Determination of Roflumilast in Pharmaceutical Formulations by Derivative Spectrophotometric Method

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Abstract

In this study, a simple, rapid, reliable and fully validated first order derivative UV spectrophotometric method was developed for determination of roflumilast in pharmaceutical formulations. The type of solvent, the degree of derivation, range of wavelength and N value were tested in order to optimize the analysis conditions. The quantitative determination of the drug was carried out using the first - derivative values measured at 220 nm (N = 4) in the wavelength range of 190 - 350 nm. The developed method was validated with respect to stability, linearity, sensitivity, specificity, precision, accuracy, robustness and ruggedness. The linear calibration range was found to be 0.75 - 35.00 μg mL⁻¹ and limit of quantitation was 0.08 μg mL⁻¹ for the proposed method. The developed and validated method was directly applied for the determination of roflumilast in its pharmaceutical dosage forms. No interference
was found from tablet excipients at the selected wavelength and analysis conditions. The results obtained from the developed method were compared with those obtained by the high performance liquid chromatographic method in the literature and no significant difference was found statistically between two methods. It was concluded that the developed method was sensitive, accurate, precise, selective, robust and rugged.

**Keywords:** Roflumilast, Derivative UV spectrophotometry, Optimization, Validation, Tablet analysis

**Özet**

**Farmasötik Formülasyonlarda Roflumilast’ın Türev Spektrofotometrik Yöntem ile Tayini**

Bu çalışmada, farmasötik formülasyonlardaki roflumilastın tayini için basit, hızlı, güvenilir ve tamamen valide edilmiş birinci derece UV spektrofotometrik yöntem geliştirilmiştir. Analiz koşullarını optimize etmek için çözücü tipi, türev derecesi, dalga boyu aralığı ve N değeri test edilmiştir. İlacın kantitatif tayini 190 -350 nm dalga boyu aralığında 220 nm (N = 4)’de birinci türev değerleri kullanılarak yapılmıştır. Geliştirilen yöntem, kararlılık, doğruluk, duyarlılık, özgünlik, kesinlik, doğruluk, sağlamlık ve tutarlılık açısından valide edilmiştir. Önerilen yöntemin doğrusal olduğu kalibrasyon aralığı 0.75 - 35.00 μg mL⁻¹ ve alt tayin sınırı 0.08 μg mL⁻¹’dir. Geliştirilen ve valide edilen yöntem, farmasötik formulasyonlarındaki roflumilastın tayini için direkt olarak uygulanmıştır. Analiz koşullarında ve seçilen dalga boyunda tablet yardımcı maddelerinden gelen bir girişim bulunmamıştır. Geliştirilen yöntemden elde edilen veriler, literatürdeki yüksek basıncılı sıvı kromatografisi yönteminden elde edilen veriler ile karşılaştırılmış ve iki yöntem arasında istatistiksel olarak anlamlı fark
Introduction

Bronchial asthma and chronic obstructive pulmonary disease (COPD) are among the most common chronic diseases, and their incidence is increasing in most industrialized countries. COPD is an inflammatory disease of the airways related mainly to smoking and characterized by airflow limitation, which manifests clinically with dyspnea, cough, and sputum production, symptoms that aggravate disease severity and disease exacerbation [1].

Following the infiltration of leukocytes into the airways and subsequent release of a variety of inflammatory mediators, many cellular events occur, including airway microvascular leakage, mucus hypersecretion, bronchoconstriction, and further attraction of inflammatory cells. Subsequently these result in abnormalities in gas exchange at the pulmonary level and respiratory failure. However, the obstruction of the airways has been found to be reversible in asthma, whereas it is largely irreversible in COPD. The need of anti-inflammatory drugs for the treatment of asthma and COPD warrants the intensive search for new drugs in these indications. One possible approach to this endeavor is the development of selective phosphodiesterase (PDE) inhibitors [2,3].

