Trade Science Inc.

June 2006 Volume 2 Issue 5-6

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 2(5-6), 2006 [167-173]

Validated Spectrophotometric Methods For The Assay Of Ciprofloxacin In Pharmaceuticals Based On Redox And Complexation Reactions

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Received: 1st May, 2006
Accepted: 11th May, 2006

Web Publication Date : 10th June, 2006

ABSTRACT

Two simple and senitive spectrophotometric methods for the determination of ciprofloxacin in pharmaceuticals were developed and validated using cerium(IV)sulphate as the oxidimetric reagent, and orthophenanthroline and thiocyanate as complexing agents. The methods involved the addition of a measured excess of cerium(IV)sulphate to ciprofloxacin in acid medium followed by determination of the residual oxidant by the different approaches. In the first procedure (method A), the residual oxidant was reduced by a known and fixed amount of iron(II), and the unreacted iron(II) was complexed with orthophenanthroline at a raised pH, and the absorbance measured at 510 nm. In the second approach (method B), the residual cerium (IV) was treated with a fixed amount of iron(II), and the resulting iron(III) was complexed with thiocyanate and the absorbance measured at 470nm. In both methods, the amount of cerium(IV)sulphate reacted corresponded to the amount of ciprofloxacin. The Beer's law was obeyed over the concentration ranges of 1.0-8.0 μg/ml (method A) and 0.5-4.0 μg/ml (method B). The calculated molar absorptivities were 6.10 x 10^4 and 4.26 x 10^4 l/mol/cm for method A and method B, respectively, and the respective limits of detection were 0.12 and 0.065 μg/ml. The methods were satisfactorily applied to the determination ciprofloxacin in tablets and no interference from excipients was observed. The validity of the methods was further ascertained by parallel assay by an established method, and by recovery studies. © 2006 Trade Science Inc. - INDIA

KEYWORDS

Ciprofloxacin;
Cerium(IV) sulphate;
Spectrophotometry;
Pharmaceuticals;
Complexation reactions.

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INTRODUCTION

Ciprofloxacin(CPF), chemically, is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7 (piperazin-1-yl) quinoline-3-carboxylic acid and belongs to the group of synthetic fluoroquinolone antibiotics with broad antimicrobial activity[1], and it is structurally related to nalidixic acid. It is believed that the mode of action of this family of drugs is through binding DNA-gyrase enzyme[2]. It is also reported that there is a direct correlation of fluoroquinolone bonding with inhibition of DNA-gyrase enzyme activity and induction of DNA breakage. Because of this special mechanism of action, fluoroquinolones are considered to be the most effective gram-positive - gram-negative pathogens to combat infections caused by micro organisms that are resistant to other microbials, such as tetracycline. Most of the methods available in the literature for the determination CPF are suitable for the assay of this drug in biological fluids[3-10]. The drug is official in British Pharmacopoeia[11] and United States Pharmacopoeia[12] which describe a high performance liquid chromatographic (HPLC) method for its assay.

Assay of CPF in pharmaceuticals has previously been achieved by several analytical techniques such as HPLC[3,13-20], HPTLC[21], TLC-fluorescence spectrodensitometry[22], capillary electrophoresis[23], high performance capillary electrophoresis[24], fluorimetry[25] spectrofluorimetry[26,27], chemiluminometry [28], potentiometry[29,30] and voltammetry[31-33]. But, many of these require expensive equipment and skilled operation. UV-spectrophotometry has also been used for the assay of CPF in single dosage forms [16,34-36] and in two component mixture[37].

Literature survey revealed that only two titrimetric metods have been proposed for the assay of CPF in dosage forms. The acid dye biphasic titration proposed by Zhang et al[38] is performed in aqueous-$CHCl_3$ medium whereas the non-aqueous titrimetric procedure[39] of Kilic et al is applicable over 15-50 mg range. Numerous visible spectrophotometric methods based on redox[40], oxidative-coupling[41], binary complexation[42-45], ternary complexation[27], charge-transfer complexation[46-48] and ion-pair complexation[49-51] reactions are found in the literature for the assay of CPF in formulations. But, most of these methods suffer from such disadvantage as poor sensitivity[40-45], heating[27, 46] or extraction step[49-51].

