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Colorimetric method for the estimation of sparfloxacin in tablet dosage forms

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ABSTRACT

A simple highly sensitive spectrophotometric method was developed for the quantification of Sparfloxacin (5-amino-1-cyclopropyl-7-[(3, 5-dimethylpiperazin-1-yl]-6, 8-difluoro-4-oxo-quinoline-3-carboxylic acid). The method involves the reaction of the target compound with copper sulphate in acetic acid in presence of sodium nitrite reagent to produce a bluish green color chromogen. The derivative chromogen exhibits absorption maxima at 404 nm. Under this reaction, no degradation occurs. The proposed method can be utilized as a stability indicating assay. The different experimental parameters affecting the derivatization reaction were carefully studied and incorporated into the procedure. Under the described conditions the proposed method is linear over the concentration range of 5-45 mcg/ml and the coefficient of determination were >0.999 with a relative standard deviation of 0.236%. The average recovery of the target compound is 99.46% with a limit of quantification (LOQ) of 0.293mcg/ml and the limit of detection (LOD) 0.096mcg/ml. The mechanism of the derivatization reaction is proposed and advantages of the proposed method are discussed.

KEYWORDS

Sparfloxacin;
Copper sulphate;
Sodium nitrite.

INTRODUCTION

The quality control of active pharmaceutical ingredients (APIs) in the formulation is always a thrust area for the pharmaceutical industries. So the development of reproducible, sensitive, simple and extremely inexpensive methods for the determination of APIs in the formulation is always challenging.

The Sparfloxacin is a broad-spectrum antibiotic. This prevents bacterial DNA from unwinding and duplicating.[1] Since bacteria and humans unwind DNA with different enzymes, most of those enzymes (topoisomerases) in humans are not affected.[2] However, recent evidence has shown that topoisomerase II is also a target for a variety of quinolone-based drugs. Thus far, most of the compounds that show high activity against the eukaryotic type II enzyme contain aromatic substituents at their C-7 positions.[3] The parent of the quinolone (aka fluoroquinolone) class is nalidixic acid. The majority of quinolones in clinical use belong to the subset of fluoroquinolones, which have a fluorine atom attached to the central ring system, typically at the 6-position or C-7 position. The term quinolone(s) refers to potent synthetic chemotherapeutic antibacterials[4-6].

Full Paper

Sparfloxacin is finding new dimensions of clinical importance. To support its investigation an appropriate analytical method (sensitive, selective, reproducible and simple) for quantification of sparfloxacin is essential. A number of analytical methods have been reported for measuring sparfloxacin. These methods involved HPLC techniques[7].

For the drugs that obey the beer Lambert's law, spectrophometric methods of analysis of single component in solution are usually rapid, sensitive and economical[8,9].

Sparfloxacin is not official in any of the pharmacopoeias, the literarture survey did not reveal any analytical method for quantitative estimation of sparfloxacin. Thus there is a need for the development of newer effective, sensitive, accurate and economical methods of analysis for quantitative estimations of sparfloxacin as an active pharmaceutical ingredient.

The aim of this work was to develop a sensitive and simple spectrophotometric method for the quantification of sparfloxacin is finding new dimensions of clinical importance. To support its investigation an appropriate analytical method (sensitive, selective, reproducible and simple) for quantification of sparfloxacin is essential.

EXPERIMENTAL

Apparatus

A model Shimadzu UV-1601 double beam spectrophotometer with a fixed slit width of 2nm using a pair of 1cm matched quartz cells was used for spectrophotometric analysis.

Materials

All the chemicals were of analytical reagent grade, and the solvents were of spectroscopic grades. A sparfloxacin (Wintac Limited, Bangalore, Karnataka State, India), Copper sulphate, acetic acid, sodium nitrite and methanol.

Spectrophometric method

Prepartion of standrad sparfloxacin solution

An accurately weighed 100 mg of pure drug sparfloxacin was taken in clean, dry 100 ml volumetric flask and dissolved in small volume of ethanol (10–20 ml). The solution is diluted to 100 ml with ethanol. Now pipette out 10 ml of this solution and dilute to 100 ml with ethanol, resulting in 100 mcg/ml of drug concentration.

Determination of absorption maxima of sparfloxacin

5 ml aliquot of standard sparfloxacin solution of 100 mcg/ml was pipetted into a 25 ml volumetric flask. To this 3 ml of 1%w/v copper sulphate in 10%v/v acetic acid and 2 ml of 2% w/v sodium nitrite solution were added and heated on a water bath for 10 minutes at 90º C. After 10 minutes, solution was cooled to room temperature. The final volume was made up to 25 ml with ethanol and solution was then scanned in the range of 400 to 800 nm against the reagent blank and graphically represented in Figure 1.

