Research article

Drug Testing and Analysis

Received: 12 December 2010 Revised: 7 February 2011 Accepted: 16 February 2011 Published online in Wiley Online Library: 28 April 2011

(www.drugtestinganalysis.com) DOI 10.1002/dta.280

Spectophotometric methods for determination of cefdinir in pharmaceutical formulations via derivatization with 1,2-naphthoquinone-4-sulfonate and 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole

Ayman A. Gouda,[a]* Hisham Hashem[b] and Wafaa Hassan[b]

Two new simple, sensitive, accurate, and precise spectrophotometric methods have been developed and validated for the determination of cefdinir (CFD) in bulk drug and in its pharmaceutical formulations. The first method was based on the reaction of CFD with 1, 2- napthaquinone-4- sulfonic acid sodium (NQS) in an alkaline medium (pH 11) to form an orange-coloured product that was measured at 490 nm. The second method depends on hydrolysis of CFD using 0.5 M NaOH at 100 °C and subsequent reaction of the formed sulfide ions with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) to form a yellow-coloured chromogen measured at 390 nm. Different variables affecting the reactions of CFD with both NQS and NBD-Cl (e.g. NaOH concentration, hydrolysis time, NQS or NBD-Cl concentration and diluting solvent) were studied and optimized. Under optimum conditions, good linear relationships with good correlation coefficients (0.9990–0.9999) were found in the range of 10–80 and 5.0–30 μg ml^{-1} for NQS and NBD-Cl, respectively. The limits of assay detection and quantitation ranged from 1.097 and 0.280 and 3.656 and 0.934 μg ml^{-1} for NQS and NBD-Cl, respectively. The accuracy and precision of the proposed methods were satisfactory. The proposed method is simple, rapid, precise and convenient and was successfully applied for analysis of CFD in its pharmaceutical formulations and the recovery percentages ranged from 99.25 to 100.20%.

Keywords: spectrophotometry; cefdinir; NQS; NBD-Cl; pharmaceutical formulations.

Introduction

Chemically, cefdinir (CFD) is [6R-[6α, 7β (Z)]]-7-[[(2- amino-4-thiazolyl) (hydroxyimino) acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (Figure 1). It is a semi-synthetic, broad-spectrum, third-generation cephalosporin. The empirical formula of CFD is $C_{14}H_{13}N_5O_5S_2$ with a molecular weight of 395.42.[1] It has a broad spectrum of activity, excellent therapeutic action against susceptible Gram-positive and Gram-negative bacteria as having potent antimicrobial activity, excellent efficacy, convenient dosing and favourable tolerability compared with other antimicrobial agents. The official monograph of the drug is presently available only in Japanese Pharmacopoeia (2001) using high performance liquid chromatography (HPLC) method for assay of CFD.[2]

In the literature, some other RP-HPLC methods have been reported for the analysis of CFD in formulations, biological samples and a few methods also offer separation of drug from related impurities.[3–11] Liquid chromatography electrospray ionization tandem mass spectrometry has been reported for analysis of the drug in human plasma[12,13] and environmental samples.[14] Other methods of analysis available in the literature are based on electrochemical technique (voltametry)[15–18] and spectrofluorimetry.[19] These sophisticated instrumental methods are not suitable for counterfeit drug assay as they are expensive and cannot be used in the field.

A simple, inexpensive, selective, and rugged visible spectrophotometric would be more appropriate for the analysis of counterfeits. The spectrophotometric methods reported in the literature for the analysis of CFD are based on UV measurement,[20–22] derivative spectrophotometric,[22–23] a method involving oxidation of CFD with excess of N-bromosuccinimide and estimating unreacted N-bromosuccinimide either with celestine blue or *p*-N-Methyl amino phenol sulfate – sulfanilamide,[24] a method based on reduction of ferric ion with CFD and complexation of resultant ferrous ions with 1, 10-phenanthroline to form a blood-red chromogen,[25] reaction of CFD with Folin-Ciocalteu reagent under alkaline condition to from a blue-coloured chromogen [22] and a method based on the hydrolysis of β-lactum ring of CFD with sodium hydroxide which subsequently reacts with iodate to liberate iodine in acidic medium.[26]

* *Correspondence to: Ayman A. Gouda, agazig University, Faculty of Science, Chemistry Department, Zagazig, 44519, Egypt.*
E-mail: aymanchimca@hotmail.com and aymanchimca@yahoo.com

a *Zagazig University, Faculty of Science, Chemistry Department, Zagazig, 44519, Egypt*

b *Zagazig University, Faculty of Pharmacy, Analytical Chemistry Department, Zagazig, Egypt*

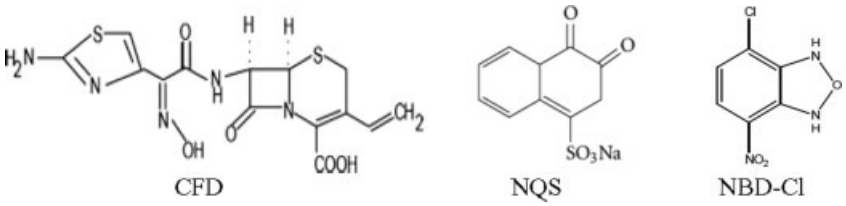


Figure 1. Structural formula of CFD, NQS, and NBD-Cl.

A non-extractive visible spectrophotometric method based on the formation of donor-acceptor complex between CFD and Fe in a buffered medium (pH 11).[27] The UV spectrophotometric method, although sensitive, is not suitable for the analysis of counterfeits due to lack of selectivity. All visible spectrophotometric methods reported are indirect and lack selectivity.

1, 2-naphthoquinone-4-sulfonic sulfonate (NQS) (Figure 1) has been used as a chromogenic reagent for the spectrophotometric determination of many pharmaceutical amines.[28–30]

The literature reveals that many spectrophotometric methods were developed for determination of some cephalosporins based on hydrolysis of these cephalasporins using alkaline degradation and subsequent reaction of the formed sulfide ions with chromogenic reagents.[31–32]

4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) as an electroactive halide reagent, which was considered as a likely target for good nuclophiles, under alkaline conditions (Figure 1) has been reported as an analytical chromogenic reagent for spectrophotometric determination of many pharmaceutical compounds.[33–44]

Visible spectrophotometry, because of simplicity and cost effectiveness, sensitivity and selectivity, fair accuracy, precision, and availability in most quality control laboratories, has remained competitive in an area of chromatographic techniques for pharmaceutical analysis. Furthermore, it does not need costly instrumentation required for published HPLC methods. On the basis of the aforementioned reasons, it was decided to develop a quantitative method for the determination of CFD based on the reaction with NQS in an alkaline medium (pH 11) and alkaline hydrolysis and subsequent reaction of the resulting hydrolysate with NBD-Cl, which may be used for simple and rapid spectrophotometric analysis of CFD either in pure form or in pharmaceutical formulations.

