



Research Article

Application of Silver Nanoparticles for the Spectrophotometric Determination of Cefdinir and Cefepime HCl in Pharmaceutical Preparations and Human Urine Samples

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Abstract

In this work, the application of silver nanoparticles (Ag NPs) for spectrophotometric determination of cefdinir (CFD) and cefepime HCl (CFP) was developed. The method was based on the ability of the alkaline degradation products of the cited drugs to reduce Ag⁺ ions to silver nanoparticles (Ag-NPs) in the presence of gelatin as a capping agent. The linear concentration ranges were 5.0 - 40 and 10 - 100 µg/mL for cefdinir and cefepime HCl, respectively. The lower detection limits were found to be 0.39 and 1.06 µg/mL for CFD and CFP, respectively, while the quantitation limits were found to be 1.18 and 3.21 µg/mL for CFD and CFP, respectively. The formed nanoparticles were characterized by ultraviolet-visible spectroscopy and transmission electron microscopy. The proposed method was applied successfully to determination of CFD and CFP in pure drugs, pharmaceutical preparations and human urine samples.

Keywords: Silver nanoparticles; Cefdinir; Cefepime HCl; Human urine samples

Introduction

Cefdinir is a third-generation oral cephalosporin antibiotic. It is chemically (6R,7R)-7-[[[(2Z)-2-(2-Amino-4-thiazolyl)-2-(hydroxyimino)acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate [1]. Cefdinir is not considerably metabolized and is excreted in the urine with an elimination half-life of 1.7 hours [2].

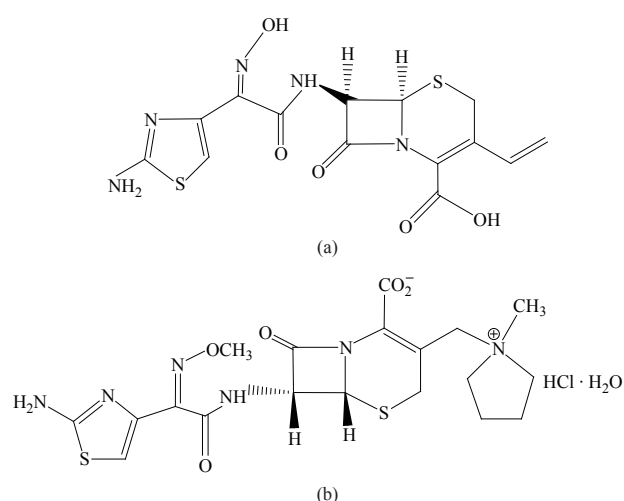
Cefepime hydrochloride is one of the fourth generation of cephalosporin antibiotics. It is chemically 1-[[[(6R,7R)-7-[2-(2-amino-4-thiazolyl)-glyoxylamido]-2-carboxy-8-oxo-5-

thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride, 7²-(Z)-(O-methyloxime), mono-hydrochloride, monohydrate [1]. Cefepime is widely distributed in body fluids and tissues; it is excreted principally by the kidneys and about 85% of a dose is recovered as intact drug in the urine [2].

Both drugs are resistant to beta-lactamase. They have an extended spectrum of activity against gram-positive and gram-negative bacteria due to binding to penicillin-binding proteins, causing thickening of the bacterial cell wall and breakage at the binding site, thereby causing cell death [3].

They are used for the treatment of moderate-to-severe infections, such as pneumonia, febrile neutropenia, skin and soft tissue infections, urinary tract infections and intra-abdominal infections [4].

Different analytical methods have been applied for the determination of cefdinir in biological fluids and pharmaceutical products such as HPLC [5, 6], HPTLC [7], spectrophotometry [3, 7], spectrofluorimetry [8], voltammetry [9, 10] and capillary electrophoresis [11].



Scheme 1 (a) Chemical structure of cefdinir; (b) Chemical structure of cefepime HCl.

