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Validation of a high-performance liquid chromatographic method for the determination of norfloxacin and its application to stability studies (photo-stability study of norfloxacin)

M. Córdoba-Borrego *, M. Córdoba-Díaz, D. Córdoba-Díaz

Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad Complutense de Madrid, E-28040 Madrid, Spain

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Abstract

The development and validation study of a sensitive, rapid, reproducible, easy and precise reversed-phase high-performance liquid chromatographic assay for norfloxacin (NFLX) samples from photo-stability of solid dosage forms, without using gradient elution, extraction methods and without using counter-ion has been carried out. The method showed excellent linearity ($r^2 \geq 0.999$) in the range 1–20 μg ml^{-1} using a Lichrosorb-RP-8 column (10 μm, 20 cm × g4.6 mm) and UV-detection (278 nm) at ambient temperature. This method showed good efficiency for the analysis of photodegraded NFLX samples, and was applied to study the photo-stability of NFLX tablets under different conditions (direct sun light, ultraviolet light and fluorescent light). It was proven that the use of a disintegrant can increase the photo-stability of the NFLX in the tablets. This effect was studied in directly compressible tablets with microcrystalline cellulose (MCC) and mannitol for direct compression.

Keywords: Norfloxacin; HPLC; Photo-stability; Direct compression; Disintegrant

1. Introduction

Norfloxacin (1-ethyl-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid) is a synthetic, broad-spectrum antibacterial agent which exhibits high antimicrobial activity in vitro against a wide variety of gram-negative and gram-positive bacteria, including gentamicin-resistant *Pseudomonas aeruginosa* and β-lactamase positive *Neisseria gonorrhoeae* [1]. It is related to nalidixic acid but its potency has been increased by a fluorine atom and by a piperazine at the 7th position. Excellent therapeutic effects have been shown in the treatment of respiratory, biliary and urinary tract infections.

Norfloxacin is photosensitive. Prolonged exposure of bulk drug and in solution under direct sunlight or under fluorescent light results in the

* Corresponding author. Fax: +34 1 3941736.

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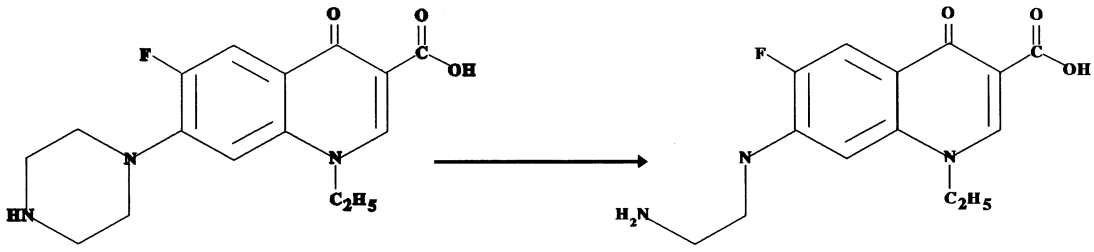

Fig. 1. Structural formula of norfloxacin and formation of the ethylenediamine degradate.

formation of ethylenediamine degradate [2]. Its structure is shown in Fig. 1. In this work, a validation of a specific HPLC method and its application to a photo-stability study is reported.

Several HPLC methods have been suggested for the determination of norfloxacin in various matrices including biologicals [3–8]; all need a counter-ion in the mobile phase, a fluorescence detector, the previous acylation of norfloxacin, the use of gradient elution or a previous extraction of samples. In the present paper, a simple and specific HPLC method is applied to the study of the photo-stability of norfloxacin contained in directly compressible tablets, due to the fact that a closely related degradate is determined. The influence of the presence of sodium starch glycolate (Explotab®) as a direct compression disintegrant in the formula on the stability of norfloxacin using different degradation conditions is reported.

