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ORIGINAL PAPER

New Spectrofluorimetric Method for Determination of Cephalosporins in Pharmaceutical Formulations

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Abstract Simple, accurate and sensitive spectrofluorimetric method has been proposed for the determination of three cephalosporins, namely; cefixime (cefi), cephalexine (ceph), cefotaxime sodium (cefo) in pharmaceutical formulations. The method is based on a reaction between cephalosporins with 1, 2-naphthoquinone-4-sulfonic (NQS) in alkaline medium, at pH values of 12.0 for cefi and 13.0 for ceph and cefo to give highly fluorescent derivatives extracted with chloroform and subsequently measured at 600,580 and 580 nm after excitation at 520,455 and 490 nm for cefi, ceph and cefo respectively. The optimum experimental conditions have been studied. Beer's law is obeyed over the concentrations of 10–35 ng/mL, 10–60 ng/mL and 20–45 ng/mL for cefi,ceph and cefo, respectively. The detection limits were 2.02 ng/mL, 2.09 ng/mL and 2.30 ng/mL for cefi, ceph and cefo, respectively, with a linear regression correlation coefficient of 0.9987, 0.9995 and 0.9991 and recoveries in range from 98.5-107.04, 95.17-101.00 and 95.00-109.55% for cefi, ceph and cefo, respectively. This method is simple and can be applied for the determination of cefi, ceph and cefo in pharmaceutical formulations in quality control laboratories.

Keywords Cephalosporins · 1,2-naphthoquinone-4-sulfonic (NQS) · Spectrofluorimetric analysis · Pharmaceutical analysis

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Introduction

Cephalosporins antibacterial are commonly used to control gram positive and gram negative activity. Cephalosporins are the second most important β-lactams after penicillin for treating infectious diseases [1]. Many of these manifestations, such as urticaria and exanthema, are cutaneous, but anaphylactic reactions have also been reported [2].

Cephalosporins are derivatives of 7-aminocephalosporanic acid (7-ACA) composed of a β-lactam ring fused with a dihydrothaizine ring Fig. 1. but differ in the nature of substituent at the 3- and/or 7-positions of the cephem ring as shown in Table 1 [3, 4].

Several methods have been described for the quantitative determination of cephalosporins included spectrophotometry [5–7], spectrofluorimetry [8], High performance liquid chromatography (HPLC) [9–15], potentiometry [16] and voltammetry [17]. These methods were time-consuming, tedious, and dedicated to sophisticated and expensive analytical instruments. Spectrophotometric methods are the most convenient techniques because of their inherent simplicity, high sensitivity, low cost, and wide availability in quality control laboratories. Unfortunately, the spectrophotometric methods that have been reported for determination

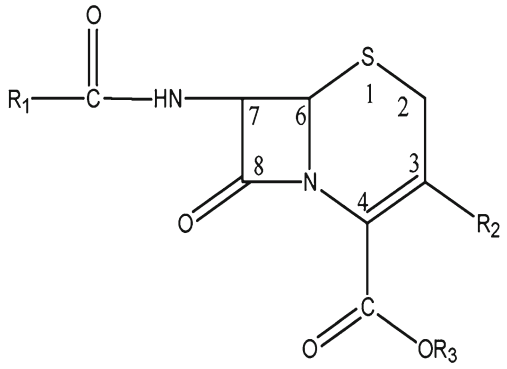


Fig. 1 Cephalosporin

of cephalosporins in their pharmaceutical formulations were associated with some major disadvantages such as the lack of selectivity, tedious extraction procedures and time-consuming. High performance liquid chromatography (HPLC) is the is the recommended method for the analysis of cephalosporins in pharmaceutical preparations [18]. Therefore, the development of new alternative spectrofluorimetric method for the determination of cephalosporins that can overcome the disadvantages of the existing methods is essential.

1,2-Naphthoquinone-4-sulfonic acid sodium salt (NQS) has been used for the determination of many compounds. It is reactive with both primary and secondary amines, and has high reaction rate [19–25].

