Determination of cefotaxime, cefoperazone, ceftazidime and cefadroxil using surface plasmon resonance band of silver nanoparticles

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The aim of this work is to evaluate simple, sensitive, effective and validated procedures for the determination of cefotaxime, cefoperazone, ceftazidime and cefadroxil. In this study, the methods based on the ability of the cited drugs to reduce Ag+ ions to silver nanoparticles (Ag-NPs) in the presence of Polyvinyl Pyrrolidone (PVP) as a stabilizing agent producing very intense surface plasmon resonance peak of Ag-NPs (λ_{max} = 410-430 nm). The plasmon absorbance of the Ag-NPs allows the quantitative spectrophotometric determination of the cited drugs. The calibration curves are linear with concentration ranges of 0.4-3.2, 1-8, 0.5-4.0 and 1.5-9.0 µg/mL for cefotaxime, cefoperazone, ceftazidime and cefadroxil, respectively. Apparent molar absorptivity, detection and quantitative limits are calculated. Applications of the proposed methods to representative pharmaceutical formulations are successfully presented. The extracellular synthesis of nanoparticles is fast, and the method doesn’t require various elaborate treatments and tedious extraction procedures.

Keywords: Silver nanoparticles. Cefotaxime. Cefoperazone. Ceftazidime. Cefadroxil

INTRODUCTION

Cefotaxime sodium (sodium (6R,7R)-3-[(acetyloxy)methyl]-7-[[2Z]-2-(2-aminothiazole-4-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-3-cephem-4-carboxylate) (Indian Pharmacopoeia Government of India, 2006) (Figure 1), is an antibiotic used to treat a number of bacterial infections. Especially, it is used to treat joint infections, pelvic inflammatory diseases, meningitis, pneumonia, urinary tract infections, sepsis, gonorrea, and cellulitis. It is given either by injection into a vein or a muscle (The American Society of Health-System Pharmacists, 2016).

Literature survey reveals several spectroscopic (Patel, Patel, Patel, 2006; Rao, Kumar, 2001), HPLC (Barker, 2003; Jolanta, Buszman, Hawranek, 2002; Victoria, Emmanouil, 2008), TLC (Nabi, Laiq, 2004) and HPTLC (Nanda et al., 2010), methods for the estimation of cefotaxime sodium individually as well as in combination with other drugs.

Cefoperazone sodium is a third-generation semisynthetic antibiotic that is used in the treatment of mild to moderate infections caused by susceptible microorganisms (Reynolds, 1998). It is official in USP (United States Pharmacopoeia, 1985). Chemically, cefoperazone sodium is 7-{[2-(4-ethyl-2,3-dioxopiperazin-1-yl carboxamide)-2-(4-hydroxyphenyl)acetamide]-3-[1-methyl-1H-tetrazol-5-yl-thiomethyl]}-3-cephem-4-carboxilate (British Pharmacopoeia, 2005) (Figure 1). Various analytical methods are applied for the determination of cefoperazone. These methods include spectrophotometry (Hoang et al., 2014; Sha et al., 2013) and HPLC (Lalitha, Puranik, Pai, 2009).

Ceftazidime is a semisynthetic cephalosporin of the third generation with a high antibacterial activity that widely used in the treatment of commonly-occurring bacterial infections, including indole-positive Proteus species and Pseudomonas aeruginosa that have been considered to be the drugs of choice for serious infections caused by Klebsiella, Enterobacter, Proteus, Providencia, Serratia and Haemophylus species (Claridge et al., 2007), Chemically, (Rodenas et al., 2008). Chemically, it is designated as (6R,7R)-7-[[2Z]-2-(2-amino-1,3-thiazol-4-yl)-2-(2-carboxypropan-2-yloxyimino)acetyl]amino]-8-oxo-3-
(pyridine-1-ium-yl)methyl)-5-thia-1-azabicyclo[4.2.0] oct-ene-2-carboxylate. Several analytical procedures are available in the literature for the analysis of cephalosporins (ceftazidime). These methods are spectrophotometry (Amin, Ragab, 2004), high performance liquid chromatography (Zivanovic et al.,), and capillary electrophoresis (Castaneda, Julien, Fabra, 1996).