The aim of the present investigation was to develop simple, rapid, sensitive and inexpensive methods for the assay of CPF in pharmaceuticals based in the use of cerium(IV)sulphate as the oxidimetric reagent, and orthophenanthroline and thiocyanate as complexing agents. The proposed methods have the advantages of accuracy and precision in addition to overcoming the limitations of most of the existing methods.

EXPERIMENTAL

A Systronics model 106-digital spectrophotometer provided with 1-cm matched quartz cells was used for all absorbance measurements. All chemicals were of analytical reagent grade and distilled water used to prepare the solutions. A 0.01 mol/l cerium (IV) sulphate solution was prepared by dissolving about 4 g of the chemical in 1.0 M sulphuric acid and diluting to 100 ml with the same acid and standardized[52]. The solution was diluted appropriately with 1.0 mol/l sulphuric acid to obtain working concentrations of 720 and 400 μg/ml, for use in method A and method B, respectively. A 1000 μg/ml ammonium ferrous sulphate solution was prepared by dissolving about 100 mg of chemical in 100 ml of water in the presence of 2 ml of dil. sulphuric acid. The solution was diluted appropriately with water to yield 700 and 400 $\mu g\ mL^{-1}$ solutions for use in method A and method B, respectively. About 250 mg of orthophenanthroline monohydrate was dissolved in 100 ml of water with the aid of heat to get 0.25 % solution. A 3 M thiocynate solution was prepared by dissolving 23g of ammonium thiocynate in 100 ml water. A 224 ml of concentrated hydrochloric acid was diluted to 500 ml with water and mixed well, to get 5M acid. A 1 M sulphuric acid was prepared by adding 28 ml of concentrated acid to 472 ml of water with cooling. Ammonia solution (1:1) was prepared by diluting 50 ml of strong ammonia with 50 ml of water. A stock standard drug solution containing 1000 μg/ml CPF was prepared by dissolving 100 mg of pure sample (Torrent Pharma-

ceuticals, Ahmedabad, India) in water and diluting to the mark in a 100 ml calibrated flask. This solution was diluted stepwise with water to obtain working concentrations of 20 and 10 μg/ml for investigations by method A and method B, respectively.

Procedures

Method A

Different aliquots (0.5, 1.0 - - 4.0 ml) of standard (20 μg/ml) CPF solution were accurately transferred into a series of 10 ml calibrated flasks by means of a micro burette and the total volume was adjusted to 5 ml by adding water. To each flask was added 1ml each of 5 M hydrochloric acid and 720 μg/ml cerium (IV) sulphate (micro burette). The contents were mixed and the flasks were let stand for 10 min. Then, 1 ml of 700 μg/ml FAS (micro burette) was added to each flask followed by 1 ml each 0.25% orthophenanthroline and 1:1 ammonia solutions after 2 min. The volume was diluted to the mark with water, mixed well and absorbance of each solution was measured at 510 nm against a reagent blank after 10 min.

Method B

Varying aliquots (0.5, 1.0….4.0 ml) of standard CPF solution (10 μg/ml) were accurately measured into a series of 10 ml calibrated flasks by means of a micro burette and the total volume was brought to 5 ml by adding water. The solution in each flask was acidified by adding 1 ml of 5M hydrochloric acid before adding 1ml of 400 μg/ml cerium(IV)sulphate (micro burette) solution. The contents were mixed well and allowed to stand for 15 min with occasional shaking. To each flask was then added 1ml of 400 μg/ml FAS, and after 2 min, 1 ml of 3 M ammonium thiocyanate solution was added and diluted to the mark with water. The absorbance of each solution was measured at 470 nm against a water blank.

