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An efficient one-pot reaction for selective fluorimetric determination of cefpodoxime and its prodrug

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ABSTRACT

Cefpodoxime proxetil (CFP), an oral third-generation cephalosporin, is a prodrug that is de-esterified *in vivo* to its active metabolite, cefpodoxime acid (CFA). Therefore, this study aimed to develop a facile and efficient one-pot reaction for selective and sensitive determination of CFA and its prodrug (CFP). The method was based on single-step reaction between CFP or CFA and 1,2-naphthoquinone-4-sulfonate (NQS) as a selective derivatizing reagent in alkaline medium without heating, extraction or reduction steps as usual for NQS derivatization reactions. The fluorescence of the formed NQS-derivative was monitored directly at emission wavelength of 440 nm after excitation at 330 nm. The method can easily be implemented in plating facilities by operators and/or incorporated in on-line derivatization reaction. The correlation coefficients of 0.9991 and 0.9984 were obtained in the concentration ranges of 50–2000 ng mL^{-1} for CFA and CFP, respectively. The detection limits were 9.17 and 9.48 ng mL^{-1} for CFA and CFP, respectively. The method was validated in accordance with the requirements of ICH guidelines and shown to be suitable for their efficient and sensitive determinations. The developed method was successfully applied for selective determination of CFP in pure form and in pharmaceutical dosage forms as well as CFA in human urine after single dose of CFP without prior need for separation. The method is valuable for quality control laboratories for monitoring of CFP and its active metabolite CFA.

1. Introduction

Cefpodoxime proxetil (CFP) (1-[(isopropoxycarbonyl)oxy]ethyl ester of (Z)-7-[2-(2-amino-1,3-thiazol-4-yl)-2-methoxyimino-acetamido]-methoxymethyl-3-cephem-4-carboxylic acid), an oral third generation cephalosporin antibiotic, is an ester prodrug with a proxetil radical attached to cefpodoxime acid (CFA) (Fig. 1). CFA is active against many gram positive and negative micro-organisms and implicated in the treatment of a variety of upper respiratory tract and urinary tract infections [1,2]. CFA exists predominantly in the ionic form at intestinal pH and thus exhibits poor permeability [3]. The free carboxylic acid moiety in CFA offers opportunities for the preparation of prodrugs. Esterification of this carboxylic acid functional group with an isopropyloxycarbonyloxyethyl group removes the ionizable group and improves lipophilicity to overcome the poor oral bioavailability of the parent drug, enabling the compound to be absorbed by passive diffusion after oral administration [4]. In the biological system, CFP undergoes ester hydrolysis by nonspecific esterases in the intestinal wall and converted into CFA to exhibit its antibiotic activity [5]. Furthermore, CFP is an official drug in British and United States Pharmacopeia [6,7].

To date, few analytical methods were reported to quantify CFP in pure form and in pharmaceutical formulations. These methods include spectrophotometry [8,9], voltammetry [10,11], high-performance liquid chromatography (HPLC) with UV detection [8,12,13], high-performance thin layer chromatography (HPTLC) [14] and densitometry [15]. On the other hand, very few HPLC methods were reported for the analysis of the active metabolite CFA in biological fluids with UV detection [16–18]. The low dosages used from CFP (100–200 mg) make blood and tissue levels of CFA difficult to measure [5].

Conventional analysis methods were neither sensitive nor selective enough to determine the studied drugs in biological fluids and in pharmacokinetic studies, which require detection levels as low as 0.1 μg mL^{-1}. Therefore, it was necessary to develop a highly sensitive and selective method for analysis of CFP in pharmaceutical formulations as well as its active form CFA in biological fluids.

The fluorimetric methods of analysis considered a convenient analytical technique, because of its simplicity, low cost, sensitivity, selectivity and wide availability in most quality control laboratories. As far as we know, no fluorimetric method was reported for the determination of CFP and/or CFA. Moreover, 1,2-naphthoquinone-4-sulphonate (NQS) was used as a derivatizing reagent for the determination of some aliphatic and aromatic amines [19–22]. The usual fluorescence derivatization reaction with NQS reagent involves three successive steps; condensation reaction by heating

