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Research paper

Development and validation of a nonaqueous titration with perchloric acid to determine sparfloxacin in tablets

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Abstract

A simple, rapid and inexpensive method for the determination of sparfloxacin in tablets is described. The procedure is based on the use of volumetric dosage in a nonaqueous medium in glacial acetic acid with 0.1 M perchloric acid. The method validation yielded good results and included precision and accuracy. It was also found that the excipients in the commercial tablet preparation did not interfere with the assay.

Keywords: Fluoroquinolone; Nonaqueous titration; Pharmaceutical analysis; Sparfloxacin

1. Introduction

Sparfloxacin (Fig. 1), an oral fluoroquinolone antibacterial agent, is active against most aerobic Gram-positive and Gram-negative organisms [1,2] and demonstrates moderate activity against anaerobes and *Mycobacteria*, which the quinolones in general have low activity [3,4].

Sparfloxacin (SPAX) has been studied in terms of therapeutic activity; however, there is no official pharmacopoeial monograph of this fluoroquinolone on the quantification of SPAX in tablets. Few reports about its analytical methods are available in the literature. High-performance liquid chromatography (HPLC) of SPAX both as a raw material and in tablets was developed in our laboratory [5]. Recent papers have reported analytical methods for determination of SPAX such as UV-spectrophotometry [6] and microbiological assay [7] which we had validated by statistical analysis.

This paper reports a procedure for the quantification of the drug in pharmaceutical forms by nonaqueous titration, providing precise and accurate results, which could be verified by statistical methods.

2. Experimental

2.1. Materials

Sparfloxacin was kindly supplied by Dainipon (Rhône-Poulenc Rorer, USA). The SPAX tablets, which were claimed to contain 200 mg of active drug, were obtained commercially. All other chemicals were analytical grade. Glacial acetic acid (Merck); perchloric acid 0.1 M, crystal violet 0.1% (in acetic acid) as indicator.

2.2. Methods

2.2.1. Tablets of sparfloxacin

In order to determine the average weight, 20 tablets were accurately weighed. Five tablets were crushed and dried at 105°C for 2 h. Each amount was accurately weighed, transferred in a 250-ml conical flask and 40 ml of glacial acetic acid and 5 drops of indicator were added. In order to determine the percentage, these solutions were titrated with perchloric acid 0.1 M until color change which indicated the final point was reached.

The percentage of SPAX was determined by applying the equation:

$$A\ (\%) = \frac{v \times \mathrm{dmEq} \times 100}{m}$$

where v is ml of perchloric acid 0.1 M, dmEq is decimal equivalent of SPAX (0.03924), and m is weight of SPAX (g).

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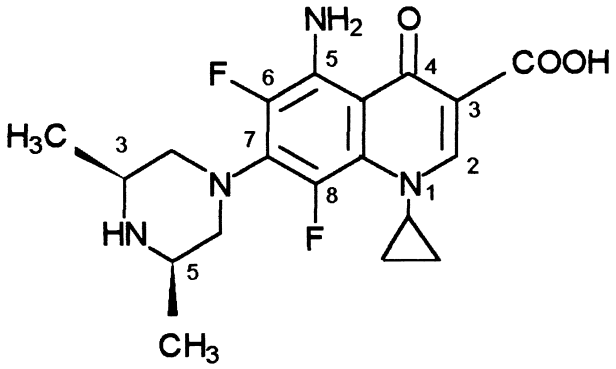

Fig. 1. Chemical structure of sparfloxacin.

A blank determination was performed at a temperature of 20°C and any necessary corrections were made.

2.2.2. Recovery test

The accuracy was determined by adding amounts of the reference substance to the samples at the beginning of this procedure according USP [8].

The recoveries were determined by adding known amounts of sparfloxacin reference substance SPAX-RS (5.0, 10.1 and 19.8 mg) to the samples at the beginning of the procedure. The SPAX-RS and tablets were dried at 105°C for 2 h. The equivalent of 200 mg SPAX in tablets was spiked with amounts of SPAX-RS and was dissolved in 40 ml of glacial acetic acid. Finally, this sample was titrated with perchloric acid 0.1 M to be quantified as described above. Three replicate determinations in three different days were carried out to test the precision of this method.

