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Determination of norfloxacin by fluorescence in the presence of different antacids: quantification of analytical interferences[1]

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Abstract

Norfloxacin is a fluorquinolone that can interfere with certain antacids (derivatives of Al and Mg) because its dissolution profiles are dependent on pH. Furthermore, it can form insoluble complexes that modify its absorption and bioavailability.

Two sensitive and selective analytical methods using fluorescence (FL) and UV spectrophotometry (UV) have been developed to study the dissolution behaviour in gastric juice of different formulations of norfloxacin in tablets. There are no significant differences when the samples are measured by both methods and their ruggedness in the presence of some excipients is proven. From this, it is concluded that they are effective for this study.

When different antacids are added to the dissolution medium, using UV and FL methods with the same samples, totally different dissolution profiles appear. Using FL, it would appear that up to 400% of the amount of norfloxacin in the tablet is released. These profiles are misleading because the uniformity of dosage units was tested before the dissolution studies.

It was proven that the antacids dissolved in gastric juice do not produce fluorescence, but cause important analytical interferences with norfloxacin. This may be because their association with Al^{3+} or Mg^{2+} forms a new compound. Nevertheless, it is observed that this effect is more important in some antacids (Almagate, Magaldrate). This may be because their ability to deliver Al to the medium is greater.

Keywords: Antacids; Chelate; Dissolution profile; Norfloxacin; Spectrofluorimetry

1. Introduction

Norfloxacin is a synthetic, broad-spectrum antibacterial agent for oral administration. This fluorquinolone is 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. Its empirical formula is $C_{16}H_{18}FN_3O_3$ and the structural formula is shown in Fig. 1. Norfloxacin features fluorine (F) at the 6th position and piperazine at the 7th position of quinolone carboxylic acid. As a result, this drug shows much higher antibacterial activity on gramnegative bacteria and covers gram-positive cocci, as compared with conventional pyridone carboxylic acids. Especially potent

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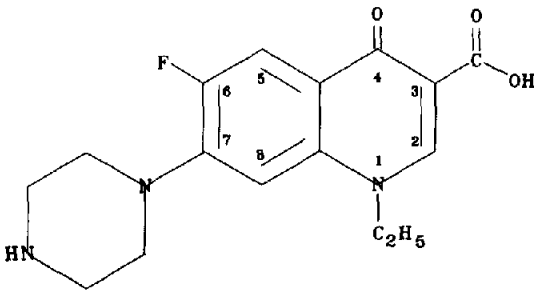

Fig. 1. Norfloxacin: numbered structural formula.

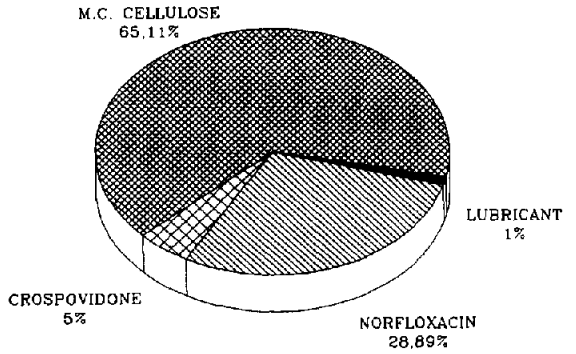

Fig. 2. Composition of the norfloxacin tablets used in the dissolution study.

antibacterial activity has been found against *Pseudomonas aeruginosa* and *Serratia sp.* [1]. Excellent therapeutic effects have been shown in the treatment of respiratory, biliary and urinary tract infections.

The bioavailability of quinolone antimicrobial agents in general and norfloxacin in particular has been shown to be less when they are ingested with antacids. The activity and solubility of norfloxacin appear to be influenced by pH [2,3] but the binding of metal ions such as Al^{3+} and Mg^{2+} contained in these preparations to the 4-keto and 3-carboxyl groups of norfloxacin and the formation of nonabsorbable chelates has been suggested as one of the possible mechanisms responsible for the reduced absorption of this quinolone [4–7].

The dissolution behaviour in gastric fluid (USP 23; Ref [8]) of norfloxacin tablets was studied using UV spectrophotometry as a simple and sensitive analytical method for the assay of norfloxacin samples.

