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Spectrofluorimetric And Spectrophotometric Determination Of Cephradine And Cefaclor

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ABSTRACT

Simple, accurate, precise and sensitive spectrofluorimetric and spectrophotometric methods were developed for determination of α-amino acyl β-lactam cephalosporines; cephradine(CPHD) and cefaclor(CFL). Both methods are based on coupling reaction of the cited drugs with 4-chloro-7-nitro-2,1,3-benzoxadiazole(NBD-Cl) in 0.1 M sodium borate buffer of pH 8-8.5 to yield yellow fluorescent products. The fluorescence was measured in methanolic-aqueous medium ($\lambda_{ex/em}$=458/528 and 490/538 nm for CPHD and CFL, respectively). The absorbance of the colored products was measured in aqueous solution at 450-455nm. All variables affecting the color development and the fluorescence emission were investigated and conditions were optimized. The calibration plots of CPHD and CFL were linear over the concentration ranges of 0.025-0.3μg ml^{-1} for the spectrofluorimetric method and of 10-60 and 5-30μg ml^{-1}, respectively for spectrophotometric method. The proposed methods were applied for the determination of CPHD and CFL in bulk and commercial dosage forms and the results obtained were compared statistically with those given by reference methods.

KEYWORDS

Spectrophotometry;
Spectrofluorimetry;
4-Chloro-7-nitro-2,1,3-benzoxadiazole;
Cephradine;
Cefaclor;
Pharmaceutical formulations.

INTRODUCTION

CPHD and CFL are β-lactam antibacterials, classified as first- and second-generation cephaospo-rins, respectively. They are used in the treatment of susceptible infections including upper and lower respiratory-tract infections, skin infections, and urinary-tract infections[1].

CPHD and CFL are official in the USP[2] and BP[3]. The pharmacopoeial monographs described HPLC

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methods for the assay of active substances in bulk drug substances and in pharmaceutical dosage forms.

CPHD and CFL are members of α-amino acyl cephalosporin having N-phenyl glycyl moiety and known to be weakly UV absorbing compound, so direct spectrophotometric measurements allow only poor sensitivity. Different spectrofluorometric and spectrophotometric methods have been widely applied for determination of CFD and CFL either through degradation reactions in alkaline[4-6] and acidic solutions[7,8] or derivatization reactions[9-13].

Also, HPLC[14-18], HPTLC[19], electro-analytical[20-23], capillary zon electrophoretic[24-26] and flow injection analytical[27,28] methods have been reported for assay of CPHD and CFL.

The present work aims to develop simple and sensitive spectrofluorimetric and spectrophotometric methods for quantitation of CPHD and CFL. The methods are based on the reaction with NBD-Cl in borate buffer of pH 8-8.5 to produce absorbing fluorescent species. The variables affecting the reaction and the experimental conditions were optimized. The applicability of the methods was verified by the analysis of the cited drugs in different commercial pharmaceutical preparations

EXPERIMENTAL

Instruments

Fluorescence spectra and measurements were taken on RF-1501 Shimadzu spectrofluorometer, equipped with 1-cm quartz cell and Xenon lamp.

For spectrophotometric measurements, a Perkin-Elmer Lambda EZ201 UV/VIS spectrophotometer with 1-cm cells was used.

Reagents

1μg ml^{-1} solution of NBD-Cl(ACROS Organics, New Jersey, USA) was prepared in methanol and kept in a refrigerator. It is stable for two weeks.

Borate buffer solution was prepared of 0.05M sodium tetraborate and the pH was adjusted with 0.1 M sodium hydroxide or 0.1M boric acid.

Standard solutions

200.0μg ml^{-1} standard solutions of CPHD and CFL were prepared in distilled water and kept in a refrigerator. The standard solutions are stable at least for one week.

Assay solutions

1. Preparation of CPHD and CFL capsules assay solutions

A quantity of the mixed contents of 20 capsules, equivalent to 20mg of either CPHD or CFL was mixed with sufficient distilled water to produce 100ml, shaken for 30 min and filtered. Dilution of the filtrate was made with distilled water to obtain a solution containing 200.0μg ml^{-1} of CPHD or CFL.