Cefadroxil is a semi-synthetic antibiotic belonging to the class of first-generation cephalosporins. Its mechanism of action is due to inhibition of the synthesis of the cell wall of mainly gram-positive bacteria being widely used for the treatment of infections such as pharyngitis, tonsillitis, gonorrhea, skin, soft tissue, ear and urinary tract (Devaliya, Jain, 2009). Chemically, it is designated as 5-thia-1-azabicyclo [4.2.0] oct-2-enecarboxylic acid, 7-[[amino[4-hydroxyphenyl] acetyl] amino]-3-methyl-8-oxo-, monohydrate, [6[R[6-alpha,7-beta(R*)]] (Figure 1), (Viswanath, Hemanth, 2017). Different methods are reported for the determination of cefadroxil including HPLC (Anjum et al., 2012), and spectrophotometry (El-Gindy, El Walily, Bedair, 2002; Shukla et al., 2008) (Figure 1).

Nanoparticles are one of the novel drug delivery systems, which can be of potential use in controlling and targeting drug delivery as well as in cosmetics textiles and paints. Nanoparticles have many advantages; they can be administered by parenteral, oral, nasal, ocular routes, by attaching specific ligands on their surfaces, nanoparticles can be used for directing the drugs to specific target cells, improving the stability and therapeutics index and reducing toxic effects. Nanoparticles have many applications, e.g. cancer therapy, intra cellular targeting, vaccine adjuvant, DNA delivery and ocular delivery.

Nanoparticles made of silver and gold have been the focus of research for many decades as a result of their intriguing optical properties. When the nanoparticles disperse in liquid media, they exhibit a strong UV-vis extinction band that is not present in the spectrum of the bulk metal. Recently, the colorimetric nanoparticles have been developed for sensitive and selective detection of nebivolol (Rahman et al., 2013), fexofenadine (Rahnama, 2013), catecholamines (Tashkhourian, Nezhad, Khodaveisi, 2011), etilefrine hydrochloride, fenoterol hydrobromide, salbutamol sulphate and estradiol valerate (Magda et al., 2015), and fluroroquinolones (Sayed et al., 2017).

In this work, nanoparticles are used in quantitative determination of drugs; a simple, sensitive, effective and validated procedures for the determination of was developed.

MATERIAL AND METHODS

Instrumentation

Shimadzu – UV 1800 double beam UV–Visible spectrophotometer (Japan) with matched 1 cm quartz cells at 200–800 nm range is used for all absorbance measurements. Spectra are automatically obtained by Shimadzu UV-Probe 2.32 system software.
Jenway 6305 UV/Visible Spectrophotometer (England).

**Material and Reagents**

The used chemicals are of the highest purity; Cefotaxime (obtained from Tabuk Pharmaceutical Company, Tabuk, Saudi Arabia), Cefoperazone (obtained from Sigma-tec Pharmaceutical Industries, Egypt), Ceftazidime (obtained from Pharco B International Company, Alexandria, Egypt), Cefadroxil (obtained from Pharco B International Company, Alexandria, Egypt) and Silver nitrate, 0.02M aqueous solution, polyvinylpyrrolidone (PVP), 0.14% aqueous solution, sodium hydroxide, 0.0025M aqueous solution.

**Pharmaceutical preparations**

Curisafe® tablets containing 1 g cefadroxil monohydrate per tablet with a batch No. 25612 (obtained from Pharco B International Company, Alexandria, Egypt).

Duricef® Capsules containing 250 mg cefadroxil monohydrate per capsule with a batch No. 19077 (obtained from Glaxosmithkline, Egypt).

Cefoperazone® vials containing 0.538 cefoperazone sodium equivalent to cefoperazone anhydrous 0.5 gm with a batch No. 26597 (obtained from Sigma-tec Pharmaceutical Industries, Egypt).

Cefzim® vials containing 1 g ceftazidime pentahydrate with a batch No. 21956 (obtained from Pharco B International Company, Alexandria, Egypt).