In either spectrophotometric method, a standard graph was prepared by plotting the increasing absorbance values in method A or decreasing absorbance values in method B versus concentration of CPF. The concentration of the unknown was read from the standard graph or computed from the respective regression equation derived using the Beer's law data.

Procedure for formulations

Twenty tablets were weighed and ground into a fine powder. An amount of powder equivalent to 100 mg of CPF was accurately weighed into a 100 ml calibrated flask, 60 ml of water added and the mixture shaken for 20 min. Then, the volume was made up to the mark with water, mixed well and filtered using a Whatman No. 42 filter paper. First 10 ml portion of the filtrate was discarded. The tablet extract (1000 μg/ml) was suitably diluted to get 20 and 10 μg/ml CPF and analysed spectrophotometrically by taking a convenient aliquot.

RESULTS AND DISCUSSION

Cerium(IV)sulphate has been a valuable oxidimetric reagent for the determination of many organic and inorganic substances and has been widely used in the assay of several pharmaceutical substances both by titrimetric and spectrophotometric methods. The present communication deals with the spectrophotometric assay of ciprofloxacin using cerium(IV)sulphate as the oxidimetric reagent. The proposed methods are indirect and are based on the determination of residual cerium(IV)sulphate after allowing the reaction between CPF and oxidant to go to completion, and rely on two different reaction schemes.

Method development

Method A

The spectrophotometric method based on the use of orthophenanthroline as the complexing agent continues to be one of the sensitive methods for the determination of iron in a variety of matrices. This reaction coupled with the oxidizing property of cerium (IV) has been made use in developing a sensitive indirect method for the assay of CPF. The drug in varying amounts, when treated with a fixed and known amount of cerium(IV)sulphate in acid medium, consumes the latter in proportionate amounts for oxidation, and there will be a concomitant decrease in the amount of the oxidant. When the decreasing amounts of oxidant are reacted with a fixed amount of iron(II) in the same acidic conditions,

Full Paper

there will be a proportional increase in the concentration of iron (II). This is indicated by the increase in absorbance of orthophenanthroline complex formed with residual iron (II). The absorbance measured at 510 nm is found to increase linearly with CPF concentration (r = 0.9919) serving as the basis for the assay procedure.

The conditions were optimized to produce a maximum colour through variation of such parameters as acid concentration, reaction time and amount of ammonia required to raise the pH to about 4. One ml of 5 M hydrochloric acid in a total volume of about 6 ml was used for the oxidation step which was complete in 10 min and the same acidic condition was used to reduce the residual cerium(IV) by iron(II). Larger amounts of acid are not desirable since they would require large quantities of ammonia to raise the pH to 4, required for iron(II)-phenanthroline complex formation.

Taking 10 μg/ml as the upper limit of iron(II) that could be determined by orthopenanthroline method, 700 μg of FAS was used in this method. Stoichiometrically, this would react with 719 μg of cerium (IV) sulphate. However, a slightly larger amount (720 μg) of cerium (IV) sulphate was used to ensure complete oxidation of iron (II) and to produce a colourless blank. The volume of 1:1 ammonia was not critical since the sensitivity and stability of the complex are unaffected over a wide pH range. However, 1 ml of freshly prepared 1:1 ammonia was used in a total volume 10 ml to raise the pH required for iron(II)–phenanthroline complex formation. The complex was stable for several days even in the presence of the reaction product.

Method B

Complex formation involving iron(III) and thiocyanate is a well known reaction that has been widely used for trace level determination of iron. The present method is based on the oxidation of CPF by a known excess of cerium(IV)sulphate in hydrochloric acid medium, reduction of the residual oxidant by a fixed amount of iron(II) and subsequent formation of iron(III)–thiocyanate complex which is measured at 470 nm. When a fixed amount of cerium(IV) is made to react with increasing amounts of CPF, there occurs a concomitant fall in the oxidant concentration. When the unreacted oxidant is reduced by a fixed amount of iron(II), there will be a proportional decrease in the concentration of iron (III). This is observed as a proportional decrease in the absorbance of iron(III)-thiocyanate complex on increasing the concentration of CPF (r = -0.9978) which formed the basis for the assay of drug by the present method.