Results and discussion

Derivatization using NQS and NBD-Cl has attracted considerable attention for quantitative analysis of many pharmaceutically active compounds. In the present investigation, NQS and NBD-Cl form coloured complexes with CFD in alkaline conditions and their absorbances were measured at 490 and 390 nm, respectively. Because of the presence of amine as chromophoric group in CFD molecule, derivatization of this compound was attempted in the present study for the development of spectrophotometric methods for its determination. NQS and NBD-Cl have been used as chromogenic reagents for primary and secondary amines; however, their reaction with CFD has not been investigated yet. Therefore, the present study was devoted to explore NQS and NBD-Cl as derivatizing reagents in the development of spectrophotometric method for the determination of CFD in pharmaceutical dosage forms.

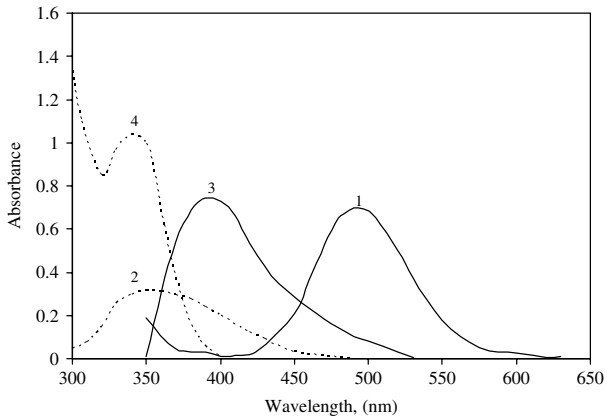


Figure 2. Absorption spectra of (1) the reaction product of CFD with NQS against reagent blank and (2) NQS 0.1% (*w*/*v*) against methanol, (3) the reaction product of CFD with NBD-Cl against reagent blank and (4) NBD-Cl 0.1% (*w*/*v*) against ethanol.

Absorption spectra

According to the procedure, the absorption spectrum of the product produced by the reaction between CFD and NQS was recorded (Figure 2). The product was orange-red-coloured exhibiting a maximum absorption peak (λ_{max}) at 490 nm, and the λ_{max} of NQS was 360 nm.

The absorption spectrum of NBD-Cl in acetone shows a maximum absorption at 340 nm. The interaction coloured product of CFD hydrolysate (sulfide ions resulted from the alkaline degradation of CFD) with NBD-Cl shows absorption maximum at 390 nm (Figure 2). In order to eliminate the interference, the measurements were carried out at 490 and 390 nm against the reagent blank for NQS and NBD-Cl, respectively.

Optimization of reaction variables

Effect of reagents concentration

The influence of the concentration of NQS and NBD-Cl was studied using different volumes of 0.5% NQS or 0.1% NBD-Cl solutions of the reagent. It was found that the reaction of NQS or NBD-Cl with CFD started upon using 0.2 ml of the reagent. Increasing the volume of the reagent produces a proportional increase in the absorbance of the reaction product up to 0.8 ml and remains constant till 1.2 ml, after which further increase produces a gradual decrease in the absorbance value or remain constant. Therefore, 1.0 ml of 0.5% NQS or 0.1% of NBD-Cl solution was chosen as the optimal volume of the reagent (Figure 3).

Effect of NaOH concentration

To generate the nucleophiles from CFD and activate the nucleophilic substitution reactions, alkaline medium was necessary.

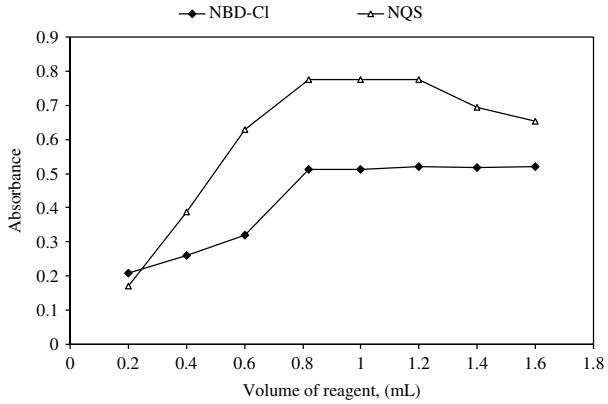


Figure 3. Effect of volume of NQS (0.5%) or NBD-Cl (0.1%) on the absorbance value of the reaction coloured product of CFD (50 μg ml^{-1}) with NQS at 490 nm or (20 μg ml^{-1}) with NBD-Cl at 390 nm.

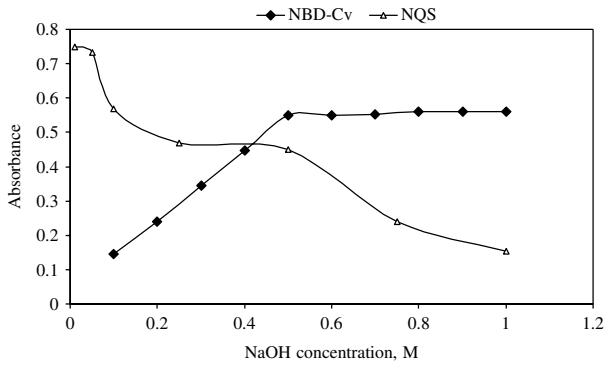


Figure 4. Effect of NaOH concentration on the absorbance of the reaction coloured product of CFD (50 μg ml^{-1}) with NQS at 490 or (20 μg ml^{-1}) with NBD-Cl at 390 nm.

Different inorganic bases were tested: sodium hydroxide, disodium hydrogen phosphate, and sodium bicarbonate, all prepared as aqueous solutions in a concentration range of 0. 01 – 1.0 M. The Best results were obtained in the case of sodium hydroxide, where, with other bases, either precipitation of white colloid occurred upon diluting the reaction solution with organic solvent, or high blank readings, non-reproducible results, and/or weak sensitivity were observed. Studies for the optimization of sodium hydroxide concentration revealed that the optimum concentration was 1.0 ml of 0.01 and 0.5 M solutions in case of NQS and NBD-Cl, respectively gives maximum absorption intensity of the coloured product between CFD and both reagents (Figure 4).

Effect of temperature and reaction time

For NQS

The effect of temperature on the reaction was studied by carrying out the reaction at different temperatures (25 – 90 °C). The results revealed that increasing the temperature had a negative effect on the absorption values of the reaction solution. This was probably attributed to the instability of the CFD-NQS derivative. For this reason, further experiments were carried out at room temperature (25 ± 5 °C). The effect of time on the formation of the reaction product was investigated by carrying out the reaction for different times. The maximum absorbance intensity was attained after 4 min, and longer reaction time up to 30 min did not affect the absorbance intensity (Figure 5). For more precise results, further experiments were carried out at 5 min.

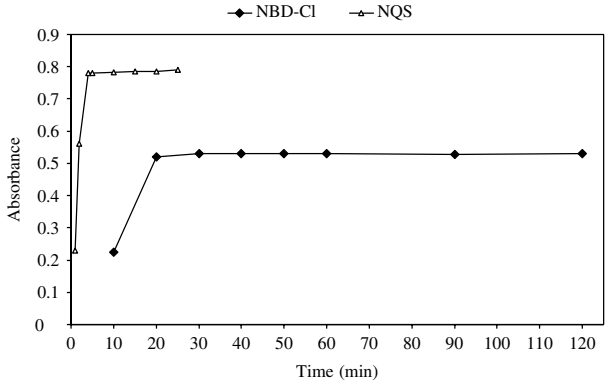


Figure 5. Effect of time on the reaction of CFD (50 μg ml^{-1}) with NQS at 490 or (20 μg ml^{-1}) with NBD-Cl at 390 nm.

