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Quantitative analysis of fluoroquinolones by ^{1}H- and ^{19}F-NMR spectroscopy

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Abstract

^{1}H- and ^{19}F-NMR assay of pefloxacin (**I**), norfloxacin (**II**) and ofloxacin (**III**) in some pharmaceutical forms has been developed. The method, based on the integration of appropriate signals of both analytes and internal standards, is simple, rapid, precise, and accurate, and can be used for quality control of these drugs.

Keywords: Antibiotic; Fluoroquinolone; Pefloxacin; Norfloxacin ; Ofloxacin; Quantification; ^{1}H-NMR; ^{19}F-NMR

The fluoroquinolones are an important new class of oral synthetic antibacterial agents used against some infections previously treated only parenterally (Rosen, 1990).

Numerous methods for fluoroquinolone analysis in biological fluids as well as in pharmaceutical preparations have been developed and, among these, spectrophotometric (e.g., Jelikic-Stankov et al., 1989) and HPLC (e.g., Chen et al., 1993) determinations are worthy of mention. NMR has been used in the fluoroquinolone field (Chenon et al., 1990; Tugnait et al., 1992; Riley et al., 1993), however, to the best of our knowledge, no NMR assay of fluoroquinolones in pharmaceutical formulations has been performed. Therefore, we applied ^{1}H- and ^{19}F-NMR spectroscopy to assay three widely used fluoroquinolones, namely, pefloxacin (**I**) [1-ethyl-6-fluoro-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid methanesulphonate dihydrate], norfloxacin (**II**) [1-ethyl-6-fluoro-7-(1-piperazinyl)-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid] and ofloxacin (**III**) [(±)3-methyl-9-fluoro-10-(4-methyl-1-piperazinyl)-2,3-dihydro-7-oxo-7H-pyrido[1,2,3,*d*,*e*](1,4)benzoxazine-6-carboxylic acid] (Scheme 1), in some commercial preparations.

The method is based on the ratios between the integrals of chosen signals of internal standards (IS) and analytes. To obtain closely comparable ^{1}H and ^{19}F results for each analyte, a unique solution containing as IS 4′-fluoroacetanilide (**IV**) for pefloxacin assay, 2-amino-3,5-dibromo-6-fluorobenzoic acid (**V**) for norfloxacin and 2-fluoro-5-aminobenzoic acid (**VI**) for ofloxacin (Scheme 1) was used for both nuclei.

Pefloxacin methanesulphonate, Peflacin® tablets and vials; norfloxacin and Noroxin® tablets; and ofloxacin and Flobacin® tablets were kindly supplied by Rhone-Poulenc Pharma I

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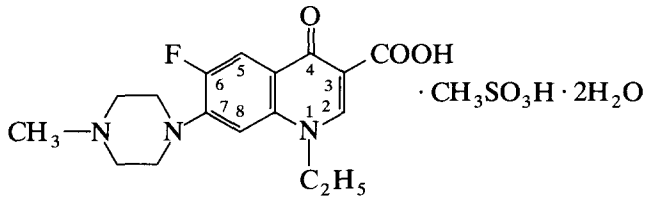
(Italy), Merck Sharp and Dohme (Italy), and Sigma Tau (Italy), respectively. 4′-Fluoroacetanilide (Jansen, Belgium), 2-amino-3,5-dibromo-6-fluorobenzoic acid and 2-fluoro-5-aminobenzoic acid (both Fluorochem Ltd, UK) were of analytical grade. D_2O, DMSO-d_6, chromium(III) acetyl acetonate, hexafluorobenzene, NaOH, NaCl and H_2SO_4 were purchased from Carlo Erba (Italy). Diethylenetriaminepentaacetic acid chromium(III) disodium salt hexahydrate was obtained from Sigma-Aldrich Srl (Italy).

The spectra were taken on a Bruker A 200 spectrometer operating at 200 MHz for ^1H and 188 MHz for ^{19}F. The samples were run in a 5 mm i.d. tube and recorded at ambient temperature. D_2O and DMSO-d_6 were used as solvents and internal locks. In the case of ^1H-NMR the reference peaks used were those of the above solvents and for ^{19}F-NMR that of hexafluorobenzene.

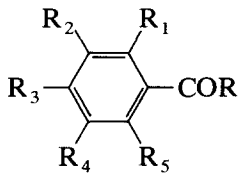
Fluorine-coupled ^1H spectra were recorded using the general conditions: 90° pulse angle (8 μs), 32 K computer points. The spectra of **I** and **II** were recorded with a spectral width (SW) of 4000 Hz, a filter width (FW) of 8000 Hz, an acquisition time (AQ) of 4.1 s, a digital resolution (DR) of 0.24 Hz/point and line broadening (LB) of 0.25 Hz before the Fourier transform. In the quantitation of **III**, SW = 3000, FW = 6000 Hz, AQ = 5.5 s, DR = 0.18 Hz/point, and LB = 0.18 Hz were used. To ensure full T_1 relaxation, an additional delay (RD) between pulses of 8 s for **I** and one of 6 s for **II** were included.