The conditions for the determination of iron(III) with thiocyanate are well established[53]. Hence, various parameters associated with the oxidation of CPF by cerium(IV) and subsequent reduction of residual oxidant by iron(II) were optimized. Although nitric acid or hydrochloric acid medium can be used for the complexation of iron(III) with thiocyanate[53], the latter was selected, since nitric acid, being an oxidizing agent itself, would interfere with the oxidation step of the reaction scheme. Sulphuric acid medium, although convenient for the oxidation step was not preferred since it is reported to reduce the colour intensity of iron(III)-thiocyanate complex. A 1.0 M hydrochloric acid for oxidation step and a 0.5 M for complexation step were used in this study.

Fixing 5.5 μg/ml as the upper limit of iron(III) that could be determined by the thiocyanate method [53], stoichiometrically, 410 μg of cerium(IV)sulphate would be required to generate it form 398 μg of FAS. However, 400 μg each of cerium(IV)sulphate and FAS were used in this investigation to ensure a quantitative reaction. Although a fixed amount of FAS is not really required, large amounts are undesirable since iron(II) tends to undergo aerial oxidation. Hence, a fixed amount (400 μg) of FAS enough to reduce the total cerium(IV) was employed. The oxidation of CPF by cerium(IV) was complete in 10 min and subsequent reduction of residual oxidant by iron(II) and complex formation reaction between the resulting iron(III) and thiocyanate were instantaneous under the described experimental conditions. Developed colour was stable for at least 60 min in the presence of reaction product.

Two blanks were prepared for this method. The reagent blank which contained the optimum concentrations of all reactants except CPF gave maximum absorbance. The other blank was prepared in the

absence of cerium(IV) and CPF, to determine the contribution of others reactants to the absorbance of the system. Since the absorbance of the second blank was negligible, all absorbance measurements were made against a water blank.

Analytical parameters of the spectrophotometric methods

A linear relation is found between absorbance and concentration in the ranges given in TABLE 1.

TABLE 1: Analytical parameters

Parameter	Method A	Method B
λ_{max}, nm	510	470
Beer's law limits, μg mL^{-1}	1.0-8.0	0.5-4.0
Molar absorptivity, L mol^{-1} cm^{-1}	2.21 x 10^4	4.26 x 10^4
Sandell sensitivity, μg cm^{-2}	0.015	0.008
Limit of detection, μg mL^{-1}	0.12	0.07
Limit of quantification, μg mL^{-1}	0.40	0.22
Regression equation, Y*		
Intercept (a)	-0.0023	0.64
Slope (b)	0.07	0.13
Correlation coefficient (r)	0.9919	-0.9978

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

In method B, Beer's law is obeyed in the inverse manner. The calibration graphs are described by the equation:

$$Y = a + b X$$

(where Y = absorbance, a = intercept, b = slope and X = concentration in μg/ml) obtained by the method of least squares. Correlation coefficients, intercepts and slopes for the calibration data are also presented in TABLE 1. Sensitivity parameters such as molar absorptivity and Sandell sensitivity values, and the limits of detection and quantification, are also presented in TABLE 1.

Method validation

Evaluation of accuracy and precision

Intra-day and inter-day precision were assessed from the results of seven replicate analyses on pure drug solution. The mean values and relative standard deviation (RSD) values for replicate analyses at three different amount/concentration levels were calculated. To calculate the inter-day precision, analysis was performed over a period of five days preparing all solutions afresh each day. The accuracy of