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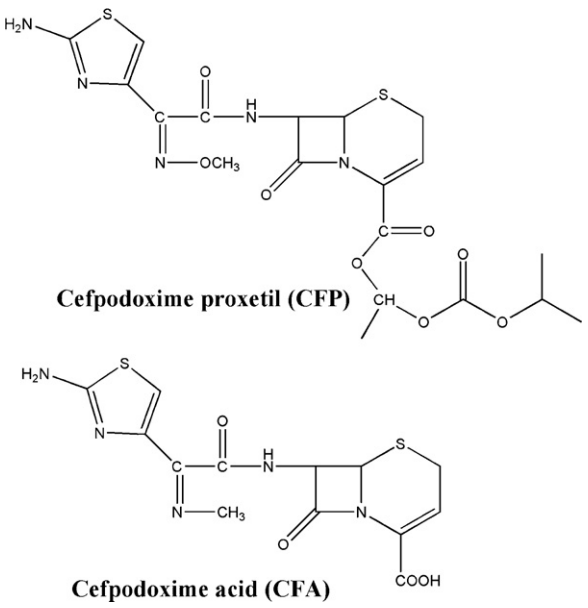

Fig. 1. Structures of CFP and its active form CFA.

in alkaline medium followed by extraction of NQS-derivative then reduction step. However, this reaction suffered from product loss during extraction and/or incomplete reduction step. One-pot reactions are more convenient for rapid and multiple sample analysis. The method can easily be implemented in plating facilities and an automated, on-line derivatization procedure is also possible

In this research, it was aimed to develop efficient, highly sensitive and selective one-pot fluorimetric method for the determination of CFP and its active form CFA in pharmaceutical preparations as well as human urine samples. The choice of NQS as a fluorigenic reagent for their determination based on the formation of highly fluorescent condensation derivative without heating, extraction or reduction steps then the reaction product was monitored fluorimetrically. The method was optimized and validated then applied for the determination of CFP in pharmaceutical dosage forms as well as CFA in human urine after a single dose of CFP without prior need for separation.

2. Experimental

2.1. Apparatus

RF-5301 PC spectrofluorimeter (Shimadzu, Japan), with 1 cm matched quartz cells, was used for all measurement. The spectrofluorimeter was set at excitation and emission bandpass of 3 nm. Super-mixer (Lab-line Instruments, Inc., USA).

2.2. Chemicals and reagents

CFP (%purity 99.2 ± 0.36) was obtained from Roussel Uclaf (Romainville, France). CFA (%purity 98.3 ± 0.25) was obtained from Toronto Research Chemicals, Inc. (Toronto, Ontario, Canada). 1,2-Naphthoquinone-4-sulphonate (%purity 100.02 ± 1.2) was obtained from (Acros Organic, NewJersy, USA). All solvents and other chemicals used throughout this study were of analytical grade.

Pharmaceutical preparations containing studied drugs were obtained from the local market. Orelox®tablets (Sanofi-Aventis, France) are labeled to contain 100 mg CFP per tablet and Orelox®suspension (Sanofi-Aventis, France) is labeled to contain 40 mg CFP per 5 mL solution.

2.3. Preparation of standard solutions

2.3.1. Preparation of standard drug solution

A stock solution of CFP or CFA reference standard ($1.0\ mg\ mL^{-1}$) was prepared in methanol. The working standard solutions were prepared by further dilution of the stock solution methanol to obtain concentration ranges from 0.05 to $2\ \mu g\ mL^{-1}$. The stock and working standard solutions were kept at $\pm 4\ ^{\circ}C$ in light protected flasks.

2.3.2. 1,2-Naphthoquinone-4-sulphonate (NQS) solution

An accurately weighed amount of NQS (25 mg) was transferred into a 50 mL calibrated flask, and dissolved in double distilled water to obtain a solution of $0.5\ mg\ mL^{-1}$. The solution was freshly prepared and protected from light during use.

2.3.3. Buffer solutions

Teorell and Stenhagen buffer solutions of the pH range 9–12 were prepared in double distilled water as reported [19].

2.4. Method validation

The validation was done according to International Conference on Harmonization (ICH) guidelines for industry on bioanalytical method validation [23]. Three days of calibration curves with five replicates of CFP and CFA solutions over concentration range of 50–2000 ng mL^{-1} were performed to assess linearity of the proposed method.

Limits of detection (LOD) and limits of quantitation (LOQ) for all the analytes were calculated as follows; LOD or LOQ = $K{\cdot}S.D{\cdot}a/b$. Where; $K = 3$ for LOD, $K = 10$ for LOQ, S.D·a is the standard deviation of intercept and b is the slope. LOD and LOQ were confirmed experimentally by evaluating the minimum levels at which the analyte could be readily detected or accurately quantified, respectively.

