Spectrophotometric determination of some fluoroquinolones antibacterial drugs in pure form and in pharmaceuticals formulations

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Received: 7th August, 2008 ; Accepted: 12th August, 2008

ABSTRACT

A simple and sensitive spectrophotometric method has been developed for the determination of gatifloxacin (GT) and moxifloxacin (MX) in pure form and in pharmaceutical formulations. The proposed method was based upon the formation of a ternary complex between palladium(II), eosin and the studied drugs in the presence of methyl cellulose as a surfactant and acetate buffer of pH (4.0-4.5). The ternary complex showed an absorption maxima at 552 and 549 nm for GT and MX, respectively. Apparent molar absorptivities were $2.4975 \times 10^4$ and $3.663 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ and Sandell’s sensitivities were 0.015 and 0.011 μg cm$^{-2}$ for GT and MX, respectively. The solution of ternary complex obeyed Beer’s law over the concentration range of 2.0-10 and 1.0-8.0 μg ml$^{-1}$ for GT and MX, respectively with minimum detection limits 0.216 and 0.184 μg ml$^{-1}$ for GT and MX, respectively. The proposed method was successfully applied to the analysis of the studied drugs in their pharmaceutical formulations. The results obtained were in good agreement with those obtained using the reported method. A proposal of the reaction pathway was presented.

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INTRODUCTION

Quinolones have been found to possess an antibiotic property. Fluorinated 4-quinolone derivatives have a broad-spectrum antibacterial activity against many gram-positive and gram negative bacteria through inhibition of their DNA gyrase$^{[1]}$.

Gatifloxacin (GTF) [1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid] and Moxifloxacin (MXF) [1-cyclopropyl-7-[(1S,6S)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-4-oxo-3-quinoline-carboxylic acid] are new additions to the class of 4-fluoroquinolones carboxylic acid antibacterials that are widely used in the treating of respiratory trace and urinary trace infections$^{[2]}$. The structure of the cited drugs are shown in (TABLE 1).

No official (pharmacopoeia) method has been found for the assay of GTF and MXF in their formulations. However, several methods have been reported for their analysis including UV-visible spectrophotometry$^{[3-11]}$, spectrofluorometry$^{[12-14]}$, atomic absorption spectrophotometry$^{[15]}$, electrophoresis$^{[16-18]}$, chromatography$^{[19-25]}$, flow injection analysis$^{[26]}$ and electrochemical methods$^{[27-39]}$.

The above mentioned techniques are sensitive but expensive and require laborious clean up procedure prior to analysis. Spectrophotometry is the technique
of choice even today due to its inherent simplicity and therefore frequently used in the laboratories of the developing countries to overcome versatile analytical problem. This led us to study its reaction through ternary complex formation with eosin and palladium in an attempt to develop simple and sensitive spectrophotometric method for the determination of gatifloxacin (GT) and moxifloxacin (MX) in pharmaceutical preparations and biological fluids.

Ternary complex formation had been used for the determination of palladium (Pd(II)) via 1,10-phenanthroline as a cationic component and eosin as an anionic counter ion\cite{30}. On the same basis, Fujita et al.\cite{31} determined a group of drugs by forming ternary complex with Pd(II) and eosin. In their studies nine cations have been tried, Pd(II) proved to be the only effective metal ion.

Colour reactions of various drugs in aqueous media were investigated utilizing the ternary complex formation such as chlorpromazine, thiamine, lincomycin, ofloxacin and theophylline\cite{31}, ciprofloxacin and norfloxacin\cite{32}, astemizole, terfenadine and flunarizine hydrochloride\cite{33}, glliclazide\cite{34} and cepharpine Na\cite{35}.

This paper reports simple, sensitive and accurate spectrophotometric method for the determination of both antibiotics (GT and MX). The two methods are based on the chelate-forming ability of the carboxylic and carbonyl groups in these quinoline derivatives with palladium(II) with the subsequent formation of a ternary complex with eosin (sodium salt of 2,4,5,7-tetabromofluorescein) in the presence of methyl cellulose. The optimum conditions (temperature, pH, etc.) were established before the application of the methods to the analysis of the drugs as bulk or in tablet forms. Moreover, the reagents used in the proposed method are stable for at least one week.