For NBD-Cl

The effect of hydrolysis time on the absorption intensity was studied using different heating times in a boiling water bath (at 100 °C) starting from 10 min until 2 h and the reaction was carried out as usual. The obtained absorbance readings were plotted against hydrolysis time. The maximum absorption intensity was attained after 20 min and remained stable for at least 100 min as shown in (Figure 5). In all subsequent experiments, 30 min hydrolysis time was used.

The reaction between CFD hydrolysate and NBD-Cl was very rapid and the interaction coloured product can survive before dilution unchanged for at least 1 h. However, measurements were achieved instantaneously.

Effect of organic solvents

It was found that the CFD-NQS or CFD- NBD-Cl coloured products are insoluble in the aqueous reaction medium. For spectrophotometric measurements, the reaction product might be dissolved in a miscible organic solvent of lower polarity than water. Different solvents were tested for dilution in order to select the most appropriate solvent for optimum colour development: methanol, ethanol, n- propanol, isopropanol, acetone, acetonitrile, dimethylformamaide and 1,4-dioxane. The results given in Table 1 show that absorption intensities were slightly influenced. Methanol and ethanol were used for dilution using NQS and NBD-Cl, respectively, throughout this work because they gave the highest absorbance readings and the most reproducible results.

Stability of the reaction coloured products

Under the aforementioned optimum conditions, the effect of time on the stability of the chromogens 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 chromogens remains stable for at least 3 and 24 h for NQS and NBD-Cl, respectively. This allowed the processing of large batches of samples and their comfortable measurement with convenience. This increased the convenience of the methods as well as making it applicable for a large number of samples.

Table 1. Effect of diluting organic solvents on the intensity of the reaction product of CFD (50 μg ml^{-1}) with NQS and (20 μg ml^{-1}) with NBD-Cl

	Absorbance[a]			
	NQS		***NBD-Cl***	
Solvent	(0.1%, *w*/*v*)	***SD***	(0.5%, *w*/*v*)	***SD***
Methanol	0.795	0.016	0.528	0.015
Ethanol	0.748	0.021	0.562	0.012
Propan-1-ol	0.760	0.019	0.487	0.008
Propan-2-ol	0.773	0.023	0.512	0.013
Acetone	0.432	0.029	0.475	0.014
Acetonitrile	0.659	0.017	0.460	0.016
Dimethylformamaide	0.410	0.012	0.455	0.010
1,4- Dioxane	0.558	0.015	0.442	0.011

[a] Values for all solvents are mean of three determinations; the RSDs for the readings were < 3.

Table 2. Effect of different acids on the absorbance readings of the reaction coloured product of CFD (20 μg ml^{-1}) with NBD-Cl (0.1%, *w*/*v*)

Acid (1.0 ml)	Absorbance[a]	SD
Hydrochloric acid	0.565	0.016
Sulfuric acid	0.450	0.012
Acetic acid	0.308	0.009
Nitric acid	0.425	0.011
Perchloric acid	0.435	0.013

[a] Average of three determinations.

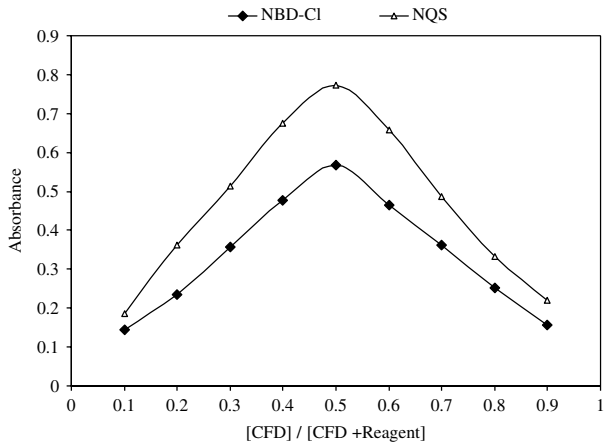


Figure 6. Job's plot for determination of stoichiometry of the reaction between CFD and NQS or NBD-Cl. [CFD]: 5 × 10^{-3} M; [NQS] or [NBD-Cl]: 5 × 10^{-3} M; [CFD]+[NQS]: 1.0 ml.

Effect of type and concentration of acid in case of NBD-Cl

Different acids such as sulfuric, hydrochloric, perchloric, nitric, and acetic acids were tested to determine the most suitable acid for the reaction. One milliliter of concentrated hydrochloric acid was selected in this study as it gave the highest absorbance readings (Table 2).

Further investigations were carried out in order to find the most suitable concentration of hydrochloric acid. It was observed that higher absorbance readings and more reproducible results were obtained upon increasing hydrochloric acid concentration. As a result of these investigations, 1.0 ml of concentrated hydrochloric acid was used for subsequent work.

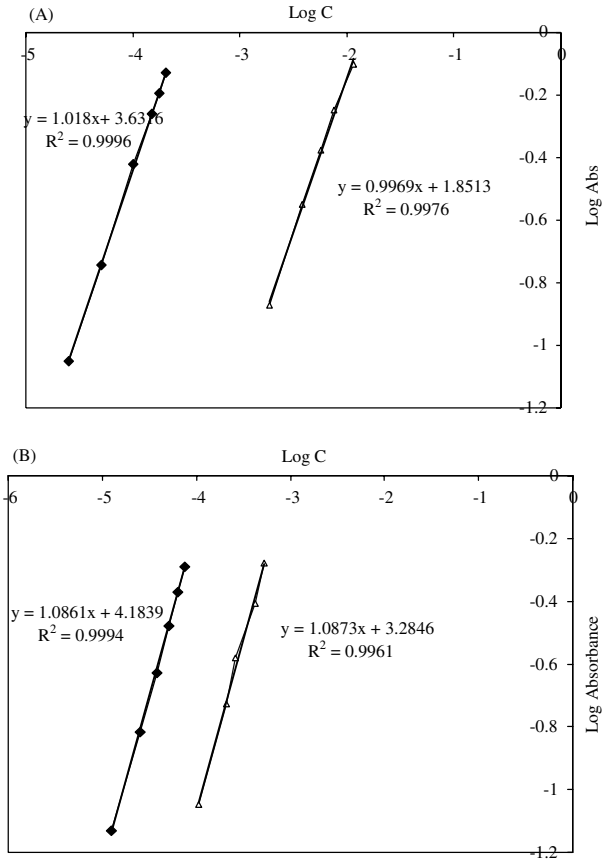


Figure 7. Limiting logarithmic plots for molar reactivity of CFD with (A) NQS and (B) NBD-Cl. *C* and *A* are the concentration and absorbance, respectively.

