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Short communication

The effect of photodegradation on the fluorescent properties of norfloxacin (Photodegradation and fluorescence of norfloxacin)

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Abstract

Norfloxacin (NFLX), a broad spectrum antibacterial quinolone, is a very thermostable but photosensitive drug, especially in solution leading to the formation of an ethylenediamine degradate. The modification of the fluorescent properties of NFLX in acid solution after exposure to fluorescent light and the degradation mechanisms were studied. Two analytical methods were previously developed and validated for NFLX, ultraviolet spectrophotometry (UV) and spectrofluorimetry (FL). Data obtained using both methods in the analysis of remaining NFLX in terms of percent recoveries revealed that there was no statistically significant modifications of the UV signal and of the recoveries obtained by the method. However, an important increase of the fluorescent signal after light exposure of NFLX solutions appeared, which led to an increase of the average recovery up to 270% over 15 months. Using a previously validated HPLC method for the photostability studies of NFLX, a loss of 5% with respect to the initial drug amount was observed. The study of UV and fluorescence spectra evidenced the formation of the degradation product, which induced significant modification of the fluorescent properties of NFLX samples. These results clearly indicated that FL analysis definitively is the method of choice and can be used to study the photodegradation of NFLX.

Keywords: Norfloxacin; Photostability; Ultraviolet spectrophotometry; Spectrofluorimetry

1. Introduction

Norfloxacin (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline-carboxylic acid) is a synthetic broad-spectrum antibacterial quino-

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Table 1
Resulting data of absorbance ($\lambda_{max} = 278$ nm) for the six solutions versus time, and mean values $\pm$ standard deviation[a]

Time (months)	Absorbance (AU)							
	Solution 1	2	3	4	5	6	Mean $\pm$ STD	Standard Solution
0.00	0.416	0.405	0.410	0.408	0.415	0.417	0.412 ± 0.005	0.658
1.00	0.422	0.415	0.410	0.410	0.416	0.414	0.415 ± 0.004	0.664
4.30	0.410	0.409	0.412	0.408	0.412	0.410	0.410 ± 0.002	0.660
10.16	0.425	0.411	0.411	0.413	0.420	0.412	0.415 ± 0.006	0.657
15.16	0.422	0.415	0.408	0.406	0.420	0.410	0.414 ± 0.007	0.648
23.28	0.410	0.403	0.410	0.405	0.413	0.412	0.409 ± 0.004	0.635
28.00	0.415	0.405	0.412	0.407	0.417	0.412	0.411 ± 0.005	0.646

[a] On the right column, data obtained for the 5 μg ml^{-1} unexposed standard solutions.

lone which is one of the drugs of first choice for the treatment of respiratory, biliary and especially complicated urinary tract infections [1–3]. It is structurally related to nalidixic acid and inhibits DNA gyrase but its potency has been increased by the introduction of a fluorine atom and by a piperazine in its structure [4].

Norfloxacin has been shown to be very thermostable but the piperazine ring at the seventh position appeared to be particularly labile after light exposure, especially in solution, leading to the formation of ethylenediamine degradate [5]. This effect was reported in a previous paper in terms of development and validation of a high-performance liquid chromatographic method and its application to photostability studies for directly compressible norfloxacin tablets [6]. On the other hand, a number of spectrophotometric and spectrofluorimetric methods for the determination of norfloxacin have been recently reported by different authors [7–9]. In this study, the modification of the fluorescent properties of norfloxacin in acid solution after prolonged exposure to fluorescent light and the possibility of using a spectrofluorimetric method to check the photodegradation of norfloxacin samples in quality control were studied.

2. Materials and methods

2.1. Chemicals and reagents

Norfloxacin was obtained from Sigma (Sigma-España) and all reagents used in the HPLC assays with a previously described analytical method [6], were HPLC-grade. Distilled and deionized water was used (Milli-Q, Millipore). All the assays using ultraviolet-visible spectrophotometry were performed with a Beckman DU-6 spectrophotometer using a previously validated method at a wavelength of 278 nm. The fluorimetric assays of norfloxacin samples were carried out using a Perkin-Elmer-204 spectrofluorimeter ($\lambda_{excit} = 330$ nm, $\lambda_{emis} = 445$ nm), adjusting the sensitivity control from 5 for standard solutions to 1 for photodegraded samples due to the dramatic increase of the fluorescence signal.

