Research Article

Colorimetric Determination of Cefadroxil and Ceftriazone in Pharmaceutical Dosage Forms

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Abstract

Purpose: To develop a simple, rapid and selective method for the spectrophotometric determination of cefadroxil and ceftriaxone using 1, 2- naphthaquinone-4- sulfonic acid sodium.

Methods: The method was based on the derivatization of cefadroxil and ceftriaxone with 1, 2-naphthaquinone-4- sulfonic acid sodium in alkaline medium to yield orange-colored products.

Results: The reaction products of cefadroxil and ceftriaxone at their respective λ max of 475 and 480 nm showed linearity in the concentration range of 10 - 100 and 25 - 175 µg/ml, respectively. Relative standard deviations of 0.82 % for cefadroxil and 0.95 % for ceftriaxone were obtained. Recoveries of cefadroxil tablets and ceftriaxone injection were in the range of 100.66 ± 0.98 and 99.38 ± 0.84 %, respectively.

Conclusion: Recovery studies gave satisfactory results indicating that none of the major additives/excipients interfered with the assay method. Therefore, the proposed method is simple, rapid, precise and convenient for the assay of cefadroxil and ceftriaxone in commercial preparations.

Keywords: Cephalosporins, Derivatization, 1, 2- Naphthaquinone 4- sulfonic acid sodium, Colorimetry, Pharmaceutical formulation.

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INTRODUCTION

Cefadroxil (CFL) is chemically designated as 8-[[2-amino-2-(4-hydroxyphenyl)-acetyl]amino-4-methyl-7-oxo-2-thia-6-azabicyclo[4.2.0]oct-4-ene-5-carboxylic acid. It is a first generation cephalosporin antibacterial drug that is the para-hydroxy derivative of cefalexin, and is used in the treatment of mild to moderate susceptible infections. It is a broad spectrum bactericidal antibiotic that is effective against many Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus piogenes*, *Moraxella catarrhalis*, *Escherichia coli*, *Klebsiella* and *Proteus mirabilis* [1].

A wide variety of analytical methods have been reported for the determination of CFL in pure form, in pharmaceutical preparations and in biological fluids. These methods mainly involve spectrophotometry [2], atomic absorption spectrophotometry [3], fluorometry [4], chemiluminescence [5], polarography [6], high performance liquid chromatography [7], and capillary electrophoresis [8]. CFL and cefotaxime have been determined by flow injection spectrophotometric method [9].

Some methods have been developed for the simultaneous determination of CFL when combined with other drugs in various dosage forms. CFL and cefotaxime in binary mixtures have been estimated by derivative spectrophotometry [10]. CFL and cephalexin in combination have been determined simultaneously by coupling technique of synchronous fluorimetry and H-point standard addition methods [11]. Similarly, CFL and cephalexin have also been determined by HPLC method [12] while CFL and cefuroxime determined in urine by measuring their first-derivative amplitude in 0.1N sodium hydroxide at 292.5 and 267.3 nm, respectively. [13].

Ceftriaxone (CFX) is (6R,7R)-7-[[((2Z)-(2-amino-4-thiazolyl)(methoxyamino)-acetyl] amino]-8-oxo-3-[1, 2, 5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl)-thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [14]. It is a cephalosporin beta-lactam antibiotic used in the treatment of bacterial infections caused by susceptible, usually Gram-positive organisms. The bactericidal activities of CFX result from the inhibition of cell wall synthesis and are mediated through CFX binding to penicillin binding proteins (PBPs). It inhibits mucopeptide synthesis in the bacterial cell wall. The beta lactam moiety of CFX binds to caboxypeptidase, endopeptidase and transpeptidase in the bacterial cytoplasmic membrane. These enzymes are involved in cell wall synthesis and cell division. The binding of CFX to these peptidases results in the formation of defective cell walls and cell death [15].

The monographs for CFX in USP [16] and BP [17] describe an HPLC method for the estimation of the drug in pharmaceutical formulation. Various methods have been developed for CFX determination using HPTLC [18] and spectrophotometry [19]. Simple liquid chromatographic method for the analysis of the blood/brain barrier permeability characteristics of ceftriaxone in an experimental rabbit meningitis model has also been carried out using lignocaine as internal standard [20].