2. Materials and methods

2.1. Norfloxacin tablets

Four different batches of norfloxacin tablets were used in this study. These formulations are described in Fig. 2. All tablets were obtained by direct compression using a 12-mm punch, from several ingredients: magnesium stearate, talc and hydrogenated vegetable oil-Lubritab® (Edward Mendell Co. Inc.) as lubricants, sodium starch glycolate-Explotab® (Edward Mendell) as super-disintegrant agent and a microcrystalline cellulose-Avicel® PH200 (FMC Corporation) and a directly compressible mannitol-Pearlitol® 500 DC (Roquette-Laisa España S.A.) as main excipients.

The physical and technological characteristics of the four batches of norfloxacin tablets are shown in Table 1. Tablets I (containing microcrystalline cellulose) had a height value of about 4.75 mm while tablets II had a height value of about 3.41 mm, due to the different compression properties of mannitol.

2.2. Instrumentation and chromatographic conditions

Chromatographic analyses were carried out using a Gilson® liquid chromatograph, with an autosampler and a UV/Vis detector set at 278 nm. Chromatograms were recorded and processed on a Spectra Physics-4270 integrator.

The choice of the conditions and the kind of column was carried out using several methods described by the US Pharmacopeia [10] and by different authors [2–9]. A Lichrosorb® C8 Chromatographic column (10 μm, 20 cm × 4.6 mm) was used. The separation of NFLX and its degradation products was achieved isocratically using a buffer solution [PO_4H_3/PO_4HNa_2] adjusted to pH 3.0–acetonitrile (85:15, v/v) as the eluent, pumped at a flow rate of 2.0 ml/min. The eluent was filtered (pore size, 0.45 μm) before use and then degassed by sonication in a ultrasound bath. The effects of the pH of the eluent and the ratio of the

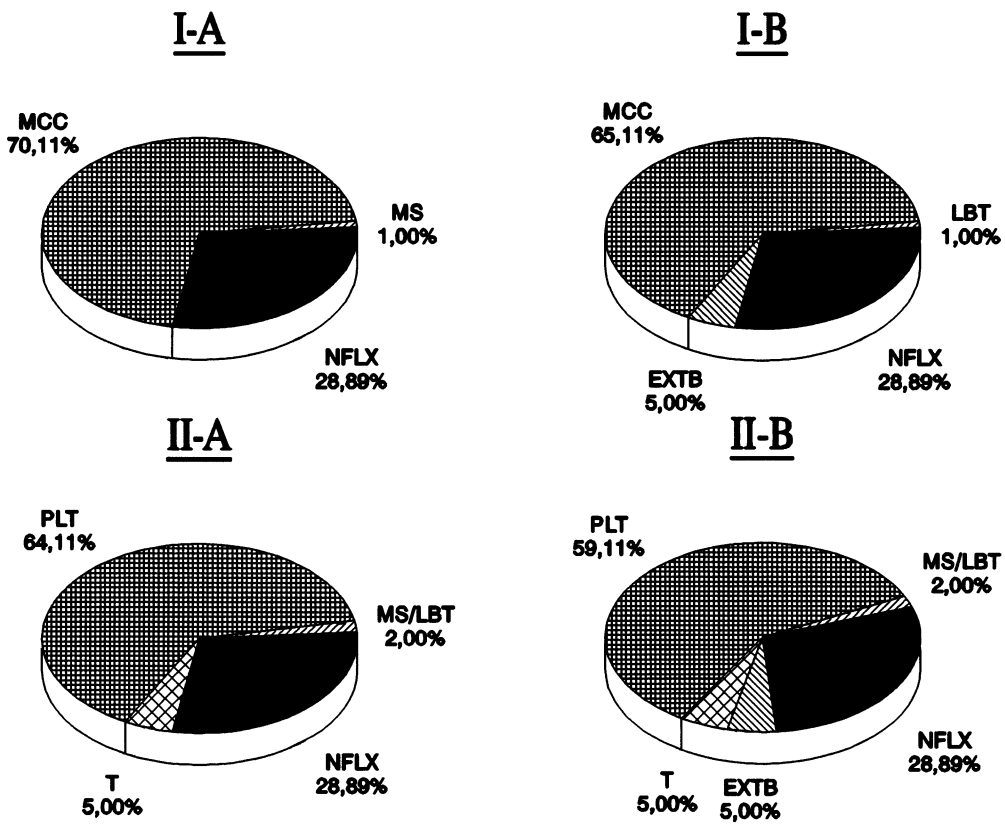

Fig. 2. Composition of the norfloxacin tablets used in the photo-stability study: norfloxacin (NFLX), magnesium stearate (MS), hydrogenated vegetable oil-Lubritab® (LBT), talc (T), sodium starch glycolate-Explotab® (EXTB), microcrystalline cellulose-Avicel®-PH200 (MCC), mannitol-Pearlitol® (PLT).