Roflumilast (ROF) (3-cyclopropylmethoxy-4-difluoromethoxy-N-[3,5-dichloropyrid-4-yl]-benzamide) (Figure 1) is a drug which acts as a selective, long-acting inhibitor of the enzyme PDE-4. It has anti-inflammatory effects and is under development as an orally administered
drug for the treatment of inflammatory conditions of the lungs such as asthma and COPD and can significantly affect disease progression. ROF is effective in inflammatory cells such as mast cells, eosinophils, neutrophils, T-lymphocytes, and macrophages [4-8].

![Chemical formula of ROF](image)

**Figure 1.** Chemical formula of ROF.

A number of analytical methods have been reported for quantitative determination of ROF in pharmaceutical dosage forms and biological fluids in the literature, including high performance liquid chromatography (HPLC) [9-16], liquid chromatography - tandem mass spectrometry [17-19], gas chromatography [20] and spectrophotometry [21,22].

There is no derivative UV spectrophotometric method for the analysis of ROF in pharmaceutical formulations has been reported in the literature. Therefore, we planned to develop a derivative spectrophotometric method for the determination of ROF.

The main task of this work was to establish novel, rapid, reliable, simple, inexpensive, sensitive, accurate, robust and reproducible derivative spectrophotometric method that meets the accepted criteria for method validation for determination of ROF in its bulk drug and commercial pharmaceutical dosage form as tablet. The proposed method was developed and validated according to the evaluation of the validation parameters. The developed method was applied to the determination of ROF in pharmaceutical formulations without the necessity of sample pre-treatment. The results obtained from this developed method were compared with those obtained by using high performance liquid chromatographic method in the literature [11].
2. Material and Methods

Apparatus
The spectrophotometric measurements were carried out using an UV - 1700 PharmaSpec model UV-VIS spectrophotometer (190-1100 nm) and an Agilent 8453 model UV-VIS spectrophotometer with a diode array detector (DAD) (190 - 1100 nm) for ruggedness test. UV spectra of standard and sample solutions were recorded in 1 cm quartz cells at the wavelength ranges of 190 - 350 nm.

Chemicals and reagents
ROF working standard was supplied from Refik Saydam Hıfzıslıha National Public Health Agency and it was used without further purification. Melting point, UV and IR spectra of ROF were evaluated to check purity and no impurities were found. A pharmaceutical formulation of ROF as Daxas Tablets® (500 µg ROF/tablet) were purchased from local pharmacy. All solvents and other chemicals were analytical reagent grade. Acetonitrile was purchased from Merck.

Standard solutions
Standard stock solution of 1000 µg mL⁻¹ ROF was prepared in acetonitrile kept in the dark and at - 20 °C maximum for 2 months. Aliquots of stock solutions were taken into 5 mL volumetric flasks and diluted with acetonitrile to get 0.75 - 35.00 µg mL⁻¹ concentration range for standard working ROF solutions. Working standard solutions were daily prepared. 6 replicate samples at 10 different concentrations were prepared and the absorbance of these solutions was measured. In measurements acetonitrile was used as a blank solution.
Tablet solutions

Ten Daxas® tablets were accurately weighed and finely powdered and mixed. A portion of the powder equivalent to the average weight of one tablet was transferred into a 10 mL volumetric flask and 5 mL of acetonitrile was added. The content of the flask was sonicated for 15 min and diluted to volume with acetonitrile. This solution was centrifuged for 15 min at 5000 rpm to separate out the insoluble excipients. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant and diluting them with acetonitrile to give final concentration (15.00 µg mL⁻¹). Then the absorbance of these solutions was measured. The amount of ROF per tablet was calculated using the calibration curve method.

3. Results and Discussion

Visible spectrophotometry is considered to be a very convenient and economical technique because of its simplicity and speed, the inexpensive equipment needed; so it has been applied for analysis of many drugs.

Derivative spectrophotometry is an analytical technique for the enhancement of sensitivity and specificity in qualitative and quantitative analysis of various compounds including pharmaceuticals. Any possible interference effect from the excipients in pharmaceutical formulation could be prevented by using this technique. It is simple and sensitive method and also it does not require any pretreatment procedure. It is an important alternative to manual analytical methods, and has clear advantages in terms of the short time required for each assay.

