Hacettepe University Journal of the Faculty of Pharmacy
Volume 36 / Number 1 / July 2016 / pp.27-49

Determination of Roflumilast in Pharmaceutical Formulations by Derivative Spectrophotometric Method

Received : 25.03.2016
Revised : 29.04.2016
Accepted : 14.06.2016

Mehmet Atmaca[*], İncilay Süslü[°]**

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

*Forensic Medicine Institutions, Ankara Group Presidency, Keçiören - Ankara, Turkey
**Hacettepe Üniversitesi, Eczacılık Fakültesi, Analitik Kimya Anabilim Dalı, Ankara
° Corresponding Author E-mail**:** isuslu@hacettepe.edu.tr

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, doğrusallık, duyarlılık, özgünlük, 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 basınçlı sıvı kromatografisi yönteminden elde edilen veriler ile karşılaştırılmış ve iki yöntem arasında istatistiksel olarak anlamlı fark

bulunmamıştır. Geliştirilmiş yöntemin duyarlı, doğru, kesin, seçici, sağlam ve tutarlı olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Roflumilast, Türev UV spektrofotometri, Optimizasyon, Validasyon, Tablet analizi

1. 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 leucocytes 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].

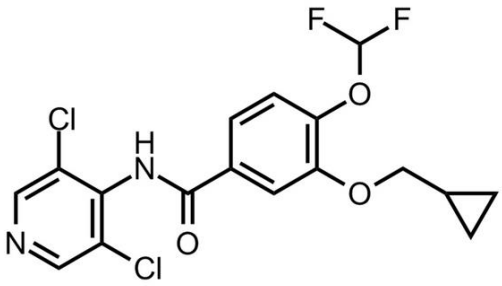

Figure 1. Chemical formula of ROF.

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 Hıfzısıhha 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 ^{o}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 HCI and 0.1 M NaOH. In chloroform, water, 0.1 M HCI 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.

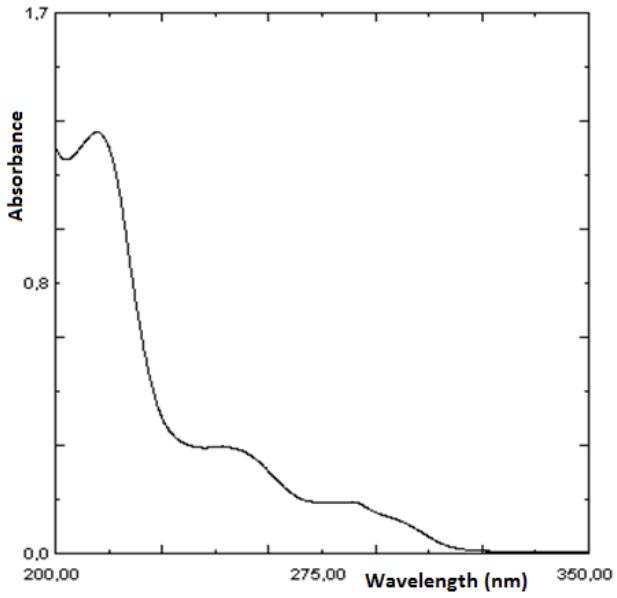

Figure 2. UV spectra of 10.00 μg mL^{-1} ROF in acetonitrile.

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 (Δλ) parameters were examined for derivative UV spectrophotometric method. The Δλ depends on the measuring wavelength range and N values. Generally, the noise decreases by increasing Δλ.

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.

