Arabian Journal of Chemistry (2015) 8, 233–239

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

New spectrophotometric method for determination of cephalosporins in pharmaceutical formulations

Shazalia M. Ali Ahmed [a], Abdalla A. Elbashir [a,*], Hassan Y. Aboul-Enein [b,*]

[a] *University of Khartoum, Faculty of Science, Chemistry Department, Khartoum, Sudan*
[b] *Pharmaceutical and Medicinal Chemistry Department, National Research Centre, Dokki, Cairo 12311, Egypt*

Received 11 May 2011; accepted 20 August 2011
Available online 27 August 2011

KEYWORDS

Cephalosporins;
1,2-Naphthoquinone-4-sulfonic (NQS);
Spectrophotometric analysis;
Pharmaceutical analysis

Abstract A simple, accurate, and precise spectrophotometric method has been proposed for the determination of three cephalosporins, namely: cefixime (cefi), cephalexine, (ceph) and cefotaxime (cefo) in pharmaceutical formulations. Proposed method is based on the derivatization of cephalosporins with 1,2-naphthoquinone-4-sulfonic (NQS). The optimum experimental conditions have been studied. Beer's law is obeyed over the concentration of 0.5–3, 0.8–2.8, and 0.2–1.2 μg/mL for cefi, ceph, and cefo, respectively.

The detection limits were 0.12, 0.168, and 0.0465 μg/mL for cefi, ceph, and cefo, respectively, with a linear regression correlation coefficient of 0.9993, 0.9993, and 0.9994 and recovery in range from 96.5–102.3, 96.04–102.22, and 97.09–99.3 for cefi, ceph, and cefo, respectively. Effects of pH, temperature, reaction time, and NQS concentration on the determination of cefi, ceph, and cefo, have been examined. This method is simple and can be applied for the determination of cefi, ceph, and cefo in pharmaceutical formulations in quality control laboratories.

1. Introduction

Cephalosporin antibacterials are commonly used to control Gram positive and Gram negative bacterial infections. Cephalosporins are the second most important β-lactams after penicillin for treating infectious diseases (Adkinson and Weiss, 1988). Many of these manifestations, such as urticaria and exanthema, are cutaneous, but anaphylactic reactions have also been reported (Pumphrey and Davis, 1999).

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 (Delgad and Wilson, 2004; Dollery,1999).

Several methods have been described for the quantitative determination of cephalosporins included spectrophotometry (Ayad et al.,1999; Saleh et al., 2001, 2003), spectrofluorimetry (Aly et al.,1996), high performance liquid chromatography (Baranowska et al., 2006; Chen et al., 2003; De Diego Glaria et al., 2005; Misztal, 1998; Moore et al.,1991; Sørensen and

* Corresponding authors. Tel.: +20 103678948; fax: +20 233370931 (H.Y. Aboul-Enein).
E-mail addresses: haboulenein@yahoo.com, hajaae@yahoo.com (A. A. Elbashir), enein@gawab.com (H.Y. Aboul-Enein).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2011.08.012

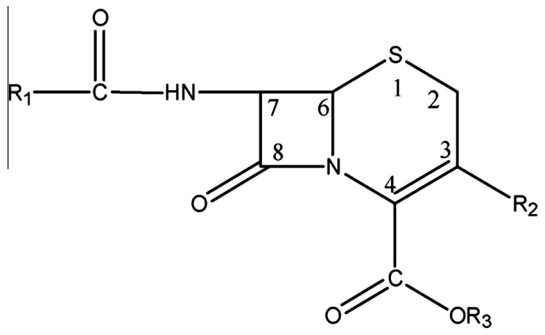


Figure 1 Cephalosporin.

Snor, 2000; Tsai and Chen, 2000), potentiometry, (Lima et al., 1998) and voltammetry (Özkan et al., 2000). 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 the determination of cephalosporins in their pharmaceutical formulations were associated with some major disadvantages, such as lack of selectivity, tedious extraction procedures, and time-consumption. The official procedures in pharmaceutical preparations utilize high performance liquid chromatography (HPLC) (United States Pharmacopoeia, 2008; United States Pharmacopoeia, 2008).Therefore, the development of new alternative spectrophotometric methods for the determination of cephalosporins that can overcome the disadvantages of the existing methods is essential.