## EXPERIMENTAL

Apparatus All absorption spectra were made using Kontron 930 (UV-Visible) spectrophotometer (German) with a scanning speed of 200 nm/min and a band width of 2.0 nm, equipped with 10 mm matched quartz cells. An Orion research Model 601A/digital ionic meter (Japan) was used for checking the pH values of a buffer solutions.

Materials and reagents All reagents and solvents were of analytical reagent grade.

- Gatifloxacin reference standard was provided by Bristol Myers Squibb Company Egypt, its potency was 99.6 ± 0.70% by HPLC method\cite{21}.
- Moxifloxacin reference standard was supplied by Bayer, Germany, its potency was 99.4 ± 0.92% by UV spectrophotometric method\cite{30}.
- The following commercial dosage forms were subjected to the analytical procedure: Tequin tablets (Bristol Myers Squibb Company, Egypt) labeled to contain 400 mg GT/tablet, Floxin tablets (Global Napi Co, Egypt) labeled to contain 400 mg GT/tablet and Avalox tablets (Bayer, Germany), labeled to contain 400 mg MX/tablet.
- Eosin (Merck, Darmstadt, Germany) was prepared as 2.0×10⁻³M, aqueous solution. The solution is stable for 2 weeks.
- PdCl₂ (Sigma, Milwaukee, WI, USA) was prepared as 2×10⁻⁴M solution by dissolving about 35.5 mg of PdCl₂ in 1 ml of hydrochloric acid, with the aid of heat, followed by the addition of 50 ml of boiled water and diluting to 100 ml with distilled water in a volumetric flask. This solution is stable for 2 weeks.
- Methyl cellulose (MC) (Prolabo, France) 1500 cP, 0.5% w/v aqueous solution, prepared by dissolving the appropriate amount in hot water (80°C) with stirring for 10 min, then chilling to 5°C for 30 min.
- Acetate buffer (pH values of 4.0 and 4.5), prepared by mixing 0.2 M acetic acid solution and 0.2 M sodium acetate solution, the pH has to be checked periodically\cite{36}.

### TABLE 2: Optical characteristics and statistical data of the regression equations for ternary complex formation with the studied drugs

<table>
<thead>
<tr>
<th>Commercial name</th>
<th>IUPAC name</th>
<th>Chemical formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gatifloxacin (GT)</td>
<td>1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl-4-oxo-3-carboxylic acid</td>
<td><img src="image" alt="Gatifloxacin structure" /></td>
</tr>
<tr>
<td>Moxifloxacin (MX)</td>
<td>[4,3,0][nony-8-yl]-6-fluoro-8-methoxy-4-oxo-quinoline-3-carboxylic acid</td>
<td><img src="image" alt="Moxifloxacin structure" /></td>
</tr>
</tbody>
</table>
A stock solution 100µg ml⁻¹ of drug was prepared by dissolving 0.01 g in 10 ml of bidistilled water and then diluted to the mark in a 100 ml calibrated flask. Working solutions of lower concentration were freshly prepared by appropriate dilution of the standard solution. The solutions were stable for at least one week if they had been stored in a cool (< 25°C) and dark place.

### General procedure

#### Calibration graph

Transfer aliquot volumes of GT and MX standard solutions into a series of 10 ml volumetric flasks. Add 1.5 ml of 0.5% MC solution, add 2 ml of acetate buffer pH (4.0 and 4.5 for GT and MX, respectively), followed by 1.0 ± 0.2 ml of eosin solution and 0.8 ± 0.1 ml of PdCl₂ solution for GT and MX, respectively. Heat at 60 ± 2°C for 10 and 15 min for GT and MX, respectively in a thermostatically-controlled water-bath, then cool for 5 min at 25°C. Complete to the mark with distilled water. Measure the absorbance of the solution at 551 and 549 nm for GT and MX against similarly prepared eosin-PdCl₂ solution (blank solution). Plot the measured absorbance vs. the final concentration to get the calibration curve. Alternatively, derive the corresponding regression equation.