Cefepime or its content of N-methyl pyrrolidine were also determined by several methods such as HPLC [12, 13], MEKC [14, 15], GC [16, 17], TLC [18, 19], ion chromatography [20, 21], spectrophotometry [22, 23], spectrofluorimetry [24, 25], voltammetry [26], capillary electrophoresis [27, 28] and chemiluminescence [29].

Nanoparticles made of silver have been the focus of interest in many researches due to their antimicrobial [30] and drug targeting applications [31]. They can be also used for drug analysis owing to their electrical [32] and optical [33] properties. These nanoparticles, in liquid media, have a strong UV-visible extinction band that is absent in the spectrum of the bulk metal.

Literature revealed that silver nanoparticles had been utilized for determination of many drugs in pure form, pharmaceutical products or in biological fluids such as cephalosporins [34], benzimidazole drugs [35], acetaminophen and gentamicin [36], teicoplanin [37], phenytoin [38], diosmin and rutin [39], 6-aminopenicillanic acid (APA) [40].

In this work, silver nanoparticles capped with gelatin [41] were firstly used in quantitative determination

of cefdinir and cefepime HCl using green, simple, sensitive, effective and validated procedures easily applied in human urine samples without interference from urine matrix components.

Experimental

Materials and reagents

Chemicals used were of analytical grade. Cefepime hydrochloride was obtained from Chemical Industrial Development (Cid), El Haram, Giza, Egypt. Cefdinir was obtained from Bristol-Myers Squibb Pharmaceutical Co., Cairo, Egypt. Silver nitrate was obtained from Aldrich Chemical Co. Ltd, used as 10^{-2} M aqueous solution. Gelatin was obtained from El-Nasr Chemical Company, Egypt, used as 1.0% w/v aqueous solution. Sodium hydroxide was obtained from El-Nasr Chemical Company, Egypt, used as 0.1, 0.5 and 1.0 M aqueous solutions. Cefepime[®] vial labelled to contain 1000 mg cefepime hydrochloride was obtained from Pharco B International, Alexandria, Egypt. Dinar[®] capsules labelled to contain 300 mg cefdinir per capsule were obtained from Kahira Pharma Co. for Adwia Co. S.A.E., 10th of Ramadan City, Egypt.

Instrumentation

A Shimadzu ultraviolet-visible (UV-VIS) spectrophotometer UV 1800 (Japan) with matched 10 mm quartz cell was employed for all absorbance measurements.

A JEOL-2100 transmission electron microscope at 200 kV (Japan) was employed for transmission electron microscopy (TEM) examination at Mansoura University.

Preparation of standard and working solutions

Stock solution of 1.0 mg/mL of cefdinir was prepared by dissolving 25 mg of the pure drug in about 2.5 mL 1.0 M NaOH and then completed to 25 mL with bidistilled water. Stock solution was further diluted with bidistilled water to prepare the working solution at 500 µg/mL.

Stock solution of 2.0 mg/mL of cefepime hydrochloride was prepared by dissolving 20 mg of the pure drug in 10 mL bidistilled water. Working solution of 1.0 mg/mL was prepared by dilution of stock solution with bidistilled water.

Preparation of calibration standards and quality control samples

Calibration standards were prepared in bidistilled water at eight concentration levels of 5.0, 10, 15, 20, 25, 30, 35 and 40 $\mu\text{g/mL}$ of cefdinir and at ten levels of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 $\mu\text{g/mL}$ of cefepime hydrochloride. Low, medium and high quality control (QC) samples were prepared at concentrations of 15, 25 and 35 $\mu\text{g/mL}$ and of 30, 50 and 70 $\mu\text{g/mL}$ for cefdinir and cefepime hydrochloride, respectively.

Urine samples were prepared in blank human urine (diluted with water 1:1 v/v) at six concentration levels of 10, 15, 25, 30, 35 and 40 $\mu\text{g/mL}$ and at eight levels of 10, 20, 30, 40, 50, 70, 80 and 100 $\mu\text{g/mL}$ for CFD and CFP, respectively.