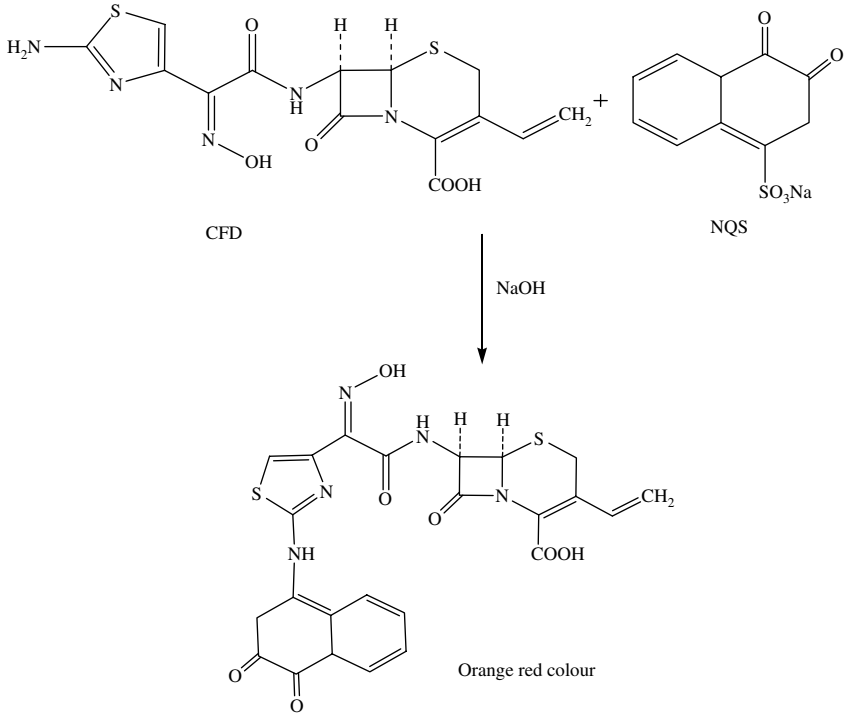
Determination of stoichiometric ratio of the reactions

Under optimum conditions, the stoichiometry of the reaction between CFD and NQS or NBD-Cl was investigated by Job[45] and limiting logarithmic[46] methods. The symmetrical bell shape of Job's plot (Figure 6) indicates that the NQS : CFD and NBD-Cl : CFD ratios were 1 : 1.

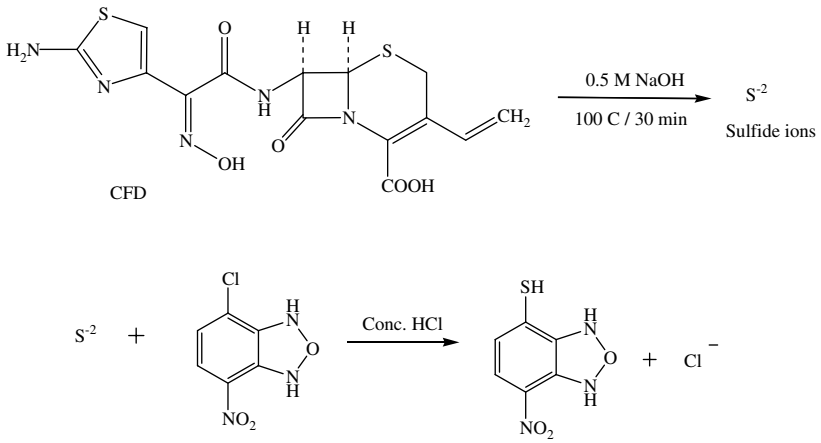
The limiting logarithmic method was employed. Two sets of experiments were carried out employing the general recommended procedures described above. The first set of experiments was carried out using varying concentrations of the analytical reagent with a fixed concentration of CFD. The second set of experiments was carried out using varying concentrations of CFD at a fixed concentration of each reagent. The logarithms of the obtained absorbances were plotted as a function of the logarithms of the concentrations of the reagent and CFD in the first and second sets of experiments, respectively. The slopes of the fitting lines in both sets of experiments were calculated.

In case of NQS, two straight lines were obtained. The values of the slopes of these lines were 0.9969 and 1.018 (Figure 7A).

In case of NBD-Cl, the values of the slopes of these lines were 1.0873 and 1.0861 (Figure 7B), confirming in both cases the 1 : 1 ratio for the reactions. Based on this ratio, and the presence of



Scheme 1A . Suggested reaction mechanism between CFD and NQS.



Scheme 1B . Suggested reaction mechanism between sulfide ions and NBD-Cl.

amino group in CFD molecule that is available for the substitution reaction, the reaction pathway was postulated to be proceeded as shown in Scheme 1 (A & B).

Validation of the methods

The method was validated according ICH guidelines on the validation of analytical methods[47] and complied with USP 31 validation guidelines.[48] All results were expressed as percentages, where *n* represents the number of values. For the statistical analysis Excel 2003 (Microsoft Office) was used. A 5% significance level was selected.

Linearity

Calibration curves for the determination of CFD by its reaction with NQS or NBD-Cl were constructed by plotting the absorbances as a function of the corresponding concentrations. The regression equations for the results were $A = -0.002 + 0.0093C$ ($r = 0.9994$) using NQS and $A = -0.0317 + 0.0182C$ ($r = 0.9997$) using NBD-Cl, where A is the absorbance at 490 and 390 nm, C is the concentration of CFD in $\mu g mL^{-1}$ in the range of 10–80 and 5.0–30 $\mu g mL^{-1}$, using NQS and NBD-Cl, respectively and r is the correlation coefficient. The molar absorptivity (ε) was 0.363×10^4 and 0.618×10^4 L mol^{-1} cm^{-1}, using NQS and NBD-Cl, respectively. For more accurate analysis, Ringbom plots[49] for optimum concentration ranges were obtained. Statistical analysis of the results obtained (Table 3), indicated that the proposed procedures were accurate and precise.

The precision of the proposed methods was determined by analyzing six replicate samples of standard CFD solution at one concentration level. The assay gave satisfactory results; the relative standard deviation (RSD) was less than 2%.

Table 3. Optical characteristics and statistical data for the regression equation of the proposed methods

Parameter	NQS	NBD-Cl
λ_{max} (nm)	490	390
Beer's Law limits (μg mL^{-1})	10-80	5.0–30
Ringbom optimum range, (μg mL^{-1})	15-76	7.5–27
Molar absorptivity (L moL^{-1} cm^{-1})	0.363×10^4	0.618×10^4
Sandell's sensitivity (ng cm^{-2})	108.93	63.98
Regression equation (y)		
Slope, *b*	0.0093	0.0182
Intercept, *c*	−0.002	−0.0317
Correlation coefficient (R)	0.9994	0.9997
Relative standard deviation%	0.451	0.463
Recovery	99.34 ± 0.448	100.05 ± 0.463
Repeatability	0.448	0.463
Limit of detection (μg mL^{-1})	1.097	0.280
Limit of quantification, (μg mL^{-1})	3.656	0.934
t-test [b]	0.52	1.63
F- test[b]	3.83	3.59

$Y = bX + c$, where X is the concentration of drug in μg mL^{-1}; Average of six determinations.
[b] The theoretical values of t and F at P = 0.05 are 2.571 and 6.39, respectively.

Sensitivity

The detection limit (LOD) for the proposed methods was calculated using the following equation:[50]

$$\text{LOD} = 3.3s/k \quad (1)$$

where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits were found to be 1.097 and 0.28 μg ml^{-1} for NQS and NBD-Cl methods, respectively, which indicate high sensitivity of the proposed methods (Table 3).