#### Procedure for tablets

Weigh and pulverize 20 tablets. Transfer a weighed quantity of the powder equivalent to 25 mg of GT and MX into a small conical flask, extract with 3×30 ml of methanol. Filter the extract into 100 ml volumetric flask. Wash the conical flask with few milliters of methanol. Pass the washings into the same conical flask and complete to the mark with the same solvent. Transfer aliquot volumes covering the working concentration range over 2.0-10 and 1.0-8.0µg mL⁻¹ for GT and MX into 10 ml measuring flasks. Proceed as described under “Calibration graph”. Determine the nominal content of the tablets either from the calibration curve or using the corresponding regression equation.

### RESULTS AND DISCUSSION

The fact that the pyridonecarboxylic acid derivatives form very stable metal chelates with different cations suggested the possibility of the utilization of this phenomenon for increasing the sensitivity of spectrophotometric measurements for the determination of GT and MX in pure form, pharmaceutical preparations and biological fluids, through the formation of a stable ternary complex of acid-palladium(II) eosin (SCHEME 1). The ternary complex formed between the metal ion: electropositive ligand and organic base often have higher values of molar extinction coefficient than binary complexes of the same components. The formation of ternary complexes improves not only the sensitivity of the method but also the selectivity as well. The absorption spectra of the binary Pd(II)-(GT) or (MX) and ternary complexes drug Pd(II)-eosin formed were scanned in the range 500-650 nm. It was found that, on addition of GT or MX to the eosin-Pd(II) solution (solution B), a difference in absorbance from eosin Pd(II)-drug (solution A) in the presence of MC and at pH 4.0 and 4.5 producing a red color with maximum absorbance value at 551 and 549 nm for GT and MX, respectively (Figure 1).

### Optimization of the experimental conditions

The spectrophotometric properties of the colored product as well as the different experimental parameters affecting the color development and its stability were carefully studied and optimized. Such factors were changed individually while the others were kept constant. The factors include pH, type of buffer, type of metal cation, temperature, time of heating, effect of different sensitizers, different surfactants, concentration of surfactants, and concentrations of eosin and Pd(II).
Effect of pH

The influence of pH on the absorbance value of the ternary complex was studied at 551 and 549 nm for GT and MX, respectively. The absorbance of the drug-Pd(II)-eosin complex solution was investigated over the pH range 3.6-5.4. Maximum absorbance value was achieved at pH 4.0 and 4.5 for GT and MX, respectively using 2 ml of acetate buffer. Other buffers having the same pH value such as phosphate and Britton Robinson buffers were tried and compared with acetate buffer. Acetate buffer was found to be superior to phosphate and Britton Robinson buffers having the same pH value since the net absorbance value was highest in case of acetate buffer (Figure 2).

Effect of eosin and Pd(II) chloride

The effect of eosin and Pd(II) concentrations on the absorbance of the ternary complex was studied keeping the concentration of the drug and Pd(II) constant and varying eosin concentration, it was found that increasing the volume of eosin \((2 \times 10^{-3} \text{ M})\) resulted in a subsequent increase in the absorbance value of the ternary complex up to 0.8 ml, and remained constant up to 1.2 ml therefore, 1.0 \(\pm\) 0.2 ml which resulted in a final concentration of \(2 \times 10^{-4}\)M was used as the optimum concentration of eosin (Figure 3).

The effect of volume of Pd(II) on the absorbance value of the ternary complex was also studied keeping the concentration of the drug and eosin constant. It was observed that increasing the volume of Pd(II) \((2 \times 10^{-3} \text{ M})\) would result in a gradual increase in the absorbance of the ternary complex up to 0.7 ml and remained constant up to 0.9 ml after which the absorbance of the complex began to decrease. Thus, 0.8 \(\pm\) 0.1 ml of \(2 \times 10^{-3}\) M of Pd(II) which resulted in a final concentration of \(1.6 \times 10^{-4}\)M was used throughout this approach (Figure 4).

Effect of temperature and heating time

In order to examine the effect of temperature and heating time on the formation rate and on the absorbance of the drug-Pd(II)-eosin ternary complex, the experiment was carried out at different temperatures settings (room temperature, 40, 50, 60 and 70\(^\circ\)C) using a thermostated water-bath for periods ranging from 5 to 30 min. Maximum and constant absorbance value
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was obtained at 60°C after 10 and 15 min for GT and MX, respectively (Figure 5). The solution was cooled under tap water for 5 min to room temperature with agitation before measuring the absorbance to solubilize jelly-like aggregates formed upon heating at 60°C. As reported by other investigators, the reproducibility was somewhat poor on using hot solution[38]. The ternary complex formed remains stable for more than 2 h.