(NQS) has been used for the determination of many compounds. It is a popular spectrophotometric reagent due to its efficient reactivity with both primary and secondary amines, and high reaction rate (Darwish, 2005; Darwish et al., 2009; El-Walily et al., 2000; Hasani et al., 2007; Li and Zhang, 2008; Li and Yang, 2007; Wang et al., 2004). NQS proved to be a useful and sensitive analytical derivatizing agent for spectrophotometric analysis of pharmaceuticals bearing a primary or secondary amino group, however the use of (NQS) for spectrophotometric determination of cephalosporins was not reported. Therefore in this work a rapid spectrophotometric 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 orange compounds, at 521, 455, and 493 nm for cefi, ceph and cefo, respectively. The chemical structure for the cephalosporins used in this study is shown in Table 1.

2. Experimental

2.1. Apparatus

All of the spectrophotometric measurements were made with a Double beam UV1800 ultraviolet–visible spectrophotometer provided with matched 1-cm quartz cells (SHIMADZU Japan) also temperature controller was used for the spectrophotometer measurements. pH meter model pH 211 (HANNA Italy) was used for adjusting pH.

2.2. Reagents and solutions

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

2.2.1. 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.

2.2.2. 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 of 0.5–3, 0.8–2.8, and 0.2–1.2 μg/mL for cefi, ceph, and cefo, respectively.

2.2.3. 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.

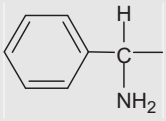
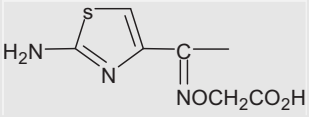
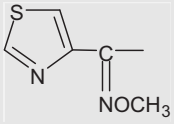
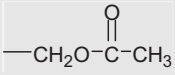
2.2.4. Buffer solutions

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 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.

2.2.5. Sample solutions

The contents of 20 capsules or the contents of 20 injection powder were evacuated and well mixed. Then an accurately weighed amount equivalent to 100 mg was transferred into a 100 mL calibrated 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 completed to volume with methanol for cefi and with double water for ceph and cefo. The contents were mixed well and filtered rejecting the first portion of the filtrate. The 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.

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

2.2.6. General recommended procedure

About 1 mL of (5–30, 8–28 and 2–12 μg/mL) for cefi, ceph, and cefo, respectively, were transferred into 10 mL 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 mixture was diluted with methanol for cefi or double distilled water for ceph and cefo. The absorbance of the solution was measured at 521, 455, 493 nm for cefi, ceph, and cefo, respectively against a reagent blank prepared in the same manner but containing no drugs.

2.2.7. Determination of the stoichiometric ratio of the reaction Job's method

The Job's method of continuous variation was employed. Equimolar (5×10^{-3} M) aqueous solutions of cefi, ceph, and cefo and (NQS) were prepared. Series of 10-mL portions of the master 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.

3. Results and discussion

3.1. 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 521, 455, and 493 nm, for cefi, ceph, and cefo, respectively and the λ_{max} of (NQS) was 360 nm. Obviously cefi, ceph, and cefo have no absorption in the range 400–800 nm, in order to eliminate interference, the determination of products are fixed at 521, 455, and 493 nm, for cefi, ceph, and cefo, respectively against the reagent blank.

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

Condition	Cefi	Ceph	Cefo
pH	12.0	13.0	13.0
Volume of buffer	2 ml	1.5 ml	1 ml
Temperature	70 °C	80 °C	Room temperature
Reaction time	20 min	10 min	25 min
NQS concentration	0.5%	0.4%	0.5%

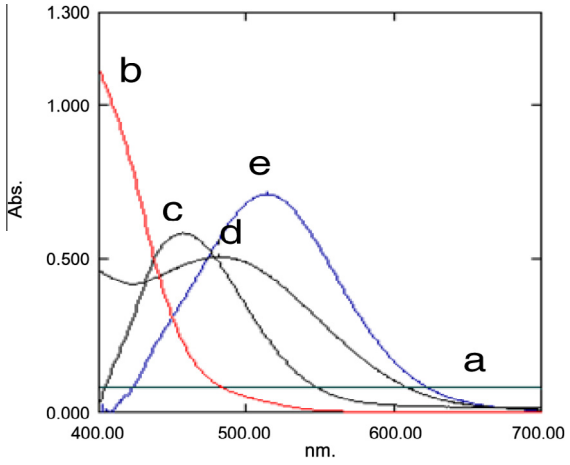


Figure 2 Absorption spectra of (a) cefi, ceph, and cefo (2, 2 and 1.5 μg/mL respectively), (b) Absorption spectra of NQS (0.5%), (c) Absorption spectra of ceph (2 μg/mL) with NQS 0.4%, (d) Absorption spectra of cefo (1.5 μg/mL) with NQS 0.5% and (e) Absorption spectra of cefi (2 μg/mL) with NQS 0.5%.