The limits of quantization, LOQ, is defined as:[50]

$$\text{LOQ} = 10\,s/k \quad (2)$$

According to Eqn 2, the limit of quantization was found to be 3.656 and 0.934 μg ml^{-1} for for NQS and NBD-Cl methods, respectively.

Reproducibility

The reproducibility of the proposed methods was determined by replicate analysis of five separate solutions of the working standards. The methods gave satisfactory results; the relative standard deviations (RSDs) were 1.505 and 1.93% for NQS and NBD-Cl methods, respectively (Table 4), indicating good reproducibility of the proposed methods. This precision level is adequate for the precision and routine analysis of CFD in quality control laboratories.

Accuracy, precision and specificity

Percentage relative standard deviation (RSD%) as precision and percentage relative error (Er %) as accuracy of the suggested methods was calculated. Accuracy and precision was carried out by six determinations at four different concentrations in the proposed spectrophotometric methods. The percentage relative error calculated using the following equation:

Table 4. Replicate analysis of CFD solution by the proposed methods

	Absorbance	
Sample number	NQS (CFD = 50 μg mL^{-1})	NBD-Cl (CFD = 20 μg mL^{-1})
1	0.463	0.335
2	0.471	0.329
3	0.458	0.341
4	0.474	0.333
5	0.459	0.330
Mean	0.465	0.3324
SD	0.007	0.0064
RSD	1.505	1.93

$$\text{Er\%} = [(\text{founded} - \textit{added})/\text{added}] \times 100 \quad (3)$$

The intra-day accuracy and precision results are shown in Table 5. The recovery values were 99.75 ± 0.72–100.20 ± 1.03 and 99.40 ± 0.96–99.73 ± 0.80 % for NQS and NBD-Cl methods. Also, the inter-day accuracy and precision results are shown in Table 4. The recovery values were 99.60 ± 0.34–100.30 ± 0.86 and 99.70 ± 0.56–100.52 ± 0.40% for NQS and NBD-Cl methods. These results indicating good accuracy and precision of the proposed methods show that the proposed methods have good repeatability and reproducibility.

The specificity of the methods was evaluated by investigating the interference liabilities from the common excipients that might be added during pharmaceutical formulation. Samples were prepared by mixing known amount (20 mg) of CFD with various amounts of the common excipients: starch, sucrose, citric acid, glucose, sodium benzoate, gum acacia, and magnesium stearate. These laboratory-prepared samples were analyzed by the proposed methods applying the general recommended procedures. The average recovery values were 99.50 ± 0.80 and 99.20 ± 0.65% for the NQS and NBD-Cl methods, respectively. These data confirmed the absence of interference from any of the common excipients with the determination of CFD by both methods.

Robustness and ruggedness

Robustness was examined by evaluating the influence of small variation in the experimental parameters on the analytical performance of the proposed methods.[51] In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. The studied parameters were: NaOH concentration, NQS or NBD-Cl concentration, heating temperature and time on the method suitability and sensitivity. It was found that small variation in the method variables did not significantly affect the procedures; recovery values were 98.60 ± 0.63–100.50 ± 0.76 % and 98.20 ± 0.90–100.60 ± 0.69 % (Table 6) which indicates the robustness of the proposed methods.

Ruggedness was also tested by applying the methods to the assay of CFD using the same operational conditions but using two

Table 5. Intra-day and inter-day precision and accuracy data for CFD obtained by the proposed methods

Method	Added concentration (μg mL^{-1})	Recovery %	Precision RSD %[a]	Accuracy Er %	Confidence limit[b]
Intra-day					
NQS	20	99.85	0.64	−0.15	19.97 ± 0.0522
	40	99.75	0.72	−0.25	39.90 ± 0.1173
	60	99.90	0.57	−0.10	59.94 ± 0.1395
	70	100.20	1.03	0.20	70.14 ± 0.2949
NBD-Cl	10	99.40	0.96	−0.60	9.94 ± 0.0390
	15	99.73	0.80	−0.27	14.96 ± 0.0489
	20	99.50	0.49	−0.50	19.90 ± 0.0398
	25	99.65	0.53	−0.35	24.91 ± 0.0539
Inter-day					
NQS	20	100.30	0.86	0.30	20.06 ± 0.0704
	40	100.05	0.63	0.05	40.02 ± 0.0555
	60	99.60	0.34	−0.40	59.76 ± 0.0829
	70	99.96	0.71	−0.04	69.97 ± 0.2028
NBD-Cl	10	99.80	1.06	−0.20	9.98 ± 0.0432
	15	100.25	0.80	0.25	15.04 ± 0.0491
	20	99.70	0.56	−0.30	19.94 ± 0.0456
	25	100.52	0.40	0.52	25.13 ± 0.0410

[a] Mean of six determination, RSD%, percentage relative standard deviation; Er%, percentage relative error.
[b] Confidence limit at 95% confidence level and five degrees of freedom ($t = 2.571$).

Table 6. Robustness of the proposed methods

Experimental parameter variation	**NQS** (CFD = 50 μg mL^{-1}) Recovery (%) ± SD[a]	**Experimental parameter variation**	**NBD-Cl** (CFD = 20 μg mL^{-1})
No variation[b]	99.25 ± 0.82	No variation[b]	99.50 ± 0.95
1- NaOH concentration		1- NaOH concentration	
0.005	99.40 ± 0.42	0.45 M	100.60 ± 0.69
0.015	98.90 ± 0.36	0.55 M	99.45 ± 0.426
2- NQS concentration		2- NBD-Cl concentration	
1.72×10^{-2} M	98.65 ± 0.51	4.5×10^{-3} M	99.70 ± 0.86
2.1×10^{-2} M	100.50 ± 0.76	5.5×10^{-3} M	99.50 ± 0.54
3- Heating temperature		3- Heating temperature	
20 °C	98.60 ± 0.63	95 °C	98.20 ± 0.90
30 °C	99.70 ± 0.83	100 °C	99.20 ± 1.25
4- Heating time		4- Heating time	
8 min	99.30 ± 0.68	25 min	99.00 ± 0.58
12 min	100.10 ± 0.78	35 min	99.70 ± 0.73

[a] Average of three determinations;
[b] Following the general assay procedure conditions.

different instruments at two different laboratories and different elapsed time. Results obtained from lab-to-lab and day-to-day variations were reproducible, as the RSD did not exceed 3%.

Applications to the analysis of pharmaceutical dosage forms

The proposed method was applied successfully for determination of CFD in its pharmaceutical dosage forms. Six replicate measurements were made in each case; the results obtained were validated by comparison with a previously reported method.[6] No significant difference was found by applying *t*- and *F*-tests at 95% confidence level indicating good accuracy and precision (Table 7). Recovery studies were also carried out by standard addition method.[52] The results in Table 8 indicate good recoveries (96.0 to 103.8%) and confirm that there is no interference from frequently encountered excipients or additives.

Suggested reaction mechanism

The NQS reagent reacts with CFD at the free NH_2 group; the reaction pathway was postulated to be processed and represented in Scheme 1A.