Effect of surfactants

The effect of surfactants on the absorbance of the ternary complex was examined using various dispersing agents, such as Benzalkonium chloride (BKC) or cetlypyridinium chloride (cationic), sodium lauryl sulfate (anionic) and Triton-X 100, Tween 20 and MC 1500 cP (non-ionic). Among the surfactants studied, best results were obtained in the presence of MC (1500 cP). Maximum and constant absorbance was obtained using 1.5 ml of 0.5% MC solution for GT and MX, respectively (Figure 6).

When the non-ionic surfactant MC was used, prior extraction steps were unnecessary. The addition of surfactants to solubilize and stabilize the ternary complex had been previously reported[31]. Cationic surfactants such as cetlypyridinium chloride decreased the color of the formed complex probably due to the formation of an ion-pair complex between eosin and the cationic surfactant. MC, which is a non-ionic water-soluble polymeric surfactant, was reported to be the best dispersing agent with respect to sensitivity[31], in addition, it increases the stability of the complex and prevent its precipitation, accordingly, MC was used in this study. The acid dissociation properties of eosin in the presence of MC were determined spectrophotometrically at ionic strength of 0.1 M at 20 ± 0.1°C[39,40].

Depending on the pH of the solution, eosin can exist in any of the following forms:

\[ \begin{align*}
    & H_3R \overset{K_{a1}}{\rightleftharpoons} H_2R^+ \overset{K_{a2}}{\rightleftharpoons} HR^- \overset{K_{a3}}{\rightleftharpoons} R^2
\end{align*} \]

where R denotes the anionic part of eosin. In this study, the pKa₁, pKa₂, pKa₃, in the presence of MC were 2.10, 2.85 and 4.95, respectively. At pH 4.3 about 80% of eosin was found to be in the form HR[41].

Figure 6: Effect of volume of (0.5%) MC (1500 cP) surfactant on the absorbance of the the ternary complex formed through reaction of 6.0µg ml⁻¹ of the studied drugs with eosin and Pd(II) chloride

Figure 7: (a) Continuous variation plots for drug : Pd(II) (5x10⁻³ M) complex ratio in the presence of excess eosin (1.5x10⁻³ M), V_i = Drug; V_m = Pd (II); (b) Continuous variation Plots for drug : Eosin (5x10⁻³ M) complex ratio in the presence of excess Pd(II), (1.5x10⁻³ M), V_i = Drug; V_m = Eosin; (c) Continuous variation plots for Pd(II) : Eosin (5x10⁻³ M) complex ratio in the presence of excess drug (2x10⁻³ M) V_i = Eosin; V_m = Pd (II)
Different sensitizers were also tried such as, fluorescein, rhodamine 6 G and quinine HCl. Eosin was found to be the best one, since it gave the highest and constant absorbance value of the complex. In the same manner various metals were studied to choose the most suitable one for formation of ternary complex, e.g. FeCl$_3$, CuSO$_4$, Pb(AC)$_2$ and PdCl$_2$, the latter was found to be the most suitable metal since it gave the highest absorbance reading.

**Composition of the ternary complex**

The nature of the ternary complex (drug-Pd(II) eosin) was determined using Job’s method of continuous variation$^{[42]}$. The results of applying this method can be summarized as follows: the Pd(II) : drug ratio in the presence of excess eosins was 1 : 1 (Figure 7a), while the eosin:drug ratio in the presence of any excess but constant amount of palladium(II) chloride was 1 : 1 (Figure 7b) and the Pd(II) : eosin ratio in the presence of excess drug was 1 : 1 (Figure 7c). Hence the composition of the ternary complex formed may be expressed as drug-Pd(II)-eosin (1:1:1) in the presence of methyl cellulose surfactant.