Optimization of experimental conditions

In the selection of the media, we paid attention to get simple sample preparation and freely solubility of ROF. The UV spectrum of ROF were obtained in different solution such as water, methanol, ethanol, acetonitrile, chloroform, 0.1 M HCl and 0.1 M NaOH. In chloroform, water, 0.1 M HCl and 0.1 M NaOH, a well-defined peak was not observed. The UV spectra of ROF
in ethanol, methanol and acetonitrile were similar. In these solutions peak shape was not changed and almost identical maximum absorbance at wavelength of 240 nm (Figure 2). The best peak shape and reproducibility of measurements was observed for acetonitrile, hence acetonitrile was used in further studies.

![Figure 2. UV spectra of 10.00 µg mL\(^{-1}\) ROF in acetonitrile.](image)

Derivative UV spectrophotometry was preferred for the analysis of ROF since the amplitude of the signal of derivative spectra was greater and the peak shape was well defined in this method. In order to propose a specific and accurate derivative method for analyzing pharmaceutical formulation containing ROF, it is essential to find optimum experimental conditions. To determine the optimized conditions, the wavelength range, derivative orders, N values (smoothing factor) and the derivative wavelength difference (\(\Delta\lambda\)) parameters were examined for derivative UV spectrophotometric method. The \(\Delta\lambda\) depends on the measuring wavelength range and N values. Generally, the noise decreases by increasing \(\Delta\lambda\).

The main disadvantage of the derivative technique is that the signal/noise ratio becomes worse as the order of the derivative increases. Therefore, in practice, the derivative technique includes
smoothing, to control the noise increase, which is an inevitable consequence of differentiation of the noise signal. The effect of smoothing of a peak-type signal is to reduce the noise. Thus, optimization of the smoothing factor is very important for obtaining the appropriate signals [23].

The derivative spectra of solutions containing the individual analytes were investigated in order to optimize the derivative order. 10.00 μg mL\(^{-1}\) of ROF solutions in acetonitrile was measured first, second, third and fourth order derivative spectra to determine the degree of the derivative spectrophotometric method. The first order derivative UV spectrum analysis of ROF gave sharper and better-defined peaks when compared with the other derivative spectrum of ROF (Figure 3). Higher derivative orders were discarded because the noise attenuation was less effective and the signal became distorted.

![Figure 3. a) First and b) second derivative (N = 4) spectrum of 10.00 μg mL\(^{-1}\) ROF.](image)

Optimal wavelength range should be chosen since the broad peaks become sharper, the ratio of signal/noise elevates and the sensitivity of the method increases by controlling the smoothing.
Therefore, a series of N values (N =1, 2, 4 and 8) were tested in the first and second order derivative spectrum of ROF in acetonitrile solution.

Smoothing factor 4 was selected in the measuring wavelength range 190 - 350 nm for first order derivative spectrophotometry, because this yielded good sensitivity, without significant sacrifice of the signal/noise ratio. 220 nm was selected as the optimum working parameter for the first order derivative UV spectrophotometric analysis of ROF (Figure 4).

**Figure 4.** First derivative spectrum of ROF at different concentrations at the optimum conditions. a) 0.75, b) 2.50, c) 5.00, d) 10.00, e) 15.00 and f) 25.00 µg mL⁻¹ of ROF.
Validation

Validation is one of the most important steps in method development for analytical determinations. The main validation parameters such as stability, linearity, sensitivity, precision, accuracy, recovery, specificity, robustness and ruggedness were evaluated in developed method [24-31].

Stability

Stability of the standard stock solutions of ROF were evaluated at various time points when kept at ambient temperature for 29 h (short term stability) and when stored refrigerator at -20 °C for 2 months (long term stability). Freshly prepared solutions and those aged solutions were analyzed by the method developed. ROF standard solutions in acetonitrile showed no spectrophotometric changes up to 7 hours at room temperature and 8 weeks when stored at -20 °C (Table 1). It is decided that ROF was highly stable in the mentioned conditions.

Table 1. The recovery values of ROF in standard solution for stability test.

