial cell wall. Then, it crosses the periplasmic space and binds with the penicillin binding proteins (PBP-1 and PBP-3) in the cell membrane. This binding then affects the peptidoglycan synthesis in cell membrane, which ultimately damages the cell **[5].**

The analytical methods for determination of CFP are based on different techniques. They include Spectrophotometric method [**6, 7, 8, 9]**, colorimetric methods **[10, 11]**, HPLC method **[12, 13, 14, 15, 16, 17, 18, 19]**, LC/MS method **[20]**. A thorough literature survey revealed that there were no colorimetric methods developed for CFP using NQS and PDAC reagents.

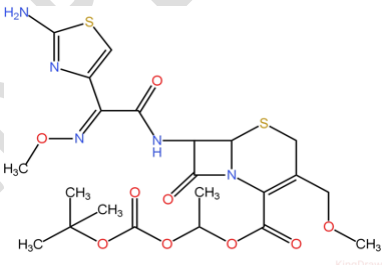

Figure 1: Structure of Cefpodoxime Proxetil

1, 2-Naphthoquinone-4-Sulfonate (Sodium-3, 4 dioxo -3, 4 dihydro naphthalene-1-sulphonate) is used as functional group reagent for amines. Readily soluble in water. Slightly soluble in 90% alcohol, moderately soluble in acetone. NQS is insoluble in ether, chloroform, benzene, and petroleum ether **[21].**

When NQS reagent was treated with any amine containing compound that will release the hydroxyl groups and the sodium sulphonate group is replaced with the aromatic amino group**.**

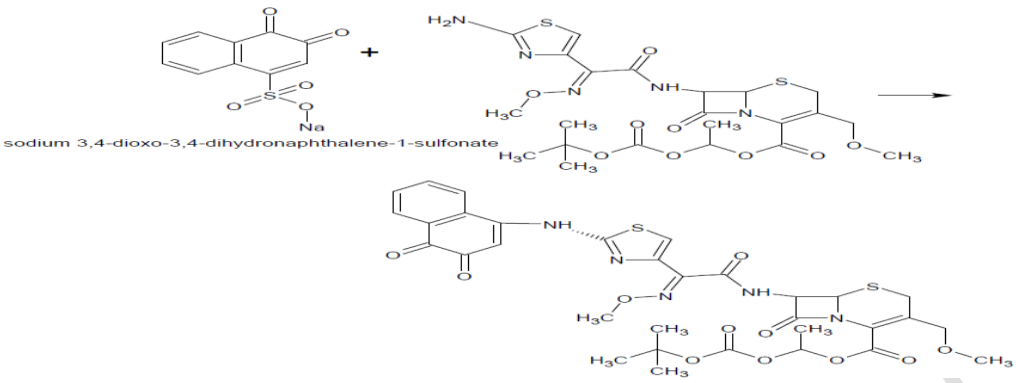

Figure 2: Chromogenic reaction of CFP with NQS Reagent

PDAC (4- (Dimethylamino) Cinnamaldehyde) is used as functional group reagent for amines. Soluble in methanol and in warm dioxide. The principle of aldehydes which condenses the aromatic amines involves the release of oxygen molecule. Then it combines with the amine group to form the yellow Schiff's base in the presence of acidic medium such as hydrochloric acid or H_2SO_4 **[22].**

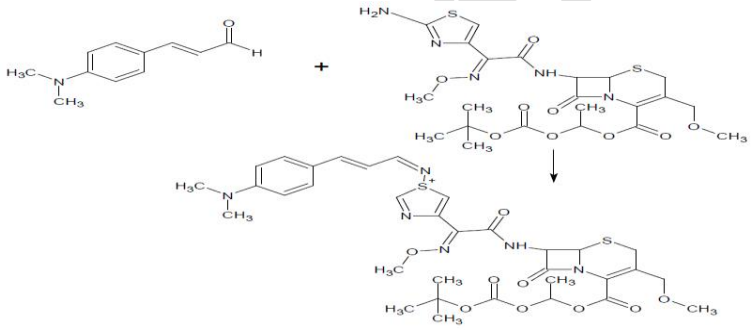

Figure 3: Chromogenic reaction of CFP with PDAC Reagent

EXPERIMENTAL PROCEDURE

Materials

Cefpodoxime Proxetil is a pure drug (API) from SEE GEE pharmaceutical Ltd. Methanol from (Qualigens), NQS, sodium hydroxide, PDAC, Hydrochloride acid from (S d fine-Chem Ltd), potassium bromide from (Fischer Scientific).