Foxime® vials containing 1 g cefotaxime sodium equivalent to 1 g cefotaxime with a batch No. 23065 (obtained from Tabuk Pharmaceutical Company, Tabuk, Saudi Arabia).

**Standard solutions**

Solutions of 100 µg/mL of cefotaxime, cefoperazone, ceftazidime and cefadroxil are prepared by dissolving 10 mg of the pure drug in bi-distilled water.

**General procedure**

In 5 mL volumetric flask, appropriate amounts of silver nitrate, PVP, different concentrations of the cited drugs and appropriate amounts of NaOH are added, completed to 5 mL with bi-distilled water, and then heated in the water bath at 90 °C for appropriate times. Absorbance is measured at the suitable wavelength against reagent blank treated similarly (Table I).

**Assay of pharmaceutical preparations**

**Assay of tablets**

For Curisafe® tablets: Ten tablets are weighed, coat removed and pulverized into fine powder, specific quantity of powdered drugs equivalent to 10 mg pure drug is dissolved in distilled water, solutions are filtered and diluted to 100 mL with distilled water then further dilution to 10 µg/mL and procedures are completed as in general procedures.

**Assay of vials**

For Cefoperazone®, Cefzim® and Foxime® vials, the content of four vials for each drug is weighed and pulverized into fine powder, specific quantity of powdered drugs equivalent to 10 mg pure drug is dissolved in distilled water, solutions are filtered and diluted to 100 mL with distilled water then further dilution to 10 µg/mL and procedures are completed as in general procedures.

**TABLE I – The analytical parameters for the determination of cefotaxime, cefoperazone, ceftazidime and cefadroxil through silver nanoparticles formation**

| Parameter                        | Cefotaxime | Cefoperazone | Ceftazidime | Cefadroxil |
|----------------------------------|------------|--------------|-------------|------------|
| $\lambda_{\text{max}}$ (nm)      | 410        | 430          | 430         | 410        |
| Volume of Silver nitrate (0.02 M), mL | 0.5       | 0.7          | 0.7         | 1.0        |
| Volume of PVP (0.14%), mL      | 1.0        | 0.7          | 0.7         | 0.5        |
| Volume of NaOH (0.0025 M), mL   | 0.7        | 1.0          | 1.0         | 0.7        |
| Temperature, °C                 | 90         | 90           | 90          | 90         |
| Time of reaction, min.          | 30         | 30           | 35          | 25         |
| Beer’s law limits (µg/mL)       | 0.4-3.2    | 1.0-8.0      | 0.5-4.0     | 1.5-9.0    |
Assay of capsules

For Duricef® Capsules, evacuating the content of ten capsules and pulverizing into fine powder, specific quantity of powdered drugs equivalent to 10 mg pure drug is dissolved in distilled water, solutions are filtered and diluted to 100 mL with distilled water then further dilution to 10 µg/mL and procedures are completed as in general procedures.

RESULTS AND DISCUSSIONS

Nanoparticles made of silver have been the focus of research for many decades due to their intriguing optical properties. The systems in this study consist of an aqueous AgNO₃ solution that includes polyvinylpyrrolidone (PVP), as a stabilizer, at an alkaline medium. Cefotaxime, cefoperazone, ceftazidime and cefadroxil act as effective reducing agents for the reduction of silver metal salt (Ag⁺) to the Ag-NPs without adding any seeds.

In the absorbance of reducing agents, there is no absorbing peak in visible region (380-700 nm). Upon the addition of the cited drugs which act as a reducing agent, silver ions reduced to silver nanoparticles and then the absorbance characteristic of the plasmon of the Ag-NPs is observed (410-430 nm) (Figure 2).