Table 1
Properties and characteristics of the four batches of norfloxacin tablets

Batch code (formulation)	Weight (mg)	Hardness (kg)	NFLX content (% deviation)
I-A	443.2 ± 5.2	5.13 ± 0.18	100.423 ± 1.446
I-B	457.6 ± 5.3	7.28 ± 0.67	102.721 ± 2.400
II-A	454.9 ± 5.1	7.33 ± 0.55	97.417 ± 1.445
II-B	449.7 ± 4.8	6.23 ± 0.60	98.971 ± 3.167

organic modifier (acetonitrile) in the mobile phase were previously studied in order to optimize the chromatographic conditions. The assays were performed at ambient temperature.

2.2.1. Standards and sample preparation

The norfloxacin standard (125 mg) was weighted accurately and transferred into a 250-ml volumetric flask and diluted with 0.1 N hydrochloric acid by sonication. This standard solution had a concentration of 500 μg/ml. Standard solution (0.5, 1, 1.5 and 2 ml) was transferred to four 50-ml volumetric flasks and diluted with mobile phase to obtain the external standard solutions having concentrations of 5, 10, 15 and 20 μg/ml of norfloxacin, respectively. For the analy-

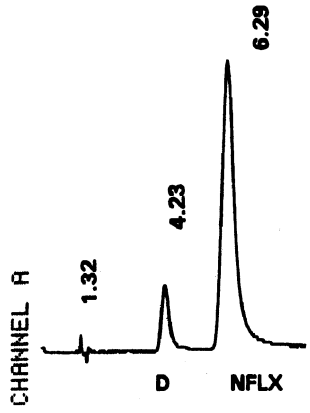

Fig. 3. Chromatogram for an acid solution of norfloxacin (10.4 μg/ml) after prolonged exposure to direct sunlight: NFLX, norfloxacin; D, degradation product.

sis of norfloxacin tablets, each tablet was weighted accurately and dissolved with sonication in 0.1 N hydrochloric acid in a 250-ml volumetric flask. One ml of this solution was diluted with mobile phase in a 50-ml volumetric flask, previously filtered using a 0.45-μm cellulose acetate filter, to obtain a solution of about 10 μg/ml.

2.3. Photo-stability studies

Norfloxacin is a very thermostable drug with a very small extent of decomposition when exposed in bulk form to high temperatures [8,9], which is the reason why the possibility of using different temperatures has not been considered in the frame of the present photo-stability study.

Formation of ethylenediamine degradate was evidenced in acid solutions of norfloxacin after prolonged exposure (6 months) to direct sunlight and fluorescent light through the presence of a new peak found in the chromatograms obtained from the analysis of these solutions. An example of these chromatograms in shown in Fig. 3.

In the present experimental work, the four batches of norfloxacin tablets were exposed under direct sunlight, fluorescent light (Philips™-TL20W/54RS, 91.56 candles) and ultraviolet radiation (continuous 254-nm UV lamp), at ambient temperature (25 ± 5°C) and 65 ± 5% relative humidity, using sealed spaces (glass-colorless under direct sunlight) in contact with a saturated aqueous solution of $NaNO_2$. In order to study kinetically the photodegradation reaction, a zero-order reaction is assumed to obtain the predicted shelf-life ($t_{10\%}$), by using Eqs. (1) and (2):

$$C = C_0 - K \cdot t \quad (1)$$

$$t_{10\%} = \frac{(100 - 90)}{K} \quad (2)$$

Where C_0 is the initial percentage of norfloxacin in the tablets ($C_0 = 100\%$), C is the ratio (%) of the amounts of norfloxacin at time t to the initial amount and K is the rate constant [9].

