ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
26 (2001) 501–504

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Short communication

Spectrophotometric determination of sparfloxacin in pharmaceutical formulations using bromothymol blue

Hérida R.N. Marona [a,*], Elfrides E.S. Schapoval [b]

[a] *Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, Rod. Araraquara-Jaú, km 1, CEP. 14801-902, Araraquara, SP, Brazil*

[b] *Curso de Pós-Graduação em Ciências Farmacêuticas, UFRGS, Porto Alegre, RS, Brazil*

Received 5 October 2000; received in revised form 5 February 2001; accepted 17 February 2001

Abstract

A visible light spectrophotometric method is described for the determination of sparfloxacin in tablets. The procedure is based on the complexation of bromothymol blue 0.5% and sparfloxacin to form a compound of yellow colour with maximum absorption at 385 nm. The Lambert–Beer law was obeyed in the concentration range of 2–12 mg/l. The present study describes a sensitive and accurate method for the determination of the concentration of sparfloxacin in tablets. It was also found that the excipients in the commercial tablet preparation did not interfere with the assay. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Fluoroquinolone; Pharmaceutical analysis; Sparfloxacin; Visible light spectrophotometry

1. Introduction

Sparfloxacin (Fig. 1) is a broad spectrum antibacterial fluoroquinolone active against some microorganisms including Gram-positive and Gram-negative bacteria [1,2] and demonstrates moderate activity against anaerobes and *Mycobacteria*, for which the quinolones in general have low activity [3,4].

Chemically, sparfloxacin is a 5-amino-1-cyclopropyl-7-(*cis*-3,5-dimethyl-piperazin-1-yl)-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid and it is not yet official in any Pharmacopeia. Sparfloxacin has been studied in terms of therapeutic activity. However, few reports about its analytical preparation are available in the literature. High performance liquid chromatography of sparfloxacin both as a raw material and in tablets was described [5]. The literature has reported analytical methods for determination of sparfloxacin such as UV-spectrophotometry [6] and microbiological assay [7]. The microbiological bioassay is

* Corresponding author. Tel.: +55-16-2320200; fax: +55-16-2220073.

E-mail address: maronah@fcfar.unesp.br (H.R.N. Marona).

PII: S0731-7085(01)00429-0

the most commonly used routine method. However, it is slow, often inaccurate, and subject to interference by other antibiotics [8]. The major disadvantages of the HPLC method include the requirement for complex and expensive equipment, provision for use and disposal of solvents, labor-intensive sample preparation procedure, and personnel skilled in chromatographic techniques.

The aim of this study was to develop a sensitive and accurate spectrophotometric method for determination of sparfloxacin. This paper reports a procedure for the quantitation of the drug in pharmaceutical forms by visible light spectrophotometry, providing precise and accurate results validated by statistical analysis as well as being a low cost method.

2. Experimental

2.1. Materials

Sparfloxacin (99.5% potency) was kindly supplied by Rhône-Poulenc Rorer (USA). Each tablet was claimed to contain 200 mg of sparfloxacin and microcrystalline cellulose, corn starch, L-hydroxypropylcellulose, magnesium stearate, colloidal silicon, hypromellose, macrogol 6000 and titanium dioxide as excipients. All other chemicals were of analytical grade. The colour reagent used was bromothymol blue 0.5%. All substances, as well as the reagents were kept at room temperature and stored protected from light. The absorbance value of each solution at 385 nm was determined in a 10-mm quartz cell using a Hitachi UV–VIS spectrophotometer.

2.2. Method

The solution of the sparfloxacin reference substance was prepared by accurately weighing 10 mg of this drug and transferring to a 100-ml volumetric flask, with addition of water to make up to volume to give a nominal concentration of 100 μg/ml.

2.2.1. Calibration curve

In a separator funnel, aliquots of sparfloxacin reference substance were added to 4 ml of bromothymol blue, 4 ml of biphthalate buffer (pH 3.2). The reaction mixture was extracted by shaking with 5 ml of dichloromethane. This extraction was conducted six times until the solution became clear. The organic layer was collected in a 50-ml volumetric flask and dichloromethane was added to make up to volume.