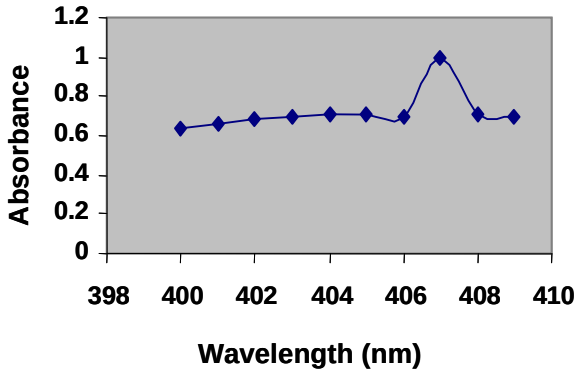

Figure 1 : Sparfloxacin: 20 mcg/ml, 3 ml 1%w/v $CuSo_4$ in 10%v/v CH_3COOH; 2 ml 2% w/v NaNO2 solution; reaction time: 10 min.

Determination of optimum strength of reagent required producing optimum chromogens having maximum absorbance

An aliquot of 7.5 ml Sparfloxacin solution of 100 mcg/ml was pipetted into five 25 ml volumetric flasks. To this 1 ml, 2 ml, 3ml and 4 ml of 1%w/v copper sulphate in 10%v/v acetic acid (reagent) was added of volumetric flasks respectively and 2 ml of 2% w/v sodium nitrite solution was added of volumetric flasks respectively. The volumetric flasks were heated on a water bath for 10 minutes at 90º C and cooled to room temperature. The final volume was made up to 25 ml with ethanol and solution was then scanned in the range of 404 nm against the reagent blank.

The final volume was made up to 25 ml with distilled water. The absorbance was measured at λmax (nm) against reagent blank. The optimum strength of

reagent required to produce stable chromogen having maximum absorbance was found to be 3 ml and represented graphically in Figure 2.

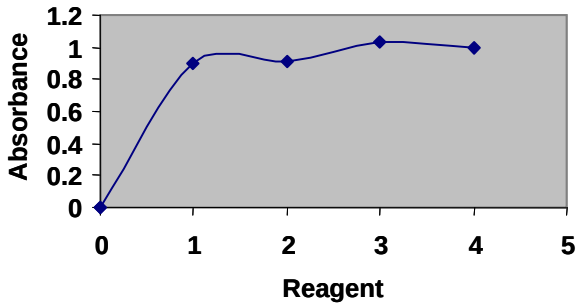

Figure 2 : Sparfloxacin: 30 mcg/ml, 1ml.2ml, 3ml & 4ml 1%w/v $CuSo_4$ in 10%v/v CH_3COOH; 2 ml, 2% w/v NaNO2 solution; reaction time: 10 min.

Determination of optimum temperature required producing chromogen

An aliquot of 7.5 ml of 100 mcg / ml of sparfloxacin solution was pipetted into four 25 ml volumetric flasks. To this 3ml of 1%w/v copper sulphate in 10%v/v acetic acid (reagent) was added of volumetric flasks respectively and 2 ml of 2% w/v sodium nitrite solution was added of volumetric flasks respectively and heated on a water bath at different times i.e., 1 minute, 5 minutes, 10 minutes and 15 minutes at 90 °C. The volumetric flasks were cooled to room temperature and the volume was made up to 25.0 ml with ethanol. The absorbance of each solution was measured at 404 against blank.The optimum strength of temperature 10 minutes required to produce stable chromogen and represented graphically in Figure 3.

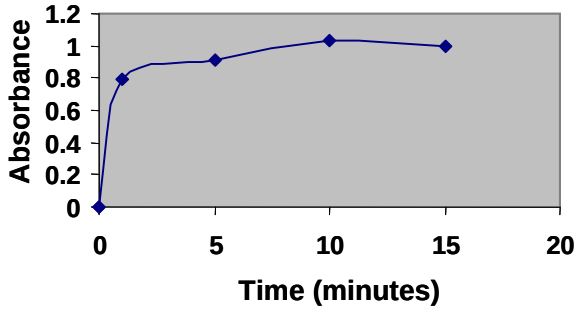

Figure 3 : Sparfloxacin: 30 mcg/ml, 3ml 1%w/v $CuSo_4$ in 10%v/v CH_3COOH; 2 ml 2% w/v NaNO2 solution; reaction time: 10 min.

