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ORIGINAL ARTICLE

Validated stability-indicating HPLC-DAD method for determination of the phosphodiesterase (PDE-4) inhibitor roflumilast

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Abstract A comprehensive stability indicating HPLC with diode array detection method was developed for determination of the recently approved phosphodiesterase type 4 (PDE-4) inhibitor roflumilast (RFL). Effective chromatographic separation was achieved using Durashell C18 column (4.6 × 250 mm, 5 μm particle size) with isocratic elution of the mobile phase composed of 0.0065 M ammonium acetate pH 6.3, methanol and acetonitrile in the ratio of 30:35:35 (by volume). The mobile phase was pumped at a flow rate of 1.3 mL/min, and quantification of RFL was based on measuring its peak areas at 251 nm. RFL eluted at retention time 6.2 min. Analytical performance of the proposed HPLC procedure was thoroughly validated with respect to system suitability, linearity, range, precision, accuracy, specificity, robustness, detection and quantification limits. The linearity range was 2.5–200 μg/mL with correlation coefficient >0.9998. The drug was subjected to stress conditions of neutral, acidic and alkaline hydrolysis, oxidation, photolysis and thermal degradation. The proposed method proved to be stability-indicating by resolution of the drug from its forced degradation products. Moreover, specificity of the method was verified by resolution of drug from more than 20 pharmaceutical compounds of various medicinal categories. The validated HPLC method was successfully applied to the analysis of the cited drug in its tablet

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dosage form. The proposed method made use of DAD as a tool for peak identity and purity confirmation.

1. Introduction

Roflumilast (RFL) (Fig. 1), chemically known as 3-(cyclopropylmethoxy)-*N*-(3,5-dichloro-4-pyridyl)-4-(difluoromethoxy) benzamide,[1] is a newly approved selective phosphodiesterase-4 (PDE-4) inhibitor for the treatment of severe chronic obstructive pulmonary disease (COPD) associated with chronic bronchitis and a history of exacerbations.[2,3] RFL is not yet an official drug in any of the famous pharmacopoeias, including the British Pharmacopoeia, European Pharmacopoeia and United States Pharmacopoeia. Furthermore, few methods can be found in the scientific literature for its analysis. Simultaneous quantification of RFL and its N-oxide metabolite in human plasma has been carried out using liquid chromatography with tandem mass spectrometry LC–MS/MS methods.[4,5] The same technique has been further applied for monitoring several PDE-4 inhibitors including RFL in sports drug testing.[6] The separation of RFL from its forced degradation products was addressed in only a single report, which described the use of RP-HPLC with a multi-step gradient mobile phase.[7] Besides, there was a lack of information about validation of the method or its application to real pharmaceutical samples.[7] Again, RP-HPLC with a multi-step gradient mobile phase method was reported for the determination of RFL in only the bulk powder.[8] Recently, a UV spectrophotometric method was described for RFL estimation in tablet dosage form.[9]

The objective of this work is to develop a simple, rapid, selective and reliable HPLC with diode array detection method for the quantitative analysis of RFL in tablet dosage form. The method was thoroughly validated and tested for its specificity and stability-indicating properties by resolution of RFL from its forced hydrolytic, oxidative, photolytic and dry heat degradation products. In addition, specificity of the proposed method was further investigated by separation of the analyte from several pharmaceutical compounds from different pharmacological categories.

2. Experimental

2.1. Instrumentation

The HPLC-DAD system consisted of Waters 2695 Alliance (quaternary pump, vacuum degasser heater, diode array and multiple wavelength detector Waters 2996) connected to a computer loaded with MILLENNIUM32 Login Version 4.00 Software. An automated injector model SM7 with loop capacity 100 μL was used. The column used was Durashell-C18 (4.6 × 250 mm, 5 μm particle size) (Bonna-Agela Technologies Inc., Wilmington, DE, USA). Filtration of solutions prior to injection to the column was done using cellulose nitrate membrane filters (0.45 μm pore size) (Sartorius Stedim Biotech GmbH, Goettingen, Germany).