Spectrofluorimetric analysis is used for the assay of a large and growing number of drugs and allows the selective and sensitive determination of low concentrations of analytes [9]. Many fluorquinolones possess native fluorescence and there is a large body of information in the literature on the analysis of quinolones using a fluorescence detector [10–13]. In this sense, a fluorimetric assay for norfloxacin samples in gastric fluid was developed and validated as an alternative analytical method.

A study of dissolution profiles obtained in the presence of some widely used antacids was carried out using both analytical methods.

2. Materials and methods

2.1. Norfloxacin tablets

The tablets of the batch used in this study contain the ingredients described in Fig. 2. These tablets were obtained by direct compression and their physical and technological characteristics are shown in Table 1. The uniformity of dosage units was tested using the requirements specified for the USP 23. The average percentage of the lab-eled amount of norfloxacin in the tablets was $101.419 \pm 1.225\%$.

2.2. Drug assay

The concentrations of norfloxacin in samples using simulated gastruc fluid (USP 23) and 0.1

Table 1
Properties and characteristics of norfloxacin tablets

Color	White/pale yellow
Tablet diameter	12 mm
Weight	452.34 ± 4.67 mg
Hardness	8.11 ± 0.56 kg
Friability	0.23%

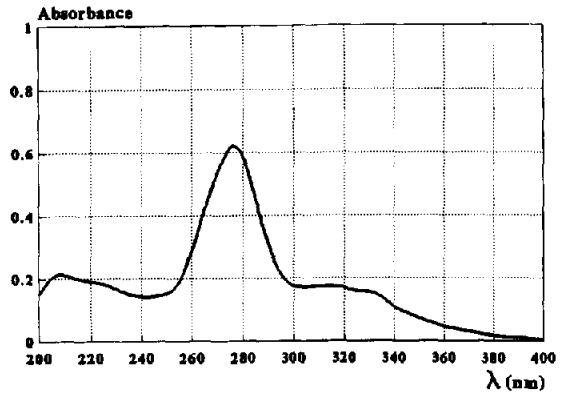

Fig. 3. Ultraviolet/visible spectrum of norfloxacin. Solvent: 0.1 N HCl.

N hydrochloric acid as solvents with a pH of about 1.2, were determined by two analytical methods: fluorimetry (FL), using a Perkin-Elmer 204 spectrofluorimeter ($\lambda_{ex} = 330$ nm, $\lambda_{em} = 445$ nm); and ultraviolet spectrophotometry (UV), using a Bechman DU-6 spectrophotometer ($\lambda_{max} = 276$ nm). Figs. 3 and 4 show the maximum absorbance λ (UV) and the $\lambda_{excitation}$ and $\lambda_{emission}$ maxima for norfloxacin. These λ_{maxima} values were checked in the presence of the antacids studied and there were no modifications to the spectra. The same concentration range (0–10.0 μg ml^{-1}) was used in both methods. All the samples were filtered using a 0.45 μm cellulose acetate filter.

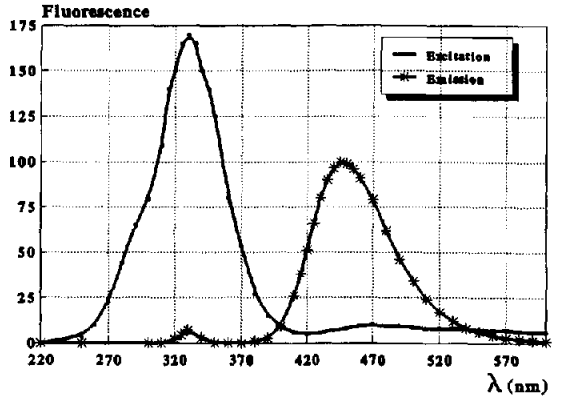

Fig. 4. Fluorescence spectrum of norfloxacin showing maximum FL excitation and emission. Solvent: 0.1 N HCl.