Effect of NaOH concentration

The influence of pH on Ag⁺ reduction by the cited drugs is expected since they have a hydroxyphenyl group which can lose H⁺ during oxidation and O-quinone formation process. Because the buffered condition failed to obtain silver nanoparticles, we added NaOH for providing enough alkalinity. By addition of NaOH, absorbance increases up to a known concentration of NaOH then decreases the formation of black precipitate which might be due to the Ag₂O formation. Thus, 0.7 mL of 0.0025 M NaOH was selected the optimum NaOH concentration for cefotaxime and cefadroxil, while 1mL of 0.0025 M NaOH is sufficient for cefoperazone and ceftazidime.

Effect of Silver nitrate concentration

Maximum absorbance values are obtained using 0.5, 0.7, 0.7 and 1.0 mL of 0.02 M, silver nitrate for cefotaxime, cefoperazone, ceftazidime and cefadroxil, respectively.

Effect of Stabilizer type and concentration

An important issue in the preparation of metal nanoparticles is the choice of the capping agent used to protect or stabilize the nanoparticle colloidal metals from agglomeration. Size and morphology of nanoparticles are depending significantly on capping materials. Nanoparticles stabilization is achieved according to the two basic modes: electrostatic and steric stabilization (Bradley, Bradely, Weinheim, 1994). Electrostatic stabilization is caused by the cumbic repulsion among particles, caused by the electrical double layer formed by ions adsorbed at the particle surface (e. g., sodium citrate) and the corresponding counter ions. Steric stabilization is achieved because of the coordination of sterically demanding organic molecules and polymers that acts as protective shields on the metallic surface (e. g., PVP). In this study, PVP and sodium citrate were selected as stabilizers for preventing of silver nanoparticles agglomeration in which the PVP is used in a better way comparing to the sodium citrate. 1.0, 0.7, 0.7 and 0.5 mL of 0.14 % PVP were optimum for cefotaxime, cefoperazone, ceftazidime and cefadroxil, respectively.

Effect of temperature and time of heating

Heating in water bath at 90 °C for 30, 30, 35 and 25 min are sufficient to produce maximum color intensities for cefotaxime, cefoperazone, ceftazidime and cefadroxil, respectively.
Method of validation

Linearity

Under the described experimental conditions, standard calibration curves with good linearity for silver nanoparticles formed using cefotaxime, cefoperazone, ceftazidime and cefadroxil are constructed by plotting absorbance against concentration.

A linear correlation is found. The concentration ranges, correlation coefficient, intercept and slope for the calibration curve are calculated. Also, relative standard deviation, detection and quantification limits are calculated and listed in tables (Tables II, III).

The validity of the proposed method was assessed by its application to the determination of the cited drugs in their pharmaceutical preparations, (Tables IV, V, VI).

Student’s t-test and F-test (at 95% confidence level) are applied to the results obtained and compared with that obtained from reported methods (Hesham et al., 2006; Hesham, 2004). The results show that there are no significant differences between the proposed and reported methods. The results of different statistical treatment of the data are shown in (Table VII).

Accuracy and precision

Accuracy and precision are carried out by six determinations at two different concentrations of the four drugs in the same day (intra-day), and in six different days (inter-day).

The results of accuracy and precision (Table VIII) show that the proposed methods have good repeatability and reproducibility.

### TABLE II – The spectral data for the determination of cefotaxime, cefoperazone, ceftazidime and cefadroxil through silver nanoparticles formation

| Parameter                        | Cefotaxime | Cefoperazone | Ceftazidime | Cefadroxil |
|----------------------------------|------------|--------------|-------------|------------|
| Linearity range (µg/mL)          | 0.4-3.2    | 1.0-8.0      | 0.5-4.0.0   | 1.5-9.0    |
| Limit of detection LOD (µg/mL)   | 0.12       | 0.33         | 0.13        | 0.33       |
| Limit of quantitation LOQ (µg/mL)| 0.35       | 0.89         | 0.39        | 1.00       |
| Slope (b)                        | 0.4069     | 0.1134       | 0.2231      | 0.0536     |
| Intercept (a)                    | -0.0009    | -0.0014      | -0.0017     | 0.0007     |
| Correlation coefficient (r)      | 0.9999     | 0.9997       | 0.9998      | 0.9999     |