2. Preparation of CPHD vials assay solutions

The content of CPHD container for injection was constituted with 100ml of distilled water and centrifuged. Further dilutions were made with distilled water to obtain a solution containing 200.0μg ml^{-1} of CPHD.

Analytical procedure for calibration graphs

Into a set of 10ml volumetric flasks, aliquots of standard working/assay solutions(to give final concentration ranges specified for fluorimetric and spectrophotometric measurements, (listed in TABLE 1) were mixed with 0.8ml borate buffer of pH 8-8.5 and 1-1.2ml NBD-Cl(1μg ml^{-1}). The reaction mixtures were heated for 30 min at 70-80^{0}C, allowed to equilibrate to room temperature and then 0.1ml of 5 M HCl was added and diluted to a final volume of 10 ml with methanol(spectrofluorimetry) or distilled water(spectrophotometry). The fluorescence intensities and absorbances were measured at the specified wavelengths(TABLE 1) using reagent blank.

RESULTS AND DISCUSSION

NBD-Cl has been widely applied in pharmaceutical analysis of amines and amino acids using different techniques, spectrofluorimetry[29,30], spectrophotometry[31-33], HPLC-fluorescence detection[34,35] and capillary electrophoresis-fluorescence detection[36].

NBD-Cl reacts with primary and secondary amines to give colored fluorescent adducts. The free α-amino acyl group of CPHD and CFL reacts readily with NBD-Cl in borate buffer of pH 8-8.5 through

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TABLE 1: Analytical features for the spectrofluorimetric and spectrophotometric methods

Parameter	Spectrofluorimetric method		Spectrophotometric method	
	CPHD	CFL	CPHD	CFL
Measurement λ, nm	458/528 ($\lambda_{ex/em}$)	490/538 ($\lambda_{ex/em}$)	450	455
Concentration range, (μg ml^{-1})	0.025-0.3	0.025-0.3	10-60	5-30
Regression equations				
Intercept (a)	3.6	7.6	-0.0016	-0.0043
Slope (b)	2808	3023	0.017	0.0355
Correlation coefficient (r)	0.9995	0.9997	0.9998	0.9997
S_a^2	68	39	6.7 E-6	5 E-6
S_b^2	1800	1020	4.8 E-9	1.3 E-8
Molar absorptivity, l mol^{-1} cm^{-1}			5.9×10^3	1.4×10^4
Sandell´s sensitivity, μg cm^{-2} (0.001A)$^{-1}$			0.059	0.028
LOD (μg ml^{-1})	0.0015	0.0015	0.45	0.24
LOQ (μg ml^{-1})	0.005	0.005	1.5	0.8
RSD%	1.1[a]	1[a]	0.7[b]	0.8[b]

S_a^2- Variance of intercept; S_b^2-Variance of slope; LOD- Limit of detection; LOQ- Limit of quantification; [a]At concentration 0.1 μg ml^{-1}; [b]At concentration 20μg ml^{-1}

the formation of meisenheimer complex[37] (Figure 1) to yield yellow fluorescent derivatives with maximum absorbance at 450-455 nm(Figure 2A) and fluorescence emission at 528 nm(λ_{ex}=458nm) and 538 nm(λ_{ex}=490nm) for CPHD and CFL, respectively (Figure 2B).

Optimization of reactions conditions

The influence of some variables on the reaction sequence was studied to establish the most favorable conditions to achieve maximum analytical sensitivity(in term of either spectrophotometric or fluorimetric measurements). The factors studied included; reaction-time/temperature, pH and volume of borate buffer, NBD-Cl concentration, and organic solvents as diluents.

