Revista de Ciências Farmacêuticas Básica e Aplicada
Journal of Basic and Applied Pharmaceutical Sciences

Rev. Ciênc. Farm. Básica Apl., v. 26, n. 1, p. 47-54, 2005
ISSN 1808-4532

Photodegradation of sparfloxacin and isolation of its degradation products by preparative HPLC

Salgado, H.R.N.[1]*; Moreno, P.R.H.[2]; Braga, A.L.[3]; Schapoval, E.E.S.[4]

[1]Depto de Fármacos e Medicamentos - Faculdade de Ciências Farmacêuticas - UNESP - Rod. Araraquara-Jaú, km 1 - CEP 14801-902 - Araraquara - SP - Brazil ; [2]Instituto de Química - USP - São Paulo - SP - Brazil; [3]Instituto de Química - UFSM - Santa Maria - RS - Brazil; [4]Faculdade de Farmácia - UFRGS- Porto Alegre - RS - Brazil;

Received 25/02/05 / Accepted 13/07/05

ABSTRACT

Sparfloxacin, a third generation fluoroquinolone derivative, is a potent antibacterial agent active against a wide range of Gram-positive and Gram-negative organisms including *Streptococcus pneumoniae, Staphylococcus aureus*, methicillin resistant *S. aureus*, *Legionella* spp., *Mycoplasma* spp., *Chlamydia* spp. and *Mycobacterium* spp. A drawback of fluoroquinolones is their photoreactivity. Sparfloxacin has been studied in terms of therapeutic activities. However, there are few published of analytical methods being applied to sparfloxacin. The aim in this study was to determine the photodegradation products of sparfloxacin, when submitted to UV light, and to characterize two of these products, designated SPAX-PDP1 and SPAX-PDP2. An accelerated study of stability in methanol solution was carried out by exposing a solution of sparfloxacin to UV light (peak wavelength 290 nm) for 36 hours at room temperature. The products were analyzed by NMR spectrophotometry, IR spectrometry and mass spectrophotometry. The results suggest that the products isolated here could be used to estimate the degradation of sparfloxacin in a stability study. However, the low activity exhibited by UV-irradiated sparfloxacin is a source of concern that demands further investigation of the mechanism of its photodegradation mechanism.

Keywords: Degradation products, fluoroquinolone, photodegradation, quality control, sparfloxacin, stability.

RESUMO

Fotodegradação de esparfloxacino e isolamento de seus produtos de degradação utilizando CLAE

Esparfloxacino, fluorquinolona de terceira geração, é um potente agente antibacteriano ativo contra Gram-positivos e Gram-negativos, incluindo *Streptococcus pneumoniae, Staphylococcus aureus, S. aureus* meticilina resistentes, *Legionella* spp., *Mycoplasma* spp., *Chlamydia* spp. e *Mycobacterium* spp. Um aspecto importante das fluorquinolonas é sua fotoinstabilidade. O esparfloxacino tem sido amplamente estudado em termos de atividades terapêuticas. Entretanto, poucos relatos a respeito de métodos analíticos para esta quinolona são disponíveis na literatura. O objetivo deste trabalho foi determinar a fotoestabilidade de esparfloxacino submetido à luz UV, bem como caracterizar dois produtos, codificados como SPAX-PDP1 e SPAX-PDP2. Um estudo acelerado de estabilidade foi realizado submetendo-se o esparfloxacino a luz UV (comprimento de onda 290 nm) durante 36 horas em temperatura ambiente. Os resultados foram analisados por espectrofotômetro de massas, de RMN de ^{1}H e de ^{13}C e espectrometria na região de infravermelho. Os resultados sugerem que os produtos isolados podem ser usados para determinar a degradação de esparfloxacino em estudos de fotoestabilidade. No entanto, a baixa atividade do esparfloxacino submetido à luz UV demonstra a necessidade de investigações a respeito do mecanismo de fotodegradação deste fármaco.

Palavras-chave: Controle de qualidade, esparfloxacino, estabilidade, fluorquinolona, fotodegradação, produtos de degradação.

INTRODUCTION

Sparfloxacin (Figure 1), a fluoroquinolone derivative, is a powerful antibacterial agent against a wide range of Gram-positive and Gram-negative organisms, including *Streptococcus pneumoniae*, *Staphylococcus aureus*, methicillin resistant *S. aureus*, *Legionella* spp., *Mycoplasma* spp., *Chlamydia* spp. and *Mycobacterium* spp. (Goa et al., 1997; Shimada J et al., 1993; Sparfloxacin, 1996; Sparfloxacin, 1999). Chemically, sparfloxacin is 5-amino-1-cyclopropyl-7-(*cis*-3,5-dimethyl-piperazin-1-yl)-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid. Although it is not yet officially listed in any pharmacopeia, several ways of measuring its content in tablets have been used: visible and UV spectrophotometry (Marona e Schapoval, 1999b; Marona e Schapoval, 2001b), bioassay (Marona e Schapoval, 1998; Marona e Schapoval, 2001a), non-aqueous titration (Marona HRN e Schapoval, 2001c) and HPLC with UV detection (Marona e Schapoval, 1999a). Sparfloxacin has been investigated mainly in terms of its therapeutic activities, and its synthesis and structure-activity relationships were described by Miyamoto and coworkers (1990).