Hashimoto et al. [26] have applied NQS for the qualitative analysis of phenethylamine derivatives. The reaction products were isolated by thin-layer chromatography and were analyzed by various techniques such as elemental analysis, NMR, IR and mass spectrometry. The results obtained proposed that the reaction products have the general structure shown in Scheme 1. NQS proved to be a useful and sensitive analytical derivatizing agent for spectrophotometric and spctrofluorometric analysis of pharmaceuticals bearing a primary or secondary amino group, however the use of (NQS) for spectrofluorimetric determination of cephalosporins was not reported. Therefore in this work a rapid spectrofluorimetric method for determining the content of cefi, ceph and cefo in pharmaceutical formulations which is based on the reaction of NQS with amino group of cefi,ceph and cefo molecules to form fluorescent derivatives.

Experimental

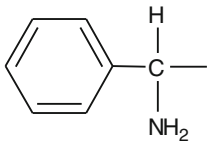
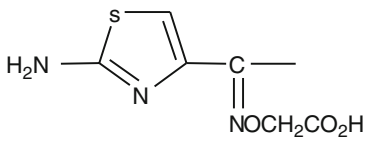
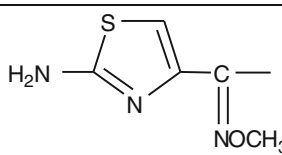
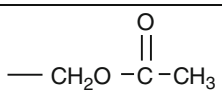
Apparatus

The spectrofluorimetric measurements were made with Shimadzu spectrofluorophotometer RF-1501 with xenon lamp. Also temperature controller was used for the spectrofluorimetric measurements. pH meter model pH 211(HANNA Italy) was used for adjusting pH.

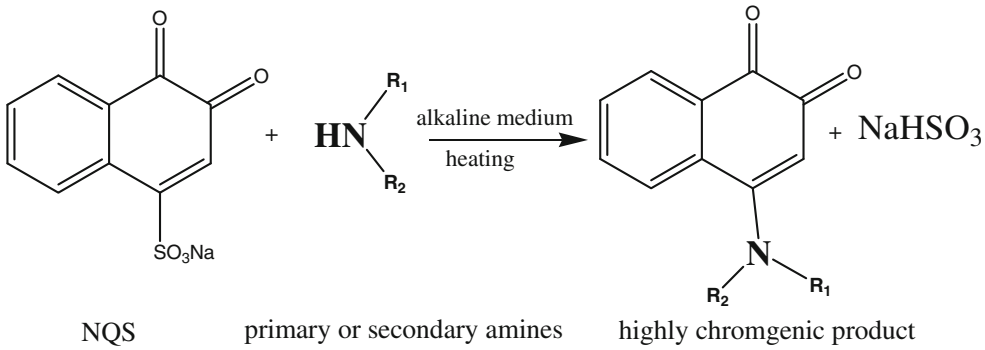
Reagents and Solutions

All reagents were of analytical reagent grade. Double distilled water was used in all experiments.

Table 1 Chemical structures of the investigated cephalosporin antibiotics

name	R1	R2	R3	generation
cephalexine		-CH_3	-H	first
cefixime		-CH=CH_2	-H	third
Cefotaxime sodium			-Na	third

Scheme 1 Reaction of amines with NQS proposed by Hashimoto et al. [26]



The standards of cefi,ceph and cefo were supplied by (Orchid Chemicals and Pharms LTD).

Pharmaceutical Formulation

The following available pharmaceutical preparations were analyzed:

(1) cefi capsules (AMIPHARMA laboratories, Sudan), labeled to contain 200 mg cefi per capsule.
(2) ceph monohydrate capsules(AMIPHARMA laboratories, Sudan), labeled to contain 500 mg ceph per capsule.
(3) cefo for injection (KILITCH drugs, India) labeled to contain 1000 mg cefo per injection.

Stock Standard Solution of Cefi,Ceph and Cefo (1000 μg/mL)

An accurately weighed 0.1 g standard sample of the three drugs was dissolved in methanol for cefi and in double distilled water for ceph and cefo, transferred into a 100 mL standard flask and diluted to the mark with methanol for cefi and with double distilled water for ceph and cefo and mixed well. This stock solution was further diluted to obtain working solutions in the ranges 10–35, 10–60 and 20–45 ng/mL for cefi,ceph and cefo, respectively.

Sodium 1, 2-Naphthoquinone-4-Sulfonic Solution (0.4%, 0.5% w/v)

An accurately weighed 0.4 g and 0.5 g of NQS was dissolved in double distilled water, transferred into a 100 ml standard flask and diluted to the mark with double distilled water and mixed well to prepare (0.4%,0.5% w/v), respectively. The solution was freshly prepared and protected from light during use in a brown calibrated flask.