<table>
<thead>
<tr>
<th>Time</th>
<th>+ 4 °C At room temperature</th>
<th>At room temperature (in the dark)</th>
<th>In daylight at room temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>2 h after</td>
<td>100.05</td>
<td>100.00</td>
<td>100.14</td>
</tr>
<tr>
<td>4 h after</td>
<td>100.31</td>
<td>100.83</td>
<td>100.00</td>
</tr>
<tr>
<td>7 h after</td>
<td>99.19</td>
<td>99.91</td>
<td>99.57</td>
</tr>
<tr>
<td>24 h after</td>
<td>97.76</td>
<td>98.46</td>
<td>97.65</td>
</tr>
<tr>
<td>29 h after</td>
<td>95.16</td>
<td>97.71</td>
<td>98.64</td>
</tr>
</tbody>
</table>

% Remaining: (Remaining ROF concentration at the end of the specified period / Initial ROF concentration) x 100.
Linearity and range

Calibration curves were constructed using the standard stock solution (100 µg mL$^{-1}$), which was taken and diluted to 5 mL with acetonitrile to obtain 10 increasing concentrations in the 0.75 to 35.00 µg mL$^{-1}$. dA/dλ values were measured in optimized conditions. Regression equations were established by plotting dA/dλ values versus concentration. Each point of the calibration graph corresponded to the mean value obtained from 6 independent measurements.

Regression analysis of the calibration curve using the method of least-squares was made to calculate the slope (b), intercept (a) and correlation coefficient (r) for first derivative spectrophotometric method and the values are presented in Table 2. Regression analysis of the Beer’s law plots at $\lambda_{\text{max}}$ reveals a good correlation. Calibration parameters were adequate for ROF determination.

The r value was found to be significant ($t_{\text{calculated}} = 115.44 > t_{\text{tabulated}} = 2.31, p < 0.05$) for proposed method.
Table 2. Analytical performance data for the first derivative spectrophotometric determination of ROF.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical wavelength (nm)</td>
<td>220</td>
</tr>
<tr>
<td>Derivative order</td>
<td>1</td>
</tr>
<tr>
<td>N (smoothing factor)</td>
<td>4</td>
</tr>
<tr>
<td>Linearity range (µg mL(^{-1}))</td>
<td>0.75 - 35.00</td>
</tr>
<tr>
<td>Limit of detection (LOD) (µg mL(^{-1}))</td>
<td>0.03</td>
</tr>
<tr>
<td>Limit of quantitation (LOQ) (µg mL(^{-1}))</td>
<td>0.08</td>
</tr>
<tr>
<td>Regression equation (y)(^a)</td>
<td>y = - 0.0075x - 0.0003</td>
</tr>
<tr>
<td>Standard error of slope</td>
<td>1.14 (x\ 10^{-4})</td>
</tr>
<tr>
<td>Standard error of intercept</td>
<td>1.02 (x\ 10^{-3})</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9997</td>
</tr>
<tr>
<td>Number of data points</td>
<td>10</td>
</tr>
</tbody>
</table>

\(^a\)\(y = bx + a; \ x = \text{concentration (µg mL}^{-1}\), \ y = dA/d\(\lambda\) value, a = intercept and b = slope.

**Sensitivity**

The sensitivity of the developed method was checked with regard to limit of detection (LOD) and limit of quantitation (LOQ) value from the calibration curves that defined linearity. The LOD is defined as the lowest concentration of an analyte in a sample can be detected. The LOQ is defined as the lowest concentration of an analyte in a sample, which can be determined
DETERMINATION OF ROFLUMILAST IN PHARMACEUTICAL FORMULATIONS
BY DERIVATIVE SPECTROPHOTOMETRIC METHOD

quantitatively with an acceptable level of accuracy and precision of the method at the optimum
conditions.

The LOD (k = 3.3) and the LOQ (k = 10) of the method were established according to the ICH
definitions (C₁ = k S₀/s, where C₁ is LOD or LOQ value, S₀ is the standard error of blank
determination, s is the slope of the standard curve and k is the constant related to the confidence
interval). The standard error of absorbance measurement for blank solution was 5.78 \times 10^{-5}
(n = 12). The calculated LOD and LOQ values for the proposed method were 0.03 \mu g \text{ mL}^{-1} and
0.08 \mu g \text{ mL}^{-1}, respectively. These LOD and LOQ data indicated that the proposed method could
be considered sensitive.

