Determination of Some Cephalosporins in Pharmaceutical Formulations by a Simple and Sensitive Spectrofluorimetric Method

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ABSTRACT

Background: Cephalosporins are among the safest and the most effective broad-spectrum bactericidal antimicrobial agents which have been prescribed by the clinician as antibiotics. Thus, the developing of simple, sensitive and rapid analytical methods for their determination can be attractive and desirable.

Methods: A simple, rapid and sensitive spectrofluorimetric method was developed for the determination of cefixime, cefalexin and ceftriaxone in pharmaceutical formulations. Proposed method is based on the oxidation of these cephalosporins with cerium (IV) to produce cerium (III), and its fluorescence was monitored at 356 ± 3 nm after excitation at 254 ± 3 nm.

Results: The variables effecting oxidation of each cephalosporin with cerium (IV) were studied and optimized. Under the experimental conditions used, the calibration graphs were linear over the range 0.1-4 µg/mL. The limit of detection and limit of quantification were in the range 0.031-0.054 and 0.102-0.172 µg/mL, respectively. Intra- and inter-day assay precisions, expressed as the relative standard deviation (RSD), were lower than 5.6 and 6.8%, respectively.

Conclusion: The proposed method was applied to the determination of studied cephalosporins in pharmaceutical formulations by good recoveries in the range 91-110%.

Introduction

Cephalosporins are among the safest and the most effective broad-spectrum bactericidal antimicrobial agents which have been prescribed by the clinician as antibiotics.1 All of these semi-synthetic antibiotics are derived from 7-aminopenicillosporanic acid and contain a β-lactam ring fused to a dihydrothiazine ring but differ in the nature of the substituents attached at the 3 and/or 7-positions of the cephem ring.1,2 Gram positive and Gram negative bacterial infections are commonly controlled by cephalosporin antibacterials. As well as, the cephalosporins are the second most important β-lactams after penicillin for treating infectious diseases.2 Cefalexin, is a first-generation cephalosporin antibiotic which has Gram-positive and Gram-negative activity and has become the most widely used antibiotic in the world.4 Cefixime, is a semi-synthetic third-generation oral cephalosporin antibiotic being prescribed for the treatment of susceptible infections such as gonorrhea, otitis media, pharyngitis, lower respiratory tract infections like bronchitis, and urinary tract infections.3 Ceftriaxone, a third generation parental cephalosporin, has a relatively long half life and is stable to β-lactamases particularly those produced by Gram-negative organisms. It is excellently effective in Gram-negative bacterial infections. Its ring system contains a highly acidic heterocyclic system on the 3-thiomethyl group, so it is believed the unique pharmacokinetic properties of ceftriaxone is due to this unusual ring system. The bacteria can be killed by its interfering in the synthesis of the cell wall. Ceftriaxone has been effective in treating infections due to other ‘difficult’ organisms such as multidrug-resistant Enterobacteriaceae.6,8 Figure 1 shows the structure of studied cephalosporins. The determination of these cephalosporins in their pharmaceutical formulations have been done by variety of methods such as, spectrophotometry,1,2,10,16 spectrofluorimetry,4,15,17 chromatography,6,8,18,22 capillary electrophoresis (CE),3 near IR23 and electrochemical methods.24,27 The chromatographic and electrophoresis methods are very sensitive and reliable, but they are relatively time-consuming and expensive. The majority of other reported methods utilize expensive or unstable reagents. Thus, the developing of simple, sensitive and rapid analytical methods as practical alternatives to above mentioned methods can be attractive and desirable. Fluorescence spectrometry has great sensitivity and selectivity as well as relatively low cost for the operation, thus it is used in quantitative analysis

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of pharmaceuticals. Due to these excellent properties, we propose here a simple, sensitive and inexpensive spectrofluorimetric method for the determination of these drugs in pharmaceutical preparations.

Figure 1. Structure of studied cephalosporins: a) cefixime, b) cefalexin, c) ceftriaxone.

Ce(IV) is a well-known oxidizing agent; it is used for the determination of some drugs. It can be easily reduced to Ce(III) that shows a characteristic fluorescence in sulfuric acid medium. The literature survey revealed that this system has not been used for the analysis of these drugs. Thus, in this work Ce(IV) has been used as an oxidizing agent for the spectrofluorimetric determination of studied cephalosporins in their pharmaceutical formulations.