Intra-day precision was determined by replicate analysis ($n = 5$) of standard solutions at low, medium, and high concentration levels (50, 500 and 1500 ng mL^{-1}). The inter-day precision was performed by repeating the analysis of CFP and CFA by the developed method over three successive days. The precision of the method was expressed as percentage relative standard deviations (%RSD). Method accuracy was determined by addition of known amounts of standard CFP and CFA to a sample solution of known concentration and comparing measured and calculated values. The accuracy was expressed as percent to the true value.

The robustness is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. For the determination of the developed method's robustness, a number of method parameters including buffer pH, NQS reagent concentration, reaction time and emission wavelength, were varied and the quantitative influence of these variables was determined.

The ruggedness is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions. The developed method's ruggedness was evaluated by applying the recommended analytical procedures using different spectrufluorimeter, different analysts, and independently on different days at different laboratories for the analysis of CFP and CFA samples. The RSD values were recorded for these different conditions.

Recovery of CFP from its formulations (tablets and suspension) were assessed ($n = 5$) by standard addition method. Known amounts of standard drugs (50, 100, 150 mg) were added to pharmaceutical formulations then samples were treated (as described in Section 2.6). The obtained relative fluorescence intensities (RFI) were compared with those obtained from analysis of equivalent quantities of pure standards.

2.5. General assay procedure

Accurately measured aliquots of CFP or CFA solution containing 50–200 ng mL^{-1} were transferred into reaction vials. One milliliter of Teorell and Stenhagen buffer solution (pH 12) was added followed by 1 mL of NQS solution (0.5 mg mL^{-1}). The reaction solution was allowed to proceed at room temperature (25 ± 5 °C) for 15 min. The reaction mixture was measured at emission wavelength of 440 nm after excitation at 330 nm against reagent blank treated similarly.

2.6. Analysis of dosage form

2.6.1. Tablets

An accurately weighed amount of powder obtained from 10 finely powdered CFP tablets equivalent to 25 mg of the drug was transferred into 50 mL volumetric flask. About 25 mL ethanol was added and the flasks were sonicated for10 min and the flasks were completed to the mark with ethanol, then the solution was filtered using 0.45 μm filter disks and the first portion of filtrate was rejected. The prepared solution was diluted quantitatively to obtain the required concentration for assay and completed as described in assay procedures.

2.6.2. Suspension

A quantity of the suspension equivalent to 25 mg of the active component was transferred into a 50 mL volumetric flask, dissolved in 25 mL ethanol, sonicated for 10 min, completed to volume with the ethanol, then the solution was filtered using 0.45 μm filter disks and the first portion of filtrate was rejected. The solution was further diluted to required concentration and completed as described in assay procedures.

2.7. Human urine sample treatment

Recovery from urine samples spiked with three different concentrations of CFA (200, 500 and 1000 ng mL^{-1}) was studied. The urine was filtered using 0.45 μm filter disks and measured directly without prior treatment against blank urine for CFA content by the developed method. In addition, the urine samples were collected from 4 healthy human volunteers (18–42 years) before and 3 h after single oral dose of 133.45 mg of CFP (Orelox® tablets) and measured for CFA content by the developed method. All human care and procedures in this experiment were approved by Assiut University Human Care Committee.

3. Results and discussion

3.1. Strategy for method development

Because of the absence of any native fluorescence for CFP and its active metabolite CFA, direct fluorimetric determination was not possible. Therefore, derivatization of CFP and CFA was attempted in the present study for the development of spectrofluorometric methods for its determination. Simple one-pot derivatization reaction was necessary for rapid determination of multiple sample analysis. The reaction of NQS with primary and secondary amines was reported for the spectrofluorimetric determination of amine compounds [19–22]. In addition, NQS was reported for spectrophotometric determination of some alpha-aminocephalosporin compounds [24]. Reaction involved condensation reaction through heating in alkaline media. The condensation products were further extracted and reduced with KBH_4 in alkaline media to give fluorescent products measured at different excitation and emission wavelengths. However, these derivatization steps were tedious, time-consuming and suffer from product loss and/or incomplete

