Determination of Norfloxacin in Human Urine Samples using Spectrofluorimetry Coupled with PARAFAC

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Abstract

Norfloxacin (NOR) is widely used in human and animal therapy, and its main route of excretion is urine. Several methods were employed to determine NOR in tablets or biological samples. Generally, in biological matrices it is performed a dilution and/or spiking of the sample. In the literature, there are some references that focus specifically the modeling using Parallel Factor Analysis (PARAFAC). Based on this, it was developed a methodology to identify and quantify NOR in urine by spectrofluorimetry coupled with PARAFAC. All urine samples were obtained from eight healthy individuals and before spiking these samples with NOR, they were diluted in two different levels (500 and 1000 times). Excitation-emission matrix (EEM) fluorescence spectra were obtained in the range from 250 – 320 nm (step of 2 nm) for excitation and 330 – 500 nm (step of 1 nm) for emission. Limit of detection (LOD) was 11.4 μg.L⁻¹ and limit of quantification (LOQ) was 34.7 μg.L⁻¹ (urine was diluted 500 times) and LOD was 8.4 μg.L⁻¹ and the LOQ was 25.6 μg.L⁻¹ (urine was diluted 1000 times). The analytical curves provided the following coefficients of correlation equal to \( r = 0.9926 \) and \( r = 0.9947 \) for urine diluted 500 times and urine diluted 1000 times, respectively.

Keywords: Parallel Factor Analysis; Spectrofluorescence; Urine samples; Pharmaceutical products; Second Order Calibration

1. Introduction

According to Carrillo (2008) fluoroquinolones are bactericide antimicrobials medicaments with excellent activity against a large aerobic bacterial spectra. They are an effective agent against infection treatment of respiratory and urinary system. There is a high interest of the pharmaceutical industry in the development of new fluoroquinolones. Gómez (2005), related that fluoroquinolones can be distributed in four categories: First generation (quinolones) that are used exclusively as the urinary antiseptic because they do not reach enough serum levels, and they are...
eliminated in the urine in its active form. The second generation (monofluoratefluoroquinolones) that has an activity more powerful than quinolones. Third generation (bi or trifluoratedfluoroquinolones) that possess a wide antibacterial spectra and in some cases includes Gram-positive and anaerobic bacteria and fourth generation that lead to a better activity related to Gram positive bacteria and vigorous activity against anaerobic bacteria.

Shao et al (2007) presented in their work that Norfloxacin (NOR) is a second generation fluoroquinolone (Fig 1) that shows high antimicrobial activity against a large variety of Gram negative and positive bacteria. According to Borrego et al (1997) it is widely used for human and animal treatment being urine its main route of excretion.

![Fig. 1. Structural formula of Norfloxacin(Borrego et al, 1997)]

Lachter et al (2009) related that the NOR is a zwitterionic fluoroquinolone in neutral pH. In its chemical formula, there are two functional groups corresponding to two chemical equilibrium of ionization: carboxylic group (position three) and the amine group. As can see in Fig 2, NOR is positively charged in acid pH and negatively charged in basic pH, because the presence of carboxylic and amine groups.

![Fig. 2. Protonation and deprotonation of fluoroquinolones]
Because of it, according Shao et al 2007, in acid pH near 4, for example, carboxylic group still does not suffer dissociation what only occurs in pH weakly acid (near pH 6 – close to pKa). In the same way, in alkaline pH, occurs the deprotonation of piperazine and in high pH values (close to pH 9), NOR is in anionic way, and for values higher than pH 9, NOR fluorescence virtually extinguish itself. Several methods are employed to determine NOR in capsules or biological samples such as high performance liquid chromatography (HPLC), fluorimetry and mass spectrometry. The main benefits of HPLC are the short time and sensitivity.

Carlucci (1998) said that due to shortly required time for analysis using HPLC and its enormous potential for separation and/or antibiotic detection, a lot of researches focused in technique for determination of a lot of biological fluids of some fluoroquinolones such as norfloxacin, ofloxacin, ciprofloxacin, perflloxacin, amiflaxacin, fleroxacin, difloxacin, lomeflaxacin and rufloxacin. Kassab et al (2005) developed a methodology for determination of ciprofloxacin and norfloxacin in pharmaceutical samples using HPLC. Aucélio et al (2009) performed a sequential determination of Norfloxacin and Levofloxacin using synchronous scanning room temperature phosphorimetry and Th (IV) as a selective signal inducer in aqueous standard and tablets. Bedor et al (2007) developed and validated method for NOR quantification by HPLC and after applied pharmacokinetic studies compared in human volunteers. It was published an extended revision containing 270 references about analytical methods for determination of quinolones and fluoroquinolones, until 1999, including NOR. According to Souza (2008), NOR determination is stated in United States of America Pharmacopeia (USP) being these official methods based in UV-Vis absorption spectrophotometry or by potentiometric titration for NOR determination in pharmaceutical formulations.