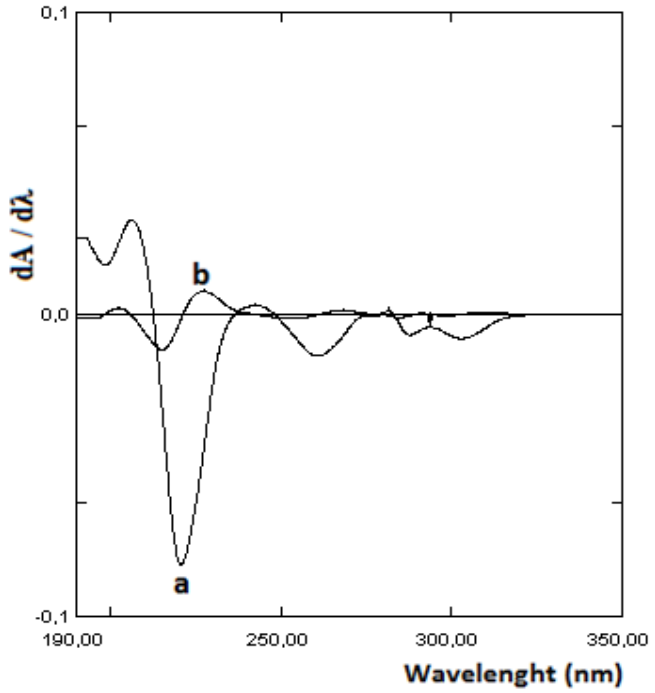

Figure 3. a) First and **b)** second derivative (N = 4) spectrum of 10.00 µg mL^{-1} ROF.

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).

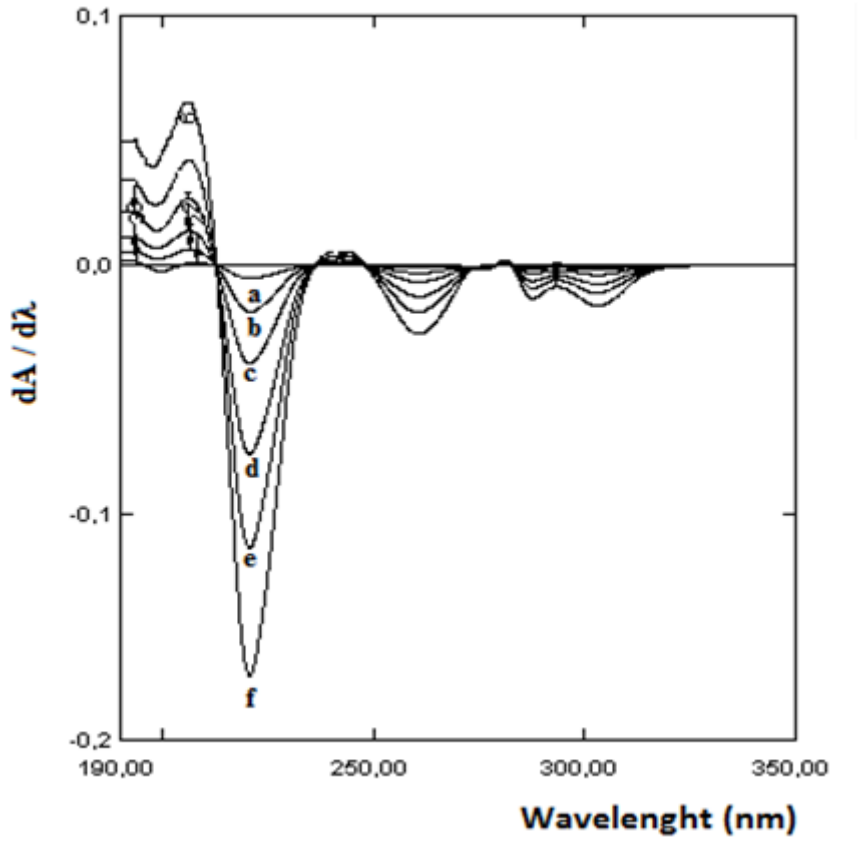

Figure 4. First derivative spectrum of ROF at different concentrations at the optimum conditions. **a)** 0.75, **b)** 2.50, **c)** 5.00, **d)** 10.00, **e)** 15.00 and **f)** 25.00 μg mL^{-1} of ROF.

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 ºC 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 ºC (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.

	% Remaining ROF			
Time	**+ 4 ºC**	**At room temperature**	**At room temperature (in the dark)**	**In dayligt at room temperature**
0 h	100.00	100.00	100.00	100.00
2 h after	100.05	100.00	100.14	100.26
4 h after	100.31	100.83	100.00	100.47
7 h after	99.19	99.91	99.57	100.43
24 h after	97.76	98.46	97.65	98.91
29 h after	95.16	97.71	98.64	97.31

% 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 λ_{max} reveals a good correlation. Calibration parameters were adequate for ROF determination.