Table 2
Composition of the antacid preparations in tablets

Name	Composition	(mg)
Almax® (Almirall)	Almagate[a]	500
Maalox® (Rhône-Poulenc-Rorer)	$Al(OH)_3$	600
	$Mg(OH)_2$	300
Bemolan® (Boehringer Mannheim)	Magaldrate[b]	400
Aligest® (Schering Plough)	$Al(OH)_3$	298
	$Mg(OH)_2$	328
	$CaCO_3$	410
	Simethicone	25

[a] Almagate (INN): $[Al_2Mg_6(OH)_{14}(CO_3)_2 \cdot 4H_2O]$. [b] Magaldrate (INN): $[Al_5Mg_{10}(OH)_{31}(SO_4)_2 \cdot xH_2O]$.

2.3. Dissolution behaviour

Dissolution assays were carried out in triplicate (three dissolution assays of one tablet each) with a modification of the method specified for norfloxacin tablets by the USP 23: apparatus, 2 (paddle); rotation speed, 50 ± 1 rev min^{-1}; temperature, 37 ± 0.1°C; medium, simulated gastric fluid (USP 23); solution ratio, 1:50; sample volume, 1 ml.

2.4. Antacids

Four widely used antacid preparations in tablets containing Al and Mg derivatives were used in our study. These preparations are described in Table 2.

3. Results

3.1. Validation of analytical methods

A prospective validation protocol described by various authors [14–16] was applied for both analytical methods.

The calibration curves obtained proved to be linear in the range 1–10 μg ml^{-1} for the analyte assayed using both methods. The same range of concentrations was used in FL and UV in order to make a comparison and to develop two methods to directly measure the samples obtained from

Table 3
Linear regression data for both methods

Regression data	UV	FL
Intercept	−0.0256	8.8056
Standard error	0.01305	1.97210
Slope	0.1329	9.6278
Standard error	0.00059	0.14689
Degrees of freedom	28	25
R_2	0.9997772	0.994206

dissolution assays. In some cases, when very high fluorescence signals resulted in the presence of antacids, the samples were diluted to bring their concentrations within the standard curve. The details of linear regression results are reported in Table 3.

3.1.1. Linearity

The UV method showed better linearity than FL, studying the relative standard deviation of response factors (RF) (see Table 4). The detection limit was also better in the UV method.

3.1.2. Precision and accuracy

Accuracy and precision were studied for both methods using 10 simulated preparations with concentration of 3, 6 and 9 $\mu g\ ml^{-1}$ (30 samples). Data analysis was carried out using Cochran's G and Student's t-test ($p = 0.05$). The average recoveries of norfloxacin are shown in Table 4.

3.1.3. Ruggedness

The influence of eluent (0.1 N hydrochloric acid or simulated gastric fluid) was studied in both methods, as well as the effect of the presence of different excipients widely used in the formulation of tablets (α-lactose, microcrystaline cellulose, povidone, crospovidone, mannitol, magnesium sterate, hydrogenated vegetable oil and sodium starch glycolate). In all cases, there were no significant differences studying the average recoveries at concentrations 3, 6 and 9 $\mu g\ ml^{-1}$ (see Table 5). The ruggedness of both methods in the presence of all excipients was proven. From this it was demonstrated that both methods are effective for a dissolution study.

Table 4
Validation data: relative standard deviation of response factors (RSD-RF) as linearity parameter, detection limits and average recoveries for both methods.

Parameter	UV	FL
RSD-RF (%)	2.67	10.17
Detection limit ($\mu g\ ml^{-1}$)	0.58	2.74
Average recoveries (%)	101.18	102.66
RSD (%)	1.65	4.84

Table 5
Ruggedness: average recoveries obtained using three different conditions in triplicate (nine samples)

Conditions	UV	FL
In 0.1 N HCl	100.230 ± 1.069	103.048 ± 0.998
In gastric fluid	100.637 ± 1.174	103.336 ± 0.864
With excipients[a]	101.109 ± 0.912	99.263 ± 0.774

[a] The same amount of excipients as in the dissolution assays: the equivalent of one tablet.