**Mechanism of the reaction**

The stoichiometry of the reaction was proceeds in the ratio of 1 : 1, confirming that one molecule of the drug denses with one molecule of PdCl$_2$. The drug reacts with the Pd(II) ion in the presence of eosin. Based on the obtained molar reactivity, the reaction pathway is proposed to proceed in SCHEME 1.

**Method validation**

**Linearity**

At described experimental conditions for GT and MX determination, standard calibration curves for GT and MX using the proposed method calibrations were constructed by plotting absorbances vs. concentrations. The linear regression equations, standard deviation, slopes and intercepts, correlation coefficients, relative standard deviation of response factors, and linearity ranges of 2.0-10 and 1.0-8.0 $\mu$g ml$^{-1}$ for GT and MX, respectively (TABLE 2) for the proposed spectrophotometric method. The molar absorptivities were calculated. Linear regression analysis of the data gave the following equation:

$$A = -2.32 \times 10^{-2} + 0.0714 C \text{ (r = 0.9999)}$$ for GT

$$A = -2.29 \times 10^{-2} + 0.0999 C \text{ (r = 0.9998)}$$ for MX

where $A$ is the absorbance in cm$^{-1}$ cell and $C$ is the concentration of the drug in $\mu$g mL$^{-1}$.

**Sensitivity**

The detection limit (LOD) for the proposed methods were calculated using the following equation$^{[37]}$:

$$\text{LOD} = \frac{3s}{k}$$

where $s$ is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and $k$ is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits obtained for the absorbance were found to be 0.216 $\mu$g ml$^{-1}$ (5.75 $\times$ 10$^{-7}$ M) and 0.184 $\mu$g ml$^{-1}$ (4.58 $\times$ 10$^{-7}$ M) for GT and MX, respectively.

The limits of quantitation, LOQ, defined as$^{[43]}$:

$$\text{LOQ} = 10 \text{ s / k}$$

According to this equation, the limit of quantitation were found to be 0.72 and 0.613 $\mu$g ml$^{-1}$ for GT and MX, respectively adopting the proposed method. It was determined by taking the concentration, which gives a reliable absorbance reading (0.028 and 0.38 A unit) for GT and MX, respectively, below which the calibration graph was not linear.

**TABLE 2: Optical characteristics and statistical data of the regression equations for ternary complex formation with the studied drugs**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GT</th>
<th>MX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction time (min)</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>pH</td>
<td>4.0</td>
<td>4.5</td>
</tr>
<tr>
<td>$\lambda_{max}$ (nm)</td>
<td>552</td>
<td>549</td>
</tr>
<tr>
<td>Beer’s conc. Range ($\mu$g ml$^{-1}$)</td>
<td>2.0-10</td>
<td>1.0-8.0</td>
</tr>
<tr>
<td>Rinbom conc. Range ($\mu$g ml$^{-1}$)</td>
<td>3.0-8.5</td>
<td>2.0-7.0</td>
</tr>
<tr>
<td>Detection limits ($\mu$g ml$^{-1}$)</td>
<td>(5.75 $\times$ 10$^{-7}$ M)</td>
<td>(4.58 $\times$ 10$^{-7}$ M)</td>
</tr>
<tr>
<td>Quantification limit ($\mu$g ml$^{-1}$)</td>
<td>0.72</td>
<td>0.613</td>
</tr>
<tr>
<td>Molar absorptivity (L mol$^{-1}$ cm$^{-1}$)</td>
<td>2.4975 $\times$ 10$^{-4}$</td>
<td>3.6634 $\times$ 10$^{-4}$</td>
</tr>
<tr>
<td>Sandell sensitivity ($\mu$g cm$^{-2}$)</td>
<td>0.015</td>
<td>0.011</td>
</tr>
<tr>
<td>Regression equation$^{[4]}$</td>
<td>$A = a + bC$, where $C$ is the concentration in $\mu$g ml$^{-1}$</td>
<td>$A = a + bC$, where $C$ is the concentration in $\mu$g ml$^{-1}$</td>
</tr>
<tr>
<td>Intercept (b)</td>
<td>-0.0232</td>
<td>-0.0229</td>
</tr>
<tr>
<td>Slope (a)</td>
<td>0.0714</td>
<td>0.0999</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9999</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

$^{[4]}$A = a + bC, where C is the concentration in $\mu$g ml$^{-1}$.
Accuracy and precision

The precision of the proposed method was evaluated by analyzing standard solutions of GT and MX. The results obtained by the proposed method were compared with those given by methods.[21,31]

Statistical analysis[43] of the results obtained by the proposed and official methods using the Student’s t-test and variance ratio F-test, showed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (TABLE 3). The selectivity of the method was investigated by observing any interference encountered from the excipients of the tablets. It was shown that these excipients did not interfere with the proposed method (TABLE 4).