In the proposed NBD-Cl method, sulfide ions were allowed to react with NBD-Cl via SN^2 mechanism. The high nucleophilicity of sulfide ions, the presence of Cl^- anion as a good leaving group at position 4, in addition to the presence of nitro group as an electron withdrawing group at position 7 of the aromatic ring in NBD-Cl, result in replacement of Cl^- anion with the attacking sulfide

Table 7. Determination of CFD in its pharmaceutical formulations

Parameter	Mean recovery %[a] ± Standard Deviation		
	Reported method[8] (n=5)	NQS	NBD-Cl
Cefdin capsules [c] (300 mg cefdinir per capsule)	99.60 ± 0.40	99.91 ± 0.18	99.65 ± 0.49
test[b]		0.93	0.10
F-ratio [b]		4.94	1.50
Cefdin suspension[c] (125 mg cefdinir per 5 ml)	99.30 ± 0.40	99.78 ± 0.31	99.73 ± 0.26
t-test[b]		1.20	1.17
F-ratio [b]		1.66	2.37

[a] Average values of six determinations were used for the proposed methods.
[b] The theoretical values for *t* and *F* at 95% confidence limit (P = 0.05) are 2.31 and 6.39, respectively.
[c] Novartis Pharma S.A.E., Cairo, Egypt.

Table 8. Standard addition method for the assay of CFD in its pharmaceutical dosage forms by the proposed methods

Pharmaceutical formulation	Taken (μg mL^{-1})	Added (μg mL^{-1})	Recovery (%) ± RSD[a]
NQS			
Cefdin capsules	30	10	100.10 ± 0.50
		30	99.87 ± 0.66
		40	99.75 ± 0.28
Cefdin suspension	30	10	99.65 ± 0.94
		30	99.73 ± 1.03
		40	99.95 ± 0.58
NBD-Cl			
Cefdin capsules	10	5	99.50 ± 0.51
		10	100.20 ± 0.74
		15	99.25 ± 0.68
Cefdin suspension	10	5	99.45 ± 0.53
		10	99.95 ± 0.61
		15	99.80 ± 0.73

[a] Average of six determinations.

ions; this in turn leads to the formation of a yellow-coloured chromophore (λmax at 390 nm). The reaction product is stable in strong acidic medium; moreover acidification could minimize possible competition between the generated sulfide nucleophile and excess OH^- which may lead to a decrease in the chromogen formed. The proposed reaction mechanism is given in Scheme 1B.

The production of sulfide ions was confirmed by carrying out specific qualitative tests such as dilute hydrochloric acid, cadmium acetate, sodium nitroprusside and methyelene blue tests[53] or by comparing λ_{max} of the formed chromogen with that obtained after applying the developed method to sodium sulfide and the same results were obtained.

Experimental

Apparatus

All the absorption spectral measurements were made using Optima UV-VIS spectrometer (SP-3000 plus) (Tokyo, Japan) with wavelength range (190–1100 nm), a band width of 1.0 nm, equipped with 10 mm matched quartz cells.

Materials and reagents

All employed chemicals and solvents were of analytical-reagent grade and high-purified water was used throughout the study. Sodium hydroxide was obtained from El-Nasr Chemical Co. (Cairo, Egypt); (0.01 and 0.5 M) aqueous solution. Concentrated hydrochloric acid was obtained from El-Nasr Chemical Co. (Cairo, Egypt).

1, 2-Naphthoquinone-4-sulfonate (NQS) 0.5% (*w*/*v*): 0.5 g of NQS (NQS; Aldrich Chemical Co., St Louis, MO, USA) was accurately weighed, transferred into a 100-ml calibrated flask, dissolved in 50-mL bidistilled water, and make up the volume up to the mark with bidistilled water to obtain a solution of 0.5% (*w*/*v*) equivalent to (1.91×10^{-2} M). 4-cholor-7-nitrobenzofurazan (NBD-Cl; Fluka Chemie AG, Switzerland) freshly prepared (5.0×10^{-3} M) equivalent to 0.1% (*w*/*v*) in acetone. Pure grade CFD reference standard was provided by (Bristol-Myers Squibb Pharmaceutical Co., Cairo, Egypt). Its purity % 99.51 ± 0.877.

Pharmaceutical formulations

The following commercial pharmaceutical formulations containing the studied drugs were purchased from local market were subjected to the analytical procedure. CFD capsules (Novartis Pharma S.A.E, Cairo, under licence from Novartis Pharma AG., Basle, Switerland, Egypt), labelled to contain 300 mg CFD per capsule. CFD suspension (Novartis Pharma S.A.E, Cairo, under licence from Novartis Pharma AG., Basle, Switerland), labelled to contain 125 mg CFD per 5.0 ml.

Preparation of standard solutions

Stock solution containing (5.0 mg ml^{-1}) of CFD was prepared in methanol. Working standard solutions of CFD containing 0.1–0.8 mg ml^{-1} (in case of NQS), 0.5–3.0 mg ml^{-1} (in case of NBD-Cl) were prepared by suitable dilution of the stock solution. The stock and working standard solutions must be freshly prepared.

Preparation of sample solutions

Capsules

Twenty capsules were weighed, finely powdered, and mixed thoroughly. An accurately weighed amount of the powder obtained from capsules equivalent to 250 mg of CFD was

transferred into a 25-ml volumetric flask, dissolved in about 10 ml methanol, sonicated for 15 min, diluted to the mark with methanol, mixed well, and filtered; the first portion of the filtrate was rejected. Further dilutions with the same solvent were made to obtain sample solution containing the specified concentration for CFD as mentioned under the preparation of standard solution and then the general procedure was followed.

Powder for oral suspension

An accurately weighed amount of powder equivalent to 250 mg of CFD was transferred into a 25-ml volumetric flask, dissolved in about 10 ml methanol, sonicated for 15 min, diluted to the mark with methanol, mixed well and filtered; the first portion of the filtrate was rejected. Further dilutions with the same solvent were made to obtain sample solution containing the specified concentration for CFD as mentioned under the preparation of standard solution and then the general procedure was followed.

General procedure

Using NQS

Accurately measured aliquots of CFD solutions containing 0.1–0.8 mg ml^{-1} were transferred into separate 10-ml calibrated flasks. One milliliter of 0.01M sodium hydroxide solution (pH 11) was added followed by 1.0 ml of NQS solution (0.5%, *w*/*v*). The reaction solution was allowed to proceed at room temperature ($25 \pm 5\,^{\circ}C$) for 10 min. The reaction mixture was completed to volume with methanol. The absorption spectrum of the complex of the resulting solution was measured at 490 nm against reagent blank treated similarly.

Using NBD-Cl

Accurately measured aliquots of CFD solutions containing 0.5–3.0 mg ml^{-1} were transferred into separate 10-ml volumetric flasks; 5.0 ml of 0.5 M NaOH were added and the flask was heated in a boiling waterbath for 30 min, cooled to room temperature, and completed to volume with bidistilled water. One milliliter of the resulting drug hydrolysate was pipetted into a 10-ml volumetric flask, 1.0 ml of 0.1 (*w*/*v*) NBD-Cl was added followed by 1.0 ml of concentrated HCl. The resulting solution was mixed well and the flask was completed to volume with ethanol. The absorbance was measured at 390 nm against reagent blank treated similarly.