According to Shao et al (2007), the progress in chemiluminescence analysis by flow injection has been fully paid attention in pharmaceutical analysis thanks to high sensitivity, agility and ease of the method. For NOR determination, different chemiluminescence systems have been proposed, including Ce (IV) – Na2SO3, Tb (III), - nitrate and H2O2 – luminol. Capillary electrophoresis is extensively used for tablets determination, but all studies have been limited to separation of few quinolones and fluoroquinolones. An investigation has been made for determination of ciprofloxacin. Dubayová et al (2003) related that to identify a substance among a lot of others in a complex mixture such as urine it is necessary to using time consuming methods such as separation, purification and chemical modification. According to Trevisan (2003) when molecular spectrofluorescence is used a problem that can happen is spectra overlapping usually when biological matrices are involved because they possess a strong background fluorescence spectrum with a lot of interferents. Caused by that it is necessary to use chemometric tools (multivariate calibration) where all spectra is used for method developing.

2. Chemometric theory

2.1. Second order calibration

According to Booksh et al (1995), the general process of calibration consists in two steps: modeling, that establishes a mathematical relationship between matrices X and Y during calibration and validation of the model that betters the analyte description. As stated by Bohoyo (2005), calibration is a procedure that relates instrumental measure with regard analyte concentration that comprises a calibration and prediction step. In calibration step instrumental signal is obtained for a set number of samples in which analyte concentration is known – standards. Following that, in the
prediction step the instrumental signal of the sample of unknown concentration is obtained, and using a defined mathematical model provided in the calibration step it is calculated. Calibration methods can be classified as linear or not linear, direct or indirect and classic or inverse and are described as mathematical or statistical process of information extraction. These calibration techniques can be univariate or multivariate due to data order. As related by Girón (2007), in second order calibration data is obtained from instruments that provide a matrix as an answer for each sample. An advantage of second order is the possibility of obtaining pure spectra for each sample component as well as the possibility of calibration, under certain circumstances, such as the presence of unknown interferent that does not need to be presented in the calibration step.

2.2. Parallel Factor Analysis (PARAFAC)
According to Bernardes (2009), PARAFAC is a decomposition method that can be compared with Principal Component Analysis (PCA) model. It was proposed in 1970 by Harshham, Carroll and Chang, whose named this method as Canonic Decomposition. Bohoyo (2005) mentioned that PARAFAC method consists in the decomposition of three or more structures. For the analysis of three-way data (second order data), the data decomposition is performed in triads or trilinear components (Fig 3). As stated by Saxberg and Kowalski (1979) instead of one loading and one score vector as PCA, each component consists in a score vector and two loading vectors.

Fig. 3. Geometric representation of PARAFAC model – decomposition of an arrangement of tridimensional data

In PARAFAC model, as related by Saxberg and Kowalski (1979) it is defined a generic element $x_{ijk}$ for tensor $X$ such as following:

$$X_{ijk} = \sum_{n=1}^{N} a_{ni} b_{nj} c_{nk} + E_{ijk}$$

(1)

where $N$ is the total number of chemical components that produces a response or signal; $E_{ijk}$ is an element of residual error of tensor $E$ (with same dimensions of $X$), $a_{ni}$, $b_{nj}$, $c_{nk}$ are element of the column vector $a_n$, $b_n$ and $c_n$ which corresponds to, relative concentrations [[1+1)x1], emissions

profiles (Jx1) and excitation profiles (Kx1), for each one of N components, respectively. Column vector \( a_n, b_n \) and \( c_n \) are associated in scores matrixes \( A \) (component relative concentration) and loadings matrices \( B \) and \( C \) (with normalized column).