Instruments

UV-Visible Spectrophotometer Shimadzu UV-1800, analytical balance.

Experimental Methods

Optimization Methods:

The main factors that affect any chemical reaction are concentration, time and temperature. Concentration: The reaction rate will vary with increase or decrease in concentration of reagent. Time: As duration increases the color of solutions will change as it effects the reaction. Temperature: with increase or decrease in temperature the intensity of colour will change.

Method A: (NQS reagent)

Effect of concentration and volume of NQS reagent

A set of 10 mL volumetric flasks held the standard stock solution of CFP; NaOH (20, 2, 0.5%) and NQS reagent (0.5, 50, 0.1%) in varying concentrations were added to each flask and stirred. They were then set aside for the color to develop for 10 to 15 minutes. Each solution was then diluted with distilled water to a volume of 10 mL. Next, using the reagent as a blank, the absorbances of the resultant solutions were measured between 350 and 700 nm. The absorbance reached its maximum at 454.9 nm when 1 mL of 0.1% NQS reagent and 2 mL of 0.5% NaOH were present.

Method B: (PDAC reagent)

Effect of concentration and volume of PDAC reagent

A set of 10 mL volumetric flasks held the standard stock solution of CFP. The PDAC reagent (0.1, 0.2%), HCl (Conc. HCl 3 drops, 0.1 N, 1 N) and varying concentrations were added to each flask and stirred. Each solution was then diluted to 10 mL with distilled water after being left aside for 10 to 15 minutes to allow the color to develop. In comparison to the reagent blank, the absorbances of the resultant solutions were measured between 350 and 700 nm. At 401.3 nm, the absorbance reached its maximum when 1 mL of PDAC (0.2%) reagent and 3 drops of conc. HCl were added.

Preparation of 0.1% NQS reagent solution:

Accurately weighed 10 mg of NQS reagent and dissolved in sufficient distilled water to produce 10 mL.

Preparation of 0.5% of NaOH solution:

Accurately weighed 0.5 gm of NaOH and dissolved in sufficient distilled water to produce 100 mL.

Preparation of stock solution (10 µg/mL)

The standard stock solution (1000 µg/mL) of CFP was prepared by dissolving 10 mg of CFP in 10 mL of methanol. From this 0.1 mL was diluted to 10 mL with methanol to obtain standard solution of CFP having final concentration of 10 µg/mL.

Preparation of 0.2% PDAC reagent solution:

Accurately weighed 20 mg of PDAC Reagent and dissolved in sufficient distilled methanol to produce 10 mL.

Preparation of stock solution (100 µg/mL):

The standard stock solution (1000 µg/mL) of CFP was prepared by dissolving 10 mg of CFP in 10 mL methanol. From this 1mL solution was diluted to 10 mL with methanol to obtained standard solution of CFP having final concentration of 100 µg/mL.

VALIDATION

The method was validated for accuracy, precision, linearity, LOD, and LOQ as per ICH guidelines the detailed procedure of which is given below **[23].**

Linearity:

Method A:

10 mL volumetric flasks were filled with aliquots of CFP standard drug solution, with concentrations ranging from 0.1 to 1 mL (0.1, 0.2, 0.4, 0.6, 0.8, and 1 µg/mL). Each flask was filled with 1 mL of the 0.1% NQS reagent and 2 mL of the 0.5% NaOH, then thoroughly shaken. After allowing the color to develop for ten to fifteen minutes, each solution was diluted with 10 mL of distilled water. After scanning the colored solutions, a calibration graph was created. Absorbance in comparison to concentration.

Method B:

A different set of 10 mL volumetric flasks were filled with aliquots of the standard drug solution of CFP, which ranged from 0.1-1 mL (1, 2, 4, 6, 8, 10 µg/mL). Each flask was filled with 1 mL of PDAC reagent, 3 drops of conc. HCl, and thoroughly shaken. After allowing the color to develop for 10 to 15 minutes, each solution was diluted with 10 mL of distilled water. After scanning the colored solutions, a calibration graph was plotted with absorbance versus concentration.

Accuracy:

The accuracy of the method was determined by calculating recoveries of Cefpodoxime Proxetil by the method of standard addition. Known amount of standard solutions of CFP were added at 80%, 100%, 120% levels to pre-quantified sample solutions of CFP using NQS (0.4 µg/mL) and PDAC (4 µg/mL). The amount of CFP was estimated by substituting the measured absorbance at 454.9 nm by using NQS and 401.3 by PDAC reagent into the regression equation obtained in the linearity studies.