Preparation of pharmaceutical tablets working solutions

Dinar[®] capsules

The content of five capsules were mixed well and a certain amount of powder equivalent to 150 mg pure drug was extracted in 50 mL flask with 1.0 mL 1.0 M NaOH, then shaken with 10 mL bidistilled water. The content of flask was diluted to the mark with bidistilled water to prepare 3.0 mg/mL stock solution, then filtered. Stock solution was further diluted with bidistilled water to give 500 $\mu\text{g/mL}$ working solution.

Cefepime[®] vial

Specific amount of powder for injection equivalent to 50 mg was dissolved in 50 mL bidistilled water to give 1.0 mg/mL stock solution.

Preparation of urine samples

Cefdinir

Blank urine (obtained from healthy non-smoking volunteers) was thawed at room temperature, mixed well, filtered through MS[®] PTFE 0.45 μm syringe filters to avoid the interference from urine components and then diluted with bidistilled water to prepare 1:1 v/v urine pool. Urine working solution of 500 $\mu\text{g/mL}$ cefdinir was prepared by mixing equal volumes of stock solution and urine pool. Urine samples were prepared by spiking drug free urine (diluted urine 1:1 v/v in bidistilled water) with different volumes of the urine working solution. 1 mL of each urine sample was subjected to the general procedures as mentioned below.

Cefepime hydrochloride

The same procedures as for cefdinir were applied, but the urine working solution was prepared at a concentration of 1.0 mg/mL cefepime HCl.

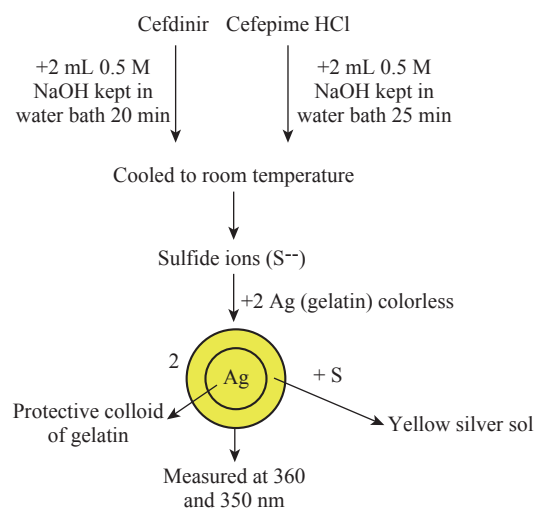
General procedure

Silver-gelatin solution was prepared by mixing 15 mL of silver nitrate (10^{-2} M) solution with 25 mL gelatin (1.0% w/v) solution in 50 mL volumetric flask. Then, pH was adjusted at 9 or 10 using 0.1 M NaOH for cefdinir and cefepime hydrochloride, respectively. The volume was completed to the mark with bidistilled water, mixed well and protected from light by aluminum foil.

In 10 mL volumetric flasks, different volumes of the cited drugs were added, followed by 2 mL of 0.5 M NaOH and heated in boiling water bath for 20 or 25 min for CFD and CFP, respectively. After cooling, specific volumes of silver-gelatin solution (3.0 or 4.0 mL for CFP and CFD, respectively) were added; flasks were shaken and kept for five minutes, and then diluted to the mark with bidistilled water. Absorbance was measured at the suitable wavelength against reagent blank treated similarly.

Results and Discussion

In this study, alkaline degradation products of cefdinir and cefepime hydrochloride acted as effective reducing agents for the reduction of silver metal salt (Ag^+) to the Ag NPs capped with gelatin in alkaline medium as shown in Scheme 2



Scheme 2 Proposed reaction mechanism of studied cephalosporins with silver-gelatin complex.