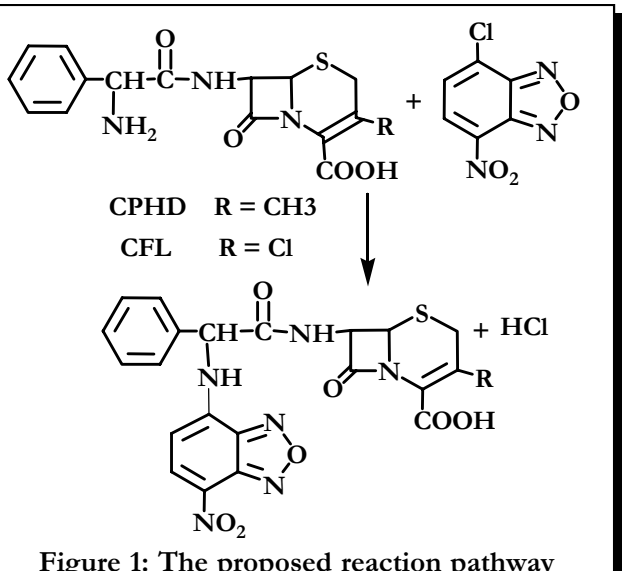

Figure 1: The proposed reaction pathway

The effect of the pH of 0.05M borate buffer solution on CPHD/NBD-Cl or CFL/NBD-Cl reaction development was investigated over the range of 7 to 10. Absorbance-pH plot was shown in figure 3.

The pHs of 8.0 and 8.5 were chosen for CPHD and CFL, respectively. Additional study on the volume of the borate buffer indicated 0.8ml as the most appropriate.

The reaction time course was studied by following a set of reaction solutions of CPHD/NBD-Cl or CFL/NBD-Cl heated between 60 and 90^{0}C for 40min. Figures 4A and 4B display the data in term of absorbance-time profile for CPHD and CFL, respectively. In either, the absorbance values reached a plateau close to 30 min.

The effect of NBD-Cl concentration(in term of x ml of 1μg ml^{-1} solution in methanol) on CPHD and CFL reactions was studied. 1.0-1.2 ml of 1μg ml^{-1} NBD-Cl was found to be sufficient for production of maximum and reproducible color intensity.

The color intensity(also the fluorescence emission) of CPHD/NBD-Cl or CFL/NBD-Cl reaction solutions, in different diluting solvents, was compared. Decreasing in absorbance was in the order;

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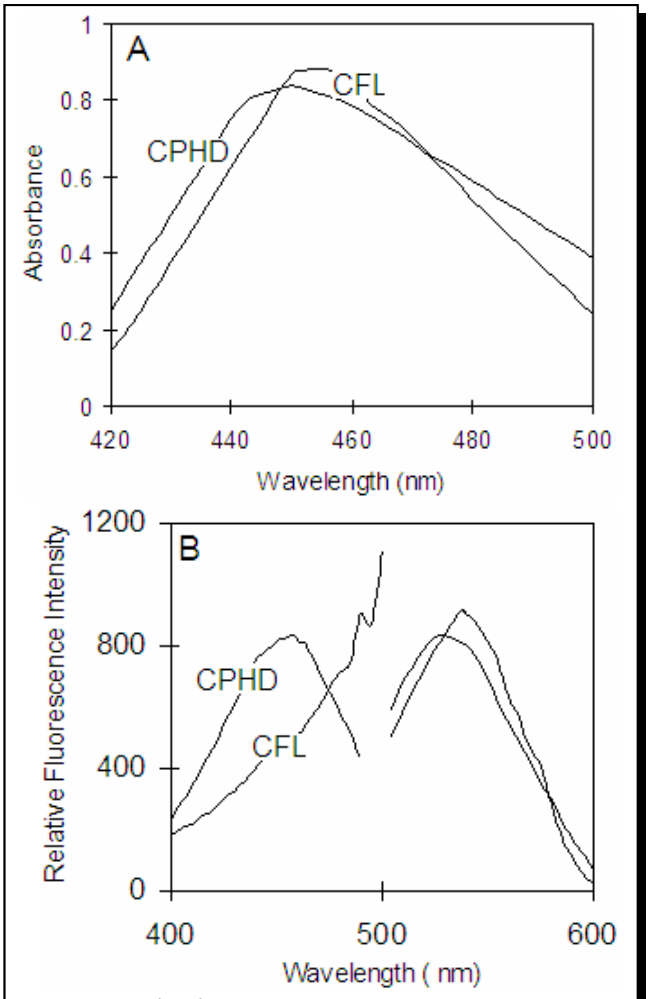