Buffer Solutions

Buffer solution of pH 12.0 was prepared by mixing 25 mL of 0.2 M KCl with 12 mL of 0.2 M NaOH, and buffer of pH 13.0 was prepared by mixing 25 mL of 0.20 M KCl solution

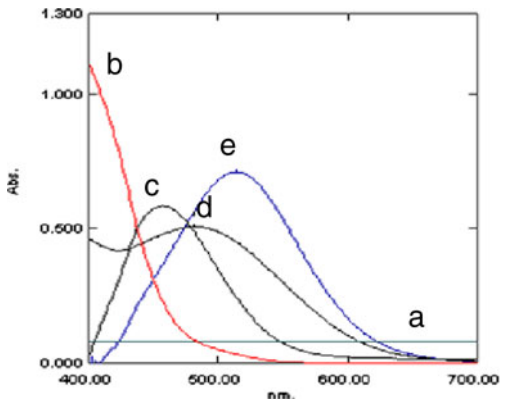


Fig. 2 Absorption spectra of (**a**) cefi, ceph and cefo (2, 2 and 1.5 μg/mL respectively) (**b**) Absorption spectra of NQS (0.5%) (**c**) Absorption spectra of ceph (2 μg/mL) with NQS 0.4% (**d**) Absorption spectra of cefo(1.5 μg/mL) with NQS 0.5% (**e**) Absorption spectra of cefi(2 μg/mL) with NQS 0.5%

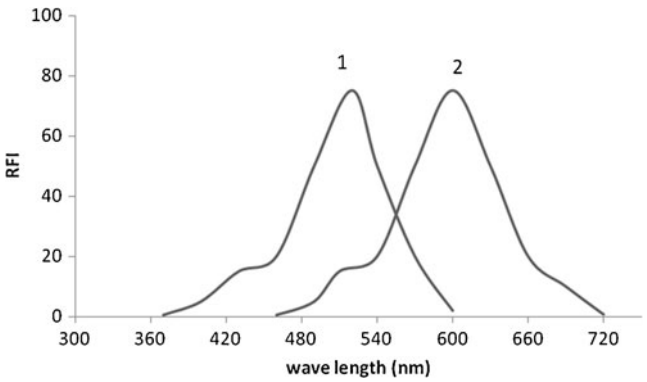


Fig. 3 Excitation (1) and Emission (2) spectra of cefi (50 ng/mL) and its reaction product with NQS (0.5% w/v) in chloroform

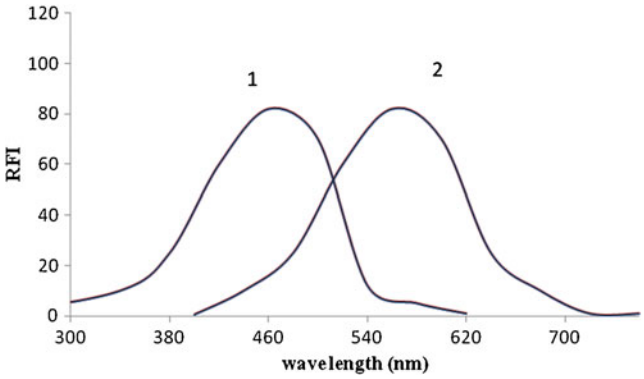


Fig. 4 Excitation (1) and Emission (2) spectra of ceph (75 ng/mL) and its reaction product with NQS (0.4% w/v) in chloroform

with 65 mL of 0.20 M NaOH solution, in 100 mL volumetric flask and adjusted by a pH meter. Buffer solutions of different pH value were also prepared according to literature method [27].

Sample Solutions

Twenty capsules or 20 injection powder were carefully evacuated; their contents were weighed and finely powdered. Then an accurately weighed amount equivalent to 100 mg was transferred into a 100 mL calibrated volumetric flask, and dissolved in about 40 mL in methanol for cefi and in double distilled water for ceph and cefo. The contents of the flask were swirled, sonicated for 5 min, and then filled to volume with methanol for cefi and with double water for ceph and cefo. The contents were mixed well and filtered. This prepared solution was diluted quantitatively with methanol for cefi and with double distilled water for ceph and cefo to obtain a suitable concentration for the analysis.