2.2. Materials and chemicals

Roflumilast was kindly donated by Pharco Pharmaceuticals Co., Alexandria, Egypt. HPLC-grade acetonitrile (Carbon Group Ringaskiddy, Cork, Ireland), HPLC-grade methanol (Lab-scan, Gliwice, Poland), HPLC-grade glacial acetic acid (Scharlau Chemie S.A., Sentmenat, Spain), reagent grade ammonium acetate (Scharlau Chemie S.A., Sentmenat, Spain), analytical grade of hydrochloric acid (BDH Laboratory Suppliers, Poole, England), sodium hydroxide (El-Nasr Pharmaceutical Chemicals Co., Egypt), 50% hydrogen peroxide (Chemajet Chemical Co., Egypt) and high purity distilled water were used. Pharmaceutical formulation assayed in the study was Dalivent® tablets (Pharco Pharmaceuticals Co., Alexandria, Egypt) labeled to contain 0.5 mg RFL per tablet.

2.3. General procedure

2.3.1. Chromatographic conditions

A mobile phase system consisting of 0.0065 M ammonium acetate pH 6.3, acetonitrile and methanol in the ratio of 30:35:35 (by volume) was used. The separation was achieved with isocratic elution. The flow rate was 1.3 mL/min. The injection volume was 35 μL. The eluant was monitored by the diode array detector (DAD) from 190 to 400 nm, and chromatograms were extracted at 251 nm. All determinations were performed at 30 °C.

2.3.2. Standard solutions and construction of the calibration curve

RFL stock solution (500 μg/mL) was prepared in HPLC-grade methanol. The working standard solutions were prepared by dilution of aliquots of the stock solution with the mobile phase to reach the concentration range 2.5–200 μg/mL. Triplicate injections were made for each concentration and chromatographed under the previously described chromatographic conditions. Peak areas were plotted against the corresponding concentrations to construct the calibration graph; in addition, the linear regression equation was calculated.

2.4. Assay of commercial tablets

Ten Dalivent® tablets were accurately weighed and finely powdered. An accurate weight of the powdered tablets equivalent to 1.25 mg RFL was extracted into 25 mL mobile phase with the aid of sonication for 10 min then filtered into a 50 mL-volumetric flask. The residue was washed with 2 × 10 mL portions of the mobile phase, washings were added to the filtrate and finally the solution was completed to volume with the mobile phase. The prepared sample solution was chromatographed using the previously described conditions. Recovered concentrations were calculated from the corresponding external standard (simultaneously prepared standard RFL solution).

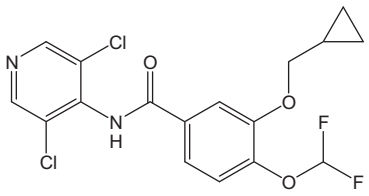

Figure 1 Chemical structure of roflumilast (RFL).

Standard addition technique was applied by spiking sample solution with a portion of RFL stock standard solution to obtain total concentration within the previously specified range then treated as previously described. Recovered concentration was calculated by comparing the drug response in tablet solution with the increment response obtained after addition of the standard.

2.5. Preparation of forced-degradation solutions

Forced degradation studies were carried out on RFL standard according to the following conditions:

2.5.1. Neutral (water) degradation

RFL solutions were treated with 5 mL of distilled water. Solutions were placed in a water bath at 90 °C for 6 h, then they were diluted to volume with the mobile phase to obtain a final concentration of 25 μg/mL.

2.5.2. Acidic and basic degradation

RFL solutions were treated with 2 mL of 1 M hydrochloric acid solution or 1 M sodium hydroxide solution. Solutions were placed in a water bath at 90 °C for 1 h. After the specified time interval, all solutions were neutralized by adjusting the pH to 7.0 and then diluted to volume with the mobile phase to obtain a final concentration of 25 μg/mL.