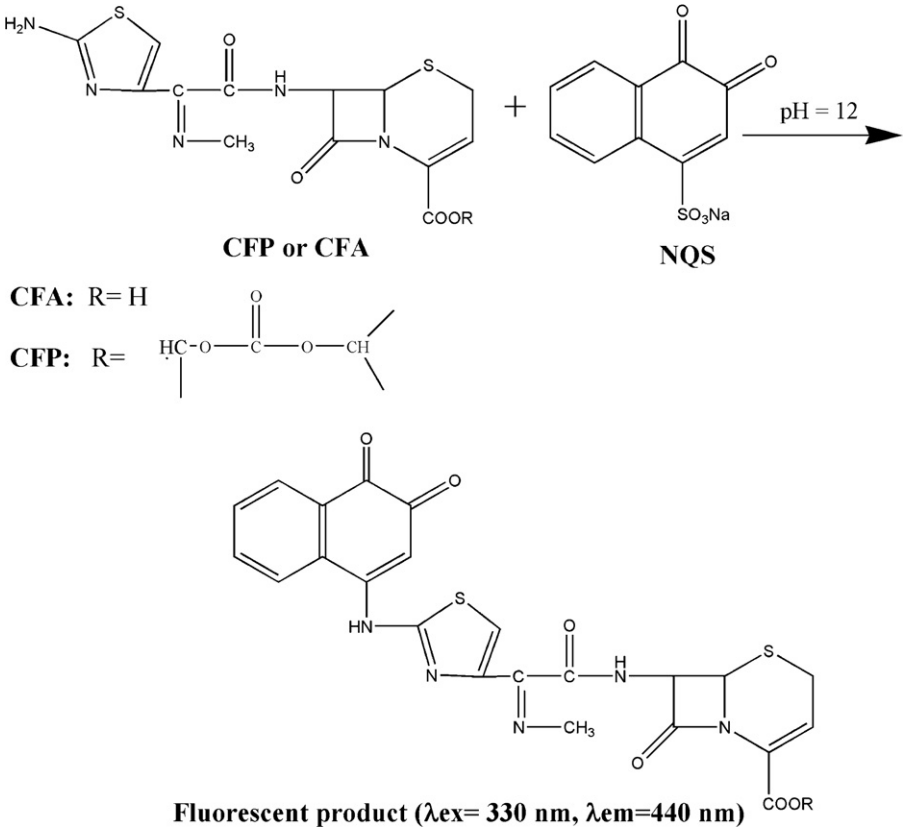

Fig. 2. Scheme for the reaction pathway of CFP and CFA with NQS.

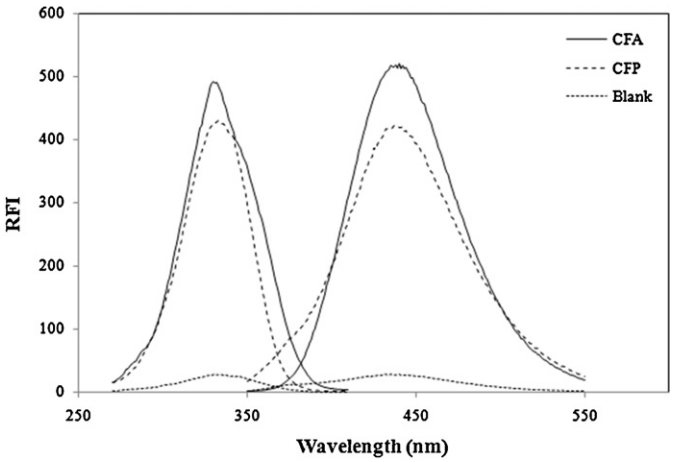

Fig. 3. Excitation and emission spectra of the reaction products of 0.6 μg mL^{-1} CFP and CFA with NQS (0.5 mg mL^{-1}) in Teorell and Stenhagen buffer (pH 12) against blank treated similarly.

reaction steps leading to inefficient analytical methodology. Therefore, the present study was devoted to explore NQS as a simple fluorescence derivatizing reagent in the development of spectrofluorometric methods for the determination of CFP and CFA. Our preliminary experiments in investigating the reaction between CFP or CFA with NQS revealed that highly fluorescent products were formed by single-step condensation reaction between free amino group of CFP or CFA and NQS reagent in alkaline medium (pH 12) at room temperature without need for heating, extraction or reduction steps. The reaction pathway was postulated to be proceeded as shown in Fig. 2. Excitation and emission spectra of the formed fluorescent products of CFP and CFA with NQS against blank treated similarly are shown in Fig. 3. The condensation products exhibit emission maxima at λ_{em} = 440 nm with excitation maxima at λ_{ex} = 330 nm for both CFP-NQS and CFA-NQS products. However, CFA-NQS gave higher fluorescence intensities compared to CFP-NQS product.

