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A high-performance liquid chromatographic assay for sparfloxacin

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Abstract

A specific and sensitive high-performance liquid chromatographic procedure was developed for the assay of sparfloxacin in raw material and tablets. It was also found that the excipients in the commercial tablet preparation did not interfere with the assay. The method validation yielded good results and included the range, linearity, precision, accuracy, specificity and recovery. This method can also be applied to stability studies. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

In recent years, there has been a growing interest in the use of fluoroquinolones as therapeutic drugs. Sparfloxacin is a potent fluoroquinolone antibacterial agent (Fig. 1) against Gram-positive and Gram-negative bacteria [1–4]. It has been reported to be more active in vitro than other quinolones against some micro-organisms including staphylococci and mycobacteria [5–7], and has a 16 h plasma half-life [8,9]. It is potentially phototoxic like the other fluoroquinolones.

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 [10,11] and is not yet official in any pharmacopeia. HPLC methods for sparfloxacin in body fluids have been reported [9,12–15]. HPLC methods are very useful in the determination of drugs, and offers a significant improve-

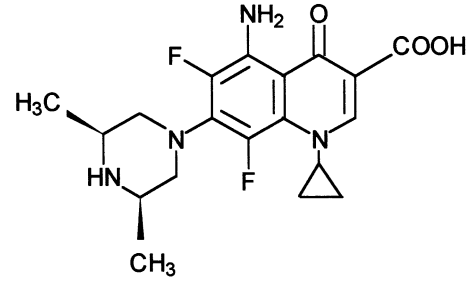

Fig. 1. The chemical structure of sparfloxacin.

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Table 1
Experimental values obtained in the recovery test for sparfloxacin tablets by HPLC

	Amount of standard (μg ml^{-1})		Recovery[a] (%)	CV (%)
	Added	Recovered		
R_1	15.0	99.67	14.95	0.27
R_2	25.0	99.60	24.90	0.39
R_3	40.0	98.45	39.38	0.43

[a] Mean of three determinations.

ment in sensitivity over previous reports. We believe that the availability of this new method, with its increased sensitivity and selectivity, will be very useful for the determination of sparfloxacin in pharmaceutical preparations. Owing to the widespread use of HPLC in routine analysis, it is important that good HPLC methods are developed and that these are thoroughly validated. The aim of this study was to develop an easy, rapid and accurate reversed-phase HPLC method for the determination of sparfloxacin in raw material and tablets. This method can also be used to identify product degradation of sparfloxacin in stability studies.

2. Experimental

2.1. Samples

The sparfloxacin reference substance (assigned purity 99.5%) was supplied by Dainippon Pharmaceutical, Osaka, Japan and Rhone-Poulenc Rorer, USA.

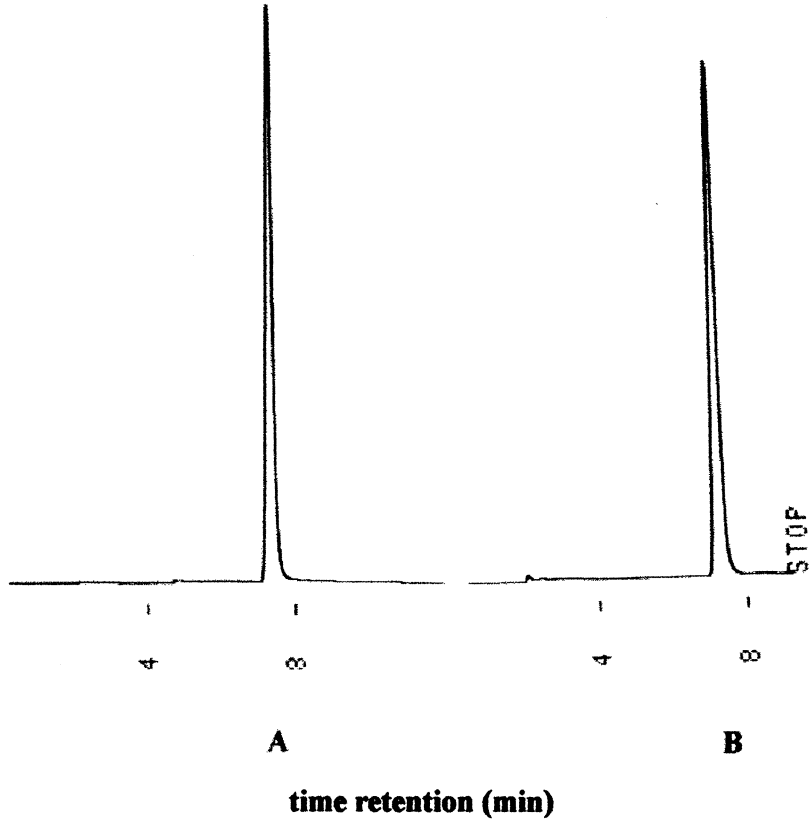

Fig. 2. HPLC chromatograms of the sparfloxacin reference substance (A) and sparfloxacin tablets (B).

Table 2
Analysis results for sparfloxacin tablets (200 mg) by HPLC

	Theoretical amount (mg)	Experimental amount[a] (mg) ± CV%	Purity (%)
1	200	188.39 ± 1.12	94.18
2	200	190.39 ± 1.24	95.19
3	200	185.89 ± 0.16	92.94

[a] Mean of three determinations.