Materials and Methods

Apparatus
Fluorescence measurements were done with a Shimadzu RF-5301 PC spectrofluorophotometer, equipped with a 150 W Xenon lamp and 1.00 cm quartz cells. Both excitation and emission slits were adjusted to 3 nm and the sensitivity adjusted to low.

Reagents
Cefalexin, cefixime and ceftriaxone were obtained as gifts from Danna Pharma Co. (Tabriz, Iran). Sulfuric acid, ethanol and Ce(IV)-sulfate-tetrahydrate were purchased from E. Merck (Darmstadt, Germany). A stock standard solution of each drug at a concentration of 500 μg/mL was prepared by dissolving appropriate amount of each drug in 5 mL absolute ethanol and diluting to 25 mL with double distilled water. These solutions were stored in refrigerator and kept from light when not in use for two weeks. Working standard solutions were obtained daily by appropriately diluting these stock solutions with double distilled water. Ceric sulfate, 0.01 mol/L was prepared in 2.0 mol/L sulfuric acid and was kept in the refrigerator at 4 °C for two week. All other reagents were of analytical-reagent grade (E. Merck) and all solutions were prepared in doubly distilled water.

Recommended procedure for calibration
An aliquot of sample solution containing each drug in the range 0.1-4.0 μg/mL was transferred into 15-mL calibrated centrifuge tubes. Then, 0.3 mL of 2.0 mol/L sulfuric acid and 60 μL of 0.01 mol/L Ce(IV) was added. The content of each tube was mixed well and diluted to 10 mL with double distilled water. The resultant solutions were left at ambient temperature for 30 min, then the fluorescence intensity of each solution was measured at 356 ± 3 nm while excited at 254 ± 3 nm against reagent's blank.

Preparation of Pharmaceutical Formulations

Tablets and capsules
Ten cefixime tablets (Pars Darou, Tehran, Iran), each containing 400 mg cefixime (as trihydrate), were accurately weighed individually and finely powdered. Powdered sample containing 40 mg cefixime was weighed and placed into a 15-mL glass tube, dissolved in 5-mL absolute ethanol and vigorously shaken on a vortex mixer for 1 min. The solution was then filtered and transferred into a 100-mL volumetric flask. The residue was washed in enough water and the solution was finally made up to the mark with double distilled water. Thus, a 400 μg/mL solution of cefixime was obtained.

The contents of five capsules of cefalexin (Jaber Ebne Hayyan, Tehran, Iran), each containing 500 mg cefalexin, were thoroughly mixed. Powdered sample containing 50 mg of cefalexin was weighed and placed into a 15-mL glass tube, dissolved in 5-mL absolute ethanol and vigorously shaken on a vortex mixer for 1 min. The solution was then filtered and transferred into a 100-mL volumetric flask. The residue was washed in enough water and the solution was finally made up to the mark with double distilled water. Thus, a 500 μg/mL solution of cefalexin was obtained. Aliquots of 10 μL of these prepared samples were used for cefixime or cefalexin determination as mentioned procedure.

Powder for Oral Suspension
Two brands for oral suspension were studied including cefixime (Exir Pharm Co., Boroojerd, Iran) and cefalexin (Danna Pharma Co., Tabriz, Iran). The contents of each vessel was completed to 100 mL with double distilled water, so each 5 mL of prepared suspension was containing 100 mg of cefixime or 250 mg cefalexin based on labeled amounts, respectively. An aliquot of 1 mL of each prepared suspension was diluted to 100 mL with water, then 40 μL of each diluted sample was used for cefixime or cefalexin determination as mentioned procedure.
**Vial**

The content of each ceftriaxone vial (Afa Shimi, Tehran, Iran), each containing 1 g ceftriaxone (as sodium), was completed to 3 mL with double distilled water. An aliquot of 250 µL of prepared vial was diluted to 100 mL with water, than 40 µL of this diluted sample was subjected to ceftriaxone determination as mentioned procedure.