**Precision**

The precision assay was investigated in terms of repeatability and intermediate precision. The
precision of the method was determined by calculating the relative standard deviation (RSD %).

The repeatability of the method was evaluated by performing 12 repeated measurements for
15.00 \mu g \text{ mL}^{-1} of ROF solution at the optimum condition on the same day. The mean of
measured dA/d\lambda was found to be 0.116 \pm 3 \times 10^{-4} with RSD of 0.87 %. These values indicated
that the proposed method have high repeatability and precision for the ROF analysis.

The intermediate precision evaluated as intra-day and inter-day precision. Intra-day precision
was tested with each 7 analysis of three samples solutions containing lower, middle and higher
linearity range (1.50, 15.00 and 30.00 \mu g \text{ mL}^{-1}) using the mentioned procedures and then RSD
% values were calculated. Inter-day precision of the method was evaluated by considering
lower, middle and higher concentration samples in linearity range on 7 consecutive days. The
RSD values of intra - day and inter - day studies varied from 0.68 - 2.18 % showed that the
intermediate precision of the method was satisfactory (Table 3).
Table 3. Precision and accuracy data of the developed method (n = 7).

<table>
<thead>
<tr>
<th>Added (μg mL⁻¹)</th>
<th>Found⁺ (μg mL⁻¹)</th>
<th>Precision RSD %</th>
<th>Accuracy⁻ (Bias %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Intra-day</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.50</td>
<td>1.50 ± 0.01</td>
<td>1.82</td>
<td>0</td>
</tr>
<tr>
<td>15.00</td>
<td>14.93 ± 0.06</td>
<td>0.99</td>
<td>-0.47</td>
</tr>
<tr>
<td>30.00</td>
<td>30.65 ± 0.26</td>
<td>0.68</td>
<td>2.17</td>
</tr>
<tr>
<td><em>Inter-day</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.50</td>
<td>1.52 ± 0.01</td>
<td>2.18</td>
<td>1.33</td>
</tr>
<tr>
<td>15.00</td>
<td>15.23 ± 0.07</td>
<td>1.19</td>
<td>1.53</td>
</tr>
<tr>
<td>30.00</td>
<td>30.25 ± 0.10</td>
<td>0.87</td>
<td>0.83</td>
</tr>
</tbody>
</table>

⁺Found = \( \bar{x} \) = mean ± standard error, RSD % = Relative standard deviation,

⁻Accuracy = \( \frac{(\text{Found} - \text{Added})}{\text{Added}} \times 100 \).

**Accuracy and Recovery**

The accuracy of a method was determined by calculating the percentage relative error (bias %) between the measured mean and added concentrations. Three different concentrations of ROF (1.50, 15.00 and 30.00 μg mL⁻¹) in the linear range were analyzed in 7 independent series on the same day (intra-day accuracy) and 7 consecutive days (inter-day accuracy). The results obtained for intra and inter-day accuracy were between -0.47 - 2.17 %. Found concentration values are in good agreement with the expected ones (Table 3).

In order to show the reliability and suitability of the proposed method, recovery experiments were performed. For this reason, in order to know whether the excipients in the pharmaceutical formulations as tablet show any interference with the analysis, the recovery test was done by the standard addition method at five concentrations (10.00, 15.00, 20.00, 25.00 and
30.00 μg mL⁻¹. A recovery study of the proposed derivative UV spectrophotometric method was carried out performed by spiking the powdered tablets with appropriate amounts of standard solution. The results were summarized in Table 4. Closeness of the results to 100 % showed that recovery of the method was very good. High recovery and low standard deviation verified the accuracy and reliability of the proposed method for the determination of ROF in different pharmaceutical formulations.