Determination of the stoichiometric ratio of the reaction

Job's method

Job's method of continuous variation[45] was employed. Master equimolar (5×10^{-3} M) aqueous solutions of CFD and NQS or NBD-Cl were prepared. A series of 10-ml portions of the master solutions of CFD and NQS or NBD-Cl was made up comprising different complementary proportions (0 : 10, 1 : 9, . . ., 9 : 1, 10 : 0, inclusive) in 10-ml calibrated flasks containing 1.0 ml of NaOH (0.01 or 0.5M) solution in case of (NQS or NBD-Cl, respectively. The solution was manipulated as described under the general procedures.

Limiting logarithmic method

In the limiting logarithmic method,[46] two sets of experiments were carried out employing the general recommended procedures described above. In the case of NQS, the first set of experiments was carried out using increasing NQS concentrations (1.9×10^{-3}–1.15×10^{-2} M) at fixed CFD concentration (1.26×10^{-4} M). The second set of experiments was carried out using increasing CFD concentrations (2.53×10^{-5}–2.02×10^{-4} M) at fixed NQS concentration (1.9×10^{-2} M). In the case of NBD-Cl, the first set of experiments was carried out using increasing NBD-Cl concentrations (1.05×10^{-4}–5.24×10^{-4} M) at fixed CFD concentration (5.06×10^{-5} M). The second set of experiments was carried out using increasing CFD concentrations (1.26×10^{-5}–7.59×10^{-5} M) at fixed NBD-Cl concentration (5.0×10^{-4} M). The logarithms of the obtained absorbances were plotted as function of the logarithms of the NQS and CFD concentration in the first and second sets of experiments, respectively. The slopes of the fitting lines in both sets of experiments were calculated.

Conclusions

The present spectrophotometric methods describe the evaluation of NQS and NBD-Cl as analytical reagents in the development of simple, sensitive, and accurate methods for the determination of CFD in bulk and pharmaceutical formulations. The reagents utilized in the proposed methods are cheap and readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The proposed methods have comparable analytical performances and devoid from any potential interference from the frequently encountered excipients and additives. Statistical analysis proves that the proposed methods could be applied for the analysis of CFD in their pure forms and in pharmaceutical formulations. Therefore, these methods can be recommended for the routine analysis of CFD in quality control laboratories.

References

[1] S. Budavari. *The Merck Index*, 12th ed. Merck Research Laboratories Division of Merck & Co., Inc.: Whitehouse Station, NJ, **1996**, pp. 316.
[2] *Japanese Pharmacopeia, Official Monograph XIV*, **2001**, pp. 324.
[3] M. Gandhimathi, A. Suganthi, T.K. Ravi, M.B. Pattasseril. RP- HPLC determination of cefdinir in capsules. *Ind. J. Pharm. Sci.* **2004**, *66*, 248.
[4] P.B. Shah, K. Pundarikakshudu. Difference spectroscopic and reverse phase HPLC methods for the estimation of cefdinir in pharmaceutical dosage forms. *Ind. J. Pharm. Sci.* **2006**, *68*, 90.
[5] G.M. Hadad, S. Emara, W.M.M. Mahmoud. Optimization and validation of an LC method for the determination of cefdinir in dosage form and human urine. *Chromatographia* **2009**, *70*, 1593.
[6] T.N. Mehta, G. Subbaiah, K. Pundarikakshudu. Determination of cefdinir by a stability-indicating liquid chromatographic method. *J. AOAC Int.* **2005**, *88*, 1661.
[7] Y. Okamoto, K. Itoh, Y. Namiki, J. Matsushita, M. Fujioka, T. Yasuda. Method development for the determination of cefdinir and its related substances by high-performance liquid chromatography. *J. Pharmaceut. Biomed.* **1996**, *14*, 739.
[8] U.C. Mashelkar, S.D. Renapurkar, An LCMS compatible stability-indicating HPLC assay method for cefdinir. *Int. J. Chem. Tech. Res.* **2010**, *2*, 114.
[9] J. Couture, A. Beaulieu, F. Vallée. Determination of cefadroxil, cefdinir, cefpodoxime, celecoxib, cephalexin, lamivudine and lamotrigine in human plasma by HPLC with ultraviolet detection. AAPS PharmSci. **2001**, 3(S1), 1055. Available at: www.aapsj.org/abstracts/AM_2001/1055.htm [2 March 2011].