**Results and Discussion**

Certain drugs have been determined based on their reaction with Ce(IV) as an oxidizing agent. The produced Ce(III) is usually more fluorescent than the oxidation products and unreacted Ce(IV) and thus the monitoring of its fluorescence has been used as a very sensitive method for determination of certain drugs. In the present work the oxidation reaction of studied drugs with Ce(IV) was performed in sulfuric acid medium and the fluorescence intensity of the produced Ce(III) was monitored in desired wavelengths. Figure 2 shows the excitation and emission spectra obtained in the optimum conditions for cefixime-Ce(IV) system. Similar spectra were obtained for cefalexin or ceftriaxone reaction systems.

![Figure 2](image)

**Effect of Ce(IV) concentration**

For investigation the influence of Ce(IV) concentration on the fluorescence intensity of the reaction product, increasing volumes of 0.01 mol/L Ce(IV) solution in the range 10-170 µL were used when other conditions kept constant. As shown in Figure 3, the maximum and constant fluorescence intensities were attained when Ce(IV) concentration was in the range 60-70 µL. (e.g. final concentration of 6.0×10⁻⁵ mol/L).

![Figure 3](image)

At concentrations lower than this range the fluorescence intensity dropped due to insufficient Ce(IV) for oxidation. On the other hand, a quenching effect has been reported in larger volume of Ce(IV). An aliquot of 60 µL of 0.01 mol/L Ce(IV) (equivalent to final concentration of 6.0x10⁻⁵ mol/L) was used for the oxidation of studied drugs in the rest of work.

![Figure 4](image)

**Effect of sulfuric acid concentration**

The effect of sulfuric acid concentration on the fluorescence intensities was studied in the range 0.02 - 0.14 mol/L and the results were presented in Figure 4. It was found that the fluorescence intensity was rapidly
increasing up to sulfuric acid concentration of 0.05 mol/L, remained approximately constant up to 0.06 mol/L and then decreased gradually. Hence, an aliquot of 300 µL of 2.0 mol/L sulfuric acid (equivalent to final concentration of 0.06 mol/L) was used in other experiments.

Effect of temperature and time
The effect of temperature on the oxidation reaction was studied in different sets ranging from 20-95 °C. Heating the reaction solution was found to increase the fluorescence intensity, so the maximum signals were obtained at 95 °C, but the results were not repeatable at this temperature. Further experiments showed that oxidation reactions of studied drugs with Ce(IV) were relatively slow but very repeatable and precise at ambient temperature, thus this temperature was used in rest of work. Thus, the oxidation reactions were done in the temperature for periods ranging from 10 to 90 min. The results revealed that standing for 30 min at this temperature was sufficient for the completion of reaction with proper sensitivity.

Analytical characteristics
In the optimum conditions, linear calibration curves (n = 11) with good correlation coefficients (r > 0.9997) were obtained for studied cephalosporins. The characteristics of proposed methods for the determination of cephalosporins have been summarized in Table 1. The limit of detection (LOD) and limit of quantification (LOQ) were defined as 3S/m and 10S/m, respectively, where S, is the standard deviation of the blank measurements and m is the slope of the calibration line. The precision of the method was determined as intra-day (n = 8) and inter-day precision (n = 4), which found to be lower than 5.6% and 6.8%, respectively. These results have been summarized in Table 2.

**Table 1.** Analytical characteristics of the method for studied cephalosporins.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>C (µg/mL)</th>
<th>Calibration equation</th>
<th>LOD (µg/mL)</th>
<th>LOQ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefixime</td>
<td>0.10-4.0</td>
<td>272.06C+3.0776</td>
<td>0.031</td>
<td>0.102</td>
</tr>
<tr>
<td>Cefalexin</td>
<td>0.17-4.0</td>
<td>156.67C+1.5706</td>
<td>0.054</td>
<td>0.172</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.15-4.0</td>
<td>178.44C+3.6978</td>
<td>0.047</td>
<td>0.146</td>
</tr>
</tbody>
</table>

Table 2. Intra- and inter-day precisions for determination of studied cephalosporins.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Cefixime Cefalexin Ceftriaxone</th>
<th>Intra-day</th>
<th>Inter-day</th>
<th>Intra-day</th>
<th>Inter-day</th>
<th>Intra-day</th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C (µg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>5.53</td>
<td>5.84</td>
<td>5.32</td>
<td>6.76</td>
<td>5.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td>3.22</td>
<td>4.25</td>
<td>4.37</td>
<td>5.25</td>
<td>2.63</td>
</tr>
</tbody>
</table>

Intra- and inter-day precisions expressed as RSD%, and for 8 and 4 replicate determinations, respectively.