Proton-coupled ^{19}F spectra were obtained using a flip angle of 90° (17 μs) and 128 K computer points. In the assay of **I**, the conditions employed were: SW = 7000 Hz, FW = 14000 Hz, DR = 0.1 s, AQ = 9.3 s, LB = 0.1 Hz, RD = 9 s; the instrumental conditions for **II** were similar to those of pefloxacin assay, the only differences being RD = 1 s, SW = 15000 Hz, AQ = 4.3 s, DR = 0.25 Hz/point and FW = 30000 Hz.

The spectra of **III** were recorded using the conditions: SW = 10000 Hz, FW = 20000 Hz, DR = 0.15 Hz, AQ = 6.6 s, LB = 0.15 Hz and RD = 1.5 s.



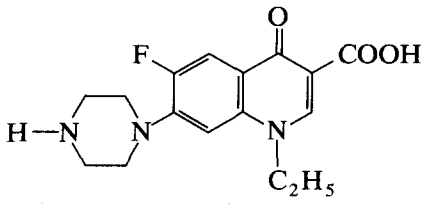
I



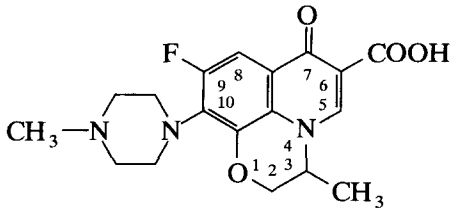
IV: $R = NH_2$; $R_1, R_2, R_4, R_5 = H$; $R_3 = F$

V: $R = OH$; $R_1 = NH_2$; $R_2, R_4 = Br$; $R_3 = H$; $R_5 = F$

VI: $R = OH$; $R_1 = F$; $R_2, R_3, R_5 = H$; $R_4 = NH_2$



II



III

Scheme 1. Structures of the fluoroquinolones analysed and the internal standards used.

Table 1
Percentage of pefloxacin recovery

Lot	From commercial tablets					From commercial vials				
	Amount declared [a]	Amount found				Amount declared/0.3 ml [a]	Amount found			
		^1H	Recovery (%)	^{19}F	Recovery (%)		^1H	Recovery (%)	^{19}F	Recovery (%)
1	16.1	16.2	100.6	16.1	100.0	16.75	16.96	101.2	16.78	100.1
2	13.5	13.7	101.5	13.7	101.5	16.75	16.63	99.3	16.79	100.2
3	15.7	15.5	98.7	15.6	99.4	16.75	16.60	99.1	16.85	100.6
4	15.0	15.2	101.3	15.1	100.7	16.75	17.01	101.5	16.92	101.0
5	14.3	14.4	100.7	14.4	100.7	16.75	16.72	99.8	16.75	100.0
Mean			100.6		100.5			99.9		100.42

[a] Weights in mg.

In both nuclei 100 free induction decays were collected for each experiment. The T_1 relaxation times were measured by the inverse recovery technique (Derome, 1988) by adding chromium(III) acetyl acetonate (10^{-4} M) to the DMSO-d_6 pefloxacin sample and diethylenetriaminepentaacetic acid chromium(III) disodium salt to NaOD norfloxacin and HCl-D_2O ofloxacin solutions as relaxation agents.

Pefloxacin assay: the ^1H-NMR spectrum of a mixture of **I** and **IV** showed the following signals:

I: 1.44 (t, $J = 6.8$ Hz, $-CH_2-\underline{CH_3}$), 2.30 (s, $N-CH_3$), 2.90 (s, $S-OCH_3$), 3.30 (bs, piperazinyl-H's), 4.60 (d, $J = 6.8$ Hz, $-\underline{CH_2}-CH_3$), 7.30 (d, $J = 7.1$ Hz, H_8); 7.90 (d, $J = 13.3$ Hz, H_5, $T_1 = 1.45$ s), 8.80 (s, H_2), 9.90 (d, $J = 8.0$ Hz, S-OH), 15.10 (d, $J = 7.5$ Hz, COOH); **IV**: 7.10 (dt, $J = 9.0$ and 9.0 Hz, $H_{3',5'}$), 7.60 (dt, $J = 9.0$ and 5.0 Hz, $H_{2',6'}$, $T_1 = 2.0$ s).