TABLE 2: Evaluation of intra-day precision and accuracy

Method A						Method B					
CPF taken, μg mL^{-1}	CPF found, μg mL^{-1}	Range, μg mL^{-1}	Relative error, %	SD, μg mL^{-1}	RSD, %	CPF taken, μg mL^{-1}	CPF found, μg mL^{-1}	Range, μg mL^{-1}	Relative error, %	SD, μg mL^{-1}	RSD, %
3.0	3.04	0.14	1.33	0.03	0.98	1.0	1.02	0.04	2.00	0.013	1.27
5.0	4.96	0.17	0.80	0.08	1.61	2.0	1.97	0.07	1.50	0.021	1.06
8.0	7.88	0.22	1.50	0.07	0.89	3.0	2.93	0.11	2.33	0.059	2.01

SD: Standard deviation; RSD: Relative standard deviation;

TABLE 3: Results of analysis of dosage forms containing CPF

Brand name$^{\Psi}$ and dosage form	Label claim, mg/tablet or mg/mL	% found* ± SD		
		Reference method	Method A	Method B
Ciprolet[a] tablets	100	99.36 ± 0.74	98.83 ± 1.08 t = 0.92 F = 2.13	100.67 ± 0.86 t = 2.58 F = 1.35
Quintor[b] tablets	250	101.72 ±1.26	99.97 ± 1.44 t = 2.05 F = 1.31	100.62 ± 1.03 t = 1.52 F = 1.50
Quintor[b] injections	2	100.28 ±0.91	101.84 ± 0.96 t = 2.64 F =0.87	98.13 ± 1.32 t =3.05 F = 1.64

*** Mean value of five determinations**
Ψ Marketed by: a. Dr. Reddy's Laboratories
b. Torrent pharmaceuticals
Tabulated t-value at 95% confidence level is 2.77
Tabulated F-value at 95% confidence level is 6.39

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the methods was determined by calculating the percentage deviation observed in the analysis of pure drug solution and expressed as the relative error (RE). TABLE 2 summarises the intra-day precision and accuracy data for the determination CPF by the proposed methods which were within 2.5%. The inter-day precision was less than 3%.

Application to tablets analysis

The Indian pharmaceutical industry has at present nearly 100 different brands of tablets in 100-750 mg doses and 20 brands of injections. Two brands of tablets and one brand of injection were assayed by the proposed methods, and the results are summarized in TABLE 3. The results obtained were compared with those obtained by an established method[16,34]. A close agreement between the results obtained by the proposed methods and the reference method interms of accuracy and precision was obtained as evident from the calculated Student's t value and F-value (TABLE 3). The results also agreed well with the label claim.

Accuracy and validity of the methods were further ascertained by performing recovery experiments *via* standard addition technique. To a fixed and known amount of CPF in tablet powder (pre analysed), pure drug was added at three levels and the total was found by the proposed methods. Each test was repeated three times. The recovery of pure CPF added to tablet powder ranged from 96.5 to 104.3 % indicating that commonly encountered tablet excipients and additives such as talk, starch, lactose, sodium alginate, magnesium stearate calcium gluconate and calcium dihydrogenorthophosphate did not interfere in the assay procedure.

Two new methods for the assay of ciprofloxacin in pharmaceuticals using cerium (IV)sulphate as the oxidimetric reagent have been developed and appropriately validated. The methods are simple, rapid and cost effective. Both methods are based on well-characterized redox-complexation reactions and are the most sensitive (in terms of molar absorptivity) over the existing methods for ciprofloxacin in pharmaceuticals. The sensitivity is better than many HPLC methods in terms of linear range of applicability and this has been achieved using as simple a technique as visible spectrophotometry. The stability of the coloured species and sensitivity of the reactions used are not critically dependent on any experimental variable unlike in many reported methods. All the methods are based on the use of cerium (IV) which is exceptionally stable in solution. These advantages coupled with a fair degree of accuracy and precision qualify the methods for use in quality control laboratories.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the receipt of pure ciprofloxacin as gift from Torrent Pharmaceuticals Ltd, Ahmedbad, India. One of the authors (PN) thanks the authorities of the University of Mysore, Mysore, for research facilities.

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Full Paper

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