Precision:

The intra-day precision of the proposed colorimetric method was determined by estimating the corresponding response three times on the same day for three different concentrations of CFP with NQS (0.2, 0.6, 1 µg/mL) and PDAC (2, 6, 10 µg/mL). The results were reported in terms of %RSD.

The inter-day precision of the proposed colorimetric method was determined by estimating the corresponding response three times on three different days for the similar concentrations used for intra-day precision. The results were reported in terms of %RSD.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The limit of detection (LOD) and the limit of quantification (LOQ) of the Cefpodoxime Proxetil were derived by calculating the signal-to-noise ratio using the following equations as per ICH guidelines.

$$LOD = 3.3 * S.D / Slope$$

$$LOQ = 10 * S.D / Slope$$

Where, S.D = Standard Deviation of the response

S = Slope of the Calibration Curve of the analyte.

RESULTS AND DISCUSSION

Optimization results:

The optimization results for effect of concentration of NaOH, NQS, PDAC was plotted in **Figure 4,5,6.**

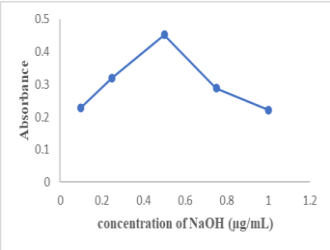

Figure 4: Effect of concentration of NaOH

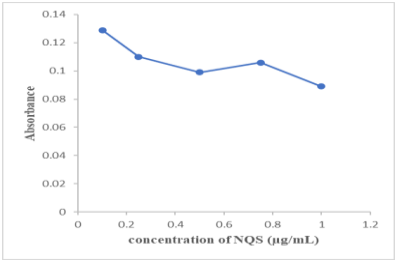

Figure 5: Effect of concentration of NQS

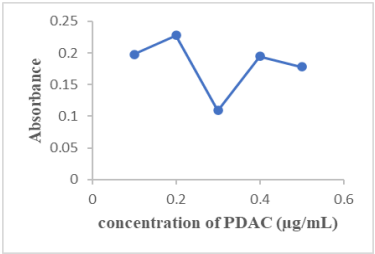

Figure 6: Effect of concentration of PDAC

UV Spectrophotometric method.

Determination of λ max:

The coloured solution of CFP obtained after chemical derivatization with NQS reagent was scanned under visible range (350-700 nm) and with PDAC (350-700 nm) and the spectrum obtained was shown in the figure 4 and figure 5. From this λ_{max} was found to be 454.9 nm for NQS and 401.3 nm for PDAC reagent is shown in **Figure 7 and Figure 8**.

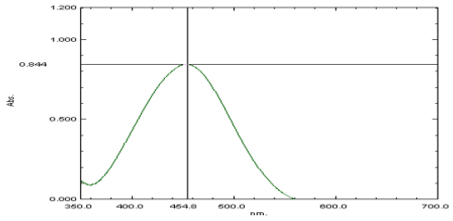

Figure: 7 Visible Spectrum of CFP (0.8 µg/mL) after chemical derivatization using NQS Reagent

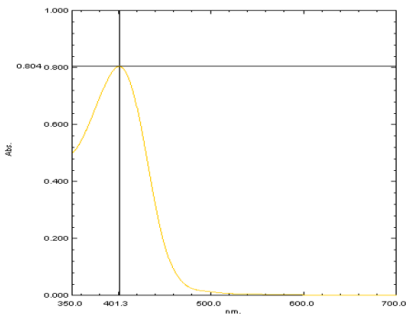

Figure 8: Visible Spectrum of CFP (8 µg/mL) after chemical derivatization using PDAC Reagent

Analytical Method Validation:

Calibration Plot for Cefpodoxime Proxetil using NQS Reagent and PDAC Reagent:

Figures 9, 9.a and 10, 10.a show the overlay spectra of Cefpodoxime Proxetil following chemical derivatization with NQS reagent and PDAC reagent, respectively. **Tables 1 and 2** provide the related linearity data. It was found that the absorbance response at 454.9 nm using NQS and 401.3 nm using PDAC reagent increased with the rise in Cefpodoxime Proxetil concentration. For Cefpodoxime Proxetil, the linearity of the calibration curve (absorbance Vs. concentration) was examined throughout a concentration range of roughly 0.1-1 µg/mL with NQS and 1-10 µg/mL with PDAC reagent. The linearity of the procedure was demonstrated by the correlation coefficient value (R2) for Cefpodoxime Proxetil utilizing NQS, which was 0.999, and for PDAC, which was 0.9991, according to the linear regression analysis.