1, 2-naphthoquinone-4-sulphonate (NQS) has been used as a chromogenic reagent for the colorimetric determination of many pharmaceutical amines [21- 23]. However, the reaction of NQS with CFL and CFX has, to the best of our knowledge, not been investigated. The present study describes the evaluation of NQS as a chromogenic reagent in the development of a simple and rapid colorimetric method for the determination of CFL and CFX in pharmaceutical dosage forms.
EXPERIMENTAL

Apparatus

A Shimadzu UV-visible spectrophotometer model 1800, with 1 cm matched quartz cells, was used for absorbance measurements. Systonics electronic balance (Shimadzu BL-220H) was used for weighing the samples.

Reagents and solutions

All the chemicals employed were of analytical grade and high-purified water was used throughout. Ceftriaxone powder (pure sample) was received as a gift from Strides Arcolab Limited, Bangalore, India while cefadroxil was also provided free of charge by Torrent Pharmaceuticals Ltd, Dist. Mehsana, India.

1, 2-Naphthoquinone-4-sulphonate (NQS) 0.5 %w/v

0.5 g of NQS (Loba Chemie Pvt. Ltd, Mumbai, India) was weighed and transferred into a 100 ml calibrated flask, dissolved in 5 ml distilled water, and made up to the mark with distilled water to obtain a solution of 0.5 %w/v. The solution was freshly prepared and protected from light during use.

Sodium hydroxide solution (0.01 M)

Sodium hydroxide (0.2 g) was weighed and transferred into a 500 ml volumetric flask and made up to the mark with distilled water.

Standard solutions

CFL and CFX stock solutions (1000 µg/ml) were prepared separately by dissolving 0.1 g of each drug in 100 ml of distilled water. Working solutions of the drug were prepared by dilution of the stock solution. The commercial tablet of CFL used in the determination was Droxyl, with a labelled strength of 500 mg, and manufactured by Torrent Pharmaceuticals Limited, Mehsana, India while the injection form of CFX used in the determination was Extacef-i with a labelled quantity of 1 g on the vial and manufactured by Nitin Life Sciences Limited, Sirmour, India.

Optimization of reaction variables

Effect of NQS concentration

The effect of NQS was studied by varying NQS concentrations (Scheme 1). The absorbance of the reaction solution increased as NQS concentration increased, and the highest absorption intensity was attained at NQS concentration of 0.25 % w/v. Further experiments were carried out using 0.5 %.

Effect of alkalinity

To generate nucleophiles from CFL/CFX and activate nucleophilic substitution reactions, alkaline medium is necessary. The different inorganic bases tested include sodium hydroxide, disodium hydrogen phosphate, and sodium bicarbonate. They were all prepared as aqueous solutions in the concentration range of 0.01 - 0.05N.

Preparation of calibration curve

Standard solutions of CFL and CFX in water, having final concentrations in the range of 10 - 100 µg/ml and 25 - 175 µg/ml, respectively, were transferred into a series of 10 ml volumetric flasks. To each of these solutions, 1 ml of 0.01M sodium hydroxide was added, followed by 1 ml of 0.5 % NQS. The mixture was then gently shaken until the appearance of orange colour. The contents were diluted to 10 ml with distilled water. The absorbance of each solution was measured at 475 nm and 480nm for CFL and CFX, respectively, against the reagent blank prepared in the same manner, without the analyte. The calibration plots and absorption spectra are represented in the Figs 1 and 2, respectively.
Fig 1: Calibration plots of CFL (●) and CFX (○) with concentration ranges of 10 - 100 and 25 - 175 µg/ml, respectively

Fig 2: Absorption spectra of NQS - CFL (●) and NQS - CFX (○), against reagent blank

Analysis of commercial pharmaceutical preparations

Tablet

Twenty tablets were weighed, crushed and their contents mixed thoroughly. An accurately weighed portion of the powder, equivalent to the labelled strength (100 mg) of CFL, was taken into a 100 ml volumetric flask containing about 75 ml of distilled water. This was shaken thoroughly for about 5 - 10 min and filtered with Whatman filter paper no. 1 (porosity 11µ) to remove insoluble matter. The filter paper was rinsed severally and the filtrate diluted to mark with distilled water to prepare 1000 µg/ml solution. An aliquot of this solution was diluted with water to obtain a concentration of 50 µg/ml. To the sample solution (0.5ml), 1 ml of 0.01M sodium hydroxide was added, followed by 1 ml of 0.5 % NQS. The mixture was then gently shaken until the appearance of orange colour. The contents were diluted to 10 ml with distilled water.