The r value was found to be significant ($t_{calculated} = 115.44 > t_{tabulated} = 2.31$, $p < 0.05$) for proposed method.

Table 2. Analytical performance data for the first derivative spectrophotometric determination of ROF.

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

[a]y = bx + a; x= concentration (μg mL^{-1}), y= dA/dλ 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

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 (C_1 = k S_0/s, where C_1 is LOD or LOQ value, S_0 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 x 10^{-5} (n = 12). The calculated LOD and LOQ values for the proposed method were 0.03 µg mL^{-1} and 0.08 µg 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 µg mL^{-1} of ROF solution at the optimum condition on the same day. The mean of measured dA/dλ was found to be 0.116 ± 3 x 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 µg 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).

Added (μg mL^{-1})	Found[a] (μg mL^{-1})	Precision RSD %	Accuracy[b] (Bias %)
Intra - day			
1.50	1.50 ± 0.01	1.82	0
15.00	14.93 ± 0.06	0.99	- 0.47
30.00	30.65 ± 0.26	0.68	2.17
Inter - day			
1.50	1.52 ± 0.01	2.18	1.33
15.00	15.23 ± 0.07	1.19	1.53
30.00	30.25 ± 0.10	0.87	0.83

[a]Found = $\bar{x}$ = mean ± standard error, RSD % = Relative standard deviation,

[b]Accuracy = [(Found - Added) / Added] x 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).

Added (μg mL^{-1})	Found[a] (μg mL^{-1})	Recovery (%)	RSD of Recovery (%)
10.00	10.17 ± 0.43	101.72 ± 1.64	1.21
15.00	15.02 ± 1.01	100.10 ± 1.79	1.74
20.00	20.00 ± 1.04	100.02 ± 1.97	1.40
25.00	25.15 ± 0.70	100.59 ± 1.06	0.72
30.00	29.86 ± 0.65	99.53 ± 0.82	0.57

[a]Found = $\bar{x}$ = 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.

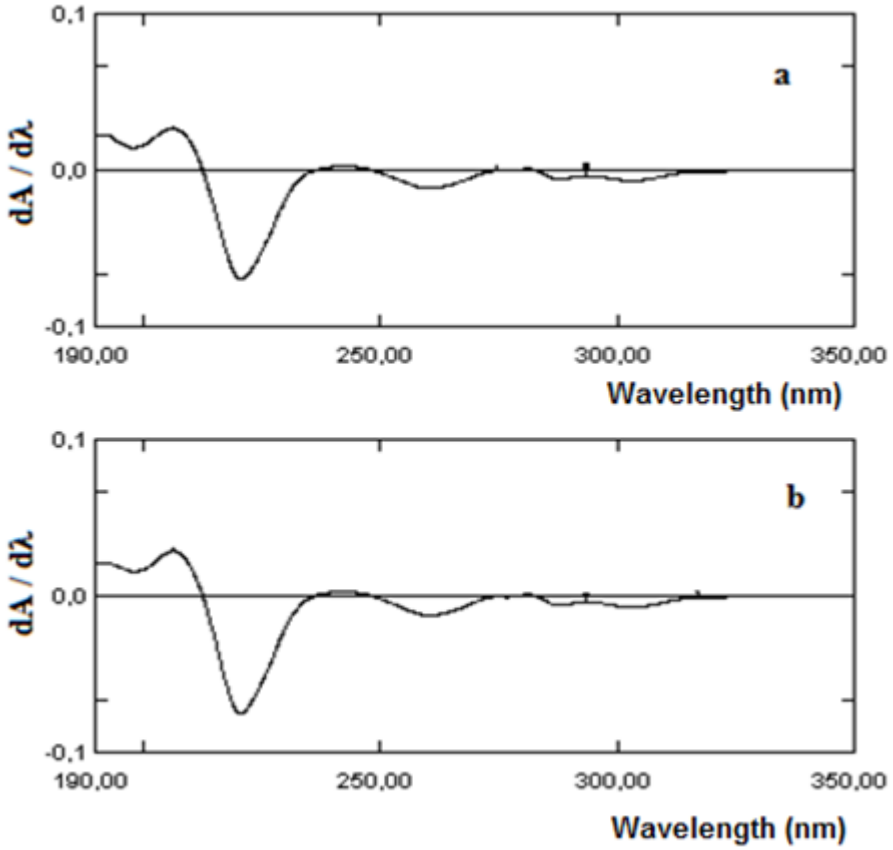

Figure 5. First derivative spectrum of 15.00 μg mL^{-1} ROF. **a)** Standard ROF solution and **b)** tablet solution.