2.2.2. Tablets

In order to determine the average weight, 20 tablets were accurately weighed. Five tablets were ground up and dried at 105°C for 2 h. An amount representing three times the average weight of each tablet (313.4 g) was transferred to a 1000-ml volumetric flask, 200 ml methanol was added and the flask shaken for 10 min followed by addition of sterile water to volume to give a nominal concentration of 600 μg/ml.

In a separator funnel, aliquots of 1 ml sparfloxacin tablet solution (600 μg/ml) were added to 4 ml of bromothymol blue and 4 ml of biphthalate buffer (pH 3.2). The reaction mixture was extracted by shaking with 5 ml of dichloromethane. This extraction was conducted six times until the solution became clear. The organic layer was collected in a 100-ml volumetric flask and dichloromethane was added to make up to volume to give a final estimated concentration of 6.0 μg/ml. This solution was prepared by six times and the absorbance of each solution was determined at 385 nm.

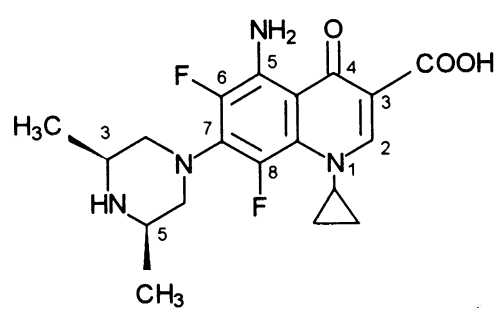

Fig. 1. Chemical structure of sparfloxacin (M.W. 392.4).

The results were analysed by linear simple regression by the least-squares method. To verify the internal validity [9] of this method, analysis of variance (ANOVA) was applied.

2.3. Recovery test

The sparfloxacin reference substance and tablets were dried at 105°C for 2 h. In order to determine the accuracy of the tablet solutions, a sparfloxacin reference substance solution of 100 μg/ml was added in 1.0-, 2.0- and 3.0-ml aliquots to a separator funnel containing 4 ml of bromothymol blue, 4 ml of biphthalate buffer (pH 3.2) and 1.0 ml of sparfloxacin tablet solution (600 μg/ml). The reaction mixture was extracted as described above and collected in a 100-ml volumetric flask and dichloromethane was added to make up to volume to give a final estimated concentration of 7.0, 8.0 and 9.0 μg/ml. Each concentration was prepared in triplicate and the absorbance of each solution was determined at 385 nm.

The final theoretical concentrations of sparfloxacin were 116.7, 133.3 and 150.0%. Three replicate determinations were carried out on three different days to test the precision of this method.

3. Results

Under the experimental conditions described, a standard calibration curve of sparfloxacin was constructed by plotting absorbance versus concentration. The UV absorption spectrum of sparfloxacin was monitored at 385 nm. Agreement with Beer's law was evident in the concentration range of the final dilution of 2.0–12.0 μg/ml. The correlation coefficient obtained for the line was 0.9955, indicating good linearity. The calibration curves of sparfloxacin were constructed by plotting concentration versus absorbance and showed good linearity (Fig. 2). The representative linear equation was: $y = 0.0304x + 0.1442$ ($n = 6$, $r = 0.9977$, $r^2 = 0.9955$). The relative deviation of the slope of the three lines was 8.6% and the relative deviation of the intercept was 11.9%.

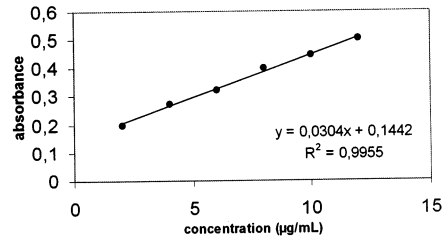

Fig. 2. Calibration curve of sparfloxacin by visible light spectrophotometry at 385 nm.

The inter-day precision was evaluated by comparing the linear regressions of three standard plots prepared on three different days. Three replicate determinations at six different concentrations were carried out to test the precision of this method. The mean percent coefficient of variation was 5.9%, indicating reasonable repeatability of the extraction method.