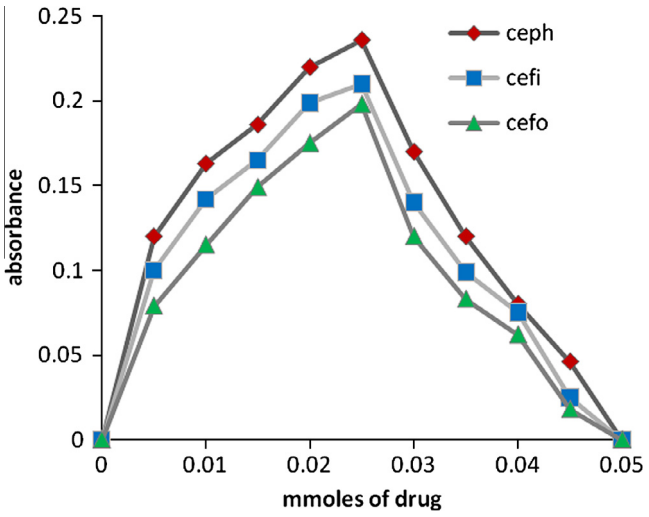


Figure 3 Job's plots of continuous variation of product: cefi; ceph; cefo; with NQS.

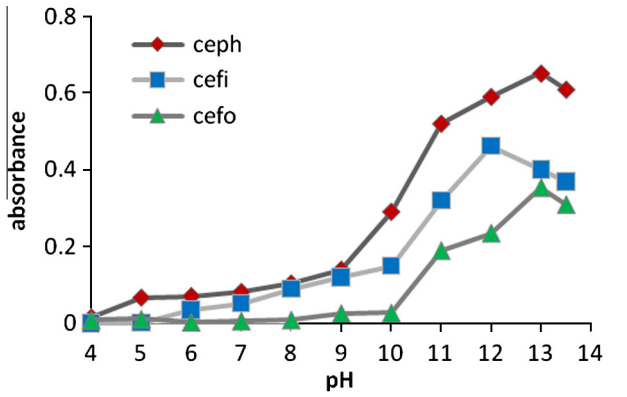


Figure 4 Effect of pH on absorbance of product cefi; ceph; cefo; with NQS.

3.2. 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. 3). Based on this ratio, the reaction pathways were postulated to be proceeded as shown in scheme 1 indicating that the cephalosporins used in this study are susceptible for reaction with NQS producing an orange color product.

3.3. Optimization of derivatization reaction and spectrophotometric procedure

3.3.1. 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. 4). 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.

3.3.2. 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. 5 and 6).

3.3.3. Effect of NQS concentration

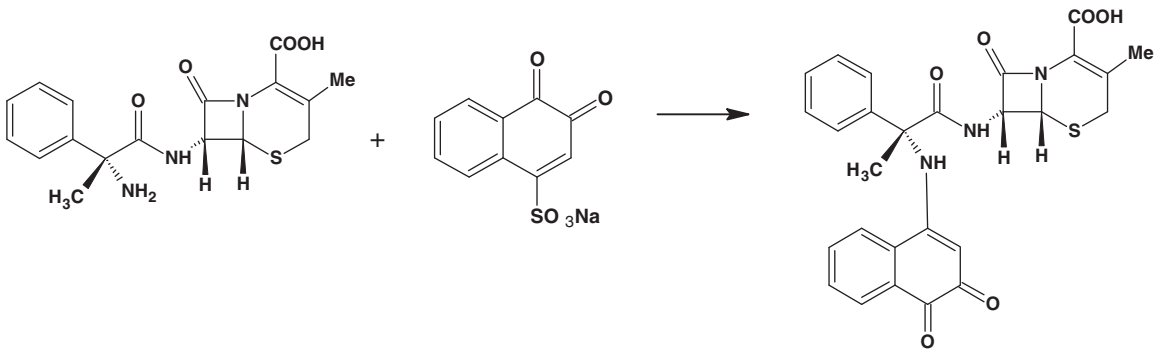
The study of NQS concentrations revealed that the reaction was dependent on NQS reagent. The highest absorption was attained when the concentration of NQS was 0.5% for cefi and cefo and 0.4% for ceph (Fig. 7).