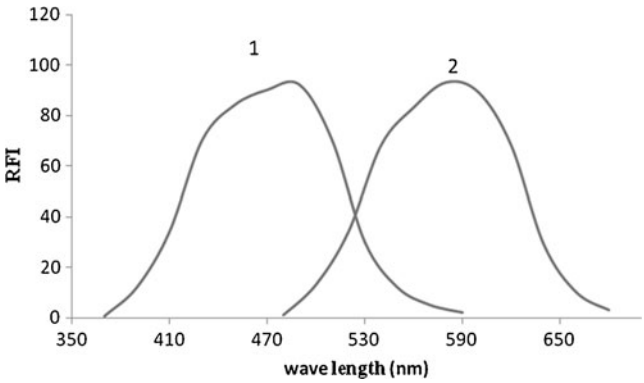


Fig. 5 Excitation (1) and Emission (2) spectra of cefo (50 ng/mL) and its reaction product with NQS (0.5% w/v) in chloroform

Table 2 Optimum conditions for the reaction of cefi, ceph and cefo with NQS

Condition	Studied range	cefi	ceph	cefo
pH	4.0–13.5	12.0	13.0	13.0
Volume of buffer (mL)	0.5–2.5	2.0	1.5	1.0
Temperature °C	30–90	70	80	Room temperature
Reaction time minutes	5–30	20	10	25
NQS concentration (w/v%)	0.1–0.6	0.5	0.4	0.5
Extraction solvents	different	chloroform	chloroform	chloroform

General Recommended Procedure

About 1 mL of (100–350, 100–600 and 200–450 ng/mL) for cefi, ceph and cefo, respectively, were transfer in to 10 mL calibrated volumetric flask subsequently, 2 mL of pH 12.0 for cefi,1.5 and 1 mL of pH 13.0 for ceph and cefo respectively were added and 1 mL of 0.5% NQS were added for cefi and cefo and 1 mL of 0.4% (NQS) solution was added for ceph, the solution was heated in a thermostat at 70 °C for 20 min and at 80 °C for 10 min for cefi and ceph respectively the solution was stood, for 25 min at room temperature for cefo. The contents of the flasks were transferred quantitatively in to separating funnels and acidified with 0.5 mL 0f 0.1 N HCl for cefi and with 1 mL of 0.1 N HCl for ceph and cefo. The solutions were extracted with two

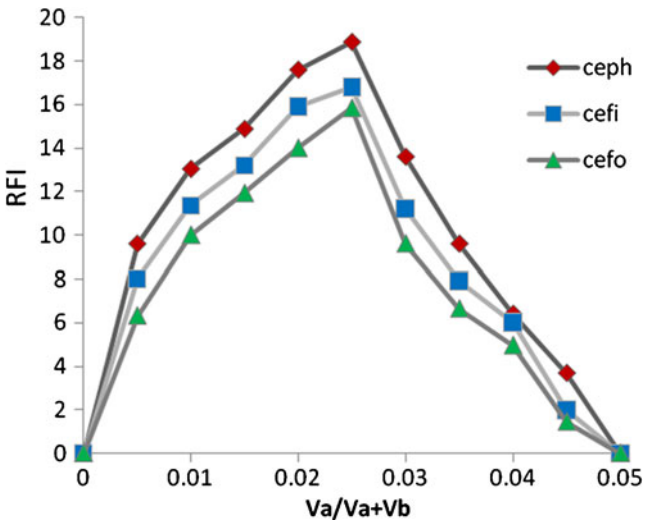
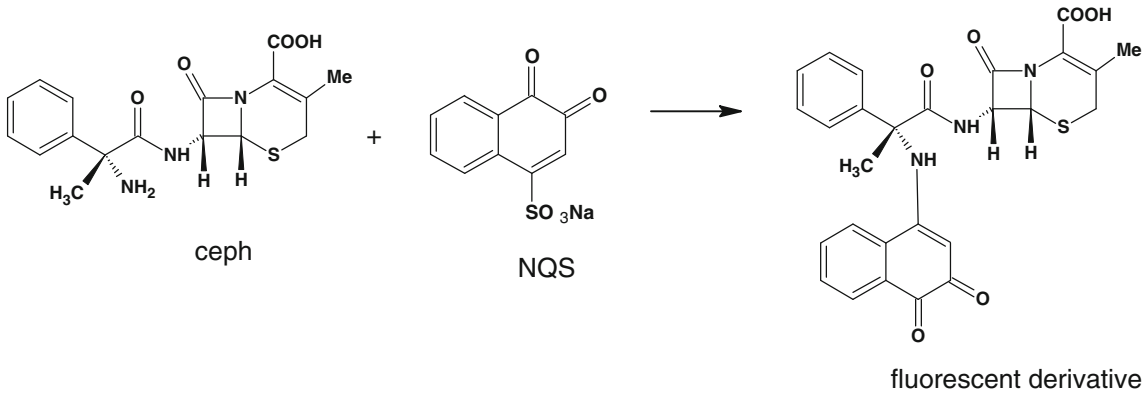