3.2. Dissolution rates

All samples were measured using both methods. There were no significant differences between profiles obtained in gastric fluid (Fig. 5). When

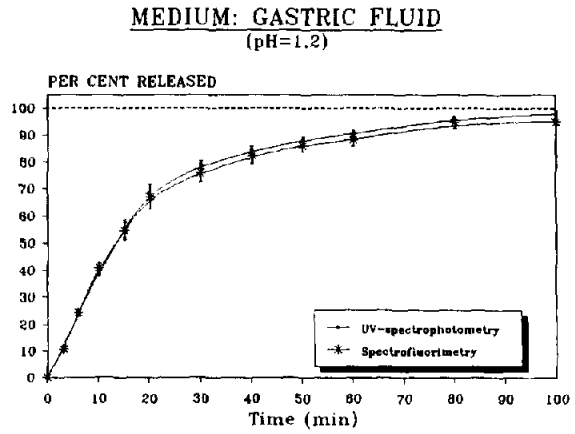

Fig. 5. Dissolution profiles of norfloxacin tablets in gastric fluid measuring the samples by both methods (mean of three assays).

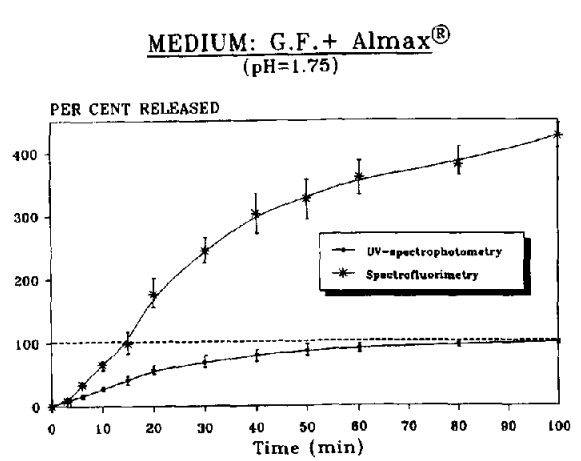

Fig. 6. Dissolution profiles in the presence of the equivalent of a half-dose of Almax® using both analytical methods (mean of three assays).

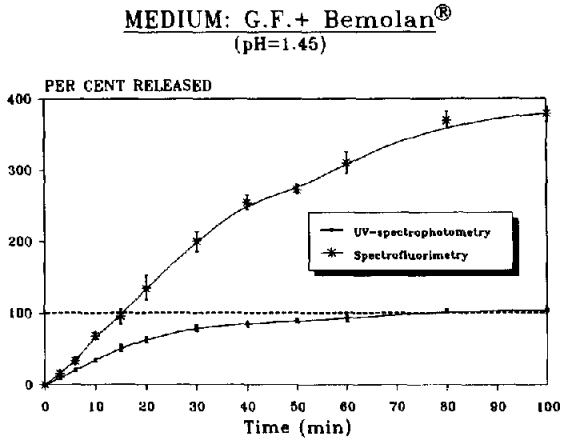

Fig. 8. Dissolution profiles in the presence of the equivalent of a half-dose of Maalox® using both analytical methods (mean of three assays).

different antacids were added to the dissolution medium, totally different dissolution profiles were found. Dissolution profiles obtained in the pres-ence of different antacids are shown in Figs. 6–9. Fig. 10 and Table 6 show the percentage of norfloxacin dissolved in 100 min using both methods.

4. Discussion

Although norfloxacin solubility is pH-dependent, these results strongly suggest that the binding of the Al^{3+} ion to the –C=O groups of norfloxacin to form nonabsorbable chelates is responsible for the interaction. It was proven that the four antacids used in this study, when dissolved in gastric juice, do not produce native fluorescence but cause important analytical interferences with norfloxacin. When different antacids are added to the dissolution medium, FL results would suggest that up to 400% of the amount of norfloxacin in the tablet is released. These results suggest that norfloxacin forms a new compound in the presence of Al^{3+} and Mg^{2+} ions, with totally different fluorescent properties, showing an