**Table 4.** Recovery data of the developed method for the analysis of ROF (n = 7).

<table>
<thead>
<tr>
<th>Added (μg mL⁻¹)</th>
<th>Found¹ (μg mL⁻¹)</th>
<th>Recovery (%)</th>
<th>RSD of Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.00</td>
<td>10.17 ± 0.43</td>
<td>101.72 ± 1.64</td>
<td>1.21</td>
</tr>
<tr>
<td>15.00</td>
<td>15.02 ± 1.01</td>
<td>100.10 ± 1.79</td>
<td>1.74</td>
</tr>
<tr>
<td>20.00</td>
<td>20.00 ± 1.04</td>
<td>100.02 ± 1.97</td>
<td>1.40</td>
</tr>
<tr>
<td>25.00</td>
<td>25.15 ± 0.70</td>
<td>100.59 ± 1.06</td>
<td>0.72</td>
</tr>
<tr>
<td>30.00</td>
<td>29.86 ± 0.65</td>
<td>99.53 ± 0.82</td>
<td>0.57</td>
</tr>
</tbody>
</table>

¹Found = $\bar{x}$ = mean ± standard error, RSD % = Relative standard deviation.

**Specificity**

The first order spectra of standard ROF standard solution and that of pharmaceutical formulation as tablet forms containing equal concentrations (15.00 μg mL⁻¹) in acetonitrile were scanned over the range 190 - 350 nm as shown in Figure 5. The first order spectra obtained from tablet solution was identical with that obtained spectrum from standard solution of ROF and the wavelength of maximum absorbance of ROF did not change. It was concluded that the excipients did not interfere with quantification of ROF in this method and the developed method could be considered specific.
In order to evaluate the excipients in this proposed method, the standard addition method was applied. The regression equation of standard addition method was found to be $y = -0.0082x - 0.0513$, $r = 0.9994$. Since the slopes of the calibration and standard addition curves were identical, it was concluded that there was no spectral interaction in the analysis of ROF in tablet dosage forms with the developed method. Therefore, the calibration curve method was used in quantitative analysis of ROF. These values showed that no significant excipients interference from tablet formulations, thus the developed method was used to determination of ROF.
ROF in the presence of excipients. In the proposed method, there was no need for pretreatment procedures and only centrifugation was applied to make the solution clear.

**Robustness**

In robustness testing of an analytical method, the aim was to find how sensitive the responses were to small changes in the experimental conditions in the proposed method. Ideally, the results should be the same for all experiments. Method robustness was evaluated by the analysis of standard solutions of ROF at different wavelengths in ± 0.2 units (218 - 222 nm). These deliberate small changes were evaluated for 15.00 µg mL⁻¹ of ROF standard solutions (n = 7) (Table 5). Only one parameter was changed in each experiment. Minor changes in the wavelength did not have any significant effect on dA/dλ of ROF.

**Table 5.** Robustness and ruggedness data of developed method (n=7).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Found (µg mL⁻¹)</th>
<th>RSD %</th>
<th>t-test Results</th>
<th>F-test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard (15.00 µg mL⁻¹)</td>
<td>15.53 ± 0.07</td>
<td>1.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wavelength (218 nm)</td>
<td>15.71 ± 0.07</td>
<td>1.14</td>
<td>t_c = 1.89</td>
<td>F_c = 1.00</td>
</tr>
<tr>
<td>Wavelength (222 nm)</td>
<td>15.69 ± 0.07</td>
<td>1.20</td>
<td>t_c = 1.68</td>
<td>F_c = 1.11</td>
</tr>
<tr>
<td>Different analyst</td>
<td>15.45 ± 0.05</td>
<td>0.92</td>
<td>t_c = 0.94</td>
<td>F_c = 1.65</td>
</tr>
<tr>
<td>Different instrument</td>
<td>15.70 ± 0.10</td>
<td>1.69</td>
<td>t_c = 1.45</td>
<td>F_c = 2.09</td>
</tr>
</tbody>
</table>

*Found = x = mean ± standard error, RSD % = Relative standard deviation, t_c = calculated t; t_t = tabulated t and F_c = calculated F; F_t = tabulated F values (t_t = 2.18 and F_t = 4.28 for n = 7, p > 0.05).*

The t- and F-tests were applied to the data to statistically examine the validity of the obtained results (Table 5). Since the calculated t- and F-values did not exceed the theoretical values, which verified there was no significant difference between the proposed and reported methods.
As a result, the obtained data from analysis confirmed that an indication of the reliability of the proposed method for the analysis of ROF. It can be said that the method developed is robust to the small changes in experimental conditions.