One of the main inconvenient that can occur in PARAFAC modeling is selecting the adequate number of component. Caused by that PARAFAC application is not so easy when compared with others calibration methods. There are some methods to achieve this selection, but due to the complexity of this problem, they can not guarantee results in all circumstances. One of these methods is called as diagnostic of model consistency or as known as Core Consistency (CC) that is applied in three-way multivariate analysis especially in PARAFAC. This parameter expression can be observed following

\[
CC = 100 \left( 1 - \frac{\sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{k=1}^{K} (C_{i,j,k} - S_{i,j,k})^2}{\sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{k=1}^{K} (C_{i,j,k})^2} \right) \tag{2}
\]

where \( c_{i,j,k} \) is an element of matrix and \( S \) is the superdiagonal matrix containing values equals one in diagonal and zero in the other position for PARAFAC adjust.

2.3. Second order standard addition
Saxberg and Kowalski (1979) proposed an extension of standard addition method to multivariate data, the \textit{generalized standard addition method} (GSAM) that requires that analyte and interfering are in the sample in a sequential way. It causes a restriction in the method. However, Booksh et al. (1995) proposed an extension of this method for multidimensional data was denoted of \textit{second order standard addition method} (SOSAM) that used direct trilinear decomposition (DTD) as a way of decomposing data. Luna et al. (2012) used a spectrofluorometry method to determine drugs (Levofloxacin) in biological fluids coupled with PARAFAC and SOSAM. Caused by that it was possible to eliminate some initial steps for sample preparation what simplified the experimental procedure.

2.4 Coefficients of determination and correlation coefficient
According to Gemperline (2006), the coefficient of correlation (\( r \)) measures the linear association degree between two variables and the coefficient of determination (\( R^2 \)) indicates the percentage of variation that is explained by regression represents total variation. Therefore, the coefficient of determination describes the fraction of the sum of squares due to the factors in relation to the sum of squares corrected for the mean. Caused by that \( R^2 \) can be determined by the following equation

\[
R^2 = \frac{SS_{tot} - SS_{resid}}{SS_{tot}} \tag{3}
\]

where \( SS_{tot} \) is the total square sum of the model and \( SS_{resid} \) is the square sum of residual. The square root of the coefficient of determination reveals the multiple correlation coefficients. The sign of the correlation coefficient is given by the slope.
2.5. Figures of Merit

2.5.1. Limit of detection and quantification
Limit of detection (LOD) is the small concentration that can be detected but not necessary quantified while limit of quantification (LOQ) is the small concentration that can be quantified according to Valderrama et al. (2009)

\[
LOD = 3.3 \frac{x S}{s}
\]

(4)

\[
LOQ = 10 \frac{x S}{s}
\]

(5)

Where S is the angular coefficient of analytical curve and s is the standard deviation

2.5.2. Sensibility, analytical sensibility and selectivity
According to Valderrama et al. (2009), the sensibility corresponds to the portion of the signal responsible by increase of the unit of concentration of interest property. The analytical sensibility presents the method sensitivity in terms of concentration unit that is used being defined as the ratio of sensitivity and standard deviation of the signal reference. Finally, the selectivity is a measure of the overlap grade between signal of analyte and interferents present in the sample indicating the part of this signal that is lost by this overlap. All equations are presented below (in order that they were mentioned in text)

\[
SEN = \frac{1}{b}
\]

(6)

\[
\gamma = \frac{SEN}{\delta k}
\]

(7)

\[
SEL = \frac{n \hat{s}_{k,i}}{x_{k,i}}
\]

(8)

where b is a vector of the regression coefficient, \( \delta k \) is referent to the standard deviation of the reference signal estimated, n\( \hat{s}_{k,i} \) is the scalar value of net analytical signal for sample i, while x\( k,i \) is a vector of the instrumental response for sample i.

2.5.3. Precision
Massart et al. (2005) mentioned that the Horwitz curve could provide an indication of the precision to be expected of a newly developed method as a function of the concentration of the analyte. The precision is a measure for the size of random errors. It measures the dispersion around the mean result. Therefore, it requires the calculation of the standard deviation of the measurement results. In this work, the precision was expressed by the repeatability, which was obtained under repeatability conditions, meaning that the operator, the instrument and the laboratory were the
same, and the time interval was kept short. Horwitz et al. (1980) proposed to express the predicted relative standard deviation (%) as a function of concentration by the following relationship:
\[
\text{Predicted } RSD_R = \sigma_H = 2^{(1-0.5\log_{10} C)}
\]
where \(C\) is the concentration expressed as a dimensionless fraction.

### 3. Experimental Procedure

The point of this work was to quantify NOR in urine samples using spectrofluorometry determination and after this method was validated using HPLC.