Figure 2: (A) Absorption spectra of the reaction products of CPHD (spectrum 1, 50µg ml-1) and CFL (spectrum 2, 25µg ml-1) with NBD-Cl in borate buffer. (B) The corresponding fluorescence spectra of 0.25µg ml-1 of either CPHD or CFL

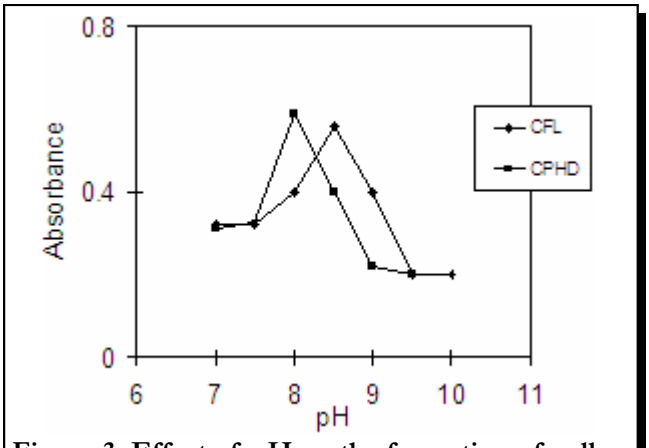

Figure 3: Effect of pH on the formation of yellow fluorescent CPHD/NBD-Cl and CFL/NBD-Cl reaction products

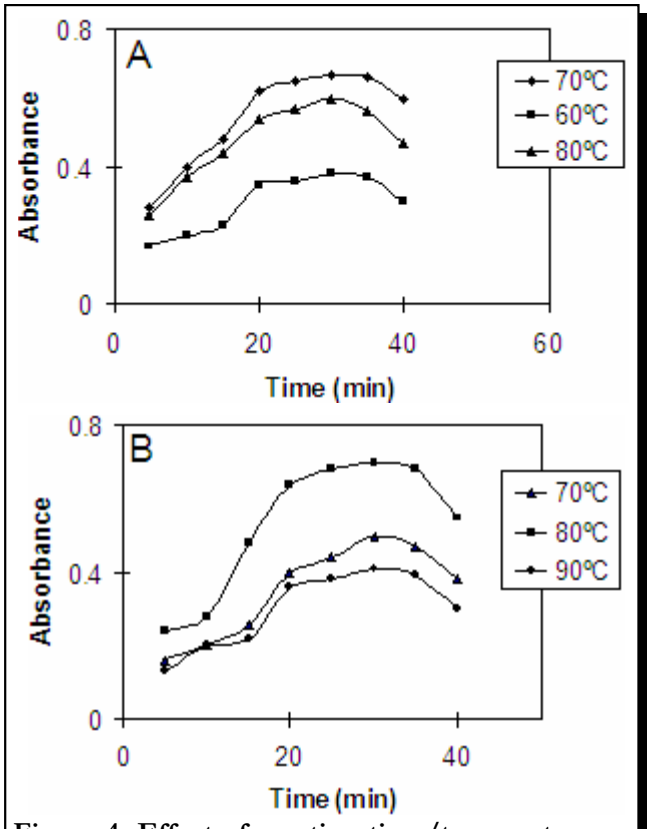

Figure 4: Effect of reaction time/temperature on the formation of yellow fluorescent CPHD/NBD-Cl (A) and CFL/NBD-Cl (B) derivatives

water, ethanol, methanol, acetonitrile, dimethyl-formamide, acetone and dioxane, while decreasing in fluorescence intensity was in the order; methanol, ethanol, water, acetonitrile, acetone, dimethyl-formamide and 1,4-dioxane. Accordingly, water and methanol were chosen as the most appropriate solvents for spectrophotometric and spectrofluorimetric measurements.