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

3.4. Validation of the methods

3.4.1. Linearity and limits of detection

In the proposed methods, linear plots ($n = 6$) with good correlation coefficients were obtained in the concentration ranges of



Scheme 1 Reaction pathway of ceph with NQS.

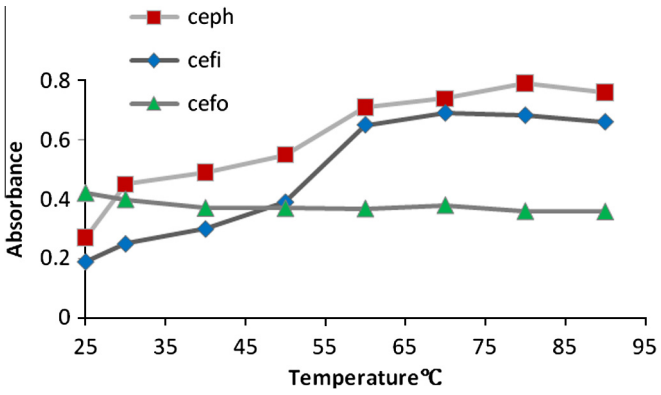


Figure 5 Effect of temperature on absorbance of product cefi; ceph; cefo with NQS.

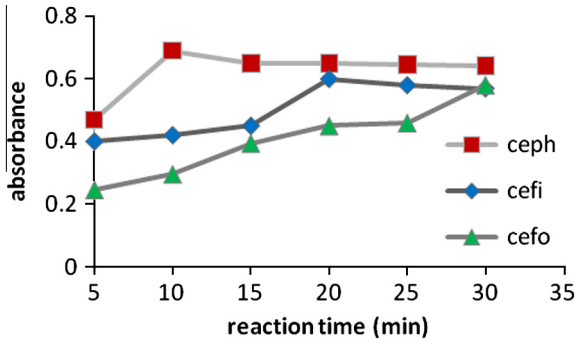


Figure 6 Effect of reaction time on absorbance of product cefi; ceph; cefo with NQS.

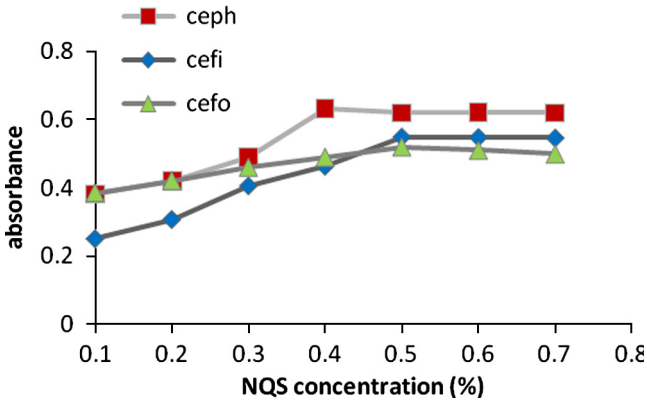


Figure 7 Effect of NQS concentration on absorbance of product cefi; ceph; cefo with NQS.

Table 3 Summary of quantitative parameters and statistical data using the proposed procedure.

Parameter	Drug NQS derivatives		
	Cefi	Ceph	Cefo
Linear range (μg/ml)	0.5–3.0	0.8–2.8	0.2–1.2
LOD (μg/ml)	0.120	0.168	0.047
LOQ (μg/ml)	0.370	0.509	0.140
Slope	0.1954	0.0893	0.3326
Intercept	0.2886	0.3829	0.1352
Correlation coefficient (r)	0.9993	0.9993	0.9994
Molar absorptivity, ε (L mol^{-1} cm^{-1})	1.63×10^5	1.95×10^5	2.9×10^5

Table 4 Recovery of the proposed methods.

Drug	Sample content (μg/mL)	Added (μg/mL)	Found (μg/mL)	Recovery (% ± RSD)*
Cefi	0.6	0.4	0.955	96.50 ± 1.80
	0.6	1.4	1.959	97.90 ± 0.46
	0.6	2.4	3.069	102.30 ± 0.97
Ceph	0.6	0.2	0.728	96.04 ± 1.10
	0.6	1.0	1.594	99.60 ± 2.00
	0.6	2.0	2.658	102.22 ± 1.60
Cefo	0.4	0.1	0.475	97.09 ± 1.77
	0.4	0.3	0.680	98.10 ± 0.69
	0.4	0.6	0.993	99.30 ± 0.54

* Recovery was calculated as the amount found/amount taken × 100. Values are mean ± R.S.D. for three determinations.