#### 3.1. Determination of norfloxacin using spectrofluorescence measurements

NOR standard used in this research was purchased from Sigma – Aldrich (Steinheim, Germany) as aforementioned. The NOR stock solution was prepared with a mixture of high purity water and ethanol 95% UV/HPLC Spectroscopic Grade (Vetec, Brazil) 50:50. The NOR standard was weighted in an analytical balance (Mettler Toledo AL204, Switzerland). An ultrasound (Ultrasonic Clear Branson 2000) was used for better dissolution of analyte, and it also either was necessary to keep stock solution from light, because NOR is photosensitive. All solution was previously filtered using with a cellulose acetate (0.75 \(\mu\)m) filter. The final solution had its pH adjusted for pH = 6. For urine tests were used samples of eight different healthy people (both gender). The analysis was carried out using a spectrofluorimeter model Fluorat 02 from Panorama (Lumex, Russia) and the solution was put in a quartz cuvette of 1 cm of the optical path. The range of the analytical curve of NOR spiked in urine was 0 – 500 \(\mu\)g. L\(^{-1}\). For each sample was performed an analytical curve, and it was investigated two dilution factor (500 and 1000 times).

Table 1 showed how each solution was prepared. For excitation wavelength, it was chosen to work in a range from 250 up to 320 nm and for emission wavelength was chosen to work in a range from 330 up to 480 nm.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Range of concentration ((\mu)g.L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 – 150</td>
</tr>
<tr>
<td>2</td>
<td>50 – 200</td>
</tr>
<tr>
<td>3</td>
<td>100 – 250</td>
</tr>
<tr>
<td>4</td>
<td>150 – 300</td>
</tr>
<tr>
<td>5</td>
<td>200 – 350</td>
</tr>
<tr>
<td>6</td>
<td>250 – 400</td>
</tr>
<tr>
<td>7</td>
<td>300 – 450</td>
</tr>
<tr>
<td>8</td>
<td>350 – 500</td>
</tr>
</tbody>
</table>

For each patient beyond analyzing solution spiked with NOR, it was made blank analysis (urine added buffer solution), and all analyzes were performed in triplicate. All patients chosen were not taking any kind of drugs. This procedure was adopted for avoiding undesirable effects in urine samples analysis (avoiding interfering of other drugs that could exhibit fluorescence presented in urine) and also to trying that all samples have a “similar” behavior.
3.2 Determination of norfloxacin using HPLC for validation

It was used an HPLC Model 1260 Infinity, Agilent Technologies, USA for carrying out the analysis. The column used was the model RP18 S 250 x 4.6 mm (5 μm) – Microsorb 100. Mobile phase was composed by water, methanol (HPLC/Spectroscopic Grade) and triethylamine (Sigma – Aldrich). The solvent proportion used in the mobile phase was 50:50:0.3, respectively. The pH of the final solution was adjusted for 3.3 with phosphoric acid (Vetec, Brazil). The mobile phase flow rate was 1 mL/min, and the volume of injection was 20 μL and temperature used was 30°C (± 2). All solution were also filtered (aqueous solvent – cellulose nitrate filter of 0.45 μm, Sartorius Stedim Biotech and for organic solvent – Durapore PVDF of 0.45 μm/ hydrophobic). The UV/Vis detection was used at 279 nm. In this case, the concentration range was 0 – 15 μg.mL⁻¹.

4. Results and Discussion

4.1 Determination of norfloxacin by spectrofluorometry method

The presence of scattering light in the fluorescence spectra produced a detrimental effect in PARAFAC model; therefore, it was necessary to remove it because it would take leading to result different and unexpected because the scattering light would be modeling together with is extremely relevant for the model. The Fig. 4 represents the excitation-emission fluorescence matrix surface free of scattering light.

![Fig. 4. (a) Excitation-emission matrix surface without scattering of light of diluted urine 500 times (b) Excitation-emission matrix surface without scattering of light of diluted urine 1000 times (where: X axis represents Emission wavelength, Y axes represents Excitation wavelength and Z axes represent Intensity)](Image)

As same as in “pure” urine spectrum, it was obtained a spectrum of a sample of urine spiked with a stock standard NOR solution (300 μg.L⁻¹) and once again it was noticed the presence of scattering of light that it was removed as can see in Fig. 5.
In the beginning, the excitation spectra range was 250 – 320 nm, and emission spectra range was 330 – 480 nm. However, all spectra were cut off in the same region, in excitation and emission range. The original excitation spectrum was from 250 – 300 nm and emission spectrum was from 380 – 480 nm. Caused by that the matrix for each sample passed from 36 x 151 (excitation step: 2 nm and emission step: 1 nm) to 25 x 101 (number of excitation wavelength x number of emission wavelength) what reduced period of analysis what is desired.