3.2. Optimization of reaction conditions

In order to obtain the best sensitivity and selectivity, the conditions of the condensation reactions were optimized. The effects of variables affecting the reaction (buffer pH, NQS concentration, and reaction time) were studied by altering each variable in turn while keeping the others constant. Alkaline medium was necessary to activate the nucleophilic substitution reaction through nucleophile formation from CFP or CFA. Teorell and Stenhagen buffer was chosen for its efficiency at wide ranges of pH (2–12). The relative fluorescence intensity (RFI) was studied as a function of buffer pH for CFP and CFA (Fig. 4). The highest RFI was attained at buffer pH 12. This was possibly due to the fact that the nucleophilic substitution reaction was activated at high pH values. Therefore, Teorell and Stenhagen buffer of pH 12 was selected for subsequent work. The studying of NQS concentrations over concentration range from 0.1 to 0.8 mg mL^{-1} revealed that the reaction was dependent on NQS concentration (Fig. 5). The RFI of the reaction product increased as the NQS concentration increased for CFP and CFA, and the highest intensities were obtained at NQS concentration of 0.5 mg mL^{-1}. Further increases in the reagent concentration led to decrease in RFI therefore, the reagent concentration of 0.50 mg mL^{-1} was chosen. The effect of temperature on the reaction was studied by carrying out the reaction at different temperatures (25–100 °C). The results revealed that increasing the temperature had negative effect on the RFI values of the reaction product for CFP and CFA. This was probably attributed to the instability of the CFP-NQS and CFA-NQS

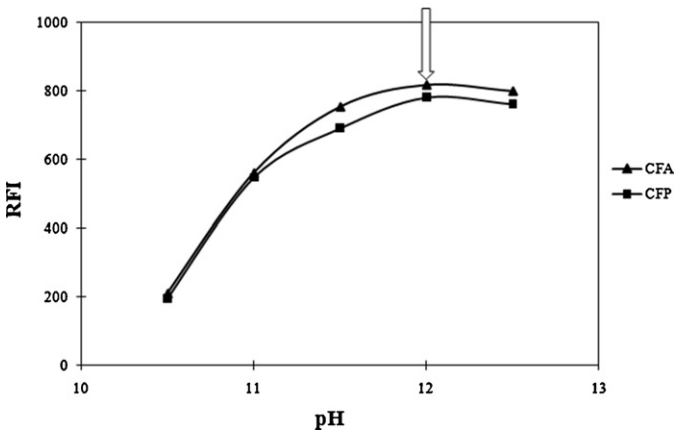

Fig. 4. Effect of buffer pH on the fluorescence intensity of the reaction product of 1 μg mL^{-1} CFP and CFA with 0.5 mg mL^{-1} NQS.

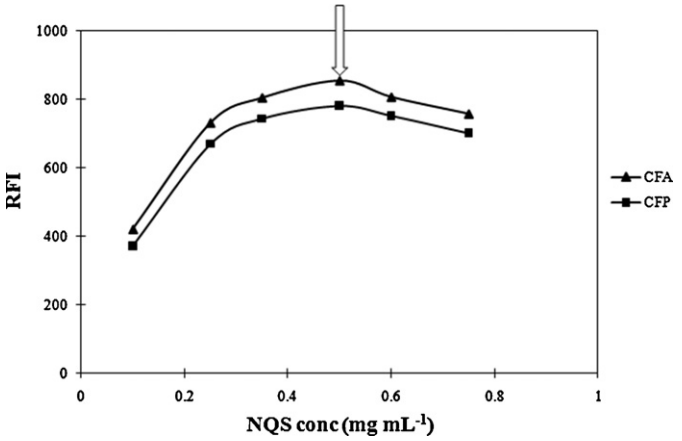

Fig. 5. Effect of NQS reagent concentrations on the fluorescence intensity of the reaction product of 1 μg mL^{-1} CFP and CFA with (0.1–0.8 mg mL^{-1}) NQS.

derivatives. For this reason, further experiments were carried out at room temperature (25 ± 5 °C). The effect of reaction time on the formation of the reaction product was investigated by carrying out the condensation reaction for different times. The maximum fluorescence intensities for CFP and CFA products were attained between 10 and 20 min (Fig. 6). So, the measurements were done within 15 min of reaction.

