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Spectrophotometric Determination of Cefadroxil in Bulk and Dosage Forms using 2,4-Dinitrophenylhydrazine.

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Abstract:

An accurate, sensitive and simple spectrophotometric method has been developed and validated for determination of cefadroxil (CFX) in bulk and pharmaceutical formulations. The method is based on the oxidation of 2,4-dinitrophenylhydrazine (DNP) using potassium periodate (PPI) to produce a diazonium salt which is coupled with CFX to form a colored chromogen which was measured at 515 nm. Appropriate conditions were examined for the reaction to obtain maximum absorptivity and sensitivity. The optimum conditions were 1.5 mL of 0.1% 2,4-DNP, 1.5 mL of 0.15% PPI and 0.5 mL of 10 M NaOH solution at room temperature. The method was linear in a concentration range between 7.5-75 µg/mL with regression coefficient of 0.998 (n=5). The limit of detection (LOD) and limit of quantification (LOQ) were 0.89 and 2.7 µg/ml, respectively. The precision was satisfactory; the values of relative standard deviation (RSD) had not exceeded 2%. The average values of recovery study at three concentration levels were found to be in the range 98.6−101.3 ± (0.28 – 0.14). The developed method was applied successfully for determination of CFX in capsules, tablets and suspension, therefore, it could be used for routine analysis of the drug in pharmaceutical dosage forms.

Keywords: Cefadroxil, 2,4-dinitrophenylhydrazine, Spectrophotometric, Derivatization.

Introduction

Cefadroxil (CFX) is an antibiotic, chemically named (6R,7R)-7-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid monohydrate (BP, 2013)[1](Figure 1). Cefadroxil is a semi-synthetic cephalosporin antibiotic. It is useful for serious infections caused by susceptible strains of micro-organisms in lower respiratory infections, genitor-urinary infections, gynecologic infections, skin infections and central nervous system infections. Cephalosporins operate by inhibiting bacterial cell wall biosynthesis which grows actively against a wide range of both gram-positive and gram-negative bacteria[2].

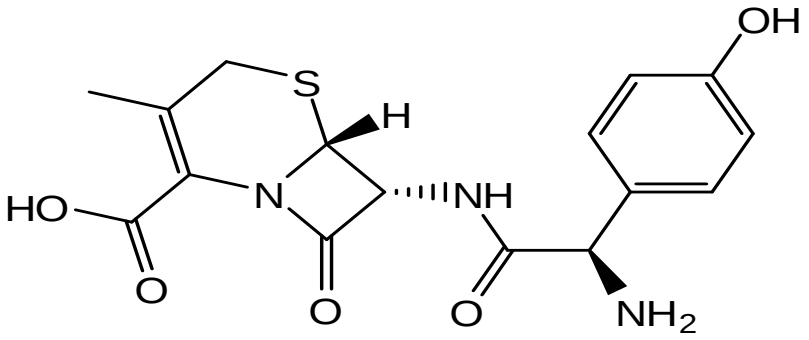

Figure 1: Chemical structure of cefadroxil.

Various analytical procedures were reported in literature for the determination of CFX. Direct spectrophotometric at 264 nm were developed[3]. Derivative spectrophotometry methods using 4-aminoantipyrene; N-bromosuccinide; TCNQ were published [4-6]. In addition, different HPLC [7-10]; capillary zone electrophoresis [11]; chemiluminescence [12]; polarographic methods were also reported [13].

Spectrophotometric analysis has the advantages of being widely availabe, simple, fast, relatively of low cost and less time consumption. Chemical derivatization increases sensitivity and selectivity [14]. 2,4-dinitrophenylhydrazine (DNP) as a derivatizing agent was used in analysis of many drugs[15-18]. This study was attained to develop an accurate spectroscopic method for CFX analysis which depends on a simple one single step reaction using DNP.

Materials and methods

Instruments:

Spectrophotometer: SHIMADZU UV-1601 (with UV-Pro software) and PerkinElmer Lambda 25 (with V5 ES software) and 1 cm quartz cells were used.

Materials and reagents

All chemicals used were of analytical grade. Cefadroxil monohydrate standard was purchased from (Merck, Germany). Biodroxil capsule (cefadroxil monohydrate 500 mg/capsule) and suspension (250mg/5mL) "Sandoz GmbH, Kundl-Austria", Cefadrox tablet (500 mg/tablet) "Birzeit Pharmaceutical Company, Birzeit, Palestine", were purchased from local pharmacy in Gaza.