^{19}F-NMR spectrum: signal **I** appeared at 40.85 ppm (dd, $J = 13.3$ and 7.1 Hz, $T_1 = 0.55$ s) and signal **IV** at 42.75 ppm (tt, $J = 9.0$ and 5.0 Hz, $T_1 = 3.15$ s).

^1H quantitative analysis was based on the integration of the signals at 7.90 ppm for **I** and at 7.60 ppm for **IV**; fluorine analysis was based on the integration of the relative signals.

Each integral value was the average of at least five readings and the quantity of pefloxacin was calculated by substituting this value in the following formula:

$$\frac{I_p}{I_{i.s.}} \cdot \frac{EW_p}{EW_{i.s.}} \cdot C$$

where I_p is the average integral value for **I**, $I_{i.s.}$ the average integral value for **IV**, EW_p the equivalent weight of **I** (molecular weight in ^1H and ^{19}F assay), $EW_{i.s.}$ the equivalent weight of **IV** (molecular weight/2 in ^1H assay and molecular weight in ^{19}F) and C the weight (in mg) of **IV**. The accuracy

Table 2
Percentage of norfloxacin recovery from commercial tablets

Lot	Amount declared [a]	Amount found			
		^1H	Recovery	^{19}F	Recovery (%)
1	15.3	15.4	100.6	15.5	101.3
2	18.2	18.3	100.5	18.0	98.9
3	14.9	14.8	99.3	14.8	99.3
4	16.2	16.3	100.6	16.2	100.0
5	15.9	16.1	101.2	16.2	100.6
Mean			100.4		100.0

[a] Weights in mg.

and linearity of the method were verified by analysing 10 synthetic mixtures containing different amounts of the analyte and approximately the same quantity of the internal standard. Five of these mixtures were prepared by dissolving the pure compounds in 0.5 ml of DMSO-d_6, the others were made by adding weighed amounts of **I** and **IV** to the excipients contained in the commercial form and extraction with DMSO-d_6 (3×0.5 ml), as described for the dosage of the solid commercial preparation (each tablet contains: pefloxacin methanesulphonate, 558.5 mg; starch, 160.0 mg; gelatin, 15.0 mg; talc, 16.9 mg; methylcellulose, 7.49 mg; ethyl cellulose, 7.32 mg; carboxymethyl starch, 2.5 mg; dibutyl sebacate, 1.46 mg; TiO_2, 131.0 mg; and polyethylene glycol 6000, 0.39 mg). From these mixtures a ^1H recovery mean of 99.9% (S.D. 0.57, c.v. 0.57) and a ^{19}F recovery mean of 100.3% (S.D. 0.35, c.v. 0.35) were obtained.

Linear regression and correlation: for ^1H assay $y = 0.0279 + 0.997x$, correlation coefficient, $r = 0.999$; for ^{19}F assay $y = -0.0403 + 1.0156x$, $r = 0.998$.

In a paired test the statistical analysis of the recovery obtained from the two different nuclei gave a t value = 0.96 with $\alpha = 0.05$ (difference not statistically significant).

Commercial tablets were assayed by mixing for each lot the powder obtained by crushing 10 tablets. A portion of powder, equivalent to approx. 15 mg of **I** was accurately weighed and **IV** (approx. 10 mg) was added. The solid mixture was extracted with DMSO-d_6 (3×0.5 ml), the combined suspensions being centrifuged and filtered. The results in Table 1 were obtained by recording the spectra of these solutions.

Each solution used for the assay of commercial vials (one vial contains: pefloxacin methanesulphonate, 558.5 mg; ascorbic acid sodium salt, 15.3 mg; and H_2O up to 5.0 ml) was prepared by transferring the content of five vials to a 50 ml Erlenmeyer flask and diluting to volume with H_2O.

0.3 ml of this solution was transferred to a test tube containing about 10 mg of the IS (accurately weighed) dissolved in 0.3 ml of DMSO-d_6. The ^1H spectra were registered using a secondary irradiation field at the water resonance frequency in order to suppress its intense signal.

Norfloxacin assay: norfloxacin is poorly soluble in organic solvents, therefore the spectra were recorded in NaOD solution with the addition of the relaxation agent.

A mixture of **II** and **V** displayed the following ^1H spectrum:

II: 1.25 (t, $J = 7.3$ Hz, $-CH_2-\underline{CH_3}$), 2.90 (b m, piperazinyl-H's), 4.0 (q, $J = 7.3$ Hz, $-\underline{CH_2}-CH_3$), 6.60 (d, $J = 7.2$ Hz, H_8, $T_1 = 0.55$ s), 7.55 (d, $J = 13.7$ Hz, H_5), 8.22 (s, H_2); **IV**: 7.62 (d, $J = 7.4$ Hz, H_4, $T_1 = 0.35$ s). The norfloxacin proton chosen for the analysis was H_8.