Injection

An appropriate amount of CFX was dissolved in water for injection to prepare 1000 µg/ml solution. An aliquot of this solution was diluted with water to obtain a concentration of 150 µg/ml. To the sample solution (1.5 ml), 1 ml of 0.01M sodium hydroxide was added, followed by 1 ml of 0.5 % NQS. The mixture was then gently shaken until the appearance of orange colour. The contents were diluted to 10 ml with distilled water.

General procedure

Series of standard solutions of CFL (0.1 to 1.0 ml) and CFX (0.25 - 1.75 ml) were taken in individual standard flasks. To each standard flask, 1 ml of 0.01M sodium hydroxide and 1 ml of 0.5 % NQS were added. The mixtures were then shaken until the appearance of orange colour. Their absorbance values were measured at λ max of 475 and 480 nm for CFL and CFX, respectively, against a blank similarly prepared by substituting the drug solution with water. The concentration of CFL and CFX in each standard flask was obtained by interpolating the corresponding absorbance value of the product from Beer’s plot of standard CFL and CFX solutions.

Quantification

The limits of Beer’s law, the molar absorptivity and the Sandell’s sensitivity values for CFL and CFX were evaluated and are given in Table 1. Regression analyses of
Table 1: Optical parameters (n = 6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cefadroxil (CFL)</th>
<th>Ceftriaxone (CFX)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>475</td>
<td>480</td>
</tr>
<tr>
<td>Beer’s law limits (µg/ml)</td>
<td>10-100</td>
<td>25-175</td>
</tr>
<tr>
<td>Molar absorptivity (L /mol/cm)</td>
<td>( 0.458 \times 10^4 )</td>
<td>( 0.295 \times 10^4 )</td>
</tr>
<tr>
<td>Correlation coefficient (R)</td>
<td>0.993</td>
<td>0.995</td>
</tr>
<tr>
<td>Sandell’s sensitivity (ng cm(^{-2}))</td>
<td>0.079</td>
<td>0.1879</td>
</tr>
<tr>
<td>Regression equation</td>
<td>( Y = 0.009X + 0.011 )</td>
<td>( Y = 0.005X + 0.010 )</td>
</tr>
<tr>
<td>Slope, ( b )</td>
<td>0.009</td>
<td>0.004</td>
</tr>
<tr>
<td>Intercept, ( c )</td>
<td>0.011</td>
<td>0.010</td>
</tr>
<tr>
<td>Relative standard deviation (%)</td>
<td>0.82</td>
<td>0.95</td>
</tr>
<tr>
<td>% Range of error (95% confidence limits)</td>
<td>0.89</td>
<td>0.92</td>
</tr>
<tr>
<td>Limit of detection (µg/ml)</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Limit of quantification(µg/ml)</td>
<td>100</td>
<td>125</td>
</tr>
</tbody>
</table>

Note: \( Y = bX + c, \) where \( X \) is the concentration of drug in µg/ml

Validation of the method

The validity of the methods for the assay of CFL and CFX were examined by determining the precision and accuracy. These were determined by analyzing six replicates of the drug within the Beer’s law limits. To study the accuracy of the methods, recovery studies were carried out by the standard addition method. For this, known quantities of pure CFL and CFX were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined; the amount of the added drug was calculated by difference and the results are given in Table 2.

Precision

The precision of the proposed methods was ascertained by actual determination of six replicates of a fixed concentration of each drug within the Beer’s law range and determining the absorbance in each case by the proposed method.

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by taking different amounts (80, 100 and 120 %) of bulk samples of CFL and CFX within the linearity range which were then added to the pre-analyzed formulation of concentrations 50 and 150 µg/ml, respectively.

Ruggedness

To ascertain the ruggedness of the methods, four replicate determinations at different concentration levels of the drugs were carried out. The within-day and inter-day RSD values were determined. The values for different concentrations of drugs obtained from the determinations are given in Table 2.