- DNP 0.1% (w/v): 0.100 g of DNP was accurately weighed and transferred into 100 mL volumetric flask, dissolved in 2.5 mL concentrated sulfuric acid and then diluted with distilled water. The solution was freshly prepared and protected from light during the use.
- Potassium periodate (PPI) 0.15% (w/v): 0.150 g of PPI was accurately weighed and transferred into a 100 mL volumetric flask, dissolved and completed the volume with distilled water.
- CFX stock standard solution (0.25 mg/mL): prepared by dissolving 25.0 mg of cefadroxil monohydrate powder in 100 mL distilled water. Working solutions were prepared by diluting the stock solution. The stock solution was freshly prepared during the use.

General procedure

An aliquot of standard stock solution was transferred into a 10 mL volumetric flask followed by 1.5 mL DNP (0.10%), 1.5 mL PPI (0.15%) and 0.50 mL NaOH (10 M). The mixture was mixed well and diluted to 10 mL with distilled water at room temperature. The absorbance was measured at λ_{max} 515 nm against blank.

Determination of stoichiometric ratio (Job's method)

Job's method of continuous variation was employed. Equimolar solutions ($3.0X10^{-3}$ M) aqueous solution of DNP and CFX were prepared. Series of 1.0 mL portions of CFX and DNP were made up compromising different complementary volumes (0.0:1.0; 0.10: 0.90; 0.20:0.80; 0.30:0.70; 0.40:0.60; 0.50:0.50; 0.60:0.40; 0.70:0.30;

0.80:0.20; 0.90:0.10; 1.0:0.0) in 10 mL volumetric flasks, respectively. The process followed the general procedure and the molar ratio of DNP was plotted against absorbance.

Optimization of reaction conditions

Different reaction parameters were studied. They included concentration and volume of DNP, PPI, and NaOH, temperature, reaction time, order of addition and stability of developed chromogen.

Method validation

Validation parameters were carried out according to ICH guidelines [19]. They included linearity range, precision, accuracy, specificity, limit of detection (LOD), limit of quantitation (LOQ), robustness, and ruggedness.

Linearity and range

Different aliquots of CFX stock solution (0.30- 3.0 mL) were transferred into a series of 10 mL volumetric flasks to produce a concentration ranged (7.5-75 µg/mL). The analysis followed the general procedure above. The absorbances were plotted against concentrations of CFX. The regression line and correlation coefficient were evaluated (n = 5).

LOD and LOQ

LOD and LOQ were calculated using the following formula

LOD = 3.3 σ / S and LOQ = 10 σ / S where σ: Residual standard deviation of regression line, S: Slope of the regression line.

Accuracy and precision

The accuracy was carried out by standard addition method. Known amount of standard CFX (50%, 100%, 150% w/v) was added to a pre-analyzed product (10, 20, 30 µg/mL) and the percent recovery was calculated.

The intra- and inter-day precisions were assessed using three different concentrations of CFX (15, 30, 50 µg/mL). Five replicates were measured on the same day and for 6 consecutive days within a week. Relative standard deviation was calculated.

Specificity

The specificity of the method was evaluated by investigating the interference liabilities from common excipients that might be added during pharmaceutical formulation. 10 and 50 mg of CFX were transferred in 100 mL volumetric flasks and mixed with various

amounts of excipients (e.g., glyceryl monostearate 50 mg, lactose 50 mg, starch 50 mg, talc 50 mg, povidone 10 mg…etc) dissolved, diluted and filtered. Analysis of the prepared samples was conducted as described under general procedure.

Robustness and Ruggedness

Robustness was examined by evaluating the influence of small variation in the method variables on the analytical performance. They included concentration and volume of reagents, temperature, and reaction time. One variable was changed while the others remained constant. Percentage recovery was calculated. On the other hand, ruggedness was assessed by replicating the procedure for three concentrations; 10, 30, 50 µg/mL of the drug three times in two different laboratories with different spectrophotometers (SHIMADZU UV-1601, Japan and PerkinElmer Lambda 25, England). The RSD was calculated for each concentration.