Optimization of reaction conditions

Effect of hydrolysis time

The studied drugs were heated for different heating times in a boiling water bath (at 100 °C), and the reaction was carried out as usual. The effect of hydrolysis time on the absorption intensity was studied. Heating for about 20 and 25 minutes for cefdinir and cefepime hydrochloride (at 100 °C) gave maximum absorption intensity (Fig. 1).

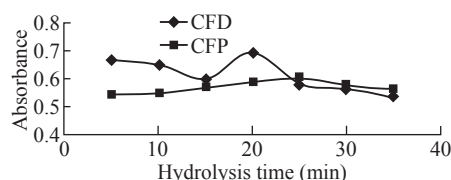


Fig. 1 Effect of hydrolysis time on the absorbance of Ag-NPs formed in the presence of 30 µg/mL CFD and 60 µg/mL CFP.

Effect of NaOH concentration

Sodium hydroxide concentration affected absorption intensity throughout the study. A wide range of NaOH molarities (0.1-4.0 M NaOH) was used and different volumes of the selected NaOH molarity were tried (0.5-5.0 mL). It was found that maximum absorption readings were obtained upon using 2.0 mL 0.5M NaOH for both analytes (Fig. 2).

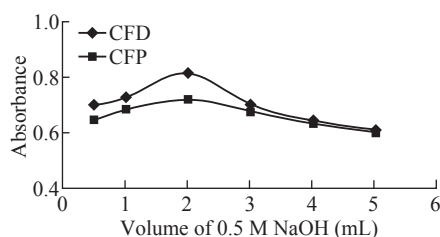


Fig. 2 Effect of volume 0.5 M NaOH on the absorbance of Ag NPs formed in the presence of 30 µg/mL CFD and 60 µg/mL CFP.

Effect of pH of the working reagent solution

The effect of pH on the reduction of silver-gelatin complex was studied over the pH range of 7 - 11. Gelatin was effectively bound to silver at pH higher than 7. Solutions of pH greater than 11 were not suitable as they caused hydrolysis of gelatin solution [42]. The optimum results were obtained at pH 9 and 10 for cefdinir and cefepime hydrochloride, respectively.

Effect of silver nitrate concentration

Different solutions of silver-gelatin complex were prepared at the selected pH by varying the

concentration constant. The silver nitrate concentration (10^{-3} - 2×10^{-2} M) keeping the gelatin optimum concentration was 10^{-2} M for both drugs.

Effect of gelatin concentration

Gelatin acts as a protective colloid to prevent the precipitation of the black silver metal and as a stabilizer for the yellow silver sol. The effect of gelatin concentration was studied by preparing working reagent solutions containing different gelatin concentrations at 0.5 - 2.5% w/v and keeping the silver nitrate concentration constant. It was found that maximum absorption readings were obtained upon using 1.0% w/v for both drugs.

Effect of working reagent solution volume

The working reagent solution (0.5 - 4.0 mL) were tried. Different volumes showed that 4.0 mL and 3.0 mL of the reagent could give the maximum color intensity for cefdinir and cefepime hydrochloride, respectively (Fig. 3).

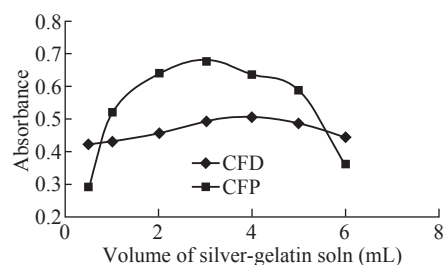


Fig. 3 Effect of volume of working reagent solution on the absorbance of Ag NPs formed in the presence of 30 µg/mL CFD and 60 µg/mL CFP.

Effect of reaction time

The time required for formation of Ag NPs was studied. It was found that after addition of the working reagent solution, flasks should stand before dilution for 5 min for both drugs which was a sufficient time for the complete reaction (Fig. 4).

