Spectrofluorimetric determination of moxifloxacin in tablets, human urine and serum

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A spectrofluorimetric method to determine the antibiotic moxifloxacin is proposed and was applied to pharmaceuticals, human urine and serum. The fluorimetric method allows the determination of 30–300 ng mL\(^{-1}\) moxifloxacin in aqueous solution containing phosphoric acid–phosphate buffer (pH 8.3) with \(\lambda_{\text{exc}} = 287\) nm and \(\lambda_{\text{em}} = 465\) nm. Detection and quantification limits were 10 and 30 ng mL\(^{-1}\), respectively, with a relative standard deviation (\(n = 10\)) of 2%. This method was applied to the determination of moxifloxacin in three Spanish commercial pharmaceutical formulations. Another variant of the method in micellar medium allows the direct measurement of moxifloxacin in human serum and urine by standard additions. The enhanced fluorescence of moxifloxacin in 8 mM sodium dodecyl sulfate (SDS) solution at pH 4.0 (acetic acid–acetate buffer) for \(\lambda_{\text{exc}} = 294\) nm and \(\lambda_{\text{em}} = 503\) nm shows the same linear range as the aqueous method with a 25% lower slope (with detection and quantification limits of 15 and 60 ng mL\(^{-1}\), respectively, and a relative standard deviation of 1.3%), but permits the background fluorescence for urine and serum blanks to be minimized. Hence, sufficient sensitivity is reached to determine therapeutic concentrations of the drug in urine (average recovery 102 ± 2%) and serum (average recovery 105 ± 2%) samples.

Introduction

Moxifloxacin (1-cyclopropyl-7-[(S,S)-2,8-diazabicyclo[4.3.0]-non-8-yl]-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolone-carboxylic acid) is a new 8-methoxyquinolone with enhanced activity in vitro against Gram-positive pathogens and maintenance of activity against Gram-negative organisms. It is active against common, less common, and atypical respiratory tract pathogens.\(^1\)

The drug is rapidly absorbed, with peak plasma concentrations reached within 1–4 h after treatment, and the long half-life (11.4–15.6 h) makes it suitable for once-daily administration. Moxifloxacin appears promising for the treatment of respiratory tract infections caused by common bacterial species.\(^2\)

Moxifloxacin is administered to patients in 400 mg day\(^{-1}\) doses. Final concentrations in serum and urine of treated patients are 2–5 and 30–60 \(\mu\)g mL\(^{-1}\), respectively.\(^3\)

Only a few reports on moxifloxacin determination have appeared in the literature. Bioassays and high performance liquid chromatography (HPLC) have been used in clinical and pharmacokinetic studies.\(^4,5\) From an analytical point of view only three papers concerning the use of HPLC\(^6,7\) and capillary electrophoresis\(^8\) for moxifloxacin determination have been found.

This work constitutes the first stage of a research project focused on the development of new analytical procedures for the determination of moxifloxacin that are less complex and faster than the HPLC methods and which do not require the use of organic solvents. This paper describes the spectrofluorimetric and micelle-enhanced spectrofluorimetric determination of moxifloxacin in spiked urine and serum and also its determination in three Spanish commercial pharmaceutical formulations first introduced onto the market in 2000.

Experimental

Reagents

Moxifloxacin and Actira® were kindly provided by Bayer. Octegra® was kindly provided by Vita Laboratories. Proflox® was kindly provided by Esteve Laboratories. Concentrated acetic acid, sodium acetate, sodium hydroxide, phosphoric acid, sodium phosphate and hydrochloric acid were of analytical-reagent grade and purchased from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS), sodium dodecylbenzenesulfonate (NaDDB), Triton® X-100 and hexa-decyltrimethylammonium bromide (HDTAB) were purchased from Fluka (Madrid, Spain). High-purity water was obtained from a Millipore (Milford, MA, USA) Milli-Q Plus System. A stock standard solution of 400 \(\mu\)g mL\(^{-1}\) was prepared by dilution of the stock standard solution with high-purity water. Stock solutions were stable for several weeks at room temperature. Urine and serum samples consisted of pooled samples obtained from several healthy volunteers, spiked with a suitable amount of the drug. To adjust the pH of the solutions, 0.1 M acetic acid–sodium acetate buffer of pH 4.0 and 0.1 M phosphoric acid–sodium phosphate buffer of pH 8.2 were used.
**Apparatus**