Analysis of commercial pharmaceutical products (capsule, tablet, suspension)

Pharmaceutical dosage forms were subjected to the analysis of their CFX content by DNP method and reference method. The content of 10 capsules and tablets were weighed, crushed and their contents mixed thoroughly. For suspension, three bottles of CFX-suspension were throughly mixed. An accurately measured portions of the dosage forms equivalent to the labeled strength (25 mg) CFX was transferred into a 100 mL volumetric flask. The mixture was shaken thoroughly with distilled water for 10 minutes then filtered to remove insoluble matter. The filtered solution was analyzed as described in the general procedure. The obtained results were compared with data of a published reference method statistically [20].

Results and discussion

The main purpose of this study was to develop a simple and accurate spectrophotometric method for the determination of cefadroxil in pharmaceutical formulations by derivatization method. Determination in pharmaceutical preparation based on direct measurement of absorption is susceptible to potential interferences. Chemical derivatization using special reagents could improve the selectivity and increase the sensitivity of a spectrophotometric assay [14].

Cefadroxil contains a phenolic ring which could be coupled with a diazonium cation in basic solution to produce a bathochromic shifted azo-derivative. The spectra of produced chromogen are shown in figure 2. An absorption maximum (λ_{max}) was recorded at 515 nm. This wavelength has been used for measuring the absorbance of the derivative, which was soluble in water. In the present work, suitable conditions were chosen for the chromogenic reaction between DNP and cefadroxil in order to reach maximum sensitivity.

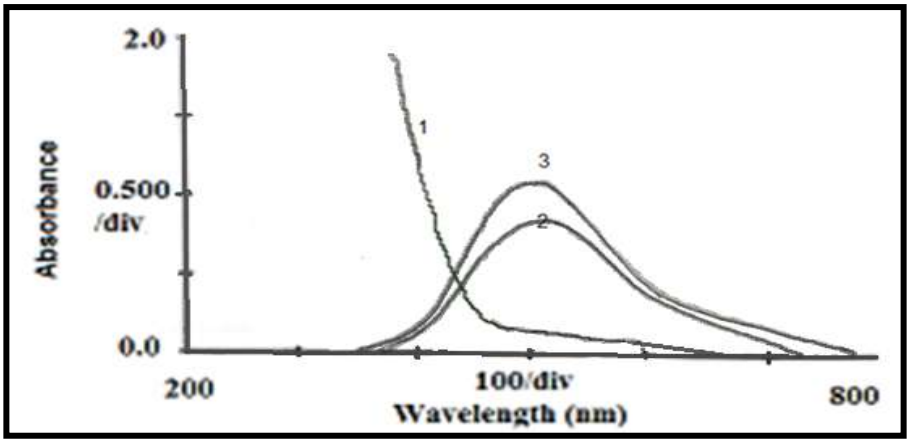

Figure 2: Absorption spectra of 1: blank spectrum against water; 2 & 3: Derivatization product against blank (cefadroxil:30 & 40 µg/ml), respectively.

Optimization of experimental conditions

To optimize the reaction variables, the effects of temperature, reaction time and the concentrations of reagents (e.g., DNP, NaOH, PPI) on the absorbance of the chromogen formed, with respect to maximum sensitivity and stability, were studied through controled experiments. The results of optimization studies are summarized in table 1. The best results were obtained by using 1.5 mL of DNP (0.10% w/v) and PPI (0.15% w/v) and 0.50 mL of NaOH (10M). The reaction was completed immediately at room temperature. In order to examine the effect of temperature and reaction time on the absorbance of the developed chromogen, the general procedure was carried out at different temperatures using thermostatic water bath. Maximum and constant absorbance was obtained at room temperature without delay

after the addition of the reaction contents. A longer reaction time has not improved the results. Under the above mentioned optimum conditions. The effect of time on the stability of the chromogen was monitored by following the absorption values of the reaction solution after dilution at different time intervals. The color formed was stable for at least 2 hours.

Furthermore, the effect of different orders of addition were studied (Table 2). The best one was adding DNP followed by PPI and NaOH to CFX solution. When NaOH was added to CFX the absorption was decreased. This can be explained by phenoxide formation and inappropriate media for diazonium salt formation [21]. Finally, a one step procedure was achieved, which is advantageous in comparison to other spectroscopic assays [22,23, 24].

Table 1: Summary of optimum conditions for cefadroxil-derivatization reaction.