3. Results

3.1. Validation of analytical HPLC method

A prospective validation protocol described by different authors [11–14] was applied for the high-performance liquid chromatographic method.

3.1.1. Chromatographic parameters

Since the analytical method was designed to work in a range of 5–20 μg/ml of concentration of norfloxacin in the samples, these four standard solutions were used to calculate the capacity factor (k') and tailing factor (T) for norfloxacin. Calculated as in USP 23, using the average of six injections, $k' = 3.75 \pm 0.02$ and $T = 1.63 \pm 0.10$ were obtained. As can be seen, a good k' was

Table 2
Linear regression data for a representative calibration curve (peak height vs. concentration) and the main validation parameters: relative standard deviation of slope (R.S.D.-slope) as linearity parameter, detection limit and average recovery (mean ± S.D.)

Intercept	−113.000
Standard error	18.000
Slope	131.140
Standard error	1.676
r^2	0.99967
R.S.D.-slope (%)	1.278
Detection limit (μg/ml)	2.185
Average recovery (%)	99.724 ± 1.288

Table 3
The ratio (%) of the amount of norfloxacin remaining in the tablet to the initial amount under fluorescent light for the four batches of norfloxacin tablets (mean ± S.D. deviation)

Time (days)	I-A	I-B	II-A	II-B
0	100.000	100.000	100.000	100.000
6	95.817 ± 2.211	97.314 ± 2.337	99.645 ± 1.414	95.708 ± 2.526
20	94.389 ± 1.015	95.610 ± 1.069	96.249 ± 2.030	94.839 ± 2.493
34	93.620 ± 1.633	95.827 ± 0.953	89.135 ± 2.147	92.135 ± 2.415
48	92.789 ± 2.149	94.098 ± 1.223	88.069 ± 1.644	91.313 ± 2.108
62	92.954 ± 1.015	93.019 ± 1.623	87.147 ± 1.354	87.663 ± 3.074
76	88.710 ± 1.884	90.047 ± 1.962	86.646 ± 1.216	82.477 ± 2.186

Table 4
The ratio (%) of the amount of norfloxacin remainding in the tablet to the initial amount under direct sunlight for the four batches of norfloxacin tablets (mean ± S.D. deviation)

Time (days)	I-A	I-B	II-A	II-B
0	100.000	100.000	100.000	100.000
4	97.474 ± 1.987	99.330 ± 0.975	97.231 ± 1.109	99.953 ± 0.713
28	93.473 ± 2.315	98.144 ± 1.530	92.049 ± 0.757	99.314 ± 0.485
42	91.888 ± 2.211	93.978 ± 1.147	89.448 ± 0.661	94.728 ± 1.271
56	85.457 ± 2.828	88.384 ± 2.121	87.630 ± 1.098	92.930 ± 1.323
77	80.234 ± 2.169	82.125 ± 1.746	86 101 ± 1.414	84.728 ± 1.728

achieved using 15% acetonitrile in the mobile phase. Reduction of the eluant acetonitrile content to 10% resulted in a marked increase in the retention times (>20 min). Due to the peak tailing, peak height was found to be better in comparison to peak area to quantify norfloxacin under the mentioned HPLC conditions.

3.1.2. Linearity

Detector response for norfloxacin was linear to at least 20 μg/ml. The resulting data was plotted as peak height (measured electronically by the integrator) versus concentration and studied by linear regression analysis. Table 2 shows linear regression data obtained. In all cases, linearity plots had correlation coefficients ≥ 0.9998.

3.1.3. Precision and accuracy

Accuracy and precision were studied using three simulated preparations with concentrations of 5, 10 and 15 μg/ml (nine samples). Data analysis were carried out by using Cochran's *G*- and Student's *t*-tests ($p = 0.05$). The average recovery of norfloxacin is shown in Table 2.