2.5.3. Oxidative degradation

RFL solutions were treated with 5 mL of H_2O_2 5% solution. Solutions were placed in a water bath at 90 °C for 1 h and 5 h. After the specified time intervals, solutions were diluted to volume with the mobile phase to obtain a final concentration of 25 μg/mL.

2.5.4. Photolytic degradation

An amount of RFL powder (100 mg) was subjected to UV irradiation (200 W h/m^2) for 6 h. After the specified time, the drug powder was dissolved in methanol, and aliquots of the methanolic stock were diluted to volume with the mobile phase to obtain a final concentration of 25 μg/mL.

2.5.5. Dry heat degradation

An amount of RFL powder (100 mg) was kept in an oven at 120 °C for 8 h. After the specified time, the powder was dissolved in methanol, and aliquots of the methanolic stock were diluted to volume with the mobile phase to obtain a final concentration of 25 μg/mL.

After the previous treatments, all forced degradation solutions were filtered with a 0.45 μm membrane filter prior to injection to the column.

3. Results and discussion

3.1. Optimization of chromatographic conditions

An isocratic liquid chromatographic method coupled with diode array detection was developed to provide a suitable and reliable procedure for the routine quality control analysis of RFL. The developed method was carefully designed and optimized to separate the cited drug from its forced degradation products as well. The most important aspect in LC method development is the achievement of sufficient resolution of the target drug from all other compounds present in the sample with acceptable peak symmetry in a reasonable analysis time. To achieve this goal, several experiments were carried out to optimize both the stationary and mobile phases. For optimization of the stationary phase, several reversed phase columns (Thermo-C18 (4.6 × 250 mm), Waters-C18 (4.6 × 250 mm) and Durashell-C18 (4.6 × 250 mm) were tested. The best clear separation between all the eluting peaks (RFL and its degradation products), better system suitability parameters, symmetric RFL peak and relatively short retention time were attained using the Durashell-C18 column; hence, it became the column of choice for this study.

Several mobile phases were evaluated using various proportions of different aqueous phases and organic modifiers. The best mobile phase combination was 0.0065 M ammonium acetate pH 6.3, methanol and acetonitrile in the ratio 30:35:35 (by volume). Methanol alone was tried as an organic modifier and ammonium acetate solution was substituted by other aqueous phases such as water. In these trials, RFL suffered from increased retention time and some chromatograms showed broad peaks and/or unresolved peaks. The mixed mobile phase was used as final dilution solvent for all working solutions since it resulted in more decent chromatograms and sharper peaks than those obtained upon using methanol or acetonitrile alone. Flow rate was kept constant at 1.3 mL/min and column temperature was adjusted at 30 °C.

The diode array detector (DAD) enhances the power of HPLC and is an elegant option for assessing method specificity by monitoring the recorded spectra during peak elution. The multiple wavelength detection offers the advantage of measuring the analyte at its maximum wavelength, thus improving sensitivity. RFL exhibits considerable absorption all over the range 200–310 nm with two maxima at 213 and 251 nm of which the latter was chosen. Accordingly, all chromatograms in this study were recorded at 251 nm. Quantification was based on peak area measurement. The previously described chromatographic conditions showed well-defined symmetric RFL peak at about 6.24 ± 0.005 min. Fig. 2 shows a typical chromatogram for RFL using the optimized conditions. Retention factor (k') is 5.25, tailing factor is 1.04 and column performance (apparent efficiency) expressed by the number of theoretical plates (N) is 9631.

3.2. Stability-indicating aspects

Forced degradation experiments were carried out on standard RFL to produce the possible relevant degradation products and test their chromatographic behavior using the developed method. Hydrolytic, using neutral (water), strong acidic (1 M HCl) and strong basic (1 M NaOH) media, oxidative (5%