Variable	Studied range	Optimum
DNP concentration (%, w/v)	0.03-0.20	0.10
Volume of 0.1% DNP solution (mL)	0.50-2.5	1.5
PPI concentration (%, w/v)	0.04-0.25	0.15
Volume of 0.15% PPI solution (mL)	0.50-2.5	1.5
NaOH concentration (molarity)	5-10	10
Volume of 10 M NaOH (mL)	0.20-2.0	0.50
Temperature (° C)	25-60	25
Time (min)	0.0 to 60	0
Order of addition	Different	*
Measuring wavelength (nm)	300-700	515

*: For best order of addition see table 2.

Table 2: Effect of order of addition on cefadroxil analysis.

Sample	First	Second	Third	Fourth	Absorbance*
1	CFX	2,4-DNP	PPI	NaOH	0.423
2	CFX	PPI	2,4-DNP	NaOH	0.411
3	CFX	NaOH	PPI	2,4-DNP	0.412
4	CFX	NaOH	2,4-DNP	PPI	0.397**
5	2,4-DNP	PPI	CFX	NaOH	0.390**
6	2,4-DNP	PPI	NaOH	CFX	0.368**

*: values were mean of three determinations; CFX 15µg/mL, 2,4-DNP 0.1% (1.5mL), PPI 0.15% (1.5mL), NaOH 10M (0.5mL), H_2O diluting solvent, at room temperature. **: error was more than 5%.

Determination of stoichiometric ratio (Job's method)

Figure 3 shows the mole fraction of DNP versus absorbances obtained. The mole ratio obtained for DNP and CFX was 1:1.The proposed reaction mechanism is illustrated in figure 4.

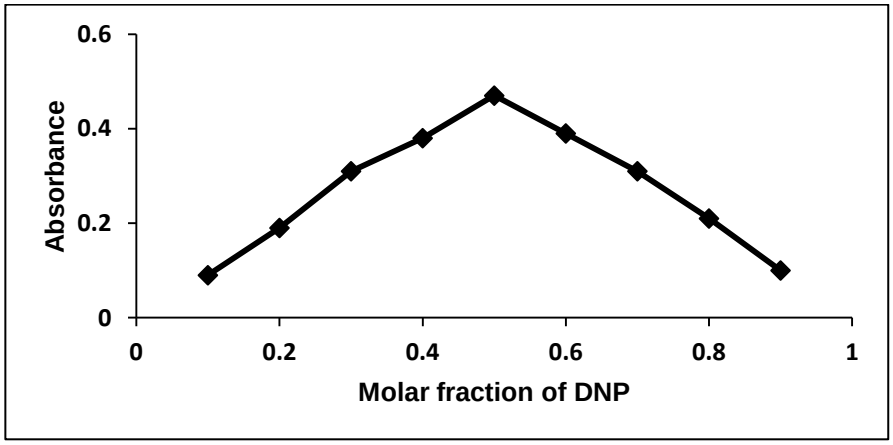

Figure 3: Determination of CFX-DNP chromogen ratio by Job's method;{CFX and DNP: $2*10^{-3}$ M, PPI 0.15% (1.50 mL), NaOH 10 M (0.50 mL), H_2O diluting solvent, at room temperature}.

