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Determination of sparfloxacin and its degradation products by HPLC-PDA


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Sparfloxacin, a quinolone carboxylic acid derivative is active, as an antimicrobial agent, against a wide range of Gram-positive and Gram-negative organisms including mycobacteria1–2. A drawback of fluoroquinolones is their photoreactivity3–5, and though sparfloxacin has been studied in terms of therapeutic activities1–2, few reports about its physicochemical analysis are available in the literature6.

Due to the photosensitivity of sparfloxacin3, the aim of this work was to develop an easy, rapid and sensitive method to determine the presence of any photodegradation products in powder. An accelerated study of stability in aqueous solution was carried out by subjecting a solution of sparfloxacin to UV light (peak wavelength 290 nm) for 5 h at room temperature. Sparfloxacin powder (purity 99.5%) was supplied by Dainippon Pharmaceutical Co., Osaka, Japan and Rhone-Poulenc Rorer, USA. All other chemicals used were of analytical grade.

HPLC analysis was performed on a Waters SCL-6A chromatograph equipped with a model LC-10AS pump; SPD-10A variable-wavelength detector (set at 292 nm); SCL-10A system controller; C-R6A integrator and Rheodyne injection valve with a 20 L loop. A Shim-pack CLC-ODS column (250 mm × 4.6 mm I.D., 5 μm particle size, 100 pore diameter) was used with aqueous acetic acid 5%:methanol:acetonitrile (80:10:10, v/v/v) isocratic as mobile phase at a flow-rate of 1.0 mL/min. The HPLC system was operated at ambient temperature (20 ± 1°C). The mobile phase was filtered by membrane filter (Supelco) 0.45 μm × 47 mm and degassed with helium sparge for 15 min. To photodegrade sparfloxacin, a fresh solution (1mg/mL) was submitted to UV light (290 nm) for 24 h and 36 h in a chamber (10 × 10 × 90cm). Sparfloxacin tablets (200 mg): Ten tablets were ground up and five times the average weight were transferred to prepare 1000 mg/L solution. This solution was placed in Petri dishes in the UV light chamber for 24 h.

The applicability of the proposed method for the determination of sparfloxacin and its degradation products was demonstrated by analysing six aliquots of reference substance. The HPLC data show that sparfloxacin is sensitive to photodegradation under the conditions used in this study. A 200–400 nm scan HPLC chromatogram, five main degradation products were detected, along with several other minor peaks with poor resolution between 1 and 3 min. The total area was made with the area for the five peaks. The UV-spectra (200–400 nm) of these peaks have an absorption profile similar to the reference substance. The second largest peak corresponded to sparfloxacin (10.3 min, 32%), followed by another more apolar at 11.5 min (Peak IV, 41%). The UV spectra for the peaks IV and II (6.7 min, 13%) are similar and show a bathochromic shift (c. 8 nm) from sparfloxacin. However, photoproducts I and III, 5.9 min (11%) and 7.6 min (4%), respectively, show a hypsochromic shift (c. 4 nm) of the absorption maximum in the UV spectrum from sparfloxacin. Peak II also shows a shoulder which possibly indicates the existence of two products. The results of these analyses are shown in the Figure. The isolation of these photoproducts will be performed by preparative HPLC and their chemical structures determined by NMR, MS, UV and IR spectra.

The results have shown that the substance studied is sensitive to photodegradation. Finally, a large decrease in the concentration of sparfloxacin after exposure to UV light was observed and detected in HPLC assay. This decrease is an important source of concern and suggests further studies about its photodegradation mechanism. The existence of photoproducts can induce side effects and toxicity as well as loss of activity expected for the treatment.

Figure 1. HPLC chromatogram of sparfloxacin and its degradation products. Detection UV- Photodiode array (scan UV total 200–400 nm).
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References