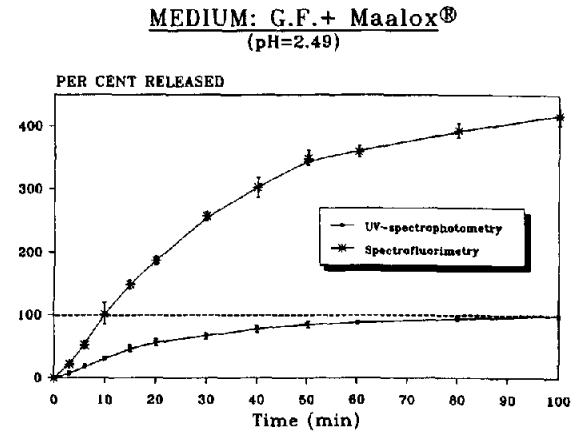

Fig. 7. Dissolution profiles in the presence of the equivalent of a half-dose of Bemolan® using both analytical methods (mean of three assays).

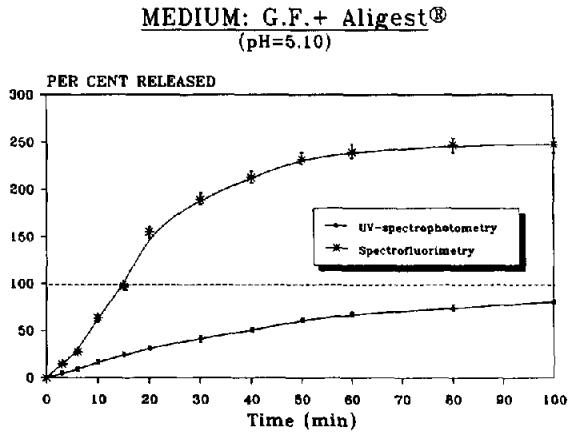

Fig. 9. Dissolution profiles in the presence of the equivalent of a half-dose of Aligest® using both analytical methods (mean of three assays).

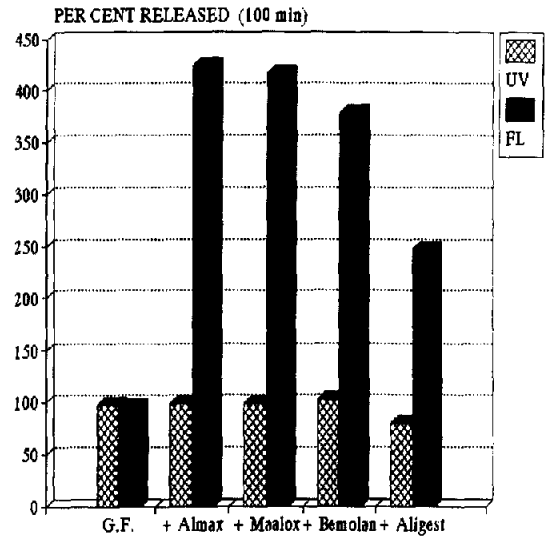

Fig. 10. Percentage of norfloxacin dissolved in 100 min using both methods.

important analytical interference. This association is not detectable using UV spectrophotometry.

It is observed that this interference is more important in some antacids such as Almagate or Magaldrate. This may be because their ability to deliver Al^{3+} to the medium is greater. In this way, FL can be directly used to measure Al^{3+} available in the dissolution medium.

Consequently, it is concluded that UV spectrophotometry is an effective method for studying the dissolution behaviour of norfloxacin tablets in gastric juice in the presence of different antacids. Fluorimetry can be used to study the dissolution profiles in gastric fluid and to compare the formation of chelates with antacids under different conditions.

It seems to be possible to relate the degree of in-vitro interaction of norfloxacin with antacids to the magnitude of the increase in the fluorescence. This hypothesis should be tested with different amounts of the antacids studied.

Table 6
Percentage released (100 min)

Medium	UV	FL
Gastric fluid (GF)	97.943 ± 2.128	96.052 ± 2.357
GF + Almax®	98.667 ± 6.842	424.723 ± 49.431
GF + Maalox®	98.382 ± 2.605	417.598 ± 28.018
GF + Bemolán®	102.335 ± 2.564	379.427 ± 19.381
GF + Aligest®	80.127 ± 1.867	247.862 ± 14.254

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