The addition of HCl is necessary for the termination of the reaction and suppression of the blank value. The fluorescence of the hydrolysis product of NBD-Cl, namely, 4-hydroxy-7-nitro-2,1,3-benzoxadiazole (NBD-OH), is quenched by decreasing the pH of the reaction medium to less than 1[38]. Also HCl results in a shift of NBD-OH absorption maximum, so, the reagent blank exhibits no significant absorption peak between 420 and 500nm[37]. Accordingly, the volume of 5 M HCl was checked to satisfy such a requirement. A 0.1ml of 5 M HCl was adequately sufficient.

The stoichiometric ratio of drug to NBD-Cl was

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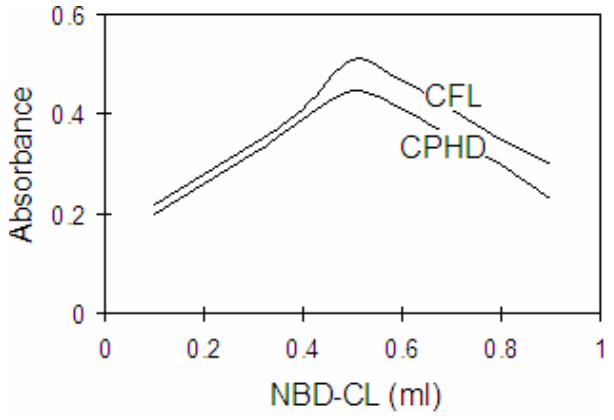

Figure 5: Job's method for determining the stoichiometry of CPHD/NBD-Cl and CFL/NBD-Cl reactions

determined using the molar ratio method. Variable volumes of equimolar solutions of either CPHD/NBD-Cl(1.5×10^{-3}M) or CFL/NBD-Cl(0.8×10^{-3}M) couples were used to obtain different ratios. The resultant data(Figure 5) indicated a drug to NBD-Cl ratio of 1:1.

Stability of the CPHD/NBD-Cl or CFL/NBD-Cl reactions product

Spectrofluorimetric and spectrophotometric measurements of CPHD/NBD-Cl or CFL/NBD-Cl reactions solutions indicated stability of the derivatives up to 24h follow up.

Statistical analysis of results

1. Concentration ranges and calibration graphs

Using the optimized reactions conditions, the relative fluorescence intensities and absorbance measured at the specified analytical wavelengths were found to be linearly correlated to the CPHD and CFL concentrations. Data recorded in TABLE 1 summarize the characteristics of the calibration plots. These include linear regression equations, concentration ranges, correlation coefficients(r), and variance of the intercept (S_a^2) and slope (S_b^2).

2. Precision

The within-day precision of the analytical response for CPHD and CFL determination was assessed using standard solutions of both drugs at a concentration of 20.0 and 0.1μg ml^{-1} for the spectrophotometric and fluorimetric measurements, respectively. The RSD ranged around 1% (TABLE 1).

3. Accuracy

The accuracy of the proposed methods was compared to that of the reported spectrofluorimetric method[8]. Accordingly, determinations of authentic CPHD and CFL were carried out. There were no significant differences between the methods compared (TABLE 2).

4. Detection and quantification limits

The limit of detection, LOD, and the limit of quantification, LOQ, were calculated in accordance to the formulas given by the official compendial methods[2], where LOD and LOQ are defined as $3s.b^{-1}$ and $10s.b^{-1}$, respectively(s is the standard deviation of replicate blank responses). Data are given in TABLE 1.