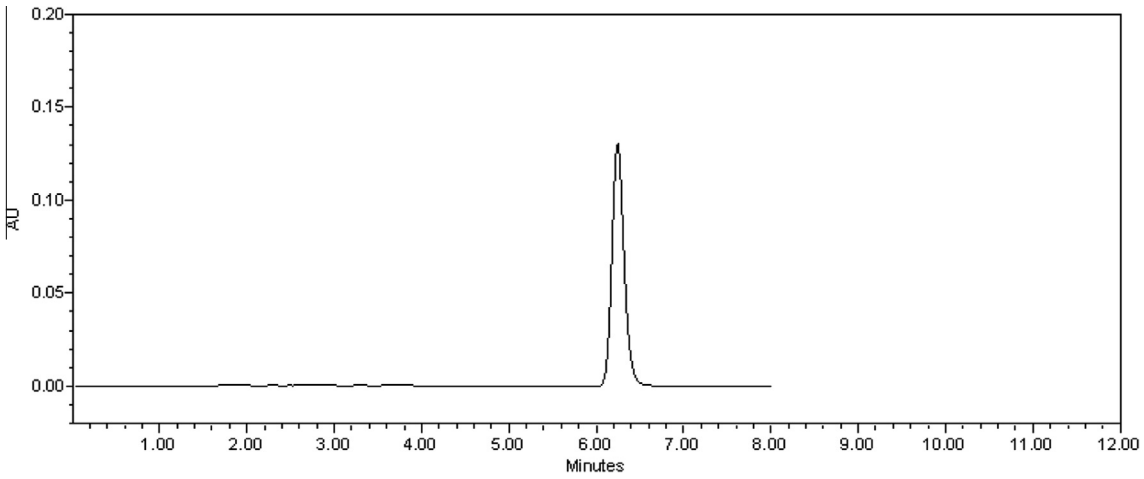

Figure 2 Representative HPLC chromatogram of RFL (25 μg/mL).

H_2O_2), photolytic and dry heat degradation experiments were conducted, and the resulting chromatograms were compared with those obtained from a standard untreated solution of the drug. No strong signs of degradation of RFL were observed after heating for 6 h at 90 °C in neutral conditions. RFL peak appeared at its specific retention time with an area almost identical (97.8%) to that of a standard of the same concentration and only small extra peaks at 2.81 and 8.29 min were observed (Fig. 3a). The situation was quite different in case of degradation under acidic conditions. About 33% decrease in the parent drug peak area as well as the appearance of new peaks for degradation products at 2.94, 3.32, 3.72 and 8.27 min was clearly noticed after heating with 1 M HCl solution at 90 °C for 1 h (Fig. 3b). Resolution was calculated between RFL and both the preceding and successive degradation peaks at 3.72 and 8.27 min, respectively. Resolution was found not less than 7.69 which implied an adequate baseline separation between RFL and either of its nearest degradation peaks. Likewise in case of degradation under basic conditions, about 18.5% decrease in the parent drug peak area as well as the appearance of new peaks for degradation products at 2.13, 2.81, 3.34 and 4.83 min was clearly detected after heating with 1 M NaOH solution at 90 °C for 1 h (Fig. 3c). Excellent resolution (R_s = 6.23) was observed between RFL and its nearest degradation peak at 4.83 min. Oxidative degradation with 5% H_2O_2 at 90 °C for 1 h revealed almost intact RFL peak. On the other hand, heating with the same reagent at 90 °C for 5 h caused about 26.4% reduction in the peak area of RFL. Several degradation peaks can be seen in the chromatogram at retention times 2.92, 3.31, 3.80, 4.18, 4.55, 4.93, 5.71 and 7.80 min (Fig. 3d). Again, resolution was calculated between RFL and the preceding degradation peak at 5.71 min, and it was found 1.78 which implied an adequate baseline separation between the two successive peaks. Irradiation of RFL powder with UV light for 6 h caused no significant change in the peak area of the parent compound. Fig. 3e shows the chromatogram of RFL after UV photolytic degradation and two well-resolved small extra peaks at 2.79 and 10.77 min are detected. Finally, RFL was found stable under thermal (dry heat) degradation conditions. Its peak appeared at its specific retention time with an area comparable to that of standard of the same concentration, and the chromatogram did not show any extra peaks (Fig. 3f). These results are in agreement with the previous study which indicated that RFL is stable to thermal and photodegradation.[7] In all these forced degradation experiments, RFL was successfully separated from the degradation products as confirmed by the resolution values calculated for each chromatogram (R_s > 1.5). Results of the forced degradation of RFL in different conditions are summarized in Table 1.