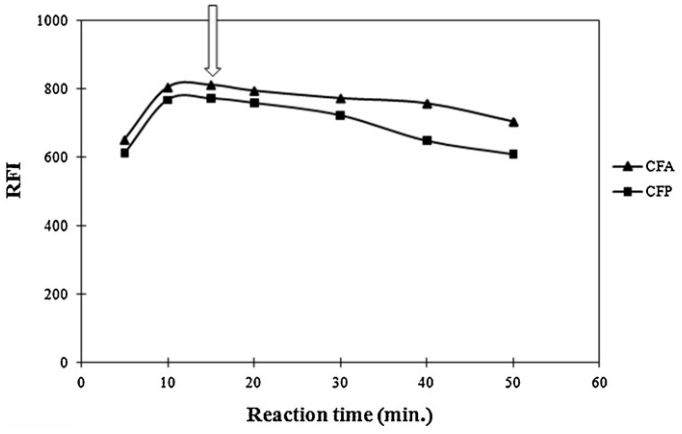

Fig. 6. Effect of reaction time on the fluorescence intensity of the reaction product of 1 μg mL^{-1} CFP and CFA with 0.5 mg mL^{-1} NQS.

Table 1
Quantitative parameters and statistical data for the analysis of CFP and CFA by the developed method.

Compound	Calibration curve[a]				Detection limit (ng mL^{-1})	Quantitation limit (ng mL^{-1})
	Range (ng mL^{-1})	Slope[a] (±SD)	Intercept[a] (±SD)	r		
CFA	50–2000	1.0 (±0.025)	9.4 ± (±3.05)	0.9984	9.17	30.56
CFP	50–2000	1.1 (±0.028)	4.7 (±3.50)	0.9991	9.48	31.48

[a] Data presented as mean ± SD of three experiments.

Table 2
Comparison between the developed method and reported methods.

Method	Target analyte	Range (μg mL^{-1})	LOD (μg mL^{-1})	% RSD	Sample	Ref.
Fluorimetry	CFA	0.05–2	0.009	0.87	Urine	This method
	CFP	0.05–2	0.009	0.79	Tablets, suspension	
Spectrophotometry	CFP	5–50	0.53	0.32	Tablets	[8]
HPLC-UV	CFP	5–60	0.79	0.46	Tablets,	[8]
HPLC-UV	CFP (R, S isomers)	5–150	0.15	3.1	Perfusate samples	[13]
HPLC-UV	CFA	0.1–3	0.02	10.1	Plasma, tissue	[16]
HPTLC	CFP	0.4–1.2 (μg spot^{-1})	0.15 (μg spot^{-1})	1.18	Tablets, self nanoemulsifying systems	[14]
Voltammetry	CFP	0.02–0.08	0.003	2.8	Urine	[11]

Table 3
Accuracy and precision of the developed spectrofluorimetric method.

Sample	Concentration (ng mL^{-1})	Intra-day assay (n = 5)		Inter-day assay (n = 3)	
		Accuracy (%)	Precision (RSD%)	Accuracy (%)	Precision (RSD%)
CFP	50	98.56	4.08	98.91	3.20
	500	100.0	1.45	99.5	1.69
	1500	99.28	0.79	99.9	0.95
CFA	50	100.1	3.82	99.3	3.45
	500	98.4	2.48	98.1	2.12
	1500	99.6	0.87	99.7	1.17

3.3. Method validation

Under the optimal reaction conditions, linear relationship with good correlation coefficient (r = 0.9991, 0.9984) were found between the RFI and drug concentration in the range 50–2000 ng mL^{-1} for CFA and CFP. Quantitative parameters and statistical data were summarized for the analysis of CFP and CFA by the developed method in Table 1. The slopes of the calibration curves reflect the sensitivity of the developed method. The detection limits (S/N = 3) obtained with the developed method were 9.48 and 9.17 ng mL^{-1} whereas quantitation limits were 31.48 and 30.56 ng mL^{-1} for CFP and CFA respectively.