[10] A. Hishida, K. Ohishi S. Nagashima. Pharmacokinetic study of an oral cephalosporin, cefdinir in hemodialysis patients. *Antimicrob. Agents Ch.* **1998**, *42*, 1718.
[11] M.V. Kumudhavalli, RP – HPLC determination of cefdinir in bulk drug and solid dosage form. *J. Pharm. Res.* **2009**, *2*, 1141.
[12] W. Franklin Smyth, V. Rodriguez. Recent studies of the electrospray ionisation behaviour of selected drugs and their application in capillary electrophoresis–mass spectrometry and liquid chromatography–mass spectrometry. *J. Chromatogr. A* **2007**, *1159*, 159.
[13] Z.J. Chen, J. Zhang, J.C. Yu, G.Y. Cao, X.J. Wu, Y.G. Shi. Selective method for the determination of cefdinir in human plasma using liquid chromatography electrospray ionization tandam mass spectrometry. *J. Chromatogr. B* **2006**, *834*, 163.
[14] R. Nageswara Rao, N. Venkateswarlu, R. Narsimha. Determination of antibiotics in aquatic environment by solid-phase extraction followed by liquid chromatography–electrospray ionization mass spectrometry. *J. Chromatogr. A* **2008**, *1187*, 151.
[15] R. Jain, K. Radhapyari, N. Jadon. Electrochemical evaluation and determination of cefdinir in dosage form and biological fluid at mercury electrode. *J. Electrochem. Soc.* **2007**, *154*, F199.
[16] R. Jain, A. Dwivedi, R. Mishra. Voltammetric behavior of cefdinir in solubilized system. *J. Colloid. Interf. Sci.* **2008**, *318*, 296.
[17] F.F. Tao, Z.Q. Yu, X.F. Han, S.P. Zheng, S.Y. Dong. Determination of cefdinir by differential pulse voltammetry. *Chin. J. Pharm. Anal.* **2008**, *28*, 1512.
[18] D. She-ying, Y. Zhu-qing, H. Xiao-feng, H. Ting-lin, Z. Jian-bin. Voltammetric behavior of degradation product and determination of cefdinir. *Chem. Res. Chin. Uni.* **2009**, *25*, 807.
[19] A. Suganthi, S. Shrikumar, M.B. Pattesseril, M. Umamaheswari, T.K. Ravi. Spectrofluorimetric estimation of cefdinir in formulation. *Ind. J. Pharm. Sci.* **2004**, *66*, 689.
[20] D.G. Sankar, M. Sudarshan, M.V. Krishna, P.V.M. Latha. UV spectrophotometric method for determination of cefdinir. *J. Inst. Chem. (India)* **2005**, *77*, 5.
[21] J. Jane, E.V.S. Subrahmanyam, D. Satyanarayana. UV visible spectroscopic methods for the estimation of cefdinir. *Indian Drugs* **2005**, *42*, 322.
[22] P.B. Shah, K. Pundarikakshudu. UV spectroscopic and colorimetric methods for the estimation of cefdinir in capsule dosage forms. *Ind. J. Pharm. Sci.* **2004**, *66*, 665.
[23] S.S. Patil, P.V. Kasture. Spectrophotometric method for estimation of cefdinir from capsule formulations. *Ind. Pharm.* **2008**, *7*, 110.
[24] L.D. Srinivas, K.V.S. Prasad Rao, B.S. Sastry. Application of n-bromosuccinimide as an oxidant for the assay of cefdinir. *Int. J. Chem. Sci. (India)* **2005**, *3*, 353.
[25] S.R. Narala, K. Saraswathi. Application of oxidants to the spectrophotometric determination of cephalosporins (cefditoren pivoxil and cefdinir) in formulations. *Asian J. Res. Chem.* **2011**, *4*, 270.
[26] B.S. Virupaxappa, K.H. Shivaprasad, M.S. Latha. A simple spectophotometric method for the determination of cefdinir in pharmaceuticals. *Anal. Chem. (An Indian J.)* **2010**, *9*,.
[27] B.K. Singh, D.V. Parwate, S. Srivastava, S.K. Shukla. Selective and non-extractive spectrophotometric determination of cefdinir in formulations based on donor-acceptor complex formation. *Quim. Nova* **2010**, *33*, 1471.
[28] Q.M. Li, Z.J. Yang. Spectrophotometric determination of amino-methylbenzoic acid using sodium 1,2-naphthoquinone-4-sulfonate as the chemical derivative chromogenic reagent. *Spectrochim. Acta A* **2007**, *66*, 656.
[29] I.A. Darwish, H.H. Abdine, S.M. Amer, L. I. Al-Rayes. Spectrophotometric study for the reaction between fluvoxamine and 1,2-naphthoquinone-4-sulfonate: Kinetic, mechanism and use for determination of fluvoxamine in its dosage forms. *Spectrochim. Acta A* **2009**, *72*, 897.
[30] I.A. Darwish. Kinetic spectrophotometric methods for determination of trimetazidine dihydrochloride. *Anal. Chim. Acta* **2005**, *551*, 222.
[31] F.A. Mohamed. Spectrophotoemtric determination of sulphide and some sulphide producing compounds. *In Proceedings of Assiut University 1st Pharmaceutical Science Conference,* Faculty of Pharmacy, Assiut, **1998**, pp. 1–18.
[32] F.H. Metwally, A.A. Alwarthan, S.A. Al-Tamimi. Flow-injection spectrophotometric determination of certain cephalosporins based on the formation of dyes. *Il Farmaco* **2001**, *56*, 601.
[33] N. Olgun, S. Erturk, S. Atmaca. Spectrofluorimetric and spectrophotometric methods for the determination of vigabatrin in tablets. *J. Pharmaceut. Biomed.* **2002**, *29*, 1.
[34] A. Onal, S.E. Kepekci, A. Oztunc. Spectrophotometric methods for the determination of antidepressant drug paroxetine hydrochloride in tablets. *J. AOAC Int.* **2005**, *88*, 490.
[35] R.O. Olojo, R.H. Xia, J.J. Abramson. Spectrophotometric and fluorometric assay of superoxide ion using 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole. *Anal. Biochem.* **2005**, *339*, 338.
[36] A.A. El-Emam, S.H. Hansen, M.A. Moustafa, S.M. El-Ashry, D.T. El-Sherbiny. Determination of lisinopril in dosage forms and spiked human plasma through derivatization with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) followed by spectrophotometry or HPLC with fluorimetric detection. *J. Pharmaceut. Biomed.* **2004**, *34*, 35.
[37] E.A. Taha. Kinetic spectrophotometric methods for the determination of dothiepin hydrochloride in bulk and in drug formulation. *Anal. Bioanal. Chem.* **2003**, *376*, 1131.
[38] A.S. Amin, G.H. Ragab, H. Saleh, Colorimetric determination of β-blockers in pharmaceutical formulations. *J. Pharmaceut. Biomed.* **2002**, *30*, 1347.
[39] H.E. Abdellatef. Kinetic spectrophotometric determination of tramadol hydrochloride in pharmaceutical formulation. *J. Pharmaceut. Biomed.* **2002**, *29*, 835.
[40] N. El-Enany, F. Belal, M. Rizk, Spectrophotometric determination of salbutamol in bulk and dosage forms after derivatization with 4 Chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD Cl). *Chem. Ana.-Warsaw* **2004**, *49*, 261.
[41] S. Ashour, M. Khateeb. Kinetic spectrophotometric determination of pravastatin in drug formulations *via* derivatization with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl), *Arabian J. Chem.* **2010**, DOI:10.1016/j.arabjc.2010.06.050.
[42] Z. Aydoğmuş, Highly sensitive and selective spectrophotometric and spectrofluorimetric methods for the determination of ropinirole hydrochloride in tablets, *Spectrochim. Acta A* **2008**, *70*, 69.
[43] A. Önal, O. Sagirli, spectrophotometric and spectrofluorimetric methods for the determination of pregabalin in bulk and pharmaceutical preparation, *Spectrochim. Acta A* **2009**, *72*, 68.
[44] H.M. Saleh, M.M. EL-Henawee, G.H. Ragab, S.S. Abd El-Hay. Utility of NBD-Cl for the spectrophotometric determination of some skeletal muscle relaxant and antihistaminic drugs. *Spectrochim. Acta A* **2007**, *67*, 1284.
[45] P. Job. *Anal. Chim. Acta,* in *Advanced Physicochemical Experiments*, 2nd ed. **1936,** *16*, 54, Oliner and Boyd: Edinburgh, UK, **1964**.
[46] J. Rose. *Advanced Physicochemical Experiments*, Pitman: London, **1964**, pp. 67.
[47] Topic Q2 (R1). Validation of analytical procedures: Text and methodology. *International Conference on Harmonisation* (*ICH*), **2005 Available**, at: http://www.emea.europa.eu/pdfs/human/ich/038195en.pdf [2 March 2011].
[48] American Pharmaceutical Association. *USP, 31 and NF 26*. Washington, DC, **2008**.
[49] A. Ringbom. Accuracy of colorimetric determinations. *Z Anal. Chem.* **1939**, *115*, 332.
[50] ICH guideline Q2(R1), *Validation of Analytical Procedures: Text and Methodology*, London, **2005**.
[51] Y.V. Heyden, A. Nijhuis, J. Smeyers-Verbeke, B.G.M. Vandeginste, D.L. Massart. Guidance for rubustness/ruggedness test in method validation. *J. Pharmaceut. Biomed.* **2004**, *24*, 723.
[52] D. Harvey. *Modern Analytical Chemistry*. McGraw-Hill: Boston, MA, **2000**.
[53] G. Svehla, *Vogel's Textbook of Macro and Semimicro Qualitative Inorganic Analysis*, 5th ed. The Chaucer Press: Great Britain, **1979**.