Fig. 6 Job's plots of continuous variation of product: cefi; ceph; cefo; with NQS. Va: NQS (5×10^{-3} M), Vb:(cefi,ceph and cefo) (5×10^{-3} M); Va+Vb=10 mL



Scheme 2 Reaction pathway of ceph with NQS

portions (5 mL) of chloroform the solutions were diluted to volume with chloroform and the fluorescence intensities of the resulting solutions were measured at 600,580 and 580 nm after excitation at 520,455 and 490 nm for cefi, ceph and cefo respectively against a reagent blank prepared in the same manner. The extraction step was necessary because the aqueous NQS was found to be fluorescence, so extraction is necessary to avoid interference [28].

Determination of the Stoichiometric Ratio of the Reaction

Job's Method The Job's method of continuous variation was employed [29]. Equimolar (5×10^{-3} M) methanolic solutions of cefi and aqueous of ceph and cefo and (NQS) were prepared. Series of 10-mL portions of the stock solutions of cefi, ceph and cefo and NQS were made up comprising different complementary proportions (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3,8:2, 9:1, 10:0), The solution was further treated as described under the general recommended procedure.

Result and Discussion

Absorption Spectra

According to the procedure the absorption spectrum of products produced by the reaction of cefi, ceph and cefo with (NQS) are recorded in (Fig. 2). The maximum absorption wave length peak (λ_{max}) at 520,455,and 490 nm, for cefi, ceph and cefo, respectively and the λ_{max} of (NQS) was 360 nm . The derivatives exhibits maximum fluorescence intensities λ_{max} at 600,580 and 580 nm after excitation at 520,455 and 490 nm for cefi, ceph and cefo respectively Figs. 3, 4 and 5.

Determination of Stoichiometric Ratio

Under the optimum conditions shown in Table 2, the stoichiometric ratio between (NQS) and each of investigated cephalosporins was found to be 1: 1 Fig. 6. Based on this ratio, the reaction pathways were postulated to be proceeded