Analysis of pharmaceutical formulations

The developed spectrophotometric and fluorimetric methods were applied to the determination of CPHD and CFL in their commercially available pharmaceutical preparations. TABLE 3 shows the results of five determinations. The methods gave satisfactory recovery data. The statistical calculations for the assay results show good precision of the proposed methods. In all cases the products tested were confirmed as being compendial quality in terms of

TABLE 2: Accuracy for the determination of CPHD and CFL by the proposed spectrofluorimetric and spectrophotometric methods

	Spectrofluorimetric method		Spectrophotometric method		Reference method[a]	
	CPHD	CFL	CPHD	CFL	CPHD	CFL
Recovery±SD[b]	99.7±0.42	99.5±0.81	100.5±1	99.6±1.1	100.4±0.9	99.9±1.1
SAE[c]	0.2	0.4	0.4	0.5		
t[d]	1.34	0.57	0.1	0.30		
F[e]	5.23	1.94	1.09	1.11		

[a][8]; [b]Mean± Standard deviation of five determinations; [c]Standard analytical error; [d]Tabulated t-value for P = 0.05 and 8 degree of freedom is 2.306, [e]Tabulated F-value for P = 0.05 and $f_1 = f_2 = 4$ is 6.38

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TABLE 3: Analysis of CPHD and CFL in some commercial pharmaceutical preparations

	Found ± SD[a]		
	Spectrofluorimetric method	Spectrophotometric method	Reference method[b]
CPHD preparations [c]			
Velosef capsules, 250 mg	99.4±0.7 t= 0.14 , F=5.44[d]	101.2±0.7 t=1.19, F=5.87[d]	99.5±1.7
Velosef capsules, 500 mg	99.3±0.8 t=, F=[d]	101.1±0.7 t=1.63, F=3.99[d]	100.2±1.4
Velosef vial, 1000 mg	99.5±0.8 t=1.2, F=3.30[d]	101.1±0.8 t=0.81, F=5.96[d]	100.4±1.7
Ultracef capsules, 500 mg	99.9±0.7 t=0.18 , F=3.72[d]	99.9±0.7 t=0.71, F=3.72[d]	100.1±1.36
Ultracef vial, 500 mg	100.1±0.6 t=0.38 , F=3.18 [d]	100.1±0.8 t=0.44, F=3.20[d]	99.9±1.26
CFL preparations [e]			
Serviclor capsules, 250 mg	100.6±0.8 t=0.77, F=4.03[d]	99.5±0.7 t=0.83, F=4.01[d]	100.1±1.4
Serviclor capsules, 500 mg	100.9±0.7 t=0.99, F=4.84[d]	99.5±0.8 t=0.99, F=4.86[d]	100.2±1.5
Bacticlor capsules, 250 mg	100.4±0.7 t=0.8, F=3.31[d]	100.7±0.7 t=1.32, F=3.30[d]	99.9±1.3
Bacticlor capsules, 500 mg	100.7±0.8 t=1.89, F=4.29[d]	100.5±0.8 t=1.63, F=4.28[d]	99.3±1.4

[a] Mean± Standard deviation of five determinations; [b][8]; [c]Cephedrine preparations: Velosef capsules and vials (Bristol Mayer Squiubb-Egypt), Ultracef capsules and vials (Misr Co., Egypt); [d] Tabulated t-value for P = 0.05 and 8 degree of freedom is 2.306, tabulated F-value for P = 0.05 and $f_1 = f_2 = 4$ is 6.38; [e]Cefaclor preparations: Serviclor capsules (Novartis under license of Biochemie), Bacticlor capsules (Pharco Co. under license of Ranbaxy)

the drug content using reported method[8]. The results obtained were compared statistically by Student's t- and variance ratio F-tests, the calculated values did not exceed the theoretical ones which indicated that there were no significant differences between the methods compared, i.e. the proposed methods are as accurate and precise as the respective reference methods.

CONCLUSION

The proposed spectrophotometric and spectrofluorimetric methods have proved to be simple, sensitive, accurate and precise and could be advantageously compared to the reported HPLC methods in term of ease of performance, economy and less time consumption. Also the sensitivity achieved by the developed methods was favorably ranked among other reported spectroscopic methods. The methods are suitable for application in quality control analysis of CFL and CPHD in various pharmaceutical preparations.

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