**Ruggedness**

In order to demonstrate the ruggedness of the method, analysis of 15.00 μg mL⁻¹ of ROF standard solutions were carried out using the same instrument by two different analysts and using different instrument under the same optimized conditions at different days. The data obtained by different analysts and instrument were evaluated by t- and F-tests and no differences were found (Table 5). The obtained results were found to reproducible and the proposed methods could be considered rugged.

**Analysis of pharmaceutical formulations**

Quantitative determination of ROF in its pharmaceutical formulations as tablets form using the optimized first order derivative UV spectrophotometric method was performed using calibration curve method without any sample extraction or filtration. The tablet solutions of ROF were prepared as 7 independent series and each series were measured two times. The amounts of ROF in tablets were calculated using the regression equations of calibration curve method. The obtained results were in good agreement with the labeled amount of ROF in its tablets (Table 6). In addition, RSD % and recovery values were found to be 1.28 and 101.89 ± 0.49 %, respectively. The good percentage recoveries confirm the suitability of the proposed methods for the routine determination of ROF in its drug formulations. Closeness of the amount found to the labeled amount and the low RSD % value showed that the proposed method was accurate and precise.

An HPLC method mentioned in the literature [11] was used as a comparison method to evaluate the validity of the method developed. The t- and F- tests were carried out and since the
calculated t- and F-values did not exceed the theoretical values, which confirmed there was no significant difference between the proposed and reported methods. (Table 6).

**Table 6.** Comparative determination of ROF in commercial drug (Daxas tablet®, 0.5 mg/tablet) by the proposed first order derivative method and reference HPLC method (n = 7).

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>( \bar{x} = 0.509 \pm 0.004 )</td>
<td>( \bar{x} = 0.506 \pm 0.004 )</td>
</tr>
<tr>
<td>SD = 0.01</td>
<td>SD = 0.01</td>
</tr>
<tr>
<td>RSD % = 1.28</td>
<td>RSD % = 1.75</td>
</tr>
<tr>
<td>Recovery % = 101.89 ± 0.49</td>
<td>Recovery % = 101.11 ± 0.67</td>
</tr>
<tr>
<td>( t_c = 0.57, t_t = 2.18, p &gt; 0.05 )</td>
<td></td>
</tr>
<tr>
<td>( F_c = 1.00, F_t = 4.28, p &gt; 0.05 )</td>
<td></td>
</tr>
</tbody>
</table>

*Found = \( \bar{x} \) = mean ± standard error, SD = Standard deviation, RSD % = Relative standard deviation. \( t_c \) = calculated t; \( t_t \) = tabulated t and \( F_c \) = calculated F; \( F_t \) = tabulated F values.

4. **Conclusion**

In this study a simple, fast and reliable first order derivative UV spectrophotometric method was developed and validated for the determination of ROF in its pharmaceutical formulations. Analytical method validation parameters were evaluated in this proposed method. The method showed good linearity, selectivity, sensitivity and repeatability. To assess the usefulness of the method, the effect of excipients and additives in pharmaceutical dosage forms of ROF was studied.

The proposed method is very simple application, less expensive and do not need any sophisticated apparatus or a special program in comparison to the mentioned techniques in its analysis methods in the literature. The proposed method has the advantage of using feasible
analytical procedure and needing only a very simple pre-treatment of the samples. Besides the proposed procedure has the lowest LOD and LOQ values and wider linear range, more sensitive and precise when compared with the already published spectrophotometric methods.

In conclusion, the proposed method could be successfully applied for the routine analysis of the studied drug in its bulk powder and in dosage form in quality control laboratories without the need for separation or complex sample preparation such as extraction steps prior to the drug analysis.

References


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BY DERIVATIVE SPECTROPHOTOMETRIC METHOD