3.1.4. Selectivity and resolution

Simulated norfloxacin samples were prepared with a concentration of 10.4 μg/ml, similar to those obtained from stability studies of norfloxacin tablets, according to the corresponding formulations, and were determined by two methods: the high-performance liquid chromatographic method under mentioned conditions and a previously validated [15] ultraviolet spectrophotometry (UV) ($\lambda_{max} = 278$ nm). The average recoveries obtained were 100.48 ± 1.05% for the HPLC assay and 99.89 ± 0.86% for UV. After prolonged exposure (6 months) to direct sunlight of the solutions, the recoveries averaged 95.64 ± 1.86% for the HPLC method and 100.56 ± 0.91% for UV. Taking into account that a new peak appeared in the chromatograms corresponding to the degradation product, with a good selectivity ($\alpha = 1.71$) and resolution among the compounds ($R_s = 2.84$), and the changes in the concentrations, these results seem to suggest that HPLC is a selective and specific method for the analysis of norfloxacin samples

Table 5
The ratio (%) of the amount of norfloxacin remaining in the tablet to the initial amount under ultraviolet light for the four batches of norfloxacin tablets (mean ± S.D. deviation)

Time (days)	I-A	I-B	II-A	II-B
0	100.000	100.000	100.000	100.000
3	101.050 ± 0.613	99.327 ± 2.071	99.801 ± 1.273	100.201 ± 0.082
6	98.012 ± 1.345	97.500 ± 1.061	96.313 ± 1.193	99.986 ± 0.485
13	96.931 ± 1.216	98.212 ± 1.971	96.625 ± 0.627	99.163 ± 0.543
20	94.003 ± 0.664	97.779 ± 1.570	95.615 ± 0.838	98.827 ± 1.537
27	92.990 ± 2.570	97.631 ± 2.082	90.260 ± 2.787	94.906 ± 1.639
34	91.038 ± 1.734	96.255 ± 1.078	88.904 ± 1.054	93.635 ± 1.934
41	90.538 ± 1.704	97.342 ± 1.580	85.077 ± 1.479	90.394 ± 1.843

from photo-stability studies, in contrast to UV-spectrophotometry, in the range of concentrations used in our work.

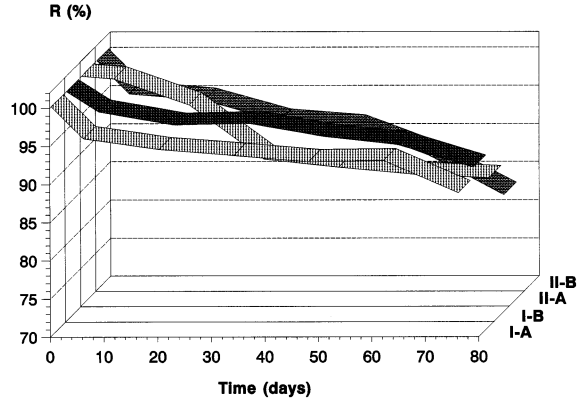

Fig. 4. Plot showing the changes in the amounts of norfloxacin (%) in the tablets under fluorescent light vs. time. Mean values.

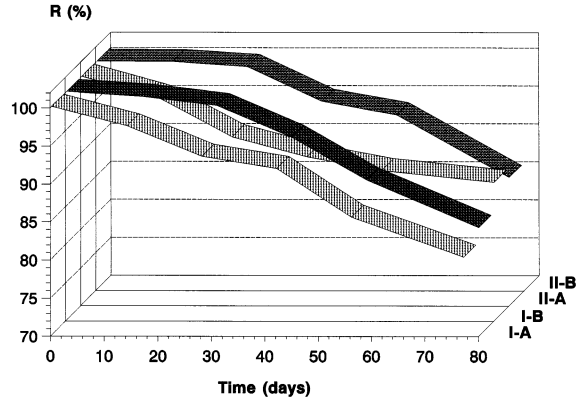

Fig. 5. Plot showing the changes in the amounts of norfloxacin (%) in the tablets under direct sunlight vs. time. Mean values.