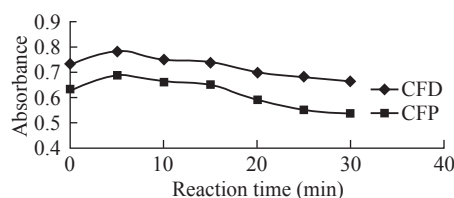


Fig. 4 Effect of volume of working reagent solution on the absorbance of Ag NPs formed in the presence of 30 µg/mL CFD and 60 µg/mL CFP.

Stability

Absorbance of Ag NPs formed in the presence

of cefdinir and cefepime hydrochloride could be measured immediately after dilution with water giving stable readings for 50 min.

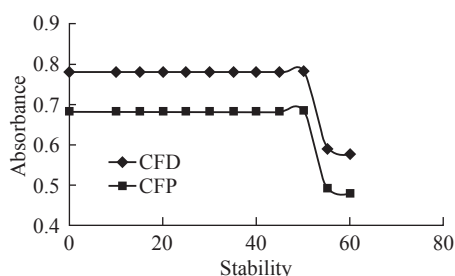


Fig. 5 Stability of Ag NPs formed in the presence of 30 µg/mL CFD and 60 µg/mL CFP.

Physical characterization of silver nanoparticles

UV-Vis spectrophotometry

Structural characterization of Ag NPs could be provided by spectrophotometric analysis as silver colloids exhibit characteristic spectra in the UV-Vis region due to the surface plasmon excitation. In the absence of reducing agents, there was no absorption peak in visible region. After reacting with the alkaline degradation products of the cited drugs, silver ions were reduced to Ag NPs which showed characteristic absorption spectra of the colloidal solution of silver as shown in Fig. 6.

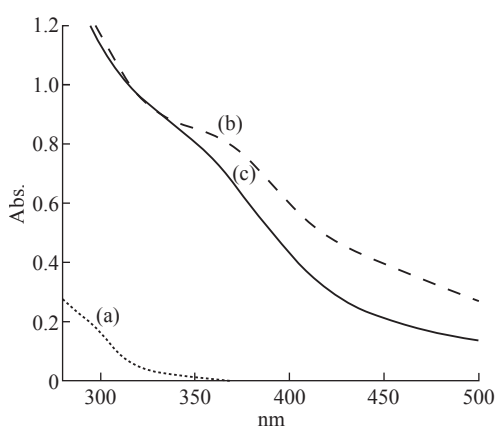


Fig. 6 (a) Absorbance of blank solution. (b) Absorbance of Ag NPs formed in the presence of 30 µg/mL cefdinir. (c) Absorbance of Ag NPs formed in the presence of 70 µg/mL cefepime HCl.

Transmission electron microscope (TEM)

The TEM image proved the formation of silver nanoparticles which were spherical in shape with smooth surface morphology and size of 14.93 ± 6.06 nm as shown in Fig. 7.

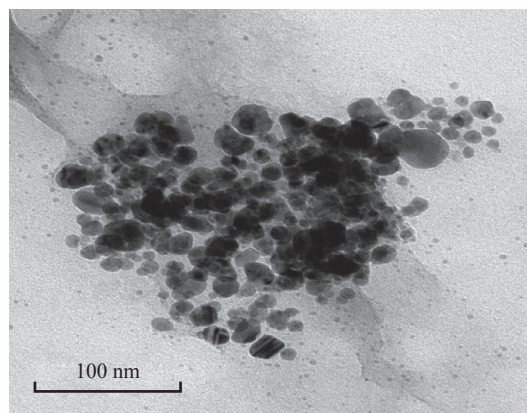


Fig. 7 TEM image of silver nanoparticles formed in the presence of CFD.

Method validation

The method was validated according to ICH guidelines on the validation of analytical methods [43].

Linearity

According to the optimized experimental conditions, standard calibration curves were constructed by plotting absorbance against concentration showing good linearity. The concentration ranges, correlation coefficient, slope and intercept for the calibration curve were calculated.