A drawback of fluoroquinolones is their photoreactivity (Chao et al., 2004; Engler et al., 1998; Ferguson et al., 1999; Goa et al., 1997; Marona e Schapoval, 1998; Marona e Schapoval, 2001a; Marona e Schapoval, 2001b; Marona e Schapoval, 2001c; Marona et al., 2002; Phillips et al., 1990; Sunderland et al., 1999; Thoma e Kübler, 1997; Tobin et al., 1997; Traynor e Gibbs, 1999). In fact, a wide range of drug types can undergo photochemical degradation. Steele (2004) reported that light instability is a problem in both the solid and solution states. Consequently, formulations have to be protected to avoid these effects.

Many substances show photoinstability. According to Tønneson (1991), these include more than a hundred of the commonest drugs in use. Many chemical groups can be unstable with respect to light, and such drugs usually contain the carbonyl group, the C=C bond, the *N*-oxide group, the nitroaromatic, polyene or phenolic groups, etc.

The International Conference on Harmonisation (ICH) has published a guideline on how to present the photostability test of new drug compounds (ICH, 1996). It requires the degradation products, which may be formed under a variety of conditions, to be identified and degradation pathways established. The stipulated

* *Corresponding author:* H.R.N. Salgado - Departamento de Fármacos e Medicamentos, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, Rod. Araraquara-Jaú, km 1, 14801-902, Araraquara, SP, Brasil. E-mail: salgadoh@fcfar.unesp.br, Fone: 55 16 33 01 69 67 - Fax: 55 16 33 01 69 60.

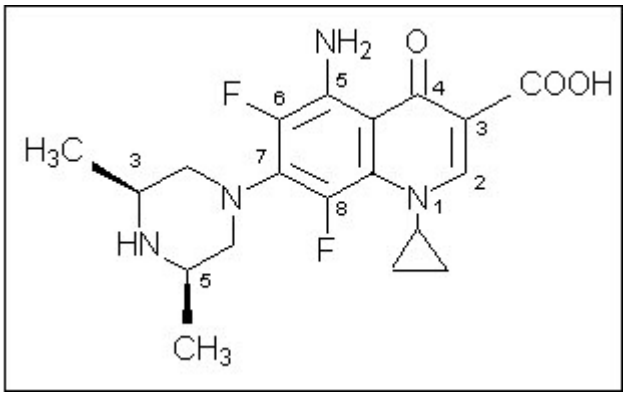

FIGURE 1 – Chemical structure of sparfloxacin (M.W. 392.4)

conditions include the temperature, photolysis, oxidation, hydrolysis and humidity when appropriate (Singh e Bakshi, 2000).

The stability data requirements for human medicines in the European Community are based on a series of regulatory guidelines adopted by the ICH. According to Matthews (1999), stability for pharmaceutical products should be taken in the sense of 'controlled, documented and acceptable change'. The most important factors are chemical, physical, pharmacotechnical, microbiological, toxicological and clinical, including bioavailability. Stability studies should include shipment and sufficient duration to cover storage.

Accelerated-stress stability tests are carried out, in which samples are stored under light conditions designed to stress the raw material or pharmaceutical preparations.

Stability studies of sparfloxacin have been described by Marona and coworkers (1999), who presented a rapid and sensitive method to determine the presence of any photodegradation products in the powder. An in vitro study was carried out to determine cytotoxic effects of sparfloxacin on mononuclear human culture cells. The results showed that sparfloxacin raw material and tablets, as well as sparfloxacin tablets under light, could reduce the number of cells significantly (according to Tukey Test). On the other hand, the two isolated products did not show this response under the same conditions (Marona et al., 2002).

The aim of this study was to determine photodegradation products of sparfloxacin submitted to UV light (254 nm) and to characterize these products by ^{1}H-NMR and ^{13}C-NMR spectrophotometry, IR spectrometry and mass spectrophotometry.

MATERIAL AND METHOD

All the chemicals used were of analytical reagent grade, and the solvents were of spectroscopic grade.

Material: Sparfloxacin reference substance (SPAX-RS), 99.5% pure, was kindly donated for the Dainippon Pharmaceutical Co. Ltd. (Suita, Osaka, Japan) and Rhone-Poulenc Rorer (U.S.A.). Sparfloxacin tablets Zagam ™ (SPAX-TAB) were purchased in France. The tablets contained 200 mg of drug according to the manufacturer. SPAX-RS and SPAX-TAB were stored protected from light.

Water and methanol were HPLC grade.

Twenty tablets of sparfloxacin were carefully crushed using a glass mortar and pestle. The crushed tablets were then quantitatively transferred to a volumetric flask and dissolved in methanol, to obtain a nominal 1 mg/mL solution of sparfloxacin.