2.2.3. Precision

The accuracy and precision of this method were determined for intra- and inter-day on three different days. The precision was expressed as the percent coefficient of variation. The analysis of variance (ANOVA) is an important statistical tool to verify the internal validity of an analytical procedure.

3. Results

The results obtained through the titrimetric analysis with SPAX tablets are displayed in Table 1, which shows mean, SD, coefficient of variation (%CV) and relative SD.

3.1. Recovery test

The results of recovery test of SPAX in tablets using the volumetric determination with perchloric acid 0.1 M are shown in Table 2.

Table 1
Titration of SPAX tablets with perchloric acid 0.1 M[a]

	Day 1	Day 2	Day 3	Day 4	Mean inter-day
I	98.66	99.32	97.65	97.86	
II	99.13	98.37	96.72	98.57	
III	98.70	99.13	98.99	99.03	
IV	98.65	99.02	98.98	99.41	
V	98.75	99.23	99.53	99.50	
Mean	98.78	99.01	98.37	98.87	98.76
SD	0.2007	0.3770	1.1557	0.6753	0.2751
%CV	0.2032	0.3808	1.1748	0.6830	0.2786
RSD	0.0897	0.1686	0.5169	0.3020	0.1376
n	5	5	5	5	$n = 4$

[a] SD, standard deviation; %CV, coefficient of variation; RSD, relative standard deviation.

4. Discussion

Sparfloxacin showing pK_{a1} 6.27 and pK_{a2} 8.80 demonstrated the character of the proton dissociation of carboxylic group in C_3 and dimethylpiperazinyl in C_7, respectively [9]. The reaction between sparfloxacin and a nonaqueous medium and glacial acetic acid is an acid–base reaction where the strong acid can donate a proton to nitrogen from the piperazinyl ring.

In preliminary studies conducted with high temperature the sample appeared to be stability-indicating. Confirmatory studies of thermal stability were carried out using thermogravimetric analysis (TGA) and differential thermal analysis (DTA), which are simple techniques to measure the weight change of a sample as function of temperature or time. The SPAX TGA involved its thermal stability by monitoring the weight change during controlled heating (20°C/min). The DTA results showed an endothermic peak related with melting temperature at 276°C, and two exothermic peaks related with product decomposition at 341 and 579°C.

The proposed analytical method in a nonaqueous medium appears to be an important tool for precision and accuracy in the quantification of sparfloxacin raw material and tablets.

No interference from the sample solvent, impurities and tablet excipients could be analyzed by nonaqueous titration using a blank sample or excipients such as cellulose microcrystalline, corn starch, L-hydroxypropylcellulose, hypromellose, macrogol 6000 and titanium dioxide. There was no evidence of interference from excipients in the tablets analyzed.

The standard deviation and the coefficient of variation

Table 2
Recovery test of sparfloxacin (RS) tablets using volumetric determination with perchloric acid 0.1 M

Spiked amount of RS (mg)	Range requirement (±5%)	Recovery amount of RS (mg)	Recovery (%)
5.0	4.75–5.25	4.80	96.0
10.1	9.60–10.61	10.58	104.7
19.8	18.81–20.79	19.38	97.4

were found to be less than 1%, indicating good repeatability of the nonaqueous titration when it is compared with other methods such as spectrophotometric analysis (1.07%) [6], HPLC (0.98%) [5] and bioassay (2.42%) [7]. The relative standard deviation observed was approximately 0.15%.

The accuracy may be expressed as percent recovery by the assay of known, added amounts of analyte [8]. The mean absolute recovery test of nonaqueous titration was 99.37% and can indicate a good accuracy. The results obtained by spectrophotometric analysis [6], HPLC [5] and bioassay [7] were 100.04, 99.24 and 98.34%, respectively.

Although nonaqueous titration as well as spectrophotometric analysis could quantify degradation products that have similar chemical structures, the nonaqueous titration is clearly the least expensive method and it does not require expensive equipment and specialized technicians when it is compared alongside spectrophotometric analysis, HPLC and bioassay. Besides, other characteristics of this method are the short time required for performance and ease of handling, also indicating this procedure as a routine laboratory method.

This volumetric method proposed is simple, rapid and inexpensive and can therefore be applied to the determination of sparfloxacin raw material and tablets. Method validation yielded good results and included precision and accuracy.

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