Limit of quantitation and detection

The limit of quantitation (LOQ) and the limit of detection (LOD) for the proposed method were calculated according to the ICH [43] using the following equations:

$$\text{LOQ} = 10 \sigma/S; \text{ and}$$

$$\text{LOD} = 3.3 \sigma/S,$$

where σ = standard deviation of replicate blank responses (under the same conditions as for sample analysis), and S = slope of the calibration curve.

According to the previous equations, LOD and LOQ were calculated as in Table 1. Their values confirmed the sensitivity of the proposed method.

Accuracy

Accuracy of the method was calculated as the recovery percentages of pure drugs at three concentration levels covering the low, medium and higher ranges of the calibration curve as shown in Table 2.

Precision

Precision was carried out by three determinations

Table 1 Characteristic parameters for determination of CFD and CFP using Ag NPs formation method

Parameter	Cefdinir	Cefepime HCl
$\lambda_{\max}$ (nm)	360	350
Volume of AgNO ₃ -gelatin complex	4.0 mL	3.0 mL
Volume of NaOH (0.5 M)	2.0 mL	2.0 mL
Heating time	20 min	25 min
Beer's law limits (µg/mL)	5.0 - 40	10 - 100
* Regression equation		
Slope (b)	0.0262	0.0113
Intercept (a)	-0.0127	0.0043
Correlation coefficient (r ²)	0.9999	0.9998
LOD (µg/mL)	0.391	1.06
LOQ (µg/mL)	1.184	3.213

* A = a + bc

Table 2 Precision data for determination of CFD and CFP using Ag NPs formation method

QC conc. (µg/mL)	Intra-day (n = 9)				Inter-day (n = 9)			
	Mean (µg/mL)	SD	RSD (%)	Er%	Mean (µg/mL)	SD	RSD (%)	Er (%)
Cefdinir								
15	14.97	0.14	0.92	-0.2	14.7	0.12	0.83	-2.03
25	25.13	0.096	0.38	0.52	25.1	0.24	0.95	0.4
35	35.19	0.23	0.66	0.54	35.17	0.28	0.8	0.49
Cefepime HCl								
30	29.86	0.18	0.62	-0.47	30.33	0.23	0.77	1.1
50	49.91	0.22	0.45	-0.18	49.94	0.34	0.67	-0.12
70	69.12	0.22	0.32	-1.26	69.27	0.23	0.34	-1.04

at three different concentrations of both drugs in the same day (intra-day), and in three different days (inter-day). Percentage of relative standard deviation (RSD%) as precision and percentage relative error (Er%) as accuracy of the suggested method were calculated. The percentage relative error was calculated using the following equation:

$$\text{Er}\% = [(\text{found} - \text{added})/\text{added}] \times 100$$

The results of precision showed that the proposed methods had good repeatability and reproducibility as shown in Table 2.

Robustness

Robustness was examined by studying the influence of small variations in one of the experimental parameters, keeping the others constant, on the analytical performance of the proposed method. The

studied parameters were working reagent solution volume, NaOH solution volume and heating times (Table 3).

Statistical analysis

The results of the proposed method were compared with those obtained from the reported methods [44, 45] using Student's t-test and F-test (at 95% confidence level). Results showed there were no significant differences between the proposed and reported methods as shown in Table 4.

Application

The proposed method could be successfully applied for the determination of the cited drugs in their pure forms, pharmaceutical preparations, and in urine samples. Good recoveries were obtained without interference from pharmaceutical preparations

excipients or urine endogenous matrix components. Monitoring of the cited drugs in urine samples did not require any extraction procedures, providing a green, simple and time consuming method (Table 5 and 6).