RFL Peak purity was checked in all the forced degradation chromatograms by using the diode array detector. Purity angle is a measure of spectral homogeneity. It is the weighted average of all Spectral Contrast Angles calculated by comparing the spectra from all data points in the integrated peak against the peak apex spectrum. If the purity angle is less than the calculated threshold angle, the peak is spectrally homogeneous. If the purity angle is greater than the calculated threshold angle, there is something within the peak that cannot be explained by noise, and consequently the peak is considered impure or not spectrally homogeneous. The obtained purity angles were within the purity threshold limits which confirm that RFL peaks are homogenous and pure in all the analyzed samples subjected to forced degradation conditions (Fig. 4).

3.3. Validation of the proposed method

The proposed HPLC method was validated as per the International Conference on Harmonization (ICH) guidelines on validation of analytical procedures.[10]

3.3.1. Linearity and concentration range

The linearity of the proposed HPLC procedure was evaluated by analyzing a series of different concentrations for the analyte (n = 14). The linear regression equation was generated by least squares treatment of the calibration data. Under the optimized conditions described above, the measured peak areas were found perfectly proportional to concentrations of RFL (Fig. 5). Table 2 presents the analytical parameters including linear regression equation, concentration range, correlation coefficient, standard deviations of the intercept (S_a), slope (S_b) and standard deviations of residuals ($S_{y/x}$). Regression analysis shows good linearity as indicated from the correlation coefficient values (>0.9998). In addition, deviation around the slope can be further evaluated by calculating the RSD% of the slope (S_b%) which was found to be