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 = - 0.0082 x - 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 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 $dA/d\lambda$ of ROF.

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

Conditions	Found (µg mL^{-1})	RSD %	t- test Results	F- test Results
Standard (15.00 µg mL^{-1})	15.53 ± 0.07	1.16		
Wavelength (218 nm)	15.71 ± 0.07	1.14	t_c = 1.89	F_c = 1.00
Wavelength (222 nm)	15.69 ± 0.07	1.20	t_c = 1.68	F_c = 1.11
Different analyst	15.45 ± 0.05	0.92	t_c = 0.94	F_c = 1.65
Different instrument	15.70 ± 0.10	1.69	t_c = 1.45	F_c = 2.09

[a]Found = $\bar{x}$ = 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).

Proposed Derivative Method	**Comparison Method [11]**
$\bar{x} = 0.509 \pm 0.004$	$\bar{x} = 0.506 \pm 0.004$
SD = 0.01	SD = 0.01
RSD % = 1.28	RSD % = 1.75
Recovery % = 101.89 ± 0.49	Recovery % = 101.11 ± 0.67
$t_c = 0.57$, $t_t = 2.18$, $p > 0.05$	
$F_c = 1.00$, $F_t = 4.28$, $p > 0.05$	

[a]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

1. Antoniu S. A.: New therapeutic options in the management of COPD - focus on roflumilast. International Journal of Chronic Obstructive Pulmonary Disease 2011, 6: 147-155.
2. Schudt AC, Gantner F, Tenor H, Hatzelmann A: Therapeutic potential of selective PDE inhibitors in asthma. Pulmonary Pharmacology and Therapeutics 1999, 12(2): 123-129.
3. Essayan D. M.: Cyclic nucleotide phosphodiesterase (PDE) inhibitors and immunomodulation. Biochemical Pharmacology 1999, 57: 965-973.
4. Hatzelmann A, Schudt C: Anti-inflammatory and immunomodulatory potential of the novel PDE4 inhibitor roflumilast in vitro. Journal of Pharmacology and Experimental Therapeutics 2001, 297: 267-279.
5. Boswell-Smith V, Spina D: PDE4 inhibitors as potential therapeutic agents in the treatment of COPD-focus on roflumilast. International Journal of Chronic Obstructive Pulmonary Disease 2007, 2 (2): 121-129.
6. Field, SK: Roflumilast: an oral, once-daily selective PDE-4 inhibitor for the management of COPD and asthma. Expert Opinion on Investigational Drugs 2008, 17 (5): 811-818.

7. Frankel SK, Cool CD, Lynch DA, et al.: Idiopathic pleuroparenchymal fibroelastosis: Description of a novel clinicopathologic entity. Chest 2004, 126: 2007-2013.

8. Yu T, Fain K, Boyd C.M, Singh S, Weiss C.O, Li TJ, Varadhan R, Puhan M.A: Benefits and harms of roflumilast in moderate to severe COPD. Thorax 2014, 69 (7): 616-622.

9. Barhate, V.D, Deosthalee Priya: Rapid liquid chromatographic method for the determination of roflumilast in the presence of degradation products. From Indian Journal of Pharmaceutical Sciences 2010, 72 (3): 401-404.

10. Barhate V.D, Deosthalee P.C: Validation of rapid liquid chromatographic method for the determination of roflumilast. Journal of Chemical and Pharmaceutical Research 2011, 3 (2): 770-775.

11. Ladani J.J, Bhimani R.D, Vyas K.B, Nimavat K.S: Analytical method development and validation of RP-HPLC for estimation of roflumilast in bulk drug and tablet dosage form. International Journal for Pharmaceutical Research Scholars 2012, 1 (3): 28-32.

12. Lakshmi B, Reddy T: RP-HPLC method for the quantification of roflumilast in formulations. South Pacific Journal of Technology and Science (SPJTS) 2013, 1 (1): 015-023.