3.2. Photo-stability tests

Degradation data under different storage conditions in terms of the ratio (%) of the amount of norfloxacin remainding in the tablets to the initial amount (100%) are shown in Table 3 (under fluorescent light), Table 4 (direct sunlight) and Table 5 (ultraviolet light) and plotted in Figs. 4–6. In all cases, six tablets were individually weighed and assayed. The weight value was taken into account in each calculation; mean ± S.D. was used for subsequent kinetic studies. The rate constant of the photodegradation reaction and the predicted shelf-life ($t_{10\%}$) were obtained under each condition assuming a zero-order reaction, by using Eqs. (1) and (2). Results of $t_{10\%}$ obtained in each formula under different exposure conditions are shown in Table 6 and in Fig. 7.

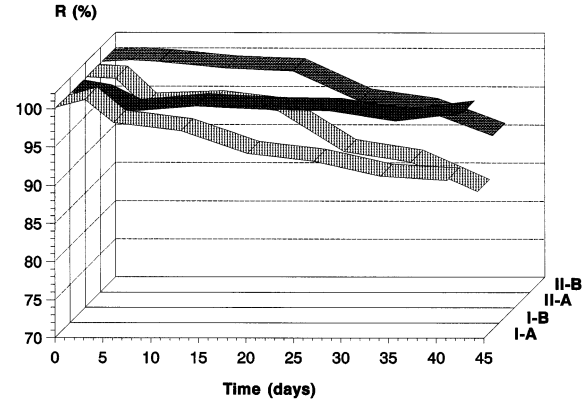

Fig. 6. Plot showing the changes in the amounts of norfloxacin (%) in the tablets under ultraviolet light vs. time. Mean values.

Table 6
Predicted $t_{10\%}$ (days) under different exposure conditions for each formula

Formulation	Fluorescent light	Direct sunlight	Ultraviolet light
I-A	72.174	41.597	39.436
I-B	83.156	51.184	148.015
II-A	47.327	47.160	29.698
II-B	45.532	42.686	48.670

4. Discussion

A general, simple and rapid procedure for the high-performance liquid chromatographic analysis of norfloxacin samples from photo-stability studies of solid dosage forms has been developed and validated. This procedure is suitable for the separation and quantitation of norfloxacin in the presence of photodegradation products. This kind of degradation reaction is not detectable by using a simple ultraviolet spectrophotometry.

Taking into account the comparative degradation data obtained with the four formulations of norfloxacin tablets, it is observed that in general lines, the use of sodium starch glycolate as a disintegrant leads to a significant increase in the photostability of norfloxacin in the tablets, under the studied exposure conditions. This effect is particularly important in those tablets exposed to ultraviolet radiation and formulated with microcrystalline cellulose (I-B). This can be due to a 'barrier-effect' of the starch granules. From data obtained in the present work, it seems to be possible to increase the photo-stability of norfloxacin in the tablets by including a 5% sodium starch glycolate by direct compression in the formula. This increase of stability resulted to be statistically significant ($p = 0.05$), specially under ultraviolet radiation.

It is observed that the use of microcrystalline cellulose instead of mannitol can produce more stable norfloxacin tablets. However, the influence of magnesium stearate on the stability of NFLX in the tablets should be prospectively studied.

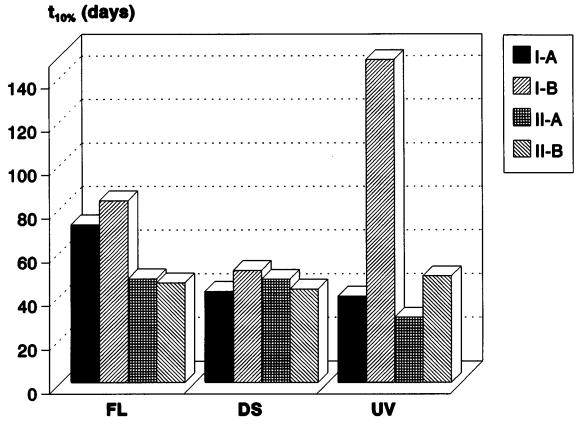

Fig. 7. Plot showing predicted shelf-life for each formula under different exposure conditions: FL, fluorescent light; DS, direct sunlight; UV, ultraviolet light.

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