The fluorescence intensity was measured on a Perkin-Elmer (Norwalk, CT, USA) LS-5 luminescence spectrometer equipped with a xenon lamp and an Acer Model 1030 computer working with the FLUORPACK software from Sciware (Mallorca, Spain). All the measurements took place in a standard 10 mm pathlength quartz cell, thermostated at 25.0 ± 0.5 °C, with 5 nm band-widths for the emission and excitation monochromators.

The pH was measured with a Crison (Barcelona, Spain) microph 2002 pH-meter.

**Sample preparation**

The proposed procedure for the determination of moxifloxacin was applied to three Spanish commercial pharmaceutical formulations: Actira®, Octegra®, and Proflox® tablets. Also, moxifloxacin in spiked human urine and serum was determined.

**Tablet treatment.** The total contents of the tablet were weighed and ground to a fine powder using a pestle and mortar. The powder was dissolved in water, and the resulting solution was filtered through an ordinary filter-paper and diluted to the mark in a 1 L calibrated flask. Suitable aliquots from this solution were taken for the determination of moxifloxacin by fluorescence measurement (0.030–0.300 µg mL⁻¹).

**Urine and serum treatment.** Urine and serum were spiked with appropriate amounts of moxifloxacin stock solution. The final moxifloxacin concentrations were in the range 30–60 and with appropriate amounts of moxifloxacin stock solution. The samples (final moxifloxacin content about 0.03-0.30 respectively, 0.5 and 2.5 mL aliquots of working prepared formed as follows: Into 25 mL calibrated flasks were pipetted, for at least 24 h. The prepared solutions remain stable in the sample was determined from a calibration graph prepared nm against a blank solution. The concentration of moxifloxacin was measured at 465 nm using an excitation wavelength of 287 nm against a blank solution. The solutions were thermostated at 25 ± 0.1 °C and the fluorescence was measured on a Perkin-Elmer LS-5 luminescence spectrometer and an Acer Model 1030 computer.

**Spectrofluorimetric determination of moxifloxacin**

For moxifloxacin determination in aqueous solution, aliquots of working solutions of moxifloxacin were pipetted into 25 mL calibrated flasks after which 5 mL of 0.1 M phosphoric acid–sodium phosphate buffer (pH 8.2) were added and the solution was diluted to the mark with high-purity water. The moxifloxacin concentration range was 0.030–1.2 µg mL⁻¹. The solutions were thermostated at 25 ± 0.1 °C and the fluorescence was measured at 465 nm using an excitation wavelength of 287 nm against a blank solution. The concentration of moxifloxacin in the sample was determined from a calibration graph prepared under identical conditions. The prepared solutions remain stable for at least 24 h.

Moxifloxacin determination in serum and urine was performed as follows: Into 25 mL calibrated flasks were pipetted, respectively, 0.5 and 2.5 mL aliquots of working prepared samples (final moxifloxacin content about 0.03-0.30 (µg mL⁻¹), followed by 5 mL of 0.1 M acetic acid–sodium acetate buffer (pH 4.0) and 2.5 mL of 0.15 M SDS solution and the mixtures were diluted to the mark with water. The solutions were thermostated at 25 ± 0.1 °C and the fluorescence was measured at 503 nm using an excitation wavelength of 294 nm against a blank solution. The moxifloxacin concentration in serum and urine was determined by the standard additions method.

**Results and discussion**

**Fluorescence properties of moxifloxacin aqueous solution**

Fig. 1 summarizes the observed optimum excitation and emission wavelengths of moxifloxacin in aqueous solution at several pH values obtained by recording the spectra. The maximum fluorescence intensity was found with λ_{exc} = 287 nm and λ_{em} = 465 nm at pH 8.2 corresponding to the carboxylic form of the moxifloxacin carboxylic group (the pK_{a} was found to be between approximately 6.0 and 7.0 by spectrophotometric measurements). When the pH was lower than 6.0 (carboxylic group neutral form), λ_{exc} and λ_{em} shifted to 294 and 503 nm, respectively, and the fluorescence intensity was 50% lower. Accordingly, λ_{exc} = 287 nm and λ_{em} = 465 nm were used in further studies in aqueous medium.