The developed method was found to be 6–50 times more sensitive than spectrophotometric methods [8,9], 15–80 times than HPLC-UV methods [8,13], 2500 times than HPTLC [14] but less sensitive than voltammetric methods [10,11]. A comparison between the developed and reported methods was presented in Table 2. However, the low reproducibility and absence of data about voltammetric behavior of the active form CFA makes these methods less convenient for routine assay of CFP and CFA in quality control laboratories. The intra-day precision of the assay was determined by replicate analysis of five separate sample solutions of the working standard of CFP and CFA at three concentration levels 50, 500 and 1500 ng mL^{-1} and the results were shown in Table 3. This study was repeated for three days to determine the inter-day precision. The relative standard deviations percentage (RSD%) were 0.79–4.08 indicating good repeatability and precision. Accuracy was determined by comparing measured concentrations of CFP and CFA with the true values and expressed as percentage. The intra-day and inter-day accuracy of the developed method was studied and the recoveries were ranged from 98.1 to 100.1% indicating good accuracy. The obtained accuracy and precision was satisfactory for quality control measurements for the CFP and CFA.

The robustness of the developed method was examined by evaluating the influence of small variations of some most important operational parameters such as buffer pH (11.5–12.5), NQS concentration (0.4–0.6 mg mL^{-1}), reaction time (13–17 min) and emission wavelength (435–445 nm) on the recovery of CFA and CFP. The recoveries obtained under the optimum experimental conditions and after small variation of these parameters were found to be 96.3–100.5%. The obtained results were shown in Table 4 and none of these variables significantly affect the determination of CFA or CFP. These data provide an indication about the reliability of the proposed method during usage in quality control laboratories therefore, the proposed procedure can be considered robust. The ruggedness of the method was evaluated by applying the recommended analytical procedures for the analysis of CFP and CFA samples by using two different spectrofluorimeters (% RSD = 2.45 for CFP, 2.36 for CFA), three different analysts (the three authors) (%

Table 4
Robustness of the developed spectrofluorimetric method.

Sample	% Recovery ± S.D.	
	CFP	CFA
No variations	98.9 ± 1.45	99.6 ± 1.94
Buffer pH		
pH 11.5	96.3 ± 1.25	97.1 ± 1.37
pH 12.5	100.5 ± 0.97	99.5 ± 1.85
NQS conc.		
0.4 mg mL^{-1}	98.1 ± 2.31	98.7 ± 0.95
0.6 mg mL^{-1}	99.9 ± 1.55	99.3 ± 1.77
Reaction time		
13 min	97.6 ± 2.75	96.5 ± 2.44
17 min	99.2 ± 0.88	99.1 ± 0.65
Emission wavelength		
435 nm	99.0 ± 1.29	99.5 ± 1.75
445 nm	98.7 ± 1.42	99.8 ± 1.38

Table 5
Analysis of CFP in pharmaceutical dosage forms by the developed and official methods.

Dosage form	Ingradient (content)	% Recovery[a] ±SD		*t*-value[c]	*F*-value[c]
		Developed method	Official method[b]		
Orelox tablet	CFP (130.45 mg)	101.77 ± 2.15	100.77 ± 2.32[b]	1.329	0.096
Orelox suspension	CFP (40 mg/5 mL)	98.93 ± 0.99	98.76 ± 0.84[c]	0.304	1.409

[a] Values are mean of six determinations.
[b] USP 2007 [7].
[c] The tabulated values of *t* and *F* at 95% confidence limit are 2.228 and 5.051, respectively.

RSD = 2.12 for CFP, 1.86 for CFA), and independently on five different days (% RSD = 2.75 for CFP, 2.66 for CFA) at two different laboratories (% RSD = 2.15 for CFP, 2.40 for CFA).

3.4. Application of the developed method for pharmaceutical formulations

The available pharmaceutical dosage forms of CFP were successfully analyzed by the proposed and official methods [7]. The obtained recovery results by proposed method were statistically compared with those obtained by official methods. The results were listed in Table 5, which indicated that common excipients and additives did not interfere with the determination. The *t*-test and *F*-test were carried out and no significant differences were found between the calculated and theoretical values of both the proposed and official method at 95% confidence level indicating that the proposed method was accurate enough for analysis of CFP in pharmaceutical formulations. Recovery of CFP from pharmaceutical dosage forms was performed by using standard addition method. The method involved the addition of different amounts of standard CFP (50, 100, 150 mg) to known fixed amount of pharmaceutical dosage forms (tablets and suspension) then samples were treated as described in experimental (Section 2.6) and the resulting solution was analyzed by the proposed method. Results presented in Table 6 indicate good recoveries. The recovery results showed that no serious interferences occurred from frequently encountered excipients and the proposed method was able to access the analyte in the presence of excipients and hence, it can be considered selective.