Calculated on the basis of the molecular weight of the drug $A = a + bc$

### TABLE III – The determination of cefotaxime, cefoperazone, ceftazidime and cefadroxil through silver nanoparticles formation

| Statistics | Cefotaxime | Cefoperazone | Ceftazidime | Cefadroxil |
|------------|------------|--------------|-------------|------------|
| Taken (µg/mL) | Recovery % | Taken (µg/mL) | Recovery % | Taken (µg/mL) | Recovery % | Taken (µg/mL) | Recovery % |
| 0.4        | 98.36      | 1.0          | 100.88      | 0.5        | 101.04      | 1.5          | 98.63      |
| 0.8        | 100.42     | 2.0          | 100.70      | 1.0        | 101.08      | 3.0          | 101.55     |
| 1.2        | 100.08     | 3.0          | 100.63      | 1.5        | 100.19      | 4.5          | 100.04     |
| 1.6        | 100.44     | 4.0          | 100.18      | 2.0        | 99.52       | 6.0          | 99.06      |
| 2.0        | 99.52      | 5.0          | 98.13       | 2.5        | 100.57      | 7.5          | 99.57      |
| 2.4        | 100.10     | 6.0          | 100.59      | 3.0        | 99.76       | 9.0          | 100.39     |
| 2.8        | 99.52      | 7.0          | 99.70       | 3.5        | 99.83       |              |            |
| 3.2        | 100.37     | 8.0          | 100.57      | 4.0        | 100.55      |              |            |
| Mean*      | 99.85      | 100.17       | 100.32      | 99.87      |
| N          | 8          | 8            | 8           | 6          |
| V          | 0.54       | 0.63         | 0.49        | 0.79       |
| ±S.D.      | 0.73       | 0.79         | 0.70        | 0.89       |
| R.S.D.     | 0.74       | 0.79         | 0.70        | 0.89       |

*Mean of three different experiments
**TABLE IV** - The determination of cefotaxime, cefoperazone, ceftazidime and cefadroxil in their pharmaceutical formulations

| Statistics | Foxime® vials | Cefoperazone® vials | Cefzim® vials | Duricef® Capsules | Curisafe® tablets |
|------------|---------------|---------------------|---------------|-------------------|-------------------|
|            | Cefotaxime    | Cefoperazone        | Ceftazidime   | Cefadroxil        | Cefadroxil        |
| T (µg/mL)  | T' %          | T (µg/mL)           | R' %          | T (µg/mL)         | R' %              |
| 0.4        | 97.81         | 1.0                 | 98.11         | 0.5               | 98.11             |
| 0.8        | 99.12         | 2.0                 | 99.51         | 1.0               | 99.14             |
| 1.2        | 99.53         | 3.0                 | 101.51        | 1.5               | 100.51            |
| 1.6        | 100.14        | 4.0                 | 100.51        | 2.0               | 99.11             |
| 2.0        | 98.95         | 5.0                 | 99.79         | 2.5               | 99.71             |
| 2.4        | 99.56         | 6.0                 | 99.51         | 3.0               | 101.01            |
| 2.8        | 99.77         | 7.0                 | 98.91         | 3.5               | 99.81             |
| 3.2        | 100.78        | 8.0                 | 99.99         | 4.0               | 100.57            |

Mean* 99.46 99.73 99.75 99.29 99.93
N 8 8 8 6 6
V 0.62 0.72 0.73 0.72 1.01
±S.D. 0.79 0.85 0.86 0.85 1.00
R.S.D. 0.79 0.85 0.86 0.85 1.00

*Mean of three different experiments

**TABLE V** - Application of standard addition technique for the determination of cefotaxime, cefoperazone, ceftazidime in their pharmaceutical formulations

| Duricef® Capsules | Curisafe® Tablets |
|-------------------|-------------------|
| Taken µg/mL       | Added µg/mL       | Recovery* % | Taken µg/mL | Added µg/mL | Recovery* % |
| 4.5               | -                 | 99.51       | 4.5         | -           | 98.75       |
| 4.5               | 100.73            |             | 4.5         | 99.09       |
| 6.0               | 99.51             | 101.51      | 6.0         | 100.74      |
| 7.5               | 101.51            |             | 7.5         | 101.11      |