Table 3. Analytical characteristics of different methods used for the determination of studied cephalosporins.

<table>
<thead>
<tr>
<th>Determination Method</th>
<th>Analyte</th>
<th>Sample</th>
<th>Concentration range (µg/mL)</th>
<th>r</th>
<th>RSD%</th>
<th>LOD (µg/mL)</th>
<th>Mean R (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinetic-S</td>
<td>Cfx &amp; Cef</td>
<td>P,F</td>
<td>10-50</td>
<td>0.9991-0.9997</td>
<td>0.53-1.66</td>
<td>0.220-1.100</td>
<td>98.0-101.9</td>
<td>1</td>
</tr>
<tr>
<td>S</td>
<td>Cfx &amp; Cef</td>
<td>P,F</td>
<td>0.5-3</td>
<td>0.9993</td>
<td>&lt;2.00</td>
<td>0.120-0.168</td>
<td>96.0-102.3</td>
<td>2</td>
</tr>
<tr>
<td>CE</td>
<td>Cfx &amp; Cft</td>
<td>P,F</td>
<td>5-100</td>
<td>0.9970-0.9980</td>
<td>0.75-1.03</td>
<td>1.420-2.730</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>F</td>
<td>Cef</td>
<td>P,F &amp; B.S</td>
<td>0.04-0.4</td>
<td>0.9900</td>
<td>&lt;5.00</td>
<td>0.013</td>
<td>98.1-100.7</td>
<td>5</td>
</tr>
<tr>
<td>HPLC</td>
<td>Cft</td>
<td>P,F &amp; B.S</td>
<td>2.5-25</td>
<td>0.9997</td>
<td>0.37</td>
<td>0.170-0.450</td>
<td>100.5101.9</td>
<td>6</td>
</tr>
<tr>
<td>S</td>
<td>Cft &amp; Cef</td>
<td>P,F</td>
<td>25-60</td>
<td>0.9982-0.9991</td>
<td>0.68-2.69</td>
<td>5.093-6.152</td>
<td>99.7-101.2</td>
<td>10</td>
</tr>
<tr>
<td>S</td>
<td>Cfx &amp; Cft</td>
<td>P,F</td>
<td>0.2-85</td>
<td>&gt;0.9999</td>
<td>0.25-3.00</td>
<td>-</td>
<td>97.4-109.8</td>
<td>14</td>
</tr>
<tr>
<td>S</td>
<td>Cft</td>
<td>P,F</td>
<td>2-100</td>
<td>0.9998-0.9999</td>
<td>0.26-0.61</td>
<td>-</td>
<td>99.6-100.2</td>
<td>16</td>
</tr>
<tr>
<td>F</td>
<td>Cfx &amp; Cef</td>
<td>P,F</td>
<td>10-60 (µg/L)</td>
<td>0.9987-0.9995</td>
<td>&lt;2.00</td>
<td>2.02-2.09 (×10^-3)</td>
<td>95.2-107.0</td>
<td>17</td>
</tr>
<tr>
<td>S</td>
<td>Cft</td>
<td>P,F</td>
<td>4-20</td>
<td>0.9992-1.0000</td>
<td>0.13-0.29</td>
<td>0.020-0.048</td>
<td>99.6-100.4</td>
<td>17</td>
</tr>
<tr>
<td>HPLC</td>
<td>Cft</td>
<td>P,F &amp; B.S</td>
<td>05-250</td>
<td>0.9998</td>
<td>4.67</td>
<td>0.017</td>
<td>98.0-103.8</td>
<td>38</td>
</tr>
<tr>
<td>F</td>
<td>Cef &amp; Cfx &amp; Cft</td>
<td>P,F</td>
<td>0.1-4.0</td>
<td>&gt;0.9997</td>
<td>&lt;5.6</td>
<td>0.031-0.054</td>
<td>91.0-110.0</td>
<td>This work</td>
</tr>
</tbody>
</table>