The sparfloxacin tablets were obtained commercially. The tablets were claimed to contain 200 mg of drug and cellulose microcristalline, corn starch, L-hydroxypropylcellulose, magnesium stearate, colloidal silicon, hypromellose, macrogol 6000 and titanium dioxide as excipients.

The sparfloxacin reference substance, as well as the tablets, were always kept protected from light.

2.2. Reagents and solvents

All other chemicals used were of analytical grade. The water used was bidistilled in an all-glass still for all analytical purposes.

2.3. Instrumentation and conditions

Quantitative HPLC was performed on a Shimadzu SCL-6A chromatograph (Japan) equipped with a model LC-10AS pump; an SPD-10A variable-wavelength detector (set at 292 nm); an SCL-10A system controller; a C-R6A integrator and Rheodine injection valve with a 20-μl loop. A Shim-pack CLC-ODS column (250 mm × 4.6 mm i.d., 5 μm particle size, 100 Å pore diameter) was used with aqueous acetic acid 5%–methanol–acetonitrile (70:15:15 v/v/v) as the isocratic mobile phase at a flow-rate of 1.0 ml min^{-1}. The sensitivity was 0.5 AUFS and the chart speed was 0.5 cm min^{-1}. The HPLC system was operated at ambient temperature (20 ± 1°C). The mobile phase was filtered by a membrane filter (Supelco) 0.45 μm × 47 mm and degassed with a helium sparge for 15 min. The analysis required less than 10 min.

2.4. Procedure

2.4.1. Sparfloxacin reference standard

Solutions of the sparfloxacin reference standard in methanol (1 mg ml^{-1}) were prepared by accurately weighing 20 mg sparfloxacin reference substance, transferring to a 20-ml volumetric flask, the addition of 10 ml methanol and shaken for 30 min by a mechanical shaker, followed by adding methanol to make up the volume. Aliquots (2 ml) of the sparfloxacin standard solution were transferred volumetrically into 100-ml flasks and water added to make up to volume to give a final concentration of 20 μg ml^{-1}.

2.4.2. Assay of sparfloxacin in tablets

Ten tablets were weighed to get the average tablet weight. The samples of the powdered tablets, claimed to contain 200 mg sparfloxacin, were transferred to a 500-ml volumetric flask, 250 ml methanol was added, and shaken for 30 min by a mechanical shaker, followed by adding methanol to make up to volume. A 5.0 ml aliquot of this solution was transferred to a 50-ml volumetric flask and water added to make up to volume to give a nominal concentration of 36 μg ml^{-1}. All determinations were conducted in triplicate.

2.4.3. Calculations

Having established the quantitative relationships between the parameters studied, and knowing the predictive performance of their association model, a linear simple regression by the least squares method was applied.

2.4.3.1. Recovery of sparfloxacin in tablets. The recoveries were determined by adding known

amounts of the sparfloxacin reference substance (15.0, 25.0 and 40.0 μg ml^{-1}) to the samples at the beginning of the process (Table 1). A recovery exercise was then performed.

2.4.4. Reproducibility and validation

The accuracy and precision of the assay, as well as the linearity of the calibration curve were determined for intra- and inter-day on three different days. The precision was expressed as the percent coefficient of variation of each curve. The statistical data were calculated by ANOVA.

3. Results and discussion

The development of methods in HPLC for the determination of drugs has received considerable attention in recent years because of their importance in quality control in pharmaceutical analysis. The goal of this study was to develop an HPLC assay for the analysis of sparfloxacin in raw material and tablets.

In order to test the column efficiency, the number of theoretical plates (N) for the separation of sparfloxacin was determined ($N = 7560$).

The retention time repeatability during the precision studies was found to be excellent for all the solutions. The retention times (t_R) of the sparfloxacin reference substance and the tablets were 7.367 and 7.308 min, respectively. The HPLC chromatogram shows the t_R (Fig. 2). From the t_R value, the capacity factor (k'') was determined for sparfloxacin ($k'' = 2.49$). According to the literature [16], the optimum k''-values lie in the range 2–5. The calibration curves for sparfloxacin were constructed by plotting the peak area versus concentration. It was found to be linear with a correlation coefficient of 0.9997, the representative linear regression equation was $y = 895145 + 41290x$, the relative deviation of the slope of the three lines was 3.7% and the relative deviation of the intercept was 1.9%. This method had good reproducibility and the results show excellent recoveries of the sparfloxacin from the spiked samples (99.0 ± 0.6). The method was validated by evaluation of the intra- and inter-day precision. In the range 10–40 μg ml^{-1}, the coefficient of variation (CV) on the basis of the peak area ratios for three replicate injections were found to be between 0.03 and 0.92%. The inter-day assay precision (3 days, $n = 6$) was expressed as CV and ranged 0.22–3.07%. The detailed accuracy and precision are shown in Table 1.

The method developed has also been used to quantify sparfloxacin in tablets. Sparfloxacin tablets (200 mg) were analyzed and the results obtained can be seen in Table 2. The average purity (%) reached was 95.19%.

No interfering peaks were found in the chromatogram due to the tablet excipients. Sparfloxacin was shown to be stable during all the procedure. However this method would also be able to identify the degradation products of sparfloxacin.

4. Conclusion

The present study proposes a rapid and precise method for the determination of sparfloxacin in raw material and tablets by HPLC. The method demonstrated acceptable linearity, sensitivity and accuracy. The results indicated that the proposed method may be recommended for determining sparfloxacin in tablets.

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