13. Fang Tan: Development and validation of stability-indicating HPLC method for roflumilast and related substances. Advanced Materials Research (Durnten-Zurich, Switzerland) 2013, 781-784.

14. Zheng Xi-zi, Guangpu S: Assay of roflumilast and the determination of its related substances by HPLC. Guangpu Shiyanshi 2013, 30 (6): 3122-3126.

15. Yan W, Chen Y.M, Ni C.L, Zheng X: Identification and quantitative analysis of related substances in roflumilast. Chinese Journal of Pharmaceutical Analysis 2013, 33 (10): 1726-1730.

16. Shah A.D, Patel C.N: Implementation of QbD approach to the RP-HPLC method development and validation of roflumilast in bulk and tablet dosage form: an application in degradation study. World Journal of Pharmacy and Pharmaceutical Sciences 2014, 3(6): 2281-2307.

17. Satheeshmanikandan R.S Thappali, Kanthikiran V.S Varanasi, Sridhar V, Swaroop Kumar V.S. Vakkalanka, Mukkanti K: Simultaneous quantitation of IC87114, roflumilast and its active metabolite roflumilast N-oxide in plasma by LC-MS/MS: application for a pharmacokinetic study. Journal of Mass Spectrometry 2012, 47 (12): 1612-1619.

18. Knebel N. G, Herzog R, Reutter Felix, Zech Karl: Sensitive quantification of roflumilast and roflumilast N-oxide in human plasma by LC-MS/MS employing parallel chromatography and electrospray ionisation. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 2012, 893-894: 82-91.

19. Thevis M, Krug O, Schaenzer Wilhelm: Monitoring phosphodiesterase-4 inhibitors using liquid chromatography/(tandem) mass spectrometry in sports drug testing. Rapid Communications in Mass Spectrometry 2013, 27 (9): 993-1004.

20. Qiu X, Wang Ai-di: Determination of residual solvents in roflumilast by headspace gas chromatography. Yingyong Huagong 2013, 42 (8): 1541-1543.

21. Ladani J.J, Bhimani R.D, Vyas K.B, Nimavat K.S: Method development and validation of roflumilast in tablet dosage form by UV spectrophotometry. Journal of Atoms and Molecules 2012, 2 (5): 369-374.

22. Patel K.R, Desai D.G, Zanwar A, Sen A.K, Seth A.K: Development and validation of UV spectroscopy method for estimation of roflumilast in bulk and tablet dosage form. Pharma Science Monitor 2013, 4 (3): 274-280.

23. Popovic G.V, Pfendt L.B, Stefanovic V.M: Analytical application of derivative spectrophotometry. Journal of Serbian Chemical Society, 2000, 65 (7): 457-472.

24. ICH Harmonized Tripartite Guideline Q2 (R1) International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Text and Methodology, International Conference on Harmonization, Geneva, Switzerland 2005.

25. International Conference on Harmonization (ICH) Topic Q2A, Validation of Analytical Procedures; Methodology, CPMP / ICH /281, 1995.

26. The United States Pharmacopoeia 29, The National Formulary 24, U.S. Pharmacopeial Convention, Rockville MD, 2006, 3050-3053.

27. Ermer J: Validation in pharmaceutical analysis. Part I: An integrated approach. Journal of Pharmaceutical Biomedical. Analysis, 2001, 24: 755-767.

28. Taverniers I, Loose D.M, Bockstaele E.V: Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. Trends in Analytical Chemistry, 2004, 23 (8): 535-552.

29. Ermer J, Ploss H.J. Validation in pharmaceutical analysis, part II: central importance of precision to establish acceptance criteria and for verifying and improving the quality of analytical data. Journal of Pharmaceutical and Biomedical Analysis, 2005, 37: 859-870.

30. Vander Heyden Y, Nijhuis A, Smeyers-Vebreke J, Vandeginste B.G.M, Massart D.L: Guidance for robustness/ruggedness tests in method validation. Journal of Pharmaceutical and Biomedical Analysis ,2001, 24: 723-753.

31. Green J.M: A practical guide to analytical method validation. Journal of Analytical Chemistry, 1996, 68: 305A-309A.