Pharmaceutical applications

The proposed method was further applied to the determination of GT and MX in its tablets. Common tablets excipients such as talc, lactose, starch, avisil, gelatin and magnesium stearate did not interfere with the assay. The results obtained were compared with those giving using a reference method.[21,31]. Statistical analysis[21] of the results using Student’s t-test and variance ratio F-test, revealed no significant difference between the two methods at the 95% confidence level regarding accuracy and precision, respectively. The results obtained are abridged in (TABLE 4).

CONCLUSION

The proposed method has the advantages of being simple, sensitive and suitable for routine analysis in control laboratories. The proposed method is considered as stability indicating method, since the side of complex formation is expect to be the side of degradation (hydrolysis). The ternary complex formed did not require prior extraction procedure and have the advantages of being suitable for the determination of GT and MX in

### TABLE 3: Statistical analysis of the results obtained using the proposed method and reference method for analysis of authentic samples

<table>
<thead>
<tr>
<th>Statistic</th>
<th>GT</th>
<th>MX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery</td>
<td>99.99 ± 0.632</td>
<td>99.60 ± 0.70</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Variance</td>
<td>0.400</td>
<td>0.49</td>
</tr>
<tr>
<td>SE</td>
<td>0.258</td>
<td>0.313</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>0.632</td>
<td>1.04</td>
</tr>
<tr>
<td>t-value</td>
<td>0.93</td>
<td>0.714</td>
</tr>
<tr>
<td>F-value</td>
<td>1.227</td>
<td>2.015</td>
</tr>
</tbody>
</table>

a Mean ± SD, b Theoretical values for t and F-values at five degree of freedom and 95 % confidence limit are (t =2.262) and (F =5.19)

### TABLE 4: Application of the standard addition technique for the determination of the studied drugs in dosage forms

<table>
<thead>
<tr>
<th></th>
<th>Tequin tablets (400mg/tablet)</th>
<th>Floxin tablets (400mg/tablet)</th>
<th>Avalox tablets (400mg/tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taken</td>
<td>Added</td>
<td>Recovery %</td>
<td>Reference method</td>
</tr>
<tr>
<td>µg ml⁻¹</td>
<td>µg ml⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>--</td>
<td>99.89</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>--</td>
<td>99.95</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>100.40</td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>4.0</td>
<td>100.05</td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>6.0</td>
<td>100.20</td>
<td></td>
<td>6.0</td>
</tr>
<tr>
<td>8.0</td>
<td>99.75</td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>Mean</td>
<td>100.04 ± 0.232</td>
<td>99.90 ± 0.30</td>
<td>100.12 ± 0.601</td>
</tr>
<tr>
<td>SD</td>
<td>0.232</td>
<td>0.30</td>
<td>0.601</td>
</tr>
<tr>
<td>V</td>
<td>0.2324</td>
<td>0.30</td>
<td>0.601</td>
</tr>
<tr>
<td>R.S.D</td>
<td>0.2323</td>
<td>0.30</td>
<td>0.60</td>
</tr>
<tr>
<td>SE</td>
<td>0.0955</td>
<td>0.120</td>
<td>0.245</td>
</tr>
<tr>
<td>t-test</td>
<td>0.825</td>
<td>0.804</td>
<td>0.22</td>
</tr>
<tr>
<td>F-value</td>
<td>1.67</td>
<td>1.78</td>
<td>1.05</td>
</tr>
</tbody>
</table>

a Mean of three different experiments; b Comparison with the official method[26,48], Values in parenthesis are the theoretical t -and F-values for five degrees of freedom and 95% confidence limits.
pure form with minimum detection limit comparable to reported values. Moreover, it could be applied to the determination of different pharmaceutical dosage forms.

REFERENCES