^{19}F spectrum: signal **II** at 38.36 ppm (dd, $J =$ 7.2 and 13.7 Hz, $T_1 = 0.305$) and signal **V** at 52.60 ppm (d, $J = 7.4$ Hz, $T_1 = 0.76$ s).

10 standard solutions of analyte and IS were prepared as previously described, but using NaOD solution as solvent: an average recovery of 99.9% (S.D. = 1.2, c.v. = 1.2%) in the quantification of ^1H and of 99.5% (S.D. = 0.7, c.v. = 0.7%) in the case of ^{19}F were obtained. Linear regression analysis of the data gave the following equations: $y = 0.0687 + 0.9931x$, correlation coefficient $r = 0.999$ for ^1H and $y = -0.058 + 1.003x$, $r = 0.998$, for ^{19}F.

The t value ($t = 0.19$) did not show significant differences in the results obtained.

The commercial tablets (each tablet contains: norfloxacin, 400.0 mg; cellulose, 87.0 mg; carboxymethylcellulose sodium salt, 10.0 mg; stearic acid magnesium salt, 3.0 mg; hydroxypropylmethylcellulose, 4.0 mg; hydroxypropylcellulose, 4.0 mg; TiO_2, 3.0 mg) were analysed according to a procedure similar to that used for **I** by dissolving the drug in the NaOD solution instead of DMSO-d_6. In Table 2 the percentage of the analyte recovery from each lot of tablets is shown.

Ofloxacin assay: like **II**, ofloxacin (**III**) is poorly soluble in organic solvents, therefore, since the excipients present in the commercial preparation (each tablet contains: ofloxacin, 300.0 mg; starch, 68.0 mg; lactose, 144.0 mg; hydroxypropylcellulose, 15.0 mg; stearic acid magnesium salt, 6.0 mg; polyvinylpyrrolidone, 30.0 mg; ethyl cellulose, 5.0 mg; dibutyl phthalate, 1.75 mg; Span, 0.60 mg; talc, 23.06 mg; TiO_2, 4.0 mg; carnauba wax, 0.04

mg) are swollen by alkali, it was dissolved in an acid medium prepared by bubbling gaseous HCl in D_2O.

The ^1H spectrum of **III** and the IS, (**VI**), showed the **III** signals: 1.10 (d, $J = 6.8$ Hz, -CH-CH_3), 2.50 (s, N-CH_3), 3.0 (m, piperazinyl-H's), 3.92 (dd, $J = 1.9$ and 11.7 Hz, H_{2b}), 4.17 (dd, $J = 1.9$ and 11.7 Hz, H_{2a}), 4.42 (m, H_3), 6.95 (d, $J = 12.0$ Hz, H_8), 8.50 (s, H_5, $T_1 = 0.85$ s) and those of the IS: 6.85 (dd, $J = 9.0$ and 8.9 Hz, H_3), 7.19 (ddd, $J = 3.0$, 4.0 and 8.9 Hz, H_4), 7.43 (dd, $J = 2.9$ and 2.9 Hz, H_6, $T_1 = 1.9$ s).

Quantitative analysis was conducted by comparing the H_5 signal of **III** with the H_6 signal of the IS. In the ^{19}F spectrum the IS signal appeared at 50.45 ppm (ddd, $J = 3.0$, 2.9 and 9.0 Hz; $T_1 = 0.90$) and that of the analyte at 44.01 ppm (d, $J = 12.0$ Hz; $T_1 = 1.25$).

By preparing 10 standard solutions in the usual way and recording the relative spectra, an average recovery of 99.7% (S.D. = 0.50, c.v. = 0.5%) in ^1H assay and of 99.8% (S.D. = 0.59, c.v. = 0.6%) in fluorine quantification were found. The equations in the linear regression analysis were: $y = -0.2002 + 1.006 \times$, $r = 0.999$, for ^1H and $y = -00536 + 0.980 \times$, $r = 0.988$, for ^{19}F; t-test = 0.726 (difference not statistically significant). Five lots of the commercial forms were prepared and analysed as previously described. An average recovery of 99.8% in the ^1H analysis and of 99.7% in ^{19}F were obtained.

The results obtained indicate that ^1H- and ^{19}F-NMR can be profitably used to quantify some fluoroquinolones from commercial forms. If sufficient care is taken to ensure quantitative instrumental conditions, the method is accurate and precise and provides a rapid and specific procedure with little sample preparation.

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