Determination of concentration range of sparfloxacin

Aliquots of 1.25 ml, 2.5 ml, 3.75 ml, 5 ml, 6.25 ml, 7.5 ml, 8.75 ml, 10 ml, 11.25 ml, 12.5 ml and 13.75 ml. of 100 mcg/ml of sparfloxacin solution was pipetted into each of eleven 25 ml volumetric flasks. To this 3 ml of 1%w/v copper sulphate in 10%v/v acetic acid and 2 ml of 2% w/v sodium nitrite solution were added and heated on a water bath for 10 minutes at 90º C. After 10 minutes, solution was cooled to room temperature. The final volume was made up to 25 ml with ethanol and solution was then scanned in the range of 400 to 800 nm against the reagent blank.

The absorbance of solutions was measured at 404 nm against blank. The abs. Vs conc. curve was found to be linear in the concentration range 5-45 mcg/ml of sparfloxacin and represented graphically in Figure 4.

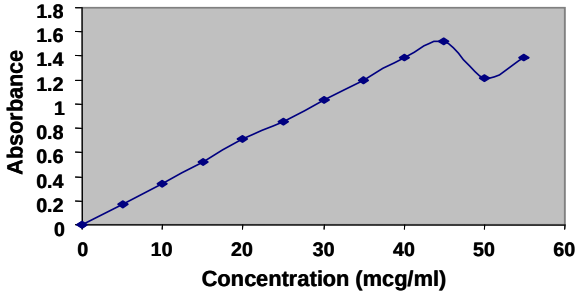

Figure 4 : Sparfloxacin: 5-55 mcg/ml, 3ml 1%w/v $CuSo_4$ in 10%v/v CH_3COOH; 2 ml 2% w/v NaNO2 solution; reaction time: 10 min.

Preparation of standard curve of sparfloxacin

Aliquots of 1.25 ml, 2.5 ml, 3.75 ml, 5 ml, 6.25 ml, 7.5 ml, 8.75 ml, 10 ml and 11.25 ml of 100 mcg/ml of sparfloxacin solution was pipetted into each of eleven 25 ml volumetric flasks. To this 3 ml of 1%w/v copper sulphate in 10%v/v acetic acid and 2 ml of 2% w/v sodium nitrite solution were added and heated on a water bath for 10 minutes at 90º C. After 10 minutes, solution

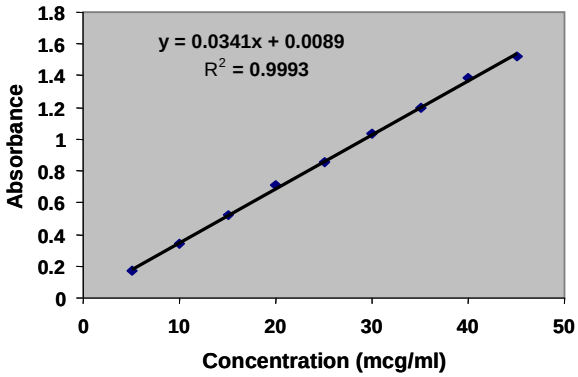

Figure 5 : Sparfloxacin: 5-45 mcg/ml, 3ml 1%w/v $CuSo_4$ in 10%v/v CH_3COOH; 2 ml 2% w/v NaNO2 solution; reaction time: 10 min.

was cooled to room temperature. The final volume was made up to 25 ml with ethanol and solution was then scanned in the range of 400 to 800 nm against the reagent blank.

The absorbance of solutions was measured at 404 nm against blank. **T**he calibration curve shows that Beer's law was obeyed in the concentration range 5-45 mcg/ml of sparfloxacin and represented graphically in Figure 5.

Optical characteristics

The optical characteristics of the proposed method have been calculated. The values are given in TABLE 1.

TABLE 1

Sr. No.	Parameters	Results
1.	Absorption maxima (nm)	404
2.	Beer's law limits (mcg/ml)	5-45
3.	Molar extinction coefficient (mole-^{1} cm-^{1})	3.445×10^{-2}
4.	Sandal's sensitivity (mcg/cm^{2}/0.001 absorbance units)	0.0290111
5.	Regression equation (y) Slope (b) Intercept (a)	0.9993 0.0341 0.0089
6.	Coefficient of variance	0.1829259
7.	Standard deviation	0.001
8.	Limit of detection (mcg/ml)	0.0967742
9.	Limit of quantitation (mcg/ml)	0.2932551

Validation parameters

Recovery studies

An aliquot of 5 ml of sample drug sparfloxacin solution 100 mcg/ml was pipetted into each of three 25 ml volumetric flasks. To this 1 ml, 1.5 ml and 2 ml of standard drug sparfloxacin solution of 100.0 mcg/ml was added respectively. To this 3 ml of 1%w/v copper sulphate in 10%v/v acetic acid and 2 ml of 2% w/v sodium nitrite solution were added and heated on a water bath for 10 minutes at 90º C. After 10 minutes, solution was cooled to room temperature. The final volume was made up to 25 ml with ethanol. The absorbance was measured at 404 nm against reagent blank. The total amount and percentage recovery of sample sparfloxacin was found to be 99.46%.