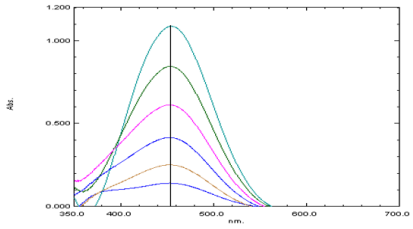

Figure 9: Overlay Spectra of CFP using NQS (0.1-1 µg/mL)

Table: 1 Linearity Data of CFP using NQS reagent

Conc. (µg/mL)	Absorbance AM ± S.D (n=3)
0.1	0.129 ± 0.003
0.2	0.245 ± 0.004
0.4	0.452 ± 0.006
0.6	0.657 ± 0.006
0.8	0.880 ± 0.005
1	1.1 ± 0.09

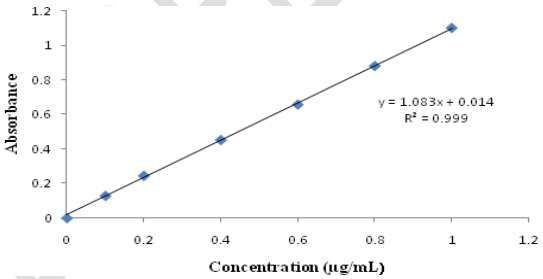

Figure 9.a: Linearity graph of CFP using NQS reagent

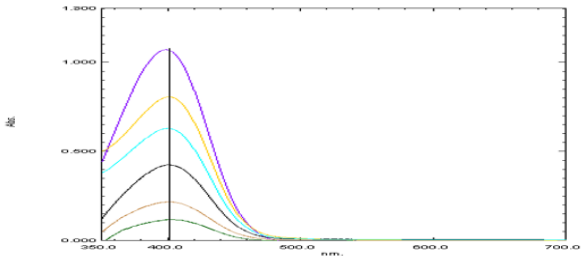

Figure 10: Overlay spectra of CFP using PDAC reagent (1-10 µg/mL)

Table 2: Linearity data of CFP using PDAC reagent

Conc. (µg/mL)	Absorbance AM ± S.D (n=3)
1	0.123 ± 0.005
2	0.228 ± 0.007
4	0.438 ± 0.005
6	0.628 ± 0.007
8	0.856 ± 0.006
10	1.09 ± 0.07

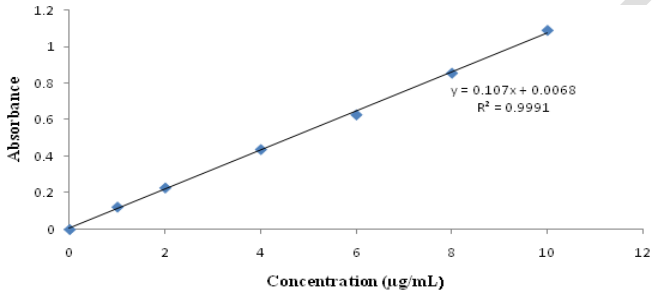

Figure 10.a: Linearity graph of CFP using PDAC reagent

Precision:

The repeatability (intra-day precision) of the method was determined by intra-day (n=3) analysis of three standard solutions of Cefpodoxime Proxetil of the concentration of 0.2, 0.6, 1µg/mL for NQS and 2, 6, 10 µg/mL for PDAC reagent. Intermediate precision was determined by the inter-day (n=3) analysis of three standard solutions of Cefpodoxime Proxetil at the above mentioned concentrations. The data obtained from precision studies are given in **Table 3 and 4**. The % RSD values for intra-day and inter-day precision study were less than 2.0, confirming that the method was precise.