Mean* 100.32 99.92
N 4 4
V 0.81 1.00
±S.D. 0.90 1.00
R.S.D. 0.89 0.99

*Mean of three different experiments

**TABLE VI** - Application of standard addition technique for the determination of cefadroxil in its pharmaceutical formulations

| Foxime® vials | Cefoperazone® vials | Cefzim® vials |
|---------------|---------------------|---------------|
| Taken µg/mL   | Added µg/mL         | Recovery* %   | Taken µg/mL | Added µg/mL | Recovery* %   | Taken µg/mL | Added µg/mL | Recovery* %   |
| 0.8           | -                   | 98.51         | 2.0         | -           | 101.45        | 1.0         | -           | 97.91         |
| 0.8           | 99.71               | 2.0           | 99.81       | 2.0         | 99.09         | 1.5         | 1.0         | 98.51         |
| 1.2           | 100.53              | 3.0           | 99.90       | 3.0         | 98.75         | 2.0         | 2.0         | 101.34        |
| 1.6           | 99.87               | 4.0           | 99.91       | 4.0         | 99.81         |             |             |               |

Mean* 99.66 100.07 100.07
N 4 4 4
V 0.57 0.65 0.69
±S.D. 0.75 0.81 0.83
R.S.D. 0.76 0.81 0.83

*Mean of three different experiments
**TABLE VII** – The statistical data for the determination of cefotaxime, cefoperazone, ceftazidime and cefadroxil through silver nanoparticles formation

| Statistics | Cefotaxime | Cefoperazone | Ceftazidime | Cefadroxil |
|------------|------------|--------------|-------------|------------|
|            | Suggested Method | Reported Method | Suggested Method | Reported Method | Suggested Method | Reported Method | Suggested Method | Reported Method |
| Mean*      | 99.85      | 99.50        | 100.17      | 99.22      | 100.32      | 99.19        | 99.87      | 99.53 |
| N          | 8          | 5            | 8           | 5          | 8           | 5            | 6          | 5    |
| V±S.D.     | 0.54       | 0.88         | 0.63        | 1.37       | 0.70        | 0.56         | 0.79       | 0.56 |
| t          | 0.33 (2.201)* | 0.45 (2.201)* | 0.09 (2.201)* | 0.35 (2.262)* |
| F          | 1.63 (4.070)* | 2.17 (4.070)* | 1.25 (4.070)* | 1.41 (4.760)* |

*Theoretical values of t and F at p =0.05

**TABLE VIII** - The intra-day and inter-day accuracy and precision data for the determination of cefotaxime, cefoperazone, ceftazidime and cefadroxil through silver nanoparticles formation

|          | Intra-day | Inter-day |
|----------|-----------|-----------|
|          | Taken (µg/mL) | Found (µg/mL) | Recovery % | RSD % | Taken (µg/mL) | Found (µg/mL) | Recovery % | RSD % |
| Cefotaxime | 1.6 | 1.58 | 99.00 | 0.87 | 1.6 | 1.61 | 100.71 | 0.96 |
| Cefoperazone | 4.0 | 3.95 | 98.75 | 1.12 | 4.0 | 3.90 | 97.53 | 0.98 |
| Ceftazidime | 2.5 | 2.52 | 100.79 | 0.78 | 2.5 | 2.49 | 99.4 | 0.83 |
| Cefadroxil | 6.0 | 5.99 | 99.85 | 1.45 | 6.0 | 5.99 | 99.76 | 1.34 |

*Mean of six different experiments

**CONCLUSION**

Application of silver nanoparticles as chromogenic agent has been demonstrated in this work for the optical detection on the cited drugs based on the seedless production of Ag-NPs.

The proposed method is simple, sensitive and inexpensive for their determination. This analytical protocol may be an important green method for the monitor and the optical determination of cefotaxime, cefoperazone, ceftazidime and cefadroxil in pure and pharmaceutical dosage forms.

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