0.5–3, 0.8–2.8, and 0.2–1.2 μg/mL for cefi, ceph, and cefo, respectively Table 3. The limits of detection (LOD) and quantitation (LOQ) were determined using the formula: LOD or LOQ = κSDa/b, where κ = 3.3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope. The LOD values were 0.120, 0.168, and 0.047 μg/mL for cefi, ceph, and cefo, respectively.

3.4.2. Accuracy and precision

The accuracy and precision of the proposed spectrophotometric method was determined at three concentration levels of cefi, ceph, and cefo by analyzing five replicate samples of each concentration. The relative standard deviations (RSD) for the results did not exceed 2% (Table 4), proving 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.

3.4.3. Selectivity

The effect of the presence of common excipients such as; starch, lactose, glucose, sucrose, and gum acacia was studied. It was found that no interference was introduced by any of them.

3.4.4. Robustness

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical

Table 5 Robustness of the proposed spectrophotometric method.

Recommended condition	Cefi	Recovery% ± SD*	Ceph	Recovery% ± SD*	Cefo	Recovery% ± SD*
Standard	12.0	98.90 ± 1.08	13.0	99.28 ± 1.60	13.0	99.60 ± 1.00
pH	11.8	102.51 ± 0.87	12.8	102.51 ± 0.79	12.8	104.51 ± 0.69
	12.2	100.34 ± 1.02	13.2	99.58 ± 0.68	13.2	99.34 ± 0.68
NQS concentration (wt/v%)	0.45	99.23 ± 0.56	0.35	99.23 ± 0.67	0.45	98.23 ± 0.87
	0.55	101.49 ± 0.39	0.45	103.59 ± 0.69	0.55	101.89 ± 0.87
Temperature (°C)	65	99.48 ± 0.98	75	106.48 ± 0.68	25	100.30 ± 0.99
	75	102.86 ± 0.88	85	105.86 ± 0.68	35	100.67 ± 0.58
Reaction time(min)	18	101.46 ± 0.88	8	101.11 ± 0.69	23	101.71 ± 1.54
	22	102.05 ± 0.98	12	103.97 ± 0.69	28	99.98 ± 1.32

* Values are mean of three determinations.

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

Drug	Pharmaceutical product	Percentage ± SD*
Cefi	200 mg of cefi/capsule	102.20% ± 0.019
Ceph	500 mg of ceph monohydrate/capsule	104.49% ± 0.009
Cefo	1000 mg of cefo/injection	101.25% ± 0.012

* Values are mean of five determinations.

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.

3.5. *Applications of the methods*

The proposed method was applied to some pharmaceutical formulations containing cefi, ceph, and cefo. The results shown in Table 6 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 102 ± 0.0186, 104 ± 0.009, 101.25 ± 0.012 for cefi, ceph, and cefo, respectively.

4. Conclusions

The present paper described the evaluation of NQS and as an analytical reagent in the development of simple, sensitive, and accurate spectrophotometric methods, 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.

References

Adkinson, N.F., Weiss, M., 1988. Immediate hypersensitivity reactions to penicillin and related antibiotics. Clin. Allergy 18, 515–540.

Aly, F.A., Hefnawy, M.M., Belal, F., 1996. A selective spectrofluorimetric method for the determination of some α-aminocephalosporins in formulations and biological fluids. Anal. Lett. 29, 117–130.

Ayad, M.M., Shalaby, A.A., Abdellatef, H.E., Elsaid, H.M., 1999. Spectrophotometric and atomic absorption spectrometric determination of certain cephalosporins. J. Pharm. Biomed. Anal. 18, 975–983.

Baranowska, I., Markowski, P., Baranowski, J., 2006. Simultaneous determination of 11 drugs belonging to four different groups in human urine samples by reversed-phase high-performance liquid chromatography method. Analytica Chimica Acta 570, 46–58.

Chen, X., Zhong, D., Huang, B., Cui, J., 2003. Determination of cefaclor in human plasma by a sensitive and specific liquid chromatographic-tandem mass spectrometric method. J. Chromatogr. B: Anal. Technologie Biomed. Sci. 784, 17–24.