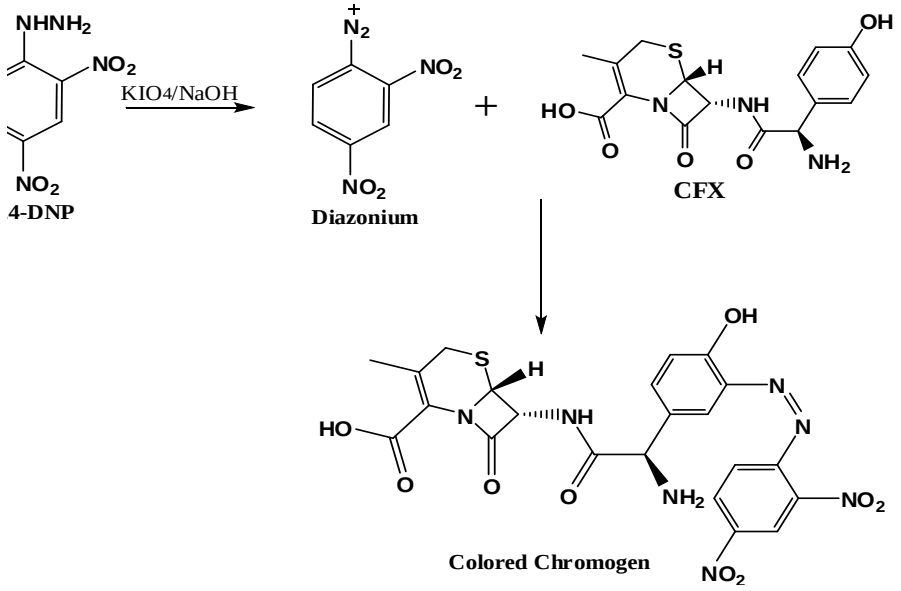

Figure 4: Suggested mechanism of the cefadroxil derivatization reaction.

Method validation

Linearity and range

Under the above optimum experimental conditions, the calibration curve was constructed by plotting the absorbance of the CFX versus the drug concentration within the specified range. A linear correlation was found between concentration and absorbance in the range given in table 1. The correlation coefficients, intercepts and slopes for the calibration data for the tested drug were calculated using least squares method (Table 3). The linear range was obtained in the concentration range 7.5-75 μg/mL. The molar absorptivity (ε) was 7.00×10^3 $L.mol^{-1}.cm^{-1}$.

Sensitivity

The limit of detection (LOD) and the limit of quantitation (LOQ) for the proposed method as well as Sandell's sensitivity were calculated and listed in table 3.

Table 3: Assay parameters and spectral data for spectrophotometric determination of cefadroxil

Parameters	**Vlaue**
$\lambda_{max,}$ nm	515
Linear range, µgmL^{-1}	7.5-75
Molar absorptivity, L mol^{-1} cm^{-1}	7.00 X 10^{3}
Sandell's sensitivity, µg/cm^{2}	0.003
Limit of detection, µg mL^{-1} (LOD)	0.016
Limit of quantitation, µg mL^{-1} (LOQ)	0.0149
Regression equation*	
Intercept (a)	− 0.030
Slope (b)	0.0294
Correlation coefficient (r)	0.9991

*Y= a + bX, where Y is the absorbance, a intercept, b slope and X concentration in µg mL^{-1}.

Accuracy and precision

Accuracy of the proposed method was confirmed by performing recovery studies at three cefadroxil concentration levels by standard addition method. The determination with each concentration was repeated three times and average percent recovery of the added standard was calculated and results are tabulated in table 4. The Intra- and inter-day precisions of the proposed method were tested using three concentrations of the tested drug (within Beer's law limits) and three replicates of each concentration. The precision of the analytical procedure is usually expressed as the relative standard deviation (RSD) of the series of measurements (table 5). The results obtained in tables 4 and 5 showed excellent mean recovery percent values, close to 100 %, and low standard deviation values (SD < 1.03) which indicate high accuracy of the proposed analytical method. Furthermore, the calculated RSD values were found to be small (< 2%) indicating good repeatability and reliability of the proposed methods.

Table 4: Results of recovery study for cefadroxil (CFX) analysis.

CFX Base level (µg mL^{-1})	CFX standard addition (µg mL^{-1})	Amount recovered * (µg mL^{-1})	% Recovery (% ± SD)
	5	4.93	98.6±0.28
	10	9.88	98.8±0.37
10	15	5.14	100.9±0.83
	10	10.13	101.3±0.14
	20	20.17	100.8±0.41
20	30	30.09	100.3±1.03
	15	15.19	101.3±0.70
	30	30.13	100.4±0.97
30	45	44.76	99.5±1.01

*: mean value of three determinations.

Table 5: Evaluation of the intra- and inter-day precision of the analytical procedure of cefadroxil

CFX conc. (µg mL^{-1})	Intra-day, (n =5)		Inter-day, (n =6)	
	Mean % recovery	RSD	Mean % recovery	RSD
15	99.9	0.57	99.5	1.38
30	100.1	0.40	99.9	0.52
50	99.9	0.51	99.8	0.54

RSD = Relative Standard Deviation

Robustness

The results of robustness are listed in table 6. It was found that small variation in the method variables did not significantly affect the procedure; recovery values were 98.2 – 102.8 (± 0.14–1.6). This indicated the reliability of the method.

Table 6: Robustness of cefadroxil analysis.