3.5. Application of the developed method for human urine samples

The prodrug CFP is rapidly and completely hydrolyzed to active CFA *in vivo* [25]. Therefore, the method was applied for the determination of CFA in human urine samples from healthy volunteers in order to test applicability of the proposed method. The high sensitivity offered by the proposed method allowed the determination of even low concentrations of CFA in biological samples. Firstly, the method was applied for the determination of CFA in spiked human urine samples from healthy adult male volunteers (Table 7). Three different concentrations of CFA (200, 500 and 1000 ng mL^{-1}) were spiked to human urine samples then CFA concentrations were determined by the proposed method against blank urine samples. The mean recovery values of CFA were 98.35–100.16% ± 1.48–1.75. It might be concluded that there was no significant interference from the components of urine matrix. NQS reacts with endogeneous amino acids and yields water soluble products measured spectrophotometrically at different wavelengths (465–475 nm) [26] whereas NQS-CFA product measured fluorimetrically in this work after excitation at 330 nm. In addition, the interferences of any other existed compounds from biological samples could be avoided by comparing results against blank urine samples. Thus, this emphasized the method selectivity. The proposed method was applied for the determination of CFA in human urine samples from four healthy adult male volunteers (18–42 years) who received a single oral dose of 133.45 mg of Orelox®tablets equivalent to 100 mg CFA. The samples of individuals were collected 3 h after administration. The peak urine concentration was expected around 3 h therefore, 3 h was chosen for urine samples collections [27]. Results are presented in Table 8. It was found that the mean concentration of CFA in human urine samples was 0.72 ± 0.12 μg mL^{-1}. The obtained results were quite similar to reported peak urine concentrations [27,28]. These results suggest that the high sensitivity and accuracy of the developed method is sufficient for clinical investigations of CFA in human urine samples.

Table 6
Percent recovery of CFP from pharmaceutical dosage forms by standard addition method.

Dosage form	Declared amount	Amount added (mg)	Recovery (%) ± SD*
Orelox tablet	130.45 mg	50	98.83 ± 1.25
		100	96.44 ± 0.15
		150	95.84 ± 0.99
Orelox suspension	40 mg/5 mL	50	97.16 ± 0.58
		100	95.92 ± 0.48
		150	98.37 ± 0.69

* Average of three determinations.

Table 7
Determination of CFA in spiked human urine samples by the developed method.

Spiked concentration	% Recovery[a] ±SD
200 ng mL^{-1}	98.35 ± 1.75
500 ng mL^{-1}	99.48 ± 1.86
1000 ng mL^{-1}	100.16 ± 1.48

[a] Values are mean of five determinations.

Table 8
Determination of CFA human urine samples from healthy human volunteers 3 hours after single dose of 133.45 mg CFP against blank urine.

Subjects	Concentration of Cefpodoxime acid (μg mL^{-1})
Volunteer 1	0.577
Volunteer 2	0.690
Volunteer 3	0.865
Volunteer 4	0.749
Mean ± SD	0.72 ± 0.12

4. Conclusion

A simple, rapid, highly sensitive one-pot reaction for spectrofluorimetric determination of CFP and its active metabolite CFA was developed and validated for their determination in dosage forms as well as human urine samples. The method involved reaction between CFP or CFA and NQS derivatizing reagent in alkaline medium without heating, extraction or reduction steps as usual for NQS derivatization reactions. The method can easily be implemented in plating facilities and/or automated on-line derivatization reactions.The simplicity and low cost make the proposed method more superior to most of the reported methods in routine

assay in quality control laboratories. High sensitivity and selectivity of the proposed method was advantageous in analysis of CFA in human urine samples. The proposed spectrofluorimetric method was successfully used to measure CFA in urine obtained from healthy human volunteers after a single dose of the prodrug CFP. Finally, the proposed method may provide a useful tool for monitoring CFA in different clinical studies of CFP pharmacokinetics as well as multiple sample analysis in quality control laboratories.

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