Table 3 Robustness of the proposed method using concentration of 25 µg/ mL CFD and 50 µg/mL CFP

Parameter	Cefdinir			Cefepime HCl		
Silver-gelatin soln volume (mL)	3.9	4.0	4.1	2.9	3.0	3.1
Recovery (%) ± SD ^a	101.48 ± 0.31	101.43 ± 0.23	99.68 ± 0.15	100.3 ± 0.47	100.06 ± 0.37	99.71 ± 0.89
NaOH volume (mL)	1.9	2.0	2.1	1.9	2.0	2.1
Recovery (%) ± SD ^a	99.29 ± 0.23	100.72 ± 0.15	100.41 ± 0.15	99.59 ± 0.18	99.83 ± 0.27	100.12 ± 0.18
Heating time (min)	18	20	22	23	25	27
Recovery (%) ± SD ^a	100.67 ± 0.38	100.41 ± 0.15	100.36 ± 0.23	100.65 ± 0.35	100.12 ± 0.35	100.83 ± 0.47

^a Average of three determinations.

Table 4 Statistical analysis of the proposed method in standard solution and the reported methods [44, 45]

	Cefdinir		Cefepime HCl	
	Proposed method	Reported method [44]**	Proposed method	Reported method [45]***
Mean	99.76	99.62	99.47	98.61
SD	0.82	0.96	0.8	0.99
RSD (%)	0.82	0.96	0.81	0.99
Variance	0.68	0.92	0.64	0.97
N	8	7	10	5
F – test	1.37 (3.87)*	--	1.51 (3.63)*	--
Student's t- test	0.32 (2.16) *	--	1.83 (2.16) *	--

*Figures between parenthesis represent the corresponding tabulated values of t and F at P = 0.05.

**Spectrophotometric method based on measurement of CFD absorbance in 0.1 N HCl at 281 nm.

***Spectrophotometric method based on condensation of CFP with NQS and measurement of absorbance at 475 nm.

Table 5 Application of standard addition method for determination of the cited drugs in pharmaceutical formulations

Pharmaceutical Preparation	Dinar [®] capsules				Cefepime [®] vials			
	Claimed taken (µg/mL)	Authentic added (µg/mL)	Found (µg/mL)	Recovery (%)	Claimed taken (µg/mL)	Authentic added (µg/mL)	Found (µg/mL)	Recovery (%)
	10	--	9.85	98.55	20	--	20.06	101.19
		5	5.03	100.53		10	10.06	98.85
		10	9.84	98.36		20	20.15	99.87
		15	14.84	98.91		30	30.77	101.98
		20	19.87	99.37		40	40.24	100.15
		30	29.65	98.82		50	49.18	99.59
						70	71.39	101.73
						80	81.57	101.74
Mean				99.09				100.64
SD				0.79				1.17
SE				0.35				0.44
RSD				0.79				1.17
Variance				0.62				1.37

Table 6 Application of the proposed method for determination of the cited drugs in spiked urine samples

Drug	Cefdinir			Cefepime HCl		
	Claimed taken (µg/mL)	Found (µg/mL)	Recovery (%)	Claimed taken (µg/mL)	Found (µg/mL)	Recovery (%)
	10	9.89	98.89	10	10.02	100.19
	15	15.03	100.19	20	19.92	99.62
	25	25.24	100.94	30	29.92	99.74
	30	30.24	100.79	40	39.53	98.83
	35	34.89	99.68	50	50.6	101.2
	40	39.99	99.98	70	70.02	100.03
				80	80.12	100.15
				100	99.73	99.73
Mean			100.08			99.94
SD			0.75			0.67
SE			0.31			0.24
RSD			0.75			0.67
Variance			0.57			0.45

Conclusions

Silver nanoparticles could be used as a chromogenic agent for optical detection of CFD and CFP in their pure forms, pharmaceutical preparations, and in urine samples. The proposed method was simple, sensitive, accurate, green and inexpensive method. This analytical protocol may be important for quality control studies and monitoring of our analytes in biological fluids.

Acknowledgments

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Conflict of Interests

There are no conflicts of interests.

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