Equipment: NMR Spectrophotometry: The NMR data were acquired with a DTX 200 MHz spectrophotometer (Bruker, Germany). Both ^{1}H NMR and ^{13}C NMR spectra were obtained at 200 MHz. All spectra were recorded in D_2O and DMSO. For the structural elucidation, the following spectra were obtained: ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY and ^{1}H-^{13}C-COSY.

Mass Spectrometry: The mass spectrometric measurements were performed in a Shimadzu 8001 (Japan) at 500 MHz.

UV-Vis Spectrophotometry: The UV-Vis data were obtained in an UV-160A at spectrophotometer (Shimadzu, Japan).

IR spectrometry: IR spectra were acquired in a Shimadzu 8001 FTIR spectrophotometer (Japan).

Semipreparative HPLC: The semiquantitative HPLC analysis was performed on a Waters Alliance 2690. A Shim-pack CLC-ODS (250 x 4.6 mm i.d., 5 µm particle size, Å100 pore diameter) was used with 5% acetic acid : methanol : acetonitrile (75:12.5:12.5, v/v/v) as the isocratic mobile phase at a flow rate of 1.0 mL/min. The HPLC system was operated at room temperature (20 ± 1°C).

Photodegradation chamber: To degrade sparfloxacin, a fresh solution (1 mg/mL) and tablets were submitted to UV light (254 nm) for 36 hours in a chamber (16 x 16 x 100 cm).

Melting point: Koefler equipment. Phenolphthalein (WHO Melting Point reference Substance – MP 263°C was used to check the temperature).

Method:

Sample irradiation

(a) SPAX-RS (99.5%) was exposed to UV-A light, in an open Petri dish, for 90 days.

(b) SPAX-RS (99.5%) was exposed to UV-C light, in an open Petri dish, for 24 hours.

(c) SPAX-RS (99.5%) was dissolved in methanol (1 mg/mL) and then exposed to UV-C light, in an open Petri dish, for 5 hours.

(d) SPAX tablets were exposed to UV-C light in an open Petri dish, for 30 days.

(e) 20 tablets of sparfloxacin were crushed to a fine powder and dissolved in methanol, to obtain a final drug concentration of 1 mg/mL. This solution was mixed thoroughly by shaking and sonication and a 25-mL aliquot was then transferred to a Petri dish without lid and exposed to UV-C light for 24 hours.

Two different irradiation sources were used:

UV-A: Samples were placed in Petri dishes at a distance of

8 cm from the light emission (320 - 400 nm) (HgV, 125 W, Tungsram, Hungary). The control sample was submitted to the same conditions, but covered with aluminum foil. The chamber internal temperature was 40ºC ± 1ºC.

UV-C: Samples were placed in Petri dishes at 10 cm from a Philips TUV germicidal lamp (254 nm) of 30 W, 96 V, (0.36) with 400 mW/cm^2, determined with a near-UV meter, model J225 (Blak-Ray). The reaction was carried out in a chamber of 100 x 16 x 16 cm, internally mirrored, maintained at 20ºC ± 1ºC.

Characterization of degradation products: The degradation of SPAX samples was characterized by TLC, using two solvent systems:

- chloroform: methanol: formic acid (18: 07: 01, v/v/v)
- dichloromethane: methanol: ammonium hydroxide: acetonitrile (4: 4: 2: 1, v/v/v/v)

RESULTS AND DISCUSSION

The development of stability tests for the determination of degradation products in drugs and medicines has received considerable attention in the last decade because of their importance in quality control in pharmaceutical analysis.

The World Health Organisation and Unicef carried out a study on the stability of essential drugs during international transport, to assure quality control and stability of these drugs in tropical countries. In this study, for the antibiotics chloramphenicol, ampicillin, benzylpenicillin, and tetracycline, the unprotected substance showed signs of degradation (Hogerzeil et al., 1992).

A recent survey of the literature revealed an excellent book on the advances made in drug degradation kinetics (Carstensen, 1995).

Degradation products of drugs are considered to be transformation products of the pharmaceutical compound, formed by the effect of heat, humidity, light, oxidizing agents, solvents, chemical reagents, etc (Görög, 2000). Tests should be performed on representative samples, for example, 20 capsules or tablets, in order to assure a homogenous distribution (Matthews, 1999).

After the extractive procedure using semipreparative-HPLC, the collected fractions from the SPAX-RS and SPAX-TAB samples were taken to dryness in a rotatory evaporator (<60ºC) under reduced pressure and visualized by TLC using chloroform: methanol: formic acid (18: 07: 01, v/v/v) and dichloromethane: methanol: ammonium hydroxide: acetonitrile (4: 4: 2: 1, v/v/v/v) as mobile phase. Because these fractions did not exhibit high purity, they were analyzed by column chromatography with silica gel as stationary phase and 5% acetic acid : methanol : acetonitrile (75:12.5:12.5, v/v/v) as eluent. Thirty fractions (5 mL) were collected, characterized by TLC, and similar fractions were aggregated. Thus, two substances were isolated, designated as SPAX-PDP1 and SPAX-PDP2.

Identifiation of the two partially-purified degradation products by NMR analysis was hindered by the low solubility of the products, as well as the of the reference standard, SPAX-RS. Consequently, the solubility of these photodegradation products was tested in different solvents, such as chloroform, benzene, trifluoracetic acid, water, methanol, pyridine and DMSO, besides evaluating effects of temperature and ultrasonic equipment.

The ^{1}H (Fig. 2) and ^{13}C NMR (Fig. 3) spectra were recorded in water; however, with previous solubilization in a small amount of NaOH solution, allowing the characterization of protons and carbons. The related lines in ^{1}H-NMR patterns of SPAX-RS in D_2O and other fluoroquinolone derivatives are presented in Table 1, while UV absorption peaks of SPAX are compared with literature data in Table 2. The totally-detached ^{13}C-NMR of SPAX-RS allowed attributions presented in Table 3 to be established. For the structural elucidation ^{1}H-^{13}C COSY is presented in Fig. 4.

For SPAX-PDP1 infra-red spectra, under the same conditions, was not observed the absorption around 1700 cm^{-1}, related to C = O stretching was not observed, possible indicating the loss of carbonyl and carboxyl (Fig. 5). In the SPAX-PDP2 IR spectrum it is possible to see peaks of the ketone and carboxyl groups at 1638 and 1713 cm^{-1}, respectively, which are present in next to those of the undergraded substance. Substances with groups C=O and H-O possess absorption peaks corresponding to intramolecular hydrogen bonds at ~ 3070 cm^{-1} (Fig. 6). However, SPAX does not show this peak. This suggests the C_5 amino group may react intramolecularly with the ketone oxygen. A second point also relates to this aspect. Compounds such as phenols, amides, nitriles and amines can react with iron (III) nitrate or copper. This reaction is used for the identification of various drugs and for qualitative and quantitative analyses. However, sparfloxacin can not be analyzed using iron (III) nitrate in 1% nitric acid, as the ferric ion does not form complex with this fluoroquinolone. Moreover, the amine group at C_5 could

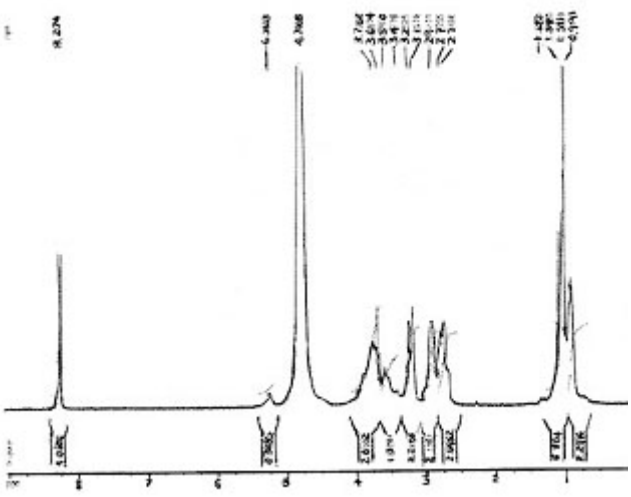

FIGURE 2 – ^{1}H NMR spectrum of SPAX in D_2O

Table 1 - Relation among ^{1}H - NMR spectra of sparfloxacin RS (D_2O) and other fluoroquinolones.

H	**SPAX D_2O** [a]	**SPAX** [b]	**COMP. 10**[c]	**CIPX**[d]	**M5**[e]
H (C2)	8.27	8.48	8.70	8.40	9.26
CH (cyclopropane)	3.77	3.97	3.77	3.61	3.5 – 4.2
CH, CH_2 (piperazinyl)	2.74-3.70	2.60-3.77	2.66-3.05	~ 3.0	-
CH_3 (piperazinyl)	1.0	0.75-1.20	0.97-1.25	-	-
CH_2 (cyclopropane)	0.92-1.34	0.75-1.20	0.97-1.25	1.05 – 1.31	1.38 – 1.64
NH_2	-	7.17	6.03	-	-

[a] SPAX RS in D_2O
[b] MIYAMOTO et al., 1990
[c] ENGLER et al., 1998
[d] MACÍAS-SANCHEZ et al., 1994
[e] GAU et al., 1986

Table 2 - Comparison of the peak wavelengths of absorption of sparfloxacin reference substance and literature data in the UV region.

compound	**λ (nm)**	**λ (nm)**	**λ (nm)**
SPAX-reference substance	368	292	226
Compound 10 [a]	350	288	242

[a] ENGLER et al., 1998

Table 3 – ^{13}C NMR lines of sparfloxacin–RS in D_2O and DMSO with ciprofloxacin data.

	SPAX (DMSO)	SPAX (D_2O)	CIPX [a]
COOH	180	178	172
CO	165	172	171
C2	150	147	166
C6	130-140	-	160
C8	130-140	-	160
C7	130-140	137	106
C8a	130-140	135	144
C4a	106	109	117
C3	105	115	114
C5	62	68	109
CH_2 –piperazine	-	-	-
CH_2 – piperazine	-	-	46-49
CH – cyclopropane	40 (1C)	39 (1C)	41 (1C)
CH_3 – piperazine	20 (2C)	17.4 (2C)	-
CH_2 – cyclopropane	7 (2C)	7.5 (2C)	10 (2C)

[a] GAU et al., 1986.

be directly interacting with the neighboring C=O, blocking the bond with (Fe^{3+}), as well as the intramolecular hydrogen bond. This might also explain the low reactivity of sparfloxacin with anti-acid formulations, compared to other fluoroquinolones. The results obtained here may demonstrate the presence of interfering groups. For this reason, these techniques are not useful to characterize the amine group of sparfloxacin.

The carboxylate ion gives two IR bands, an intense one at 1650 to 1550 cm^{-1}, associated with anti-symmetrical axial deformation, and a weaker band around 1400 cm^{-1} due to symmetrical axial deformation. The SPAX-PDP2 shows a broad band in the region of 1700 cm^{-1}, whereas it is not possible to verify this characteristic band in the SPAX-PDP1 spectrum. The analysis by NMR of partially-purified SPAX-PDP2 discloses the presence of H on C-2 and the

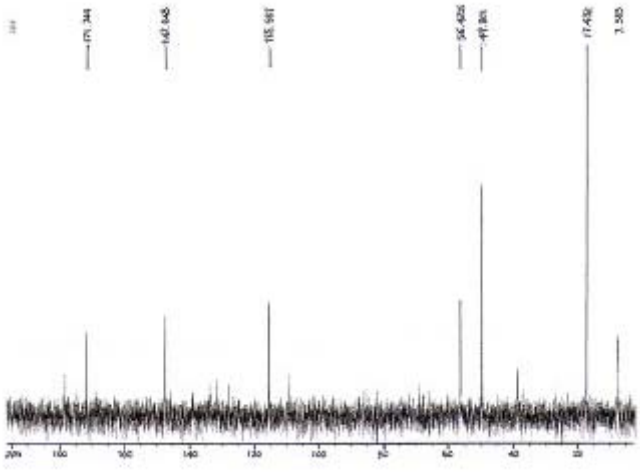

FIGURE 3 - ^{13}C NMR spectrum of SPAX in D_2O

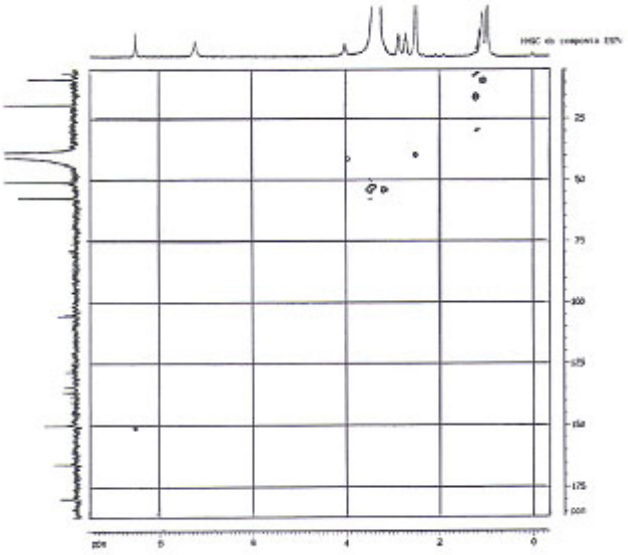

FIGURE 4 - ^{1}H -^{13}C NMR COSY of SPAX in DMSO

loss of the cyclopropane and the piperazine rings (Fig. 7). The lines in ^{1}H-NMR spectrum of SPAX-RS were attributed to groups as presented in Table 2.

The interaction of sparfloxacin with β-cyclodextrin has been shown to increase the stability of this fluoroquinolone, by several analytical procedures, including ^{1}H-NMR, ^{13}C-NMR, fluorescence spectroscopy, infrared spectroscopy, thermal analysis, and scanning electron microscopy (Chao et al., 2004). NMR techniques can provide not only quantitative information but also detailed information about the geometry of the complex. 2D nuclear effect spectroscopy, one of the many NMR tools, has proven to be a powerful technique for investigating intermolecular interaction (Chao et al., 2004).

The products of degradation of a drug can be responsible for its toxicity. On the other hand, very stable drugs can become environmental pollutants. Consequently, the study of the stability of drugs has stimulated important discussions in recent decades (Yoshida et al., 1993). Yoshida and coworkers (1993) carried out the degradation of levofloxacin, obtaining 10 products with modifications in the piperazine group. From these results, those authors concluded that the fluoroquinolone in question was sensitive to photodegradation, with oxidation in the substituent on the C-7, and that levofloxacin, being degraded by sunlight, does not come to be recognized as a pollutant. SPAX-PDP1 and SPAX-PDP2 exhibited melting points > 300ºC. Such temperatures are also reached by quinolonic derivatives, as reported by Koga and coworkers (1980).

Ciprofloxacin metabolites have been isolated and their structure elucidated by Gau et al. (1986). Phillips and coworkers (1990) confirmed the loss of antibiotic activity of ciprofloxacin. However, some studies have reported antimicrobial activity in fluoroquinolone degradation products such as moxifloxacin and levofloxacin (Thoma e

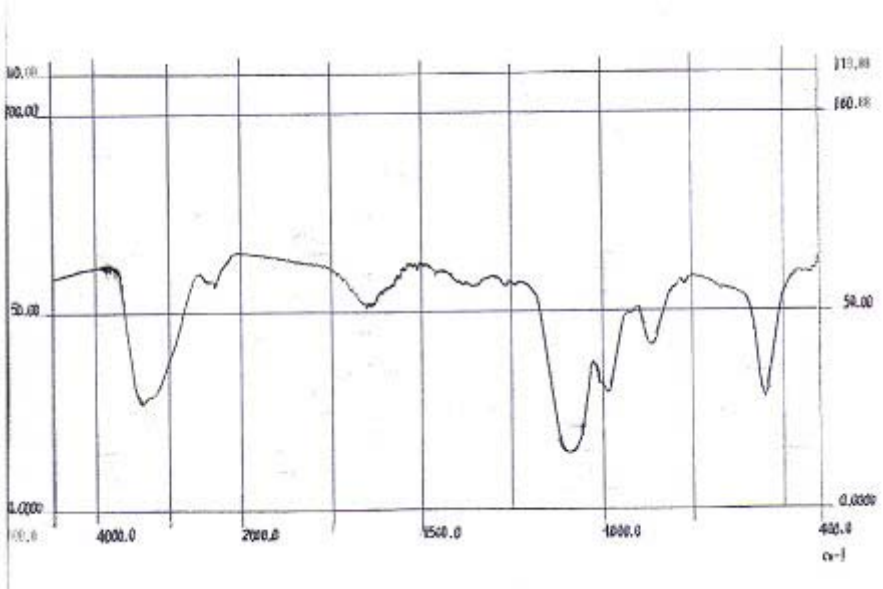

FIGURE 5 - Infrared spectrum of SPAX-PDP1 in KBr.

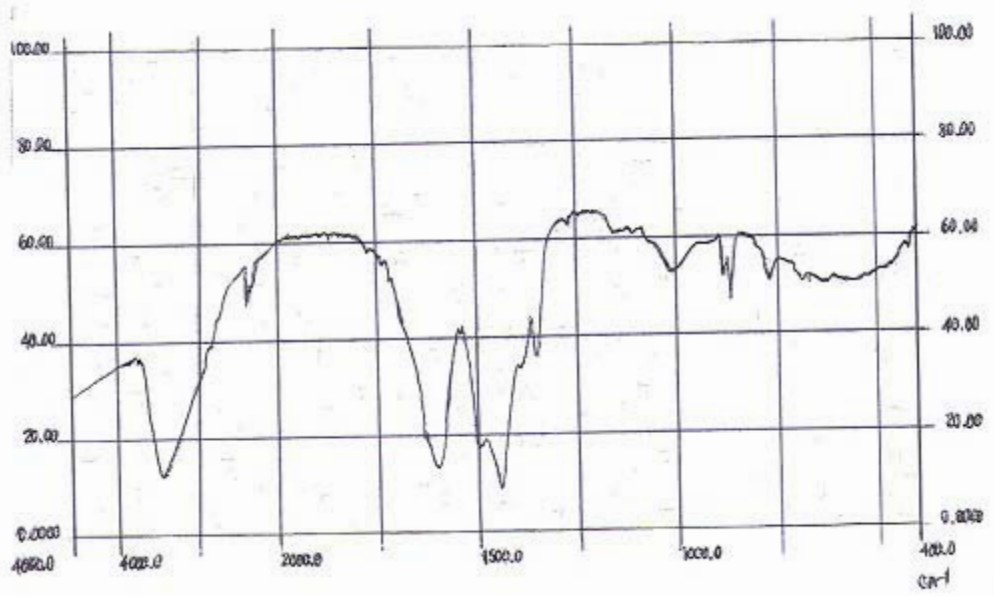

FIGURE 6 – Infrared spectrum of SPAX-PDP2 in KBr

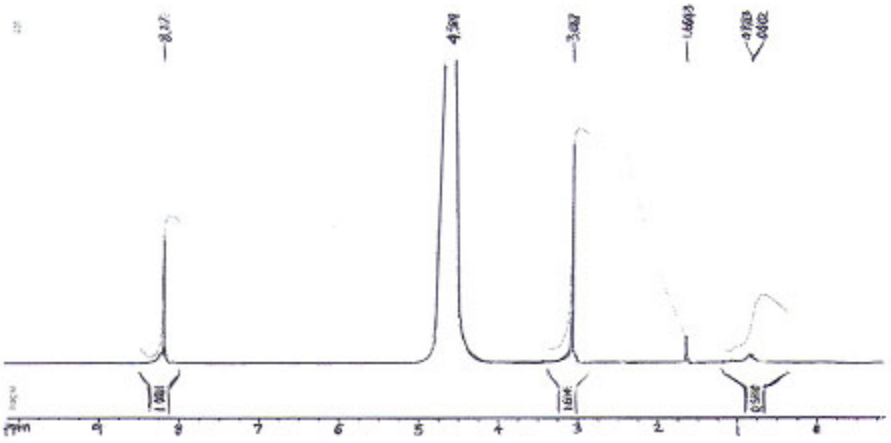

FIGURE 7 - ^{1}H NMR spectrum of fractions submitted to UV-C light in D_2O

Kübler, 1997; Sunderland et al., 1999). Macías-Sanchez (1994) described a physico-chemical study of interaction between fluoroquinolones such as ciprofloxacin and ofloxacin and polyvalent cations. Bioassay and liquid chromatographic methods with UV detection have been used to study the stability of sparfloxacin in the raw material and tablets (Marona et al., 1999; Marona e Schapoval, 2001a). The bioassays were carried out to test for antibacterial activity in sparfloxacin and its degradation products (Marona e Schapoval, 2001a). A significant loss of antibacterial activity of sparfloxacin against *E. coli* NCTC 10418 was observed after exposure to UV-C light, confirming that sparfloxacin was sensitive to photodegradation.

According to Engler and coworkers (1998), low stability is related to the high degree of fluorination, and this favored the choice of SPAX for the photodegradation study. The results demonstrate the photochemical instability of SPAX, when exposed to UV-C; however, the product proved be stable to UV-A light UV over 90 days.

The existence of photodegradation products could lead to side effects as well as toxicity during antibiotic therapy. This finding is a source of great concern and suggests the need for research on the mechanism of photodegradation.

An important factor in the improvement of pharmaceutical stability testing has been the development of analytical methods that are suitable for routine use in a stability-indicating assay (Kommanaboyina e Rhodes, 1999).

The light used in this stability study was chosen in relation to a basic law of photochemistry, that only absorbed light could start a photochemical process. The sparfloxacin spectra show two characteristic bands between 300 and 200 nm. This range is typical of germicidal light emitted by a low-pressure mercury TUV lamp. This fact was used to define the irradiation source. UV-A did not induce

degradation of any sample after exposure for 90 days. For this reason, the study of photodegradation was carried out with UV-C light.

Finally, sparfloxacin was sensitive to photodegradation under the experimental conditions described. Our results showed the presence of photodegradation products when sparfloxacin was exposed to UV-C light. The applicability of the proposed method for the determination of sparfloxacin and its degradation products was demonstrated by analyzing six aliquots of each sample. HPLC, LC and TLC were carried out, resulting in the isolation of two photodegradation products, whose chemical structures were partially characterized analyzing by ^{1}H-NMR, ^{13}C-NMR, MS, UV and IR spectra. It is of vital importance to develop techniques that facilitate quality control in the manufacture of drugs and medicines, and allow the ideal storage conditions of pharmaceutical products to be determined.

In view of the very complex nature of photochemical decomposition of these drugs, further studies will be performed on the identification and structure elucidation of the products.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Sayuri Kitada (Dainippon Co., Japan) and Dr. Michael Pease (Rhône-Poulenc Rorer, U.S.A.) for providing the sparfloxacin reference substance and Dr. Helder Teixeira for providing the tablets. This work was supported by CAPES program (Brasília-Brasil).

This work was presented at The Third International Postgraduate Research Symposium on Pharmaceutics, in Istanbul, Turkey, 15 - 18 September 2002.

REFERENCES

Carstensen JT. *Drug Stability.* 2nd.ed. New York, Marcel Dekker, 1995.

Chao JB, Tong HB, Huang SP, Liu DS. Preparation and study on the solid inclusion complex of sparfloxacin with β-cyclodextrin. *Spectrochim Acta Part A* 2004;60:161-166.

Engler M, Rüsing G, Sörgel F, Holzgrabe U. Defluorinated sparfloxacin as a new photoproduct identified by liquid chromatography coupled with UV detection and tandem mass spectrometry. *Antimicrob Agents Chemother* 1998;42:1151-1159.

Ferguson J, McEwen J, Gohler K, Mignot A, Watson D. Phototoxic potential of gatifloxacin, a new fluoroquinolone antimicrobial. *Drugs* 1999;58:397-399.

Gau W, Kurz J, Petersen U, Ploschke HJ, Wuensche C. Isolation and structural elucidation of urinary metabolites of ciprofloxacin. *Arzneimittel Forschung Drug Res* 1986;36:1545-1549.

Goa KL, Bryson HM, Markham A. Sparfloxacin: a review of its antibacterial activity, pharmacokinetic properties, clinical efficacy and tolerability in lower respiratory tract infections. *Drugs* 1997;53:700-725.

Görög S. *Identification and determination of impurities in drugs. Progress in Pharmaceutical and Biomedical Analysis,* 2000. v.4. 772 p.

Hogerzeil HV, Battersby A, Srdanovic V, Stjernstrom NE. Stability of essential drugs during shipment to the tropics. *Brit Med J* 1992;304:210-212.

ICH-Harmonised Tripartity Guideline. International Conference on Harmonisation OF Technical Requirements for Registration of Pharmaceuticals for Human Use. Stability Testing: Photostability Testing of New Drug Substances and Products. Geneva: IFPMA, 1996, p. 1–10.

Koga H, Itoh A, Murayama S, Suzue S, Irikura T. Structure-activity relationships of antibacterial 6,7- and 7,8-disubstituted 1-alkyl-1,4-dihidro-4-oxoquinoline-3-carboxylic acids. *J Med Chem* 1980;23:1358-1363.

Kommanaboyina B, Rhodes CT. Trends in stability testing, with emphasis on stability during distribution and storage. *Drug Dev Ind Pharm* 1999;25:857-868.

Macías-Sanchez B, Cabarga MM, Navarro AS, Hurlé ADG. A physico-chemical study of the interaction of ciprofloxacin and ofloxacin with polyvalent cations. *Int J Pharm* 1994;106:229-235.

Marona HRN, Schapoval EES. Desarrollo de análisis microbiológico para la determinación de esparfloxacino en polvo y en comprimidos de 200 mg. *Inf Tecnol* 1998;9:251-254.

Marona HRN, Zuanazzi JAS, Schapoval EES. Determination of sparfloxacin and its degradation products by HPLC. *J Antimicrob Chemother* 1999;44:301-302.

Marona HRN, Schapoval EES. A high-performance liquid chromatographic assay of sparfloxacin. *J Pharm Biomed Anal* 1999;20:413-417.

Marona HRN, Schapoval EES. Spectrophotometric determination of sparfloxacin in tablets. *J Antimicrob Chemother* 1999;44:136-137.

Marona HRN, Schapoval EES. Analysis of sparfloxacin and its degradation products by bioassay. *Acta Pharm Turcica* 2001;43:7-9.

Marona HRN, Schapoval EES. Spectrophotometric determination of sparfloxacin in pharmaceutical formulations using bromothymol blue. *J Pharm Biomed Anal* 2001;26:501-504.

Marona HRN, Schapoval EES. Development and validation of a nonaqueous titration with perchloric acid to determine sparfloxacin in tablets. *Eur J Pharm Biopharm* 2001;52:227-229.

Marona HRN, Schapoval EES, Nardi NB. Análise citotóxica de esparfloxacino e seus proutos de degradação em células mononucleares humanas. *Rev Ciênc Farm*

2002;23:227-238.

Matthews BR. Regulatory aspects of stability testing in Europe. *Drug Dev Ind Pharm* 1999;25:831-856.

Miyamoto T, Matsumoto JI, Chiba K, Egawa H, Shibamori K, Minamida A, Nishimura Y, Okada H, Kataoka M, Fujita M, Hirose T, Nakano J. Synthesis and structure-activity relationships of 5-substituted 6,8-difluoroquinolones, including sparfloxacin, a new quinolone antibacterial agent with improved potency. *J Med Chem* 1990;33:1645-1656.

Phillips G, Johnson BE, Ferguson J. The loss of antibiotic activity of ciprofloxacin by photodegradation. *J Antimicrob Chemother* 1990;26:783-789.

Shimada J, Nogita T, Ishibashi Y. Clinical pharmacokinetics of sparfloxacin. *Clin Pharmacok* 1993;25:358-369.

Singh S, Bakshi M. Stress test to determine inherent stability of drugs. *Pharm Technol* 2000; 4:1-14.

Sparfloxacin Spara™ Zagam™. *Drugs Fut* 1999;24:581.

Sparfloxacin: focus on clinical performance. *J Antimicrob Chemother* 1996;37. (Whole issue).

Steele G. Preformulation predictions from small amounts of compound as an aid to candidate drug selection. In: Gibson M. *Pharmaceutical Preformulation and Formulation.* Boca Raton: Interpharm/CRC, 2004. Cap. 3, p.21-95.

Sunderland J, Tobin CM, White LO, MacGowan AP, Hedges AJ. Ofloxacin photodegradation products possess antimicrobial activity. *Drugs* 1999;58:171-172.

Thoma K, Kübler N. Untersuchungen zur Photostabilität von Gyrazehemmern. *Pharmazie* 1997;52:519-529.

Tobin CM, Sunderland J, MacGowan AP, Hedges AJ, White LO. Antimicrobial activity of moxifloxacin and levofloxacin photodegradation products. *Drugs* 1999;58:144-145.

Tønneson HH. Photochemical degradation of components in drug formulations. Part 1: An approach to the standardization of degradation studies. *Pharmazie* 1991;46:263-265.

Traynor NJ, Gibbs NK. Fluorquinolone photogenotoxicity in human keratinocytes: involvement of photoproducts and H_2O_2. *Brit J Dermatol* 1999;140:784-787.

Yoshida Y, Sato E, Moroi R. Photodegradation products of levofloxacin in aqueous solution. *Arzneimittel Forschung Drug Res* 1993;43:601-606.