2.2. Standards and sample preparations

125 mg norfloxacin accurately weighed were transferred into a 250 ml volumetric flask and diluted with 0.1 N hydrochloric acid by sonication. A total of 1 ml of this solution was transferred to a 100 ml volumetric flask and diluted with the same solvent to obtain a standard solution having a concentration of 5μg ml^{-1}. This standard solution was prepared in each measuring of the photo-degraded samples and was used to adjust the 100% of fluorescence in the spectrofluorimeter as well as to compare the UV-signal obtained with the photodegraded solutions.

130 mg norfloxacin standard accurately weighed was transferred into a 250 ml volumetric flask in order to reach a concentration of 520 μg ml^{-1}. Six solutions of 520 μg ml^{-1} were exposed to a direct

Table 2
Resulting data of fluorescence (%) versus time for the six solutions exposed[a]

Time (months)	Fluorescence signal (%)						
	Solution 1	2	3	4	5	6	Mean ± STD
0.00	65.00	65.50	64.50	66.00	65.00	65.50	65.25 ± 0.52
1.00	73.00	74.00	69.00	71.50	71.50	72.00	71.83 ± 1.69
4.30	83.00	84.50	86.00	82.00	79.00	77.00	81.91 ± 3.98
10.16	90.00	92.50	98.50	96.50	89.50	93.00	93.33 ± 3.56
15.16	165.00	166.00	168.00	164.00	160.00	152.00	162.50 ± 5.79
23.28	164.00	165.00	169.00	164.00	162.00	158.00	163.67 ± 3.61
28.00	165.00	165.00	168.00	164.00	161.00	157.00	163.33 ± 3.85

[a] In all cases, the fluorescence scale was adjusted to 100% with standard solutions of 5 μg ml^{-1}.

fluorescent light source (Philips™ TL20w/54RS, 91.56 candles) at a distance of 60 cm. A total of 6 ml from each solution was removed over the course of incubation and diluted with 0.1N HCl solution in a 100 ml volumetric flask; and 5 ml of this solution was transferred to a 50 ml volumetric flask and diluted in order to obtain a theoretical 3.12 μg ml^{-1} solution It was then assayed using the above mentioned analytical techniques in comparison to the corresponding unexposed standard solutions.

2.3. Photodegradation studies

In the present work, all samples were exposed to the above mentioned fluorescent light for a period of 28 months at ambient temperature and measured by both UV-spectrophotometric and fluorimetric previously validated methods [10]. All values are presented as mean percent recoveries ± STD. In the present paper, the increase of the fluorescence signal, which evidenced a degradation of norfloxacin, was studied kinetically assuming a first-order reaction to obtain the rate constant (k) by using the following equation:

$$\ln F = \ln F_0 + kt \quad (1)$$

where F is the fluorescence signal at time t and F_0 is the fluorescence signal obtained at the beginning of the study ($t = 0$). Thus, a plot of ln F versus time should be linear [11].

3. Results

It was observed that along the exposure time, all solutions started to become slightly yellow in appearance after 1.5 months. This yellow colour increased progressively among the experiment. Data obtained from the ultraviolet spectrophotometric assay of exposed samples, with a theoretic initial concentration of 3.12 μg ml^{-1}, are shown in

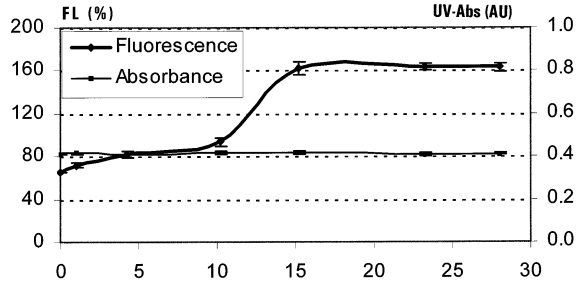

Fig. 1. Plot showing the changes in the fluorescent properties of norfloxacin during light exposure in contrast to UV signal, which is not significantly altered.

Table 3
Linear regression data corresponding to the evolution of fluorescence signal versus time and estimated values for the mean initial signal (F_0) and $t_{130\%}$ (time in which the fluorescence signal is double)

Intercept (ln F_0)	4.1718
Standard error	0.08108
Slope (k)	0.05322
Standard error	0.00966
R	0.954002
F_0	64.379%
$t_{130\%}$	13.087 months

Table 4
Average percent recoveries (mean ± STD), obtained using both analytical techniques along the time exposure

Time (months)	UV-spectrophotometry R (%)	Spectrofluorimetry R (%)
0.00	102.805 ± 1.146	99.886 ± 0.902
1.00	103.549 ± 0.917	111.209 ± 2.914
4.30	102.374 ± 0.458	128.555 ± 5.863
10.16	103.549 ± 1.375	148.207 ± 6.175
15.16	103.314 ± 1.604	267.239 ± 10.043
23.28	102.139 ± 0.916	269.252 ± 6.262
28.00	102.609 ± 1.147	268.667 ± 6.678

Table 1. The absorbance data obtained for the standard solutions with a theoretical concentration of 5.0 μg ml^{-1} are also given. For all the freshly prepared standard solutions, a mean percent recovery of 102.00 ± 1.51 was obtained. These solutions were used to check the ultraviolet absorbance in comparison to the exposed samples and also to calibrate the spectrofluorimeter adjusting the 100% value of fluorescence under the above mentioned conditions.