The influence of pH on the fluorescence intensity of 1 µg mL⁻¹ moxifloxacin solution at selected wavelengths is shown in Fig. 2A. The fluorescence intensity exhibits a maximum within the pH range 8–9. Thus, a phosphoric acid–sodium phosphate buffer of pH 8.2 was chosen for the spectrofluorimetric determination of moxifloxacin.

The effect of the buffer solution added was studied. The fluorescence intensity remains stable with less than 20 mL of 0.1 M buffer solution for a 25 mL total volume. Accordingly, a 5 mL aliquot of 0.1 M buffer solution in a 25 mL total volume was selected as a suitable volume for the recommended procedure.

With the aim of studying the influence of the ionic strength, aqueous solutions of moxifloxacin containing buffer at various concentrations of KCl were prepared. The results showed no fluorescence intensity variations for concentrations < 1 M KCl.

The influence of the temperature on the fluorescence intensity shows a nearly linear (negative) relationship between temperature and fluorescence intensity for moxifloxacin. When the temperature is decreased the fluorescence is enhanced, as expected, with a temperature coefficient of 1.06%. This value showed that internal conversion was probably the main non-radiative process. In further studies in aqueous medium, the influence of the temperature on the fluorescence intensity was examined. The results showed that fluorescence intensity decreases with increasing temperature. The temperature coefficient of fluorescence intensity was found to be 1.06% per °C in aqueous solutions of moxifloxacin containing buffer at various concentrations of KCl.

**Fig. 1** Excitation (A) spectra (λ_{em} = 465 nm) and emission (B) spectra (λ_{exc} = 287 nm) of moxifloxacin (0.2 µg mL⁻¹) in aqueous solution at pH 3.0, 6.0, 7.5 and 9.0.

**Fig. 2** Influence of pH on the fluorescence intensity for 1 µg mL⁻¹ moxifloxacin. A: Aqueous solution (λ_{exc} = 287 nm; λ_{em} = 465 nm). B: Micellar media (λ_{exc} = 294 nm; λ_{em} = 503 nm).
Study of the emission features of the moxifloxacin micelle-enhanced fluorescence

The fluorescence of moxifloxacin in micellar media was studied, by preparing 1 mg mL$^{-1}$ moxifloxacin solutions with increasing concentrations of SDS, Triton® X-100, NaDDB and HDTAB at several pH values. A fluorescence enhancement (measurements performed at $\lambda_{\text{exc}} = 294$ nm and $\lambda_{\text{em}} = 503$ nm) was found only for SDS addition and a pH lower than 6.0. The fluorescence intensity increased with SDS concentration, until it reached a stable level with a 8 mM concentration of SDS (Fig. 3). Further additions of SDS provoked no increment of fluorescence intensity; thus, a 10 mM SDS concentration was selected as a suitable concentration. As can be seen in Fig. 2, the influence of pH on the micelle-enhanced fluorescence intensity shows a maximum for a pH between 2 and 5 (Fig. 2B), and pH 4.0 was selected for further measurements in micellar medium. This shift in optimum pH value suggests that moxifloxacin was solubilized in SDS micelles only when the carboxylic group was neutral, with the optimum $\lambda_{\text{exc}}$ and $\lambda_{\text{em}}$ corresponding to the values found for this form (Fig. 1).

The fluorescence intensity improvement due to SDS addition partially compensates for the decrease caused by working in a non-optimum pH range (4.0 instead of 8.2), but in principle a micellar medium represents no advantage because the final fluorescence is still lower than that found in aqueous medium. However, a micellar medium was demonstrated to be more suitable for urine and serum moxifloxacin determinations, as fluorescence blanks were minimal under these working conditions. Nevertheless, the method of choice for pharmaceutical samples is that in aqueous media, which ought to be simpler and more sensitive.