The remotion of the scattering light from the excitation-emission matrix of the urine samples was made because it caused a deleterious effect on the NOR spectrum. Lately, the PARAFAC was used to produce a deconvolution of the spectra, and it allowed identifying the NOR. The results on the Table 2 showed that, three component models were adequate for both dilution factors because the core consistency values were higher than 50% of the explained variance. For PARAFAC models with a superior number of components, the core consistency drops down to 23% or lower; it means that the noise was incorporated to the model. Therefore, the number of components was maintained in two for both dilution factors models.

Table 2 Number of components obtained by PARAFAC model

<table>
<thead>
<tr>
<th>Dilution Factor</th>
<th>Number of component</th>
<th>Variance (%)</th>
<th>Core Consistency</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>2</td>
<td>99.68</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>99.95</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>99.98</td>
<td>&lt;0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>99.69</td>
<td>91</td>
</tr>
<tr>
<td>1000</td>
<td>3</td>
<td>99.96</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>99.98</td>
<td>23</td>
</tr>
</tbody>
</table>
In Fig. 6 it is possible to observe PARAFAC overlap excitation loadings for urine diluted 1000 times and in Fig. 7 it is possible to observe PARAFAC overlap emission loading for urine diluted with the same dilution.

**Fig. 6.** Excitation loadings of PARAFAC (Load 1: NOR in urine; Load 2 and 3: some compounds in urine; NOR A.S. – spectrum overlap of NOR aqueous standard)

**Fig. 7.** Emission loadings of PARAFAC (Load 2 and 3 – compounds in urine; Load 1 – NOR in urine; NOR A.S. – Overlap of NOR aqueous standard spectra)
The pseudo-univariate linear regression curve was created with PARAFAC scores values associated with predicted NOR concentration values (Fig. 8).

**Fig. 8.** Predicted concentration x Scores PARAFAC for urine diluted 1000 x

In addition, it is common to use the analysis of residuals plot as a diagnostic tool to evaluate the model. The residual denotes the difference between the observed value and the value estimated by the model. In the case of a valid model, the residuals explain the random error of the regression model. In Fig 9, it is possible to observe residual values. Therefore, it could be concluded that the variances of y values were similar for all x values, i.e., they are homoscedastic, and the residuals were, then, independent, normally distributed and homoscedastic.
Caused by that based on Table 3 values, it is possible to say that there is a strong correlation between predicted NOR concentration and PARAFAC scores for urine samples.

**Table 3** Data of PARAFAC model for urine

<table>
<thead>
<tr>
<th>Factor of dilution</th>
<th>Equation of model</th>
<th>$R^2$</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>$Y = 3.87x + 49.77$</td>
<td>0.9852</td>
<td>0.9926</td>
</tr>
<tr>
<td>1000</td>
<td>$Y = 3.50x + 45.14$</td>
<td>0.9995</td>
<td>0.9947</td>
</tr>
</tbody>
</table>

where $Y$ represents the predicted NOR concentration and $x$ denotes the PARAFAC scores.

Based on the previous table, it can be noticed that when urine was diluted 1000 times the results were slightly better than diluted urine samples 500 times.

The initial thought was putting the calibration group (standard aqueous NOR) together with spiked urine sample group for creating a tensor. After performing the deconvolution of spectra using PARAFAC scores, the analytical curves were created. However, when plot was built it was noticed there were two groups of data as following (Fig. 10)
According to this previous figure, it was noticed that calibration proposed group consisted in a NOR standard aqueous curve and spiked urine samples another one. In order to circumvent this problem, it was decided to remove calibration group that was composed only by standard aqueous NOR. After this, the new tensor composed exclusively by urine and spiked urine samples was deconvoluted for each urine sample. The figures of merit (FOM) were obtained and can be seen in Table 4.