Darwish, I.A., 2005. Kinetic spectrophotometric methods for determination of trimetazidine dihydrochloride. Analytica Chimica Acta 551, 222–231.

Darwish, I.A., Abdine, H.H., Amer, S.M., Al-Rayes, L.I., 2009. Spectrophotometric stud y for the reaction between fluvoxamine and 1,2-naphthoquinone-4-sulphonate: kinetic, mechanism and use for determination of fluvoxamine in its dosage forms. Spectrochimica Acta – Part A: Mol. Biomol. Spectrosc. 72, 897–902.

De Diego Glaría, M., Moscciati, G.G., Ramos, R.G., 2005. Determination of ceftriaxone in cerebrospinal fluid by ion-pair liquid chromatography. J. AOAC Intl. 88, 436–439.

Delgad, J.N., Wilson, W.A., 2004. Textbook of Organic Medicinal and Pharmaceutical Chemistry. Lippincott Williams & Wilkins, New York (tenth ed.).

Dollery, C., 1999. Therapeutic drugs. Vol. I. third ed. Churchill Livingstone, Edinburgh.

El-Walily, A.F.M., Gazy, A.A., Belal, S.F., Khamis, E.F., 2000. Quantitative determination of some thiazole cephalosporins through complexation with palladium (II) chloride. J. Pharm. Biomed. Anal. 22, 385–392.

Hasani, M., Yaghoubi, L., Abdollahi, H., 2007. A kinetic spectrophotometric method for simultaneous determination of glycine and lysine by artificial neural networks. Anal. Biochem. 365, 74–81.

Li, Q., Zhang, H., 2008. A novel spectrophotometric method for the determination of aminophylline in pharmaceutical samples in the presence of methanol. Spectrochimica Acta – Part A: Mol. Biomol. Spectrosc. 70, 284–289.

Li, Q.-M., Yang, Z.-J., 2007. Spectrophotometric determination of aminomethylbenzoic acid using sodium 1, 2-naphthoquinone-4-sulfonate as the chemical derivative chromogenic reagent. Spectrochimica Acta – Part A: Mol. Biomol. Spectrosc. 66, 656–661.

Lima, J.L.F.C., Montenegro, M.C.B.S.M., Sales, M.G.F., 1998. Cefuroxime selective electrodes for batch and FIA determinations in pharmaceutical preparations. J. Pharm. Biomed. Anal. 18, 93–103.

Misztal, G., 1998. Determination of cefotaxime and ceftriaxone in pharmaceuticals by HPLC. Pharmazie 53, 723–724.

Moore, C.M., Sato, K., Katsumata, Y., 1991. High-performance liquid chromatographic determination of cephalosporin antibiotics using 0.3 mm I.D. columns. J. Chromatogr. 539, 215–220.

Özkan, S.A., Erk, N., Uslu, B., Yilmaz, N., Biryol, I., 2000. Study on electrooxidation of cefadroxil monohydrate and its determination by differential pulse voltammetry. J. Pharm. Biomed. 23, 263–273.

Pumphrey, R.S.H., Davis, S., 1999. Under-reporting of antibiotic anaphylaxis may put patients at risk. Lance 353, 1157–1158.

Saleh, G.A., Askal, H.F., Darwish, I.A., El-Shorbagi, A.N.A., 2003. Spectroscopic analytical study for the charge-transfer complexation of certain cephalosporins with chloranilic acid. Anal. Sci. 19, 281–287.

Saleh, G.A., Askal, H.F., Radwan, M.F., Omar, M.A., 2001. Use of charge-transfer complexation in the spectrophotometric analysis of certain cephalosporins. Talanta 54, 1205–1215.

Sørensen, L.K., Snor, L.K., 2000. Determination of cephalosporins in raw bovine milk by high-performance liquid chromatography. J. Chromatogr. A 882, 145–151.

Tsai, T.H., Chen, Y.F., 2000. Simultaneous determination of cefazolin in rat blood and brain by microdialysis and microbore liquid chromatography. Biomed. Chromatogr. 14, 274–278.

United States Pharmacopoeia, 2008. 31 and NF 26, American Pharmaceutical Association, Washington, DC.

Wang, H.Y., Xu, L.X., Xiao, Y., Han, J., 2004. Spectrophotometric determination of dapsone in pharmaceutical products using sodium 1,2-naphthoquinone-4-sulfonic as the chromogenic reagent. Spectrochimica Acta – Part A: Mol. Biomol. Spectrosc. 60, 2933–2939.