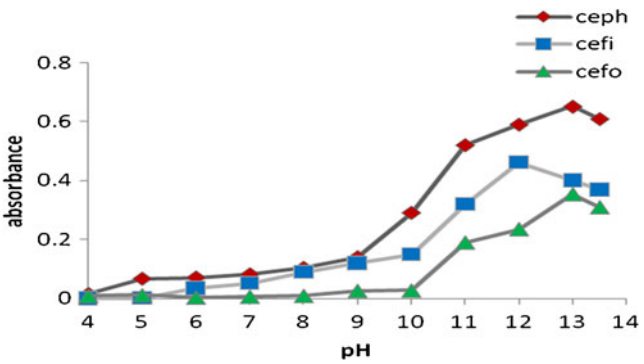


Fig. 7 Effect of pH on absorbance of product cefi; ceph; cefo; with NQS

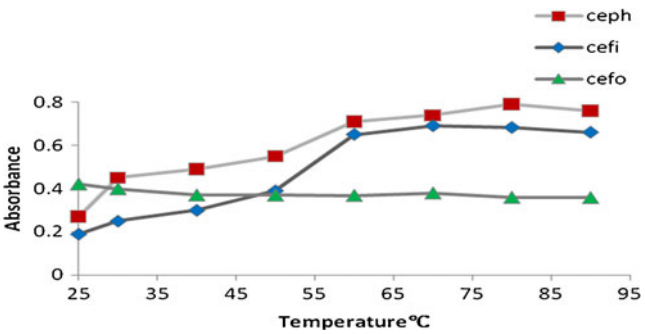


Fig. 8 Effect of temperature on absorbance of product cefi; ceph; cefo with NQS

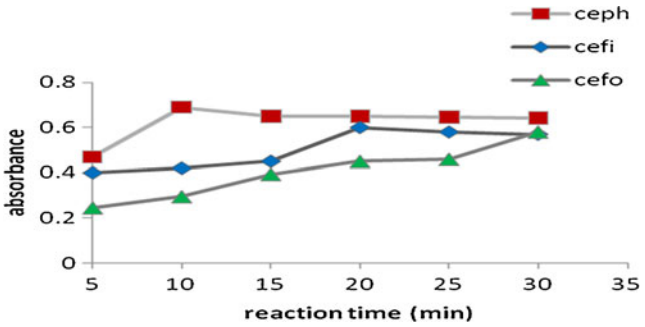


Fig. 9 Effect of reaction time on absorbance of product cefi; ceph; cefo with NQS

as shown in Scheme 2 indicating that the cephalosporins used in this study are susceptible for reaction with NQS producing fluorescent products.

Optimization of Derivatization Reaction and Spectrophotometric Procedure

In order to optimize the reaction conditions between the NQS and cephalosporins the following parameters were investigated: pH of the buffer, reaction time and temperature and NQS concentration.

Effect of pH

The effects of pH on the reaction of cefi,ceph and cefo with (NQS) were examined by varying the pH from 4.0 to 13.0, The results revealed that cefi,ceph and cefo have difficulty to react with (NQS) in acidic media (Fig. 7).This was possibly due to the existence of the amino group of cefi, ceph and cefo in the form of hydrochloride salt, thus it loses its nucleophilic substitution capability. As the pH increased, the readings increased rapidly, as the amino group of cefi, ceph and cefo (in the hydrochloride salt) turns into the free amino group, thus facilitating the nucleophilic substitution. The maximum readings were attained at pH values of 12.0 for cefi and 13.0 for ceph and cefo. At pH values more than 12.0 for cefi and more than 13.0 for ceph and cefo, a decrease in the readings occurred. This was attributed probably to the increase in the amount of hydroxide ion that holds back the reaction of cefi, ceph and cefo with NQS.

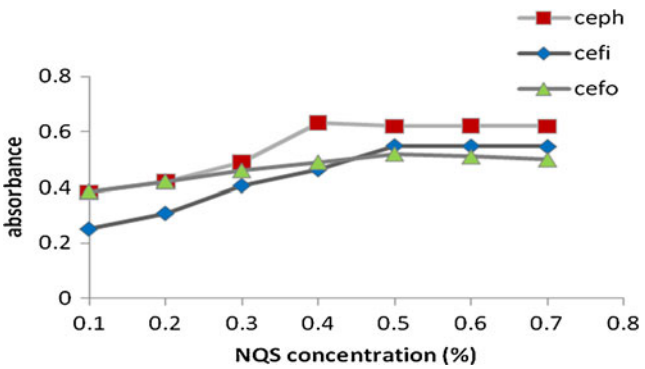


Fig. 10 Effect of NQS concentration on absorbance of product cefi; ceph; cefo with NQS

Table 3 Summary of quantitative parameters of the proposed method

parameter	Drug NQS derivitives		
	Cefi	ceph	cefo
λex/λem	520/600	455/560	490/580
Linear range(ng/ml)	10_35	10_60	20_45
LOD(ng/ml)	2.02	2.09	2.30
LOQ (ng/ml)	6.114	6.350	7.000
Slope	0.4567	0.2911	0.2120
Intercept	24.7950	35.3600	25.0261
Correlation coefficient(r)	0.99871	0.99947	0.99913

Effect of Reaction Temperature and Time

The effect of temperature on the reaction was also studied by varying the temperature from 25 °C to 90 °C for cefi and ceph. The reaction does not proceed at room temperature and the highest absorbance is obtained at 70 °C for 20 min for cefi and at 80 °C for 10 min for ceph. However, for cefo it was found that the reaction with NQS was not affected by increasing the temperature, and the reaction at room temperature (25±5 °C) went to completion in 25 min (Figs. 8 and 9).