Cefixime = Cef, cefalexin = Cfx, Ceftriaxone = Cft, R = recovery; S = spectrophotometry; F = fluorometry; CE = capillary electrophoresis; HPLC = high performance liquid chromatography; B.S = biological sample; P.F = pharmaceutical formulation.
The performance of the proposed method was compared with the performances of other methods to highlight the distinct features of the proposed method (see Table 3). Compared with references which use HPLC or CE for the determination of studied drugs, the proposed method does not require high investment and maintenance costs of the instruments. More importantly, our figures of merit were comparable to or even better than some of these methods.

### Recovery experiments and interference study

Aliquot volumes of each prepared pharmaceutical preparation according to the section of “Preparation of Pharmaceutical Formulations”, were transferred to clean centrifuge tubes and spiked with drug at two test concentrations and then analyzed following the optimized procedure. The obtained recoveries have presented in Table 4, which ranged from 91 to 110% and seem to be satisfactory. On the other hand, typical excitation and emission spectra for blank sample, cefixime standard solution, two pharmaceutical preparations and the last spiked with cefixime standard solution were plotted.

As shown in Figure 1, no additional peaks, caused by interfering compounds, were observed at the emission wavelengths used in this work. Thus, the similarities in the excitation and emission spectra along with reasonable recoveries that were achieved in this work, indicated that there was no significant matrix effect.

As well as, the effect of frequently encountered excipients such as: starch, talc, lactose, glucose, sucrose, magnesium-stearate and gum acacia on the determination of studied drugs were studied. The tolerance limit was taken as the concentration causing an error less than 8% in the determination of the drug. No interference effect from these excipients and additives was observed at concentrations up to at least 500-fold excess related to the studied drug. So, the proposed method can be considered a selective one.

### Analysis of Pharmaceutical Formulations

The proposed method was applied successfully for determination of studied drugs in the pharmaceutical formulations and the results are presented in Table 5.

### Table 5. Determination of the used drugs in their pharmaceutical formulations using proposed method

<table>
<thead>
<tr>
<th>Sample</th>
<th>Labeled amount(mg)</th>
<th>Found amount ± SD (n = 3) (mg)</th>
<th>Experimental t-values</th>
<th>R%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefixime</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tablet</td>
<td>400</td>
<td>432.0 ± 14.0</td>
<td>3.95</td>
<td>108</td>
</tr>
<tr>
<td>Powder for Oral Suspension</td>
<td>Each 5 mL containing 100 mg</td>
<td>Each 5 mL containing 92.0 ± 3.6</td>
<td>3.84</td>
<td>92.0</td>
</tr>
<tr>
<td>Cefalexin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capsule</td>
<td>500</td>
<td>455.0 ± 21.1</td>
<td>3.69</td>
<td>91.0</td>
</tr>
<tr>
<td>Powder for Oral Suspension</td>
<td>Each 5 mL containing 250 mg</td>
<td>Each 5 mL containing 231.0 ± 10.2</td>
<td>3.22</td>
<td>92.4</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vial</td>
<td>1000 mg</td>
<td>943.0 ± 25.7</td>
<td>3.84</td>
<td>94.3</td>
</tr>
</tbody>
</table>

Tabulate t-test at P=0.05, t = 4.3

### Conclusion

This report describes a validated spectrofluorimetric method for the assay of cefixime, cefalexin and ceftriaxone in their pharmaceutical formulations. Method validation using spiked real samples demonstrated that the method is capable of determining studied cephalosporins with adequate trueness and precision. In addition, the obtained LODs and LOQs are comparable or better than those of other methods reported in Table 3. Although, HPLC or CE methods are precise and sensitive (in the case of HPLC), but they use high sophisticated and expensive instruments.
Therefore, from the economical point of view, the proposed method is simple, rapid, sensitive and inexpensive thus can be used as an alternative method for quality control or pharmaceutical analysis purposes.

Conflict of interests
The authors claim that there is no conflict of interest.

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