Analytical figures of merit

Linearity of the response. For the aqueous fluorescence method, a series of 15 standard solutions (three replicates for each) of moxifloxacin were measured by following the procedure described under Experimental. Table 1 summarizes the results obtained from a statistical analysis of the data. The calibration graph of fluorescence intensity ($Y$ versus moxifloxacin concentration expressed in $\mu$g mL$^{-1}$) ($X$) was found to be linear in the range 0.030-0.300 $\mu$g mL$^{-1}$ [eqn. (1)].

For the micelle enhanced fluorescence method, a series of moxifloxacin standard solutions (three replicates) were prepared as indicated in the procedure described under Experimental. The response was found to be linear in the same range, viz., 0.030-0.300 $\mu$g mL$^{-1}$ [eqn. (2)] but with a 25% lower slope than that found with aqueous fluorescence method (Table 1).

Detection and quantification limits and precision. Table 1 summarizes the detection and quantification limits found for the aqueous and micelle-enhanced fluorescence methods, calculated according to the Analytical Methods Committee. As can be seen, the aqueous fluorescence method shows better detection and quantification limits than the micelle-enhanced fluorescence method.

Ten replicates of target solutions of 150 ng mL$^{-1}$, prepared on different days within a period of 1 month, were determined by using the proposed aqueous and micellar methods. The results were 150 ± 3 and 149 ± 2 ng mL$^{-1}$, respectively, with between-day relative standard deviations (RSD) of 2 and 1.3%, respectively. The described HPLC method shows a better detection limit (2 ng mL$^{-1}$) but a worse RSD (<8%).

Interference studies

The interference of foreign compounds (soluble excipients used in Octega®. Actira® and Proflax® tablets, and some typical co-administered drugs) was studied by adding increasing concentrations of these compounds to a 0.30 $\mu$g mL$^{-1}$ moxifloxacin solution until a greater than 5% variation in fluorescence intensity was achieved. Table 2 shows the maximum tolerable weight ratio for these compounds when using both the aqueous and micellar fluorescence methods. As can be seen, the selectivity achieved by the aqueous method is better than, or similar to, that of the micellar fluorescence method, but both have sufficient selectivity to determine moxifloxacin in the presence of most of the compounds tested.

Table 1 Calibration equations [eqn. (1) and (2)] for moxifloxacin in aqueous and micellar solutions (results from least-squares regression)

<table>
<thead>
<tr>
<th>Concentration range/(\mu\text{g mL}^{-1})</th>
<th>Intercept on the ordinate ((a))</th>
<th>Standard deviation of the intercept on the ordinate ((s_a))</th>
<th>Slope ((b))</th>
<th>Standard deviation of slope ((s_b))</th>
<th>Number of points ((n))</th>
<th>Correlation coefficient ((r))</th>
<th>Detection limit/(\mu\text{g mL}^{-1})</th>
<th>Quantification limit/(\mu\text{g mL}^{-1})</th>
<th>RSD (%) ((n = 10))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous solution eqn. (1)</td>
<td>0.03–0.30</td>
<td>0.6</td>
<td>260</td>
<td>2</td>
<td>15</td>
<td>0.999</td>
<td>10</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>Micellar solution eqn. (2)</td>
<td>0.03–0.30</td>
<td>0.1</td>
<td>195</td>
<td>1</td>
<td>15</td>
<td>0.999</td>
<td>15</td>
<td>60</td>
<td>1.3</td>
</tr>
</tbody>
</table>

*Calculated according to the Analytical Methods Committee.