RESULTS

The test method was based on the reaction between NQS and cephalosporin molecules. The NQS reacted with cephalosporin at the free \( \text{NH}_2 \) group as represented in Scheme 1. The reaction was carried out in alkaline medium and best results were obtained with sodium hydroxide. With other bases, either precipitation of a white colloid occurred upon diluting the reaction solution with organic
solvent, or high blank readings, non-reproducible results, and/or weak sensitivity, were observed. Studies for optimization of sodium hydroxide concentration revealed that the optimum concentration was 0.01 M.

### Table 2: Analysis, recovery and ruggedness data (± SEM, n = 6) for CFL tablets and CFX injection by the proposed standard addition method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Drug strength (mg)</th>
<th>NQS method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefadroxil</td>
<td>500</td>
<td>100.12±0.70</td>
</tr>
<tr>
<td>(Droxil-500)</td>
<td>500</td>
<td>100.66±0.98</td>
</tr>
<tr>
<td>Recovery</td>
<td>500</td>
<td>100.33±0.52</td>
</tr>
<tr>
<td>Intraday analysis</td>
<td>500</td>
<td>100.53±0.65</td>
</tr>
<tr>
<td>Interday analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>1000</td>
<td>99.49±0.55</td>
</tr>
<tr>
<td>injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Extacef ®)</td>
<td>1000</td>
<td>99.38±0.84</td>
</tr>
<tr>
<td>Recovery</td>
<td>1000</td>
<td>99.12±0.96</td>
</tr>
<tr>
<td>Intraday analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interday analysis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The reagent blank had negligible absorbance in the range used for the detection of the cephalosporins. As Fig 1 shows, Beer’s law was obeyed in the range of 10 - 100 µg/ml for CFL and 25 - 175 µg/ml for CFX. Plots of absorbance versus concentration showed the intercept values of about 0.011 and 0.010 for CFL and CFX, respectively; the plots are described by the regression equation, \( Y = bX + c \) (where \( Y \) is the absorbance of a 1 cm layer, \( b \) is the slope, \( c \) is the intercept and \( X \) is the concentration of the drug in µg/ml) obtained by the least-squares method. The average percent recoveries obtained were quantitative, indicating good accuracy of the methods. The low values of the relative standard deviation (R.S.D.) indicate good precision of the methods. The data indicate that the proposed method has reasonable ruggedness.

### DISCUSSION

Derivatisation using NQS has attracted considerable attention for the quantitative analysis of many pharmaceutically active compounds. In the present investigation, NQS formed a coloured complex with CFL and CFX in alkaline medium and their absorbance maxima were 475 and 480 nm, respectively. Because of the presence of amine as a chromophoric group in CFL and CFX molecules, derivatization of these compounds was attempted in the present study for the development of a colorimetric method for their determination. NQS has been used as a chromogenic and fluorogenic reagent for primary and secondary amines [20]. Therefore, the present study was devoted to explore NQS as a derivatizing reagent in the development of a colorimetric method for the determination of CFL and CFX in pharmaceutical dosage forms. Optimisation of the spectrophotometric conditions was intended to take into account the various goals of method development. Analytical conditions were optimised via a number of preliminary experiments. The effect of NQS concentration was studied and it was found that 0.5 % gave good absorbance values, and so further experiments were carried out using 0.5 % NQS. To generate nucleophiles from CFL and CFX, and activate the nucleophilic substitution reactions, alkaline medium was necessary. Though various inorganic bases were tested such as sodium hydroxide, disodium hydrogen phosphate, and sodium bicarbonate, all were prepared as aqueous solutions in a concentration range of 0.01 - 0.05N. The best results were obtained with sodium hydroxide in a concentration of 0.01 N solution (1 ml).

Under optimum conditions, the reaction between CFL/CFX and NQS was completed within 2 min at room temperature, and the absorbance remained unchanged after standing for up to 40 min. The effect of time on the stability of the chromogen was studied by following the absorption intensity of the
reaction solution (after dilution) at different time intervals. It was found that the absorbance of the chromogen remained stable for at least 4 h. This allowed the processing of large batches of samples and their measurement with convenience. In addition to convenience, the methods would also facilitate their applicability to a large number of samples.

**CONCLUSION**

The reagents used in the proposed methods are low-cost, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Furthermore, the methods are free from interference by common additives and excipients. The wide applicability of the new procedures in routine quality control was well established by the assay of the cephalosporins (CFL and CFX) in pure form and in pharmaceutical dosage forms.

**ACKNOWLEDGEMENT**

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