### Table 4 Figures of merit obtained for norfloxacin using PARAFAC scores

<table>
<thead>
<tr>
<th>FOM</th>
<th>Diluted urine 500 X</th>
<th>Diluted urine 1000 X</th>
</tr>
</thead>
<tbody>
<tr>
<td>REP (%)</td>
<td>16.3</td>
<td>12.6</td>
</tr>
<tr>
<td>SEN (FU L $\mu$g$^{-1}$)</td>
<td>0.020</td>
<td>0.022</td>
</tr>
<tr>
<td>SEL</td>
<td>0.087</td>
<td>0.088</td>
</tr>
<tr>
<td>SENA</td>
<td>0.29</td>
<td>0.39</td>
</tr>
<tr>
<td>LOD ($\mu$g.L$^{-1}$)</td>
<td>11.4</td>
<td>8.4</td>
</tr>
<tr>
<td>LOQ ($\mu$g.L$^{-1}$)</td>
<td>34.7</td>
<td>25.6</td>
</tr>
</tbody>
</table>

Based on values from Table 4, it is possible once again to accept the model gets better with the increasing of dilution. The precision was expressed by repeatability. It was lower than the predicted relative standard deviation ($\sigma_{R}$), according to Horwitz, for this level of concentration. It can be observed that the sensitivity and selectivity not showed a significance difference for both dilutions. The LOD and LOQ are minor with a higher dilution as expected because the dilution decreases the interference of matrix. It is also better because is possible to determine NOR in urine samples with the dilution factor of 1000 times.

4.2 Validation of the methodology for determination of norfloxacin by HPLC

In Fig. 11, it is possible to observe a chromatogram of NOR standard with 10 mg. L$^{-1}$. 
The first peak that appears is attributed to the mobile phase and the second peak was attributed to NOR. It was proved when was performed an analysis only with mobile phase. In urine samples, it was worked with two dilution factors (500 and 1000 times), and it was spiked different amounts of NOR (5, 10 and 15 mg.L⁻¹) and the blank solution was only diluted urine. In Fig. 12, it is possible to observe a chromatogram of 1000 x diluted urine spiked with 15 mg.L⁻¹ of NOR.

Table 5 shows results for the analytical curves obtained for norfloxacin determination in urine using HPLC.

**Table 5** Results of NOR determination in urine using HPLC

<table>
<thead>
<tr>
<th>Dilution Factor</th>
<th>Model equation</th>
<th>R²</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>$Y = 141.56C - 164.35$</td>
<td>0.9990</td>
<td>0.9995</td>
</tr>
<tr>
<td>1000</td>
<td>$Y = 142.58C - 147.21$</td>
<td>0.9998</td>
<td>0.9999</td>
</tr>
</tbody>
</table>

where C represents the concentration of norfloxacin in mg.L⁻¹.
As it can be seen methodology used for norfloxacin, the quantification is useful for all patients \((R > 0.999)\). The dilution factor influences in results because the urine sample was more diluted offered better results when it compared with concentrated ones as expected. **Table 6** shows results of precision of HPLC methodology.

**Table 6** Results of precision for determination of norfloxacin using HPLC

<table>
<thead>
<tr>
<th>Expected Concentration (mg.L(^{-1}))</th>
<th>Predicted Concentration (mg.L(^{-1}))</th>
<th>Residual Standard Deviation (RSD,%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5.04 ± 0.07</td>
<td>1.38</td>
</tr>
<tr>
<td>10</td>
<td>9.90 ± 0.14</td>
<td>1.41</td>
</tr>
<tr>
<td>15</td>
<td>15.81 ± 0.72</td>
<td>4.55</td>
</tr>
</tbody>
</table>

In spite of the high performance liquid chromatography with UV detector needed sample more concentrated than spectrofluorometry technique, the results obtained for NOR, in urine samples, were adequate for both techniques. In addition, the figures of merit obtained showed good results for both methodologies. Therefore, it can be concluded that spectrofluorimetric determination using PARAFAC methodology can replace HPLC determination of NOR in urine samples.

**5. Conclusions**

It was possible to say that norfloxacin quantification using spectrofluorescence coupled with PARAFAC it is a potent method. Although in most of the cases, the technique most used is HPLC, spectrofluorescence also showed results with a good precision. The figures of merit were estimated showing once again this new method was acceptable. It is worth to mention that this new method offered the advantage of being faster in terms of time of analysis, because, in HPLC, it is needed a large time (at least one hour) only for stabilization of the column and also its cleaning process what is not needed for spectrofluorescence. In this technique, it is not necessary using organic solvents as methanol what in environmental terms it desirable. It is also worth to mention that the concentration range for spectrofluorescence was in order of \(\mu g. L^{-1}\) while for HPLC this range was in order of mg.L\(^{-1}\). Even urine is relatively complex matrix when second order multivariate calibration is used it is possible to produce deconvolution of spectra.

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