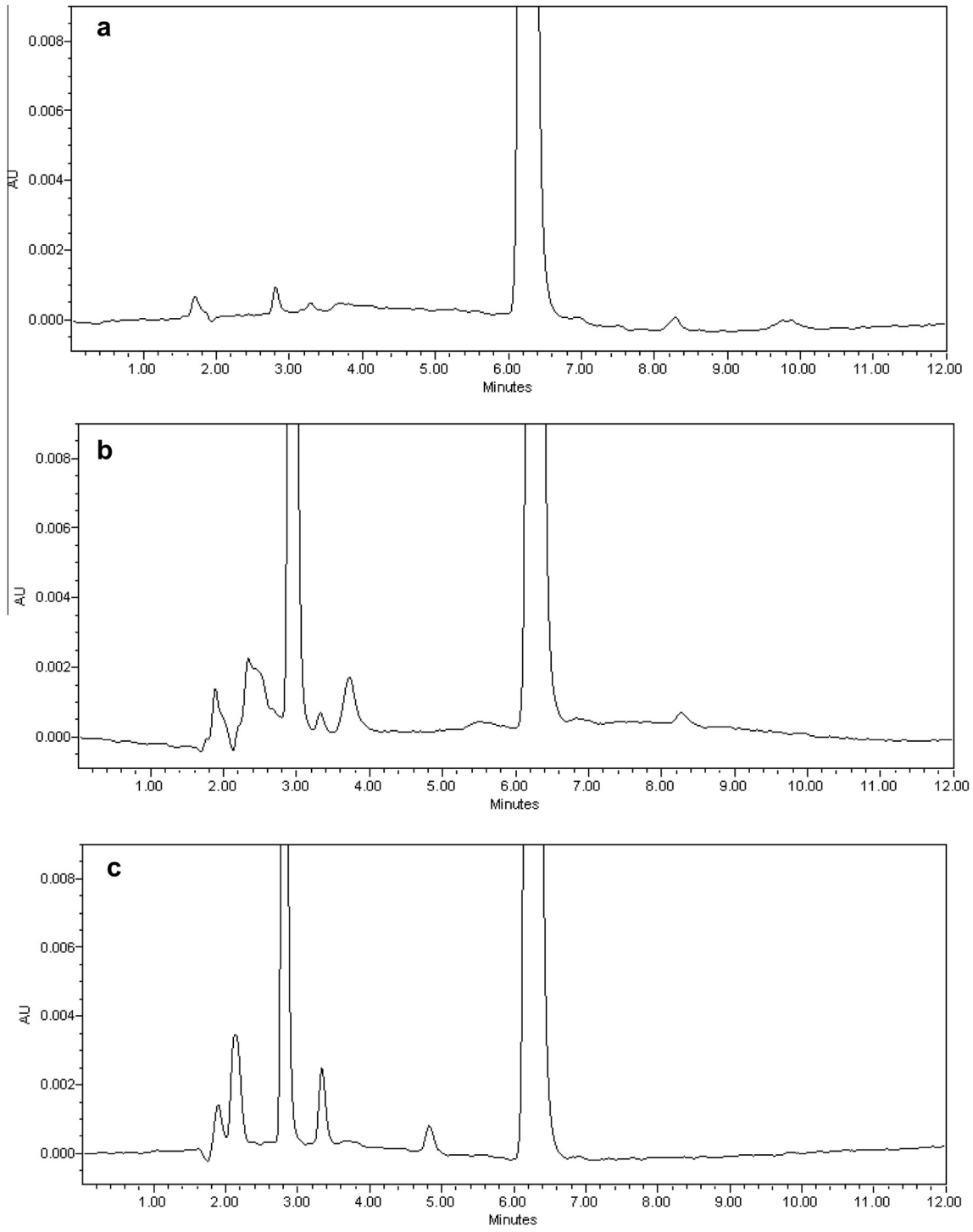

Figure 3 HPLC chromatograms of RFL (25 μg/mL) after exposure to neutral (a), acid (b), base (c), oxidative (d), photolytic UV (e) and thermal dry heat degradation (f).

less than 0.5%. Linearity can be further guaranteed by the analysis of variance (ANOVA) test. The most important statistic in this test is the *F*-value which is the ratio of the mean of squares due to regression divided by the mean of squares due to residuals. High *F* value reveals an increase in the mean of squares due to regression and a decrease in the mean of squares due to residuals. The greater the mean of squares due to regression, the steeper is the regression line. The smaller the mean of squares due to residuals, the less is the scatter of experimental points around the regression line. Consequently, regression lines with high *F* values (low significance *F*) are much better than those with lower ones. Good regression lines show high values for both *r* and *F* statistical parameters.