Effect of NQS Concentration

The studying of NQS concentrations revealed that the reaction was dependent on NQS reagent. The highest absorption

Table 4 Recovery of the proposed methods

drug	Sample content (ng/mL)	Added (ng/mL)	found (ng/mL)	Recovery (% ± RSD)[a]
cefi	10	10	19.10	95.50±1.10
	10	15	26.76	107.04±2.00
	10	25	35.72	102.05±0.51
ceph	20	10	30.30	101.00±0.18
	20	20	38.06	95.17±0.69
	20	40	57.60	96.01±0.29
cefo	10	10	19.00	95.00±1.74
	10	20	32.22	107.40±0.67
	10	20	43.82	109.55±0.75

[a] Recovery was calculated as the amount found/amount taken × 100. ± R.S.D. Values are mean for triplicate runs.

Values are mean of 3 determinations

Table 5 Robustness of the proposed method

condition	Cefi concenration (20 ng/mL)	Recovery% ± SD[a]	Ceph concentration (30 ng/mL)	Recovery% ± SD[a]	Cefo concentration (20 ng/mL)	Recovery% ± SD[a]
Standard condition		96.23±0.53		99.28±1.60		96.64±0.23
pH	11.8	96.77±0.10	12.8	96.63±0.10	12.8	100.04±0.20
	12.2	98.74±0.40	13.2	94.70±0.25	13.2	98.63±0.56
NQS concentration (wt/v%)	0.45	102.24 ±0 .15	0.35	95.03±0.51	0.45	97.06±0.46
	0.55	97.10±0.72	0.45	108.88±0.50	0.55	104.926±0.44
temperature °C	65	96.33±0.81	75	95.49±0.54	25	96.59±0.64
	75	99.62±0.53	85	101.67±0.75	35	103.35±0.44
Reaction time(min)	18	94.80±0.49	8	95.02±0.74	23	96.12±0.54
	22	101.80±0.56	12	104.18±0.87	28	103.51±0.25

[a] values are mean of 3 determinations

was attained when the concentration of NQS was 0.5% for cefi and cefo and 0.4% for ceph (Fig. 10).

From the previously described experiments the optimum conditions for the reaction of NQS with cefi, ceph and cefo are summarized in Table 2.

Validation of the Methods

Linearity and Limits of Detection

In the proposed method, linear plots (n=6) with good correlation coefficients were obtained in 10–35, 10–60 and 20–45 ng/mL for cefi,ceph and cefo, respectively. As shown in Table 3. The limit of detection (LOD) was calculated based on the standard deviation of response and the slope of the calibration curve [30]. The limit of detection was expressed as:

$$LOD = 3.3Q/S$$

Where Q is the standard deviation of intercept and S is the slope of the calibration curve. The LOD values were 2.018, 2.090 and 2.30 for cefi, ceph and cefo, respectively.

Accuracy and Precision

The accuracy and precision of the proposed method were determined at three concentration levels of cefi, ceph and cefo by analyzing three replicate samples of each concentration. The relative standard deviations (RSD.) for the results did not exceed 2% as shown in Table 4, indicating the high reproducibility of the results and the precision of the method. This good level of precision was suitable for quality control analysis of cefi, ceph and cefo in their pharmaceutical formulations.

Robustness

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the procedures; recovery values were shown in Table 5.

Applications of the Methods

The proposed method was applied to some pharmaceutical formulations containing cefi, ceph and cefo. The results are shown in Table 5, Indicate the high accuracy of the proposed method for the determination of the studied drugs. The proposed method has the advantage of being virtually free from interferences by excipients. The percentages were 109.24±0.207, 101.08±0.180 and 102.85%±0.424 for cefi, ceph and cefo, respectively Table 6.

Conclusions

The present paper described the evaluation of NQS as an analytical reagent in the development of simple, sensitive,

Table 6 Determination of the studied drugs in their pharmaceutical dosage forms

drug	Pharmaceutical product	Percentage ± SD[a]
cefi	200 mg of cefi/capsule	109.24±0.207
ceph	500 mg of ceph monohydrate/capsule	101.08±0.180
Cefo	1000 mg of cefo/injection	102.85%±0.424

[a] values are mean of 5 determinations

and accurate spectrofluorimetric method, for the determination of cefi, ceph and cefo in pharmaceutical formulations. The described method is superior to the previously reported spectrophotometric methods in terms of the simplicity and sensitivity. The proposed method has comparable analytical performances and devoid from any potential interference. This gives the advantage of flexibility in performing the analysis on any available instrument. Therefore, this method can be recommended for the routine analysis of cefi, ceph and cefo in quality control laboratories.

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