The experimental results obtained for the determination of sparfloxacin tablets are shown in Table 1. The S.D. was found to be less than 0.02, indicating good repeatability.

3.1. Recovery test

The recoveries determined by spiking known amounts of the sparfloxacin reference substance to the samples at the beginning of the procedure are shown in Table 2. This method had good reproducibility and the results show excellent recoveries of sparfloxacin from the spiked samples.

Table 1
Analysis of sparfloxacin tablets (200 mg) by visible light spectrophotometric method[a]

	A	Mean ± S.D.	CV (%)
1	0.288		
2	0.315	0.293 ± 0.02	6.67
3	0.277		

[a] CV, coefficient of variation; S.D., standard deviation.

Table 2
Recovery test of sparfloxacin tablets using spectrophotometry at 385 nm

Spiked amount of SR (μg)	Recovery amount of SR (mg)	Recovery[a] (%)
100.0	98.0	98.0
200.0	200.0	100.0
300.0	278.1	92.7

[a] Mean of three replicate analyses.

4. Discussion

Sparfloxacin could be analysed by visible light spectrophotometry both as a raw material and as a pharmaceutical formulation in tablet form.

In general, extractive methods can give results with more variation when compared with other methods. This may be due to a loss of pharmaceutical substance during handling. The amount of dichloromethane extractions was investigated and it was found that six times gave the highest absorbance and a satisfactory coefficient of variance. There was no evidence of interference from excipients in the tablets analysed. The S.D. and the coefficient of variation were found to be less than 0.02 and 7%, respectively, indicating acceptable repeatability for an extractive method followed by spectrophotometry. Good specificity was obtained with this method. The assay range was 2.0–12.0 μg/ml. The mean absolute recovery was found to be 96.9%.

Although this procedure cannot be classified as having ease of handling, this visible light spectrophotometric method proposed is simple and can therefore be applied to the determination of sparfloxacin raw material and tablets. Being partially automated, the procedure consists of three extractions before submission of the sample into a spectrophotometer. HPLC and microbiological bioassay involve more procedural steps and take more operator time and expertise. No interfering absorbances were found due to the tablet excipients and solvent. The analyte in solution was stable during the analytical procedure and the time taken for assay was approximately 2 h.

5. Conclusion

The visible light spectrophotometric method developed in this study demonstrated acceptable linearity, sensitivity and accuracy. Moreover, this method provides precise and accurate results validated by statistical analysis as well as being a low cost method.

The method validation yielded good results and included linearity, repeatability/reproducibility, sensitivity, recovery and accuracy.

Acknowledgements

We thank the Rhône-Poulenc Rorer (USA) for providing the sparfloxacin reference substance used in this study. This work was supported by the CAPES/PICDT program (Brasília-Brasil).

References

[1] S. Nakamura, A. Minami, K. Nakata, N. Kurobe, K. Kouno, Y. Sakaguchi, S. Kashimoto, H. Yoshida, T. Kojima, T. Ohue, K. Fujimoto, M. Nakamura, M. Hashimoto, M. Shimizu, Antimicrob. Agents Chemother. 33 (1989) 1167–1173.
[2] K.L. Goa, H.M. Bryson, A. Markham, Drugs 53 (1997) 700–725.
[3] K. Borner, E. Borner, H. Lode, J. Chromatogr. Biomed. Appl. 579 (1992) 285–289.
[4] T. Kojima, M. Inoue, S. Mitsuhashi, Antimicrob. Agents Chemother. 33 (1989) 1980–1988.
[5] H.R.N. Marona, E.E.S. Schapoval, J. Pharm. Biomed. Anal. 20 (1999) 413–417.
[6] H.R.N. Marona, E.E.S. Schapoval, J. Antimicrob. Chemother. 44 (1999) 136–137.
[7] H.R.N. Marona, E.E.S. Schapoval, Inf. Technol. 9 (1998) 251–254.
[8] D.S. Reeves, M.J. Bywater, J. Antimicrob. Chemother. 1 (1975) 103–116.
[9] United States Pharmacopoeia, 24th edn., United States Pharmacopeial Convention, Rockville, 1999, pp. 2149–2152.