Table 3: Precision Data for Cefpodoxime Proxetil using NQS reagent

Theoritical Amount (µg/mL)	Intra-day		Inter-day	
	Amt found (µg/mL) AM ± S.D (n=3)	%RSD	Amt found (µg/mL) AM ± S.D (n=3)	%RSD
0.2	0.218 ± 0.004	1.79	0.210 ± 0.002	0.95

0.6	0.608 ± 0.007	1.06	0.58 ± 0.005	0.85
1.0	1.086 ± 0.007	0.58	1.09 ± 0.008	0.66

Acceptance Criteria: % RSD should not be more than 2

Table 4: Precision Data for Cefpodoxime Proxetil using PDAC reagent

Theoritical Amount (µg/mL)	**Intra-day**		**Inter-day**	
	Amt found (µg/mL) AM ± S.D (n=3)	**% RSD**	**Amt found (µg/mL) AM ± S.D (n=3)**	**% RSD**
2	2.2 ± 0.002	0.82	1.9 ± 0.0036	1.6
6	6.2 ± 0.007	1.03	5.8 ± 0.01	1.5
10	9.4 ± 0.015	1.4	9.65 ± 0.015	1.44

Acceptance Criteria: % RSD should not be more than 2

Accuracy (Recovery Studies)

The accuracy was determined by standard addition method. Three different levels (80%, 100% and 120%) of standards were spiked to commercial powder in triplicate. The mean of percentage recoveries and % RSD values were calculated and reported in **Table 5**. The %recovery of Cefpodoxime Proxetil was found to be in the range 100.5-101.4% for NQS and 98.9-100.7% for PDAC reagent which are satisfactory.

Table 5: Accuracy data for Cefpodoxime Proxetil using PDAC and NQS reagent

Spiking Level	**Theoritical Content (µg/mL)**		**Amt found (µg/mL)AM ± S.D (n=3)**		**%Recovery**		**%RSD**	
Method	**PDAC**	**NQS**	**PDAC**	**NQS**	**PDAC**	**NQS**	**PDAC**	**NQS**
80%	7.2	0.72	7.31 ± 0.01	0.720 ± 0.002	101.6	100.6	1.4	0.27
100%	8	0.8	7.9 ± 0.006	0.811 ± 0.001	98.9	101.4	0.76	0.123
120%	8.8	0.88	8.86 ± 0.007	0.885 ± 0.007	100.7	100.5	0.79	0.8

Acceptance Criteria: % RSD should not be more than 2

Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD was found to be 0.009 µg/mL for NQS and 0.154 µg/mL for PDAC reagent and LOQ was found to be 0.027 µg/mL using NQS and 0.467 µg/mL using PDAC reagent for Cefpodoxime Proxetil respectively.

Analysis of Marketed Formulations (Assay):

The assay of commercially available tablets (Cepodem® 100) containing 100 mg of CFP was used to assess the accuracy of the suggested approach. **Table 6** present the comparison between the Cefpodoxime Proxetil results and the corresponding indicated quantities. The assay value was determined to be 100.32% using PDAC reagent and 102.1 mg using NQS and 100.32 mg using NQS; the amount of CFP was found to be 102.1 mg using NQS and 100.32 mg using PDAC reagent. These sums fell inside the permitted range. The assay result's percent RSD was determined to be less than 2, indicating the suggested method's correctness.

Table 6: Analysis of Commercial tablets using NQS and MBTH reagent (assay)

Formulation with label Claim	Reagents	Amt found (mg)AM ± S.D (n=3)	% Assay	%RSD
CEPODEM® 100mg	NQS	102.1 ± 0.0049	102.1 %	0.722
	MBTH	100.32 ± 0.004	100.32 %	0.613

CONCLUSION

Due to its ease of use, sensitivity, and selectivity, visible spectrophotometry has maintained its competitiveness in the field of chromatographic techniques for pharmaceutical analysis. Using NQS and PDAC Reagents, two straightforward, accurate, and exact visible spectrophotometric techniques were created to measure CFP. The absorbance maxima in the linearity range of 0.1-1 (µg/mL) NQS and 1-10 (µg/mL) PDAC reagent were found at the λ max of 454.9 nm using NQS and 401.3 nm using PDAC reagent. Precision was determined to be RSD<2 and Assay obtained 102.1% for NQS and 100.3% PDAC reagent; accuracy was found to be 100.5-101.4% NQS and 98.9-101.6 PDAC reagents. 0.009 NQS and 0.154 PDAC and 0.027 NQS and 0.467 PDAC, respectively, were the LOD and LOQ values found in the CFP regression equation. For NQS and PDAC of Cefpodoxime Proxetil, the regression equations are y = 1.083x + 0.014 and y = 0.107x + 0.0068 respective. The research findings

demonstrated that the colorimtery method that was created is straightforward, linear, accurate, exact, and selective. In order to ensure Ceftriaxone quality control in API and pharmaceutical dosage forms, the established colorimetric approach can be utilized.

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