Table 2 Tolerance of different compounds in the determination of 0.3 $\mu$g mL$^{-1}$ moxifloxacin by aqueous and micellar fluorescence methods

<table>
<thead>
<tr>
<th>Compound added</th>
<th>Aqueous fluorescence</th>
<th>Micellar fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, lactose, fructose, saccharose</td>
<td>2000*</td>
<td>2000*</td>
</tr>
<tr>
<td>Imidemem</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Amilakina</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Sulbactam</td>
<td>40*</td>
<td>10</td>
</tr>
<tr>
<td>Penicillin</td>
<td>40*</td>
<td>20</td>
</tr>
<tr>
<td>Aspirin</td>
<td>30*</td>
<td>20</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

*Maximum weight ratio tested.
Moxifloxacin fluorescence was also measured in the presence of some cations occurring typically in urine and serum samples. For the aqueous fluorescence method Ca$^{2+}$, Na$^+$, Al$^{3+}$, K$^+$ and Mg$^{2+}$ do not cause interference at molar ratios of cation:moxifloxacin > 60. Zn$^{2+}$ has a negative interference at molar ratios of cation:moxifloxacin > 30. Fe$^{3+}$ interferes negatively at molar ratios > 10 (due to the formation of a coloured co-ordination complex between Fe$^{3+}$ and moxifloxacin). The micellar fluorescence method shows similar tolerance values except for Al$^{3+}$ and Fe$^{3+}$ (which interfere at molar ratios > 20 and > 3, respectively).

### Analysis of pharmaceutical samples

A tablet of each of the commercial pharmaceuticals Octegra®, Actira® and Proflox® (with a nominal moxifloxacin content of 400 mg) was treated according to the procedure described under Sample preparation. The prepared samples were analysed by the aqueous solution fluorescence method (five replicates). The results obtained were compared with those obtained using an independent method (direct spectrophotometric determination at pH 4.0 at 292 nm). Table 3 summarizes the results obtained for Octegra®, Actira® and Proflox® tablets. As can be observed there is a good agreement between the two methods (statistically proved according to the paired $t$-test\(^{11}\)), and excellent concordance between the nominal and experimental moxifloxacin contents. The fluorescence method was found to be 100 times more sensitive than the spectrophotometric method.

### Analysis of spiked urine and serum

Urine and serum blanks show a noticeable fluorescence with $\lambda_{\text{exc}} = 287$ nm and $\lambda_{\text{em}} = 465$ nm at pH 6.5–8.5. This fluorescence blank decreases to less than 30% of its original value with $\lambda_{\text{exc}} = 294$ nm and $\lambda_{\text{em}} = 503$ nm in micellar medium at pH 4.0. Thus, the micelle-enhanced fluorescence method was chosen as the more convenient for moxifloxacin determination in these samples.

Urine samples were prepared as described under Experimental. In order to avoid matrix effects affecting the results obtained by applying external calibration, the standard additions method was used. The average recovery obtained for five replicates was $102 \pm 2\%$. For serum samples (prepared as described under Experimental), the standard additions method was also used, obtaining a final recovery of $105 \pm 2\%$ (five replicates). These values were similar to those found in the literature for the HPLC method.\(^6\)

### Conclusions

Fluorescence measurement is a simple, accurate, fast and precise procedure to determine moxifloxacin in aqueous solution. The method was applied to the determination of the active constituent in three commercial pharmaceuticals, obtaining an excellent concordance between the experimental results and the nominal values. The fluorescence method was found to be 100 times more sensitive than spectrophotometric measurement.

The micelle-enhanced fluorescence method minimized blank signals for both urine and serum samples. Thus, this method was applied to moxifloxacin determination in these biological fluids, spiked at levels found after drug administration at normal clinical doses, without the need for complex pre-treatment. The recommended procedure is an excellent and easier alternative to conventional determinations made by HPLC,\(^6\) with an equivalent sensitivity.

### Acknowledgements

The authors acknowledge Bayer for supplying moxifloxacin and Actira®, Vita Laboratories for supplying Octegra® and Esteve Laboratories for supplying Proflox®.

### References


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### Table 3

Results of moxifloxacin determination in the pharmaceuticals Octegra®, Actira® and Proflox® by fluorescence and spectrophotometric methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fluorescence method</th>
<th>Spectrophotometric method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moxifloxacin content/mg</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Octegra®</td>
<td>405</td>
<td>0.5</td>
</tr>
<tr>
<td>Actira®</td>
<td>402</td>
<td>0.3</td>
</tr>
<tr>
<td>Proflox®</td>
<td>400</td>
<td>0.4</td>
</tr>
</tbody>
</table>