Table 2 shows the data obtained for norfloxacin solutions as a percentage of fluorescence signal versus exposure time to fluorescent light. The change of the fluorescence signal of norfloxacin exposed solutions versus time, as well as the UV-absorbance values are shown in Fig. 1. As can be seen, the fluorescence of all solutions increased,

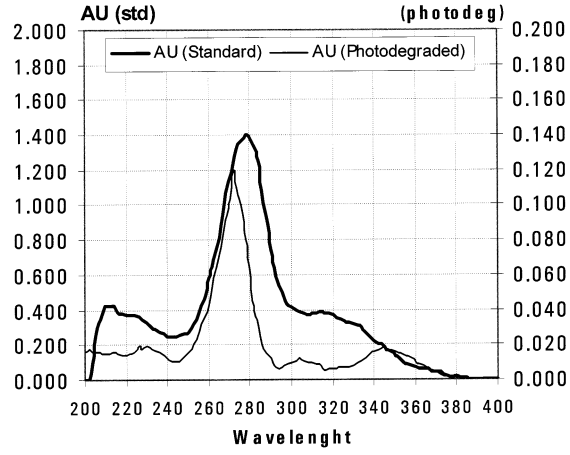

Fig. 2. UV/Vis spectra of a norfloxacin photodegraded solution (maximum absorbance at 273 nm) and a standard solution (maximum absorbance at 278 nm).

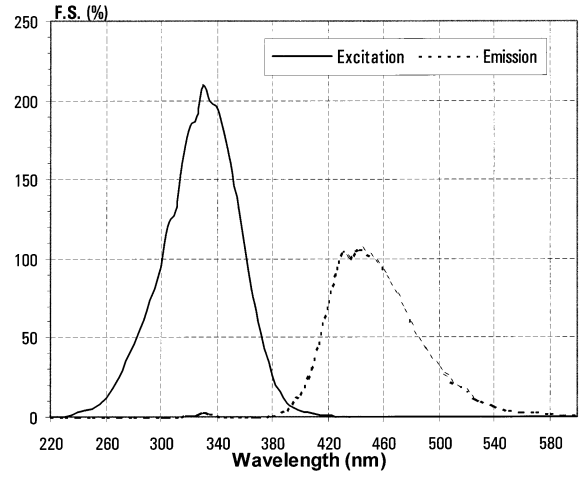

Fig. 3. Fluorescence spectrum of the 9.92 μg ml^{-1} norfloxacin standard solution using as a background solution the solvent (HCl 0.1N). Sensitivity, 5.

even at the 1st month of exposure in which all solutions started to became slightly yellow in appearance, because of the degradation reaction. On the contrary, ultraviolet signals were not significantly modified. This fact evidenced the difference between the two analytical methods in terms of selectivity in order to monitor the degradation process of norfloxacin in solution.

The rate constant corresponding to the evolution of the fluorescence signal versus time as well as the regression data obtained assuming a first order reaction by using Eq. (1), are shown in Table 3. The F_0, mean initial fluorescence signal, was obtained from the intercept value, and the $t_{130\%}$ value was obtained from the linear regression in order to determine the time in which the mean fluorescence signal of norfloxacin solution was twice the initial value. As can be seen, in ≈13 months the fluorescence reached values of 130% under normal laboratory exposure conditions. Norfloxacin concentration values were calculated for each response and the average percent recoveries obtained using both methods are shown in Table 4.