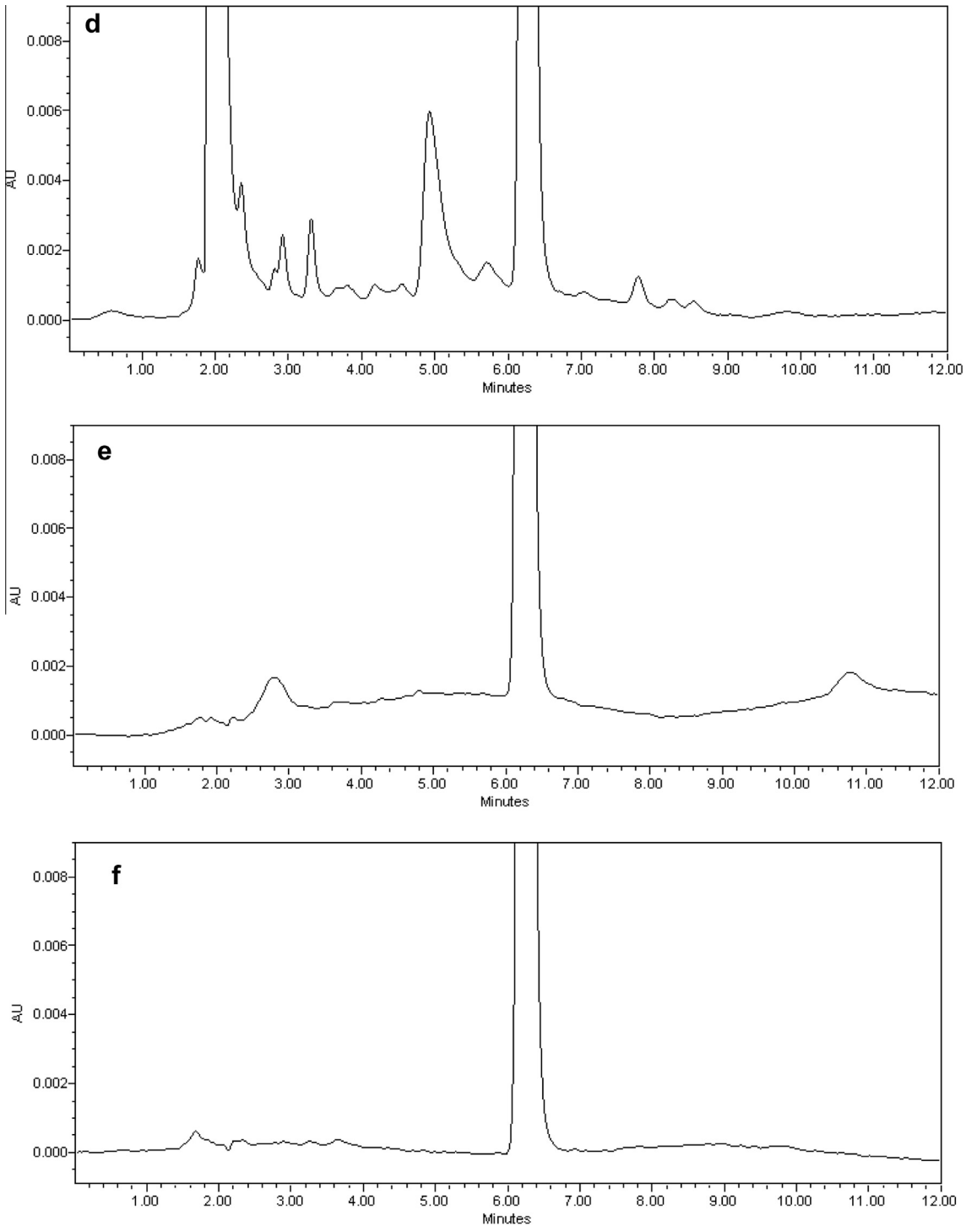

Figure 3 (*continued*)

3.3.2. Detection and quantification limits

The limit of detection (LOD) is defined as the concentration of the analyte which has a signal-to-noise ratio of 3:1. For the limit of quantification (LOQ), the ratio considered is 10:1. The LOD and LOQ values of RFL were calculated using the signal-to-noise ratio method and are given in Table 2. Both LOD and LOQ values confirm the sensitivity of the proposed HPLC method.

3.3.3. Accuracy and precision

The within-day (intra-day) precision and accuracy for the proposed method were studied at four concentration levels for RFL using three replicate determinations for each concentration within 1 day. Similarly, the between day (inter-day) precision and accuracy were tested by analyzing the same four concentrations using three replicate determinations repeated on 3 days. Recoveries were calculated using

Table 1 Summary of the degradation results of RFL.

Degradation type	Degradation conditions	% Remaining of RFL	Retention times of the degradation peaks (min)
Neutral (water)	90 °C for 6 h	97.8	2.81, 8.29
Acidic (1 M HCl)	90 °C for 1 h	67.0	2.94, 3.32, 3.72, 8.27
Basic (1 M NaOH)	90 °C for 1 h	81.5	2.13, 2.81, 3.34, 4.83
Oxidation (5% H_2O_2)	90 °C for 5 h	73.6	2.92, 3.31, 3.80, 4.18, 4.55, 4.93, 5.71, 7.80
Photo-degradation	UV irradiation for 6 h	98.0	2.79, 10.77
Thermal (dry heat)	120 °C for 8 h	98.5	–