Variable	Normal	Variation	Recovery (% ± SD)*
2,4-DNP concentration (%, w/v)	0.10	0.08	99.97 ± 0.33
		0.12	99.2 ± 1.5
Volume of 0.10% 2,4-DNP solution (mL)	1.5	1.4	101.5 ± 1.5
		1.6	100.4 ± 0.68
PPI concentration (%, w/v)	0.15	0.13	99.92 ± 1.6
		0.17	98.2 ± 0.52
Volume of 0.15% PPI solution (mL)	1.5	1.4	98.67 ± 0.48
		1.6	102.78 ± 1.3
NaOH concentration (Molarity)	10	9.8	98.9 ± 0.4
		10.2	99.44 ± 0.24
Volume of 10M NaOH (mL)	0.50	0.40	99.97 ± 0.31
		0.60	99.2 ± 1.5
Temperature (° C)	25	23	99.28 ± 0.14
		27	100.65 ± 0.39

*: mean value of three determinations. The concentration of CFX was 50 µg/mL.

Ruggedness

Results obtained from lab-to-lab variations were reproducible, as RSD did not exceed 1.43% (Table 7).

Table 7: Ruggedness of the spectrophotometric method of cefadroxil analysis.

CFX actual conc. (µg/mL)	Relative Standard Deviation (RSD)*	
	Shimaduz UV-1601	PerkinElmer Lambda 25
10	0.568	0.653
30	0.700	0.502
50	0.421	0.466

*: RSD value of three determinations

Specificity

The degree of interference by some excipients which often accompany pharmaceutical preparations were evaluated by measuring the percentage recovery of 10 mg of CFX with various amounts of diverse exipients. The average recovery value obtained was 99.96 ± 0.49% (Table 8). These results confirm the absence of interferences from common excipients.

Table 8: Interferences liabilities from excipients on CFX analysis.

Excipients	Amount of excipients added (mg)	Recovery (% ± SD)*
Glyceryl monostearate	50	99.32 ± 0.15
Lactose	50	98.12 ± 0.04
Starch	50	101.32 ± 0.32
Talc	50	101.72 ± 0.4
Titanium dioxide	50	100.12 ± 0.24
Povidone	10	99.72 ± 1.1
Magnesium stearate	10	98.32 ±1.0
Silicon dioxide (50)	10	99.22 ± 0.15
Saccharin	50	98.8 ± 0.69
Sucrose	50	99.61 ± 0.83
Peach Flavoring powder	10	99.43 ± 0.92
Polysorbate 80	50	101.20 ± 0.24
Sodiun benzoate	50	99.32 ± 0.18

Xanthan gum	50	101.52 ± 0.24
FD&C yellow no 6	10	101.66 ± 0.22
Average % recovery ± SD		**99.96 ± 0.49**

*: values were mean of three determinations.

Application to pharmaceutical dosage forms

The proposed method has been successfully applied to the determination of the CFX in commercial dosage forms: capsules, tablets and suspensions. The recovery percentage of the drug content with low values of standard deviation established the precision of the proposed method. The results obtained are shown in Table 9. The recovery percentage obtained by the proposed method was comply with the BP 2013 specifications of CFX content in capsules and suspension [1] and comparable to the results obtained in several validated published methods [24].

Upon comparing the obtained recovery results with the results of a validated published spectrophotometric method [20], using t-test (Table 9), no significant difference was found at 95% confidence level providing similar accuracy in the determination of CFX [20].

Table 9: Determination of CFX in dosage forms by developed method and comparison with validated published method.

Dosage form[a]	Recovery (% ± SD)[b]		
	DNP method	**Published method[c]**	**p- value[d]**
Capsule	99.41±0.95	99.98± 0.12	0.2199
Tablet	101.05±0.15	99.98 ± 0.96	0.2101
Suspension	99.91±0.08	100.14± 0.10	0.1845

[a]: labeled to contain 500 mg CFX per capsule, 500 mg CFX per tablet, and 250 mg CFX per 5 mL suspension; [b]: mean values of five determinations; [c]: published method: see Ref 20. [d]: p-value > 0.05 insignificant difference.

Conclusion:

The present work described a successful derivatization of CFX using DNP in a single step procedure. In addition, it was advantageous due to avoiding heating, expensive instrumentation or usage of toxic organic solvents. The developed method was simple, accurate and precise. It was successfully applied for determination of CFX in dosage forms.

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