Precision

Repeatability

An aliquot of 5 ml of sample drug sparfloxacin solution 100 mcg/ml was pipetted into each of three 25 ml volumetric flasks. To this 3 ml of 1%w/v copper sulphate in 10%v/v acetic acid and 2 ml of 2% w/v sodium nitrite solution were added and heated on a water bath for 10 minutes at 90º C. After 10 minutes, solution was cooled to room temperature. The final volume was made up to 25 ml with ethanol. The absorbance was measured at 404 nm against reagent blank. The average percentage recovery of sample sparfloxacin was found to be 100.06.

Stability

7.5 ml aliquot of standard sparfloxacin solution of 100 mcg/ml was pipetted into a 25 ml volumetric flask. To this 3 ml of 1%w/v copper sulphate in 10%v/v acetic acid and 2 ml of 2% w/v sodium nitrite solution were added and heated on a water bath for 10 minutes at 90º C. After 10 minutes, solution was cooled to room temperature. The final volume was made up to 25 ml with ethanol and solution was then scanned in the range of 404 nm against the reagent blank and graphically represented in Figure 6. The colour of the derivative chromogen of sparfloxacin was found to be stable for 30 minutes after which the absorbance decreases slowly.

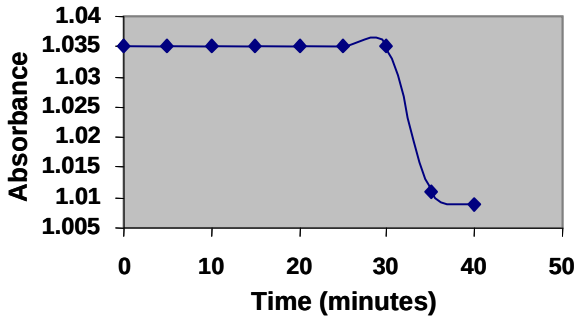

Figure 6 : Sparfloxacin: 30 mcg/ml, 3ml 1%w/v $CuSo_4$ in 10%v/v CH_3COOH; 2 ml 2% w/v NaNO2 solution; reaction time: 40 min.

RESULT AND DISCUSSIONS

The experimental conditions affecting the development and stability of the colored chromogens produced were carefully studied. The colored chromogens are stable for at least 30 min which permits the convenient application of the proposed method.

Different experimental conditions, especially temperature and reagent concentration were carefully se-

lected as they could greatly affect the quantification of the target compound.

The effect of reagent concentration on the derivative chromogen formation was observed by measuring the absorbance of sparfloxacin concentration ratios, while all other experimental conditions were kept constant at the optimum values. Figure 2. In order to obtain optimum derivative chromogen with highest and most stable absorbance, the effect of the reaction time and heating temperature on the absorbance of the reaction product was studied. The reaction was carried out at different temperatures (60 ^{O}C, 80.0 ^{O}C, and 90.0 ^{O}C) using a thermostated water bath for periods ranging from 0.0 to 75.0 min. Maximum and constant absorbance was obtained at 90 ^{O}C after 10min. the colored product was stable for at least 30 min.

Calibration, sensitivity and precision

From the results obtained in the experimental section, the absorbance of the sparfloxacin derivatized with reagent was proportional to the concentration of the sparfloxacin over the range 5-45 mcg/ml (Figure 5) and the total concentration of sparfloxacin can be calculated using the corresponding correlation equation with a correlation coefficient (r) = 0.999 for n=6 with the detection limit of 0.0967742 mcg/ml.

The precision of the proposed method was studied by determination of the drug in six replicates, individually derivatized with reagent at concentration of 20 mcg/ml obtaining relative standard deviations of 0.236.

Coefficient of variance was found to be 0.1829259. The standard deviation of 0.001, LOD and LOQ was found to be 0.0967742 mcg/ml and 0.2932551 mcg/ml, indicated accuracy and reproducibility in color development. The method was extended for the determination in formulation. It was observed that the results obtained were comparable to that of label claim. The recovery studies of the standard drug when performed in the preanalysed formulation gave Percentage recovery of 99.72% to 100.12% indicating practically no interference of formulation excipients with the proposed method.

CONCLUSION

It was found that this method developed was new simple, accurate, sensitivity, economical and reproducible which could provide satisfactory results. The methods can be used for routine analysis of sparfloxacin in formulation. The methods are practical and valuable.

The described methods have many advantages

It does not need expensive apparatus; it is simple and quick; its linear range is relatively wide, it has good selectivity. Furthermore, the proposed method may be successfully used to determine sparfloxacin in pharmaceutical formulations. Accordingly, the method is practical and valuable.

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