As can be seen, there was a very high increase of the fluorescence signal, which provokes an important increase in the mean percent recoveries obtained, in comparison to those data obtained using UV-spectrophotometry in which the mean

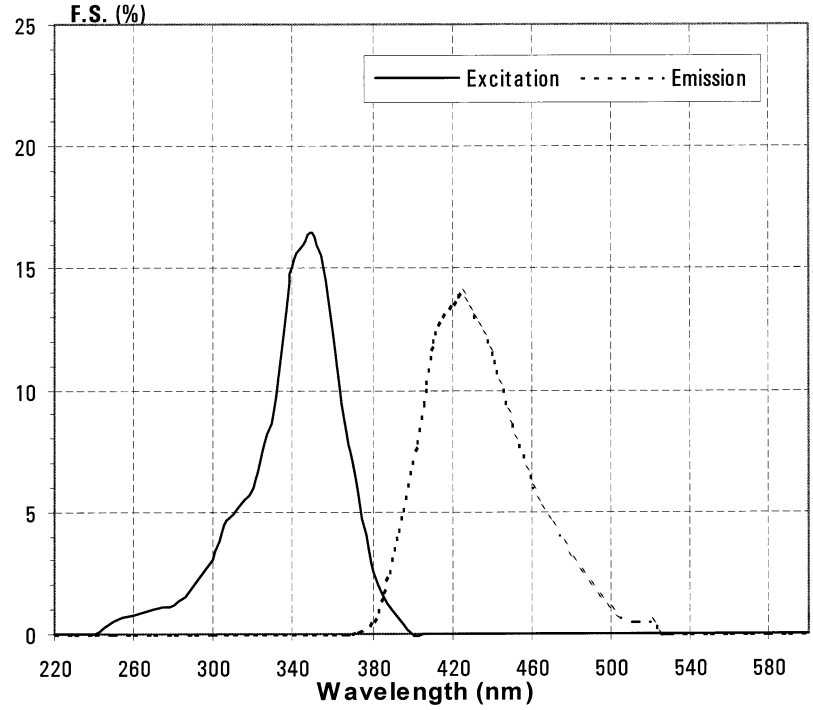

Fig. 4. Fluorescence spectrum of the photodegraded norfloxacin solution using as a background the 9.92 μg ml^{-1} standard solution. Sensitivity, 1.

percent recoveries are not significantly altered along time exposure.

The real concentration of norfloxacin remainder in the photo-degraded solutions was calculated using a previously validated HPLC method. After 10 months, the real mean percent recovery obtained was $95.1 \pm 1.5\%$. After 15.2 months, the mean percent recovery reached values of $85.3 \pm 2.5\%$. Using an aliquot (1 ml) of one of the exposed solutions during 10.16 months, a concentration of 9.92 ± 0.04 μg ml^{-1} was obtained using the previously mentioned HPLC method.

A standard solution of 9.92 μg ml^{-1} was prepared and used as a background solution in order to assay the exposed photodegraded sample using both the UV-spectrophotometric and the spectrofluorimetric methods. Fig. 2, shows the ultraviolet/visible scan of the photodegraded solution in comparison to the standard solution. In the scanning of the photodegraded sample, the 9.92 μg ml^{-1} standard solution was used as a background solution. In the scanning of the standard solution, only the solvent (0.1 N hydrochloric acid) was used as a background. A new substance was observed in the photodegraded sample, when the UV absorbance corresponding to the norfloxacin remainder was eliminated with the background solution, a new peak with a maximum absorbance at 273 nm appeared. These results could indicate that the degradation product of norfloxacin has different UV spectral properties in comparison to the initial norfloxacin whose spectrum shows a maximum absorbance at 278 nm. The same standard and photodegraded solutions were used under the same conditions and the fluorescence spectra were registered out for both solutions. These spectra are shown in Figs. 3 and 4. Not only the percentage of fluorescent signal increased but was also modified in terms of maximum excitation and emission wavelengths. The differences of sensitivity have also to be taken into account to compare the two plots: with the standard unexposed solution, the sensitivity control of the spectrofluorimeter was adjusted to 5, as in the

previously validated method to assay norfloxacin samples, while the sensitivity of the spectrofluorimeter had to be reduced from 5 to 1 in order to measure the photodegraded sample due to the dramatic increase of the fluorescent signal.

4. Discussion

Data obtained in the present work strongly suggests that photodegradation of norfloxacin in solution could be detected using a rapid and simple spectrofluorimetric procedure. The fluorescence values under a long-term stability testing conditions, indicated that norfloxacin is photolabile in acid solution. This effect was detected while studying the fluorescent properties of norfloxacin in comparison to an unexposed solution.

It was observed that the photodegradation of norfloxacin could also modify the ultraviolet spectrum. Nevertheless, UV/Vis-spectrophotometry is not a suitable method to detect the presence of norfloxacin photodegradation products in solution because of the lack of selectivity of this technique, but it can be used in the frame of an HPLC assay.

The linear relationship found between the evolution of fluorescence signal versus time indicated that the photodegradation of norfloxacin in acid solution as well as the increase of the fluorescent signal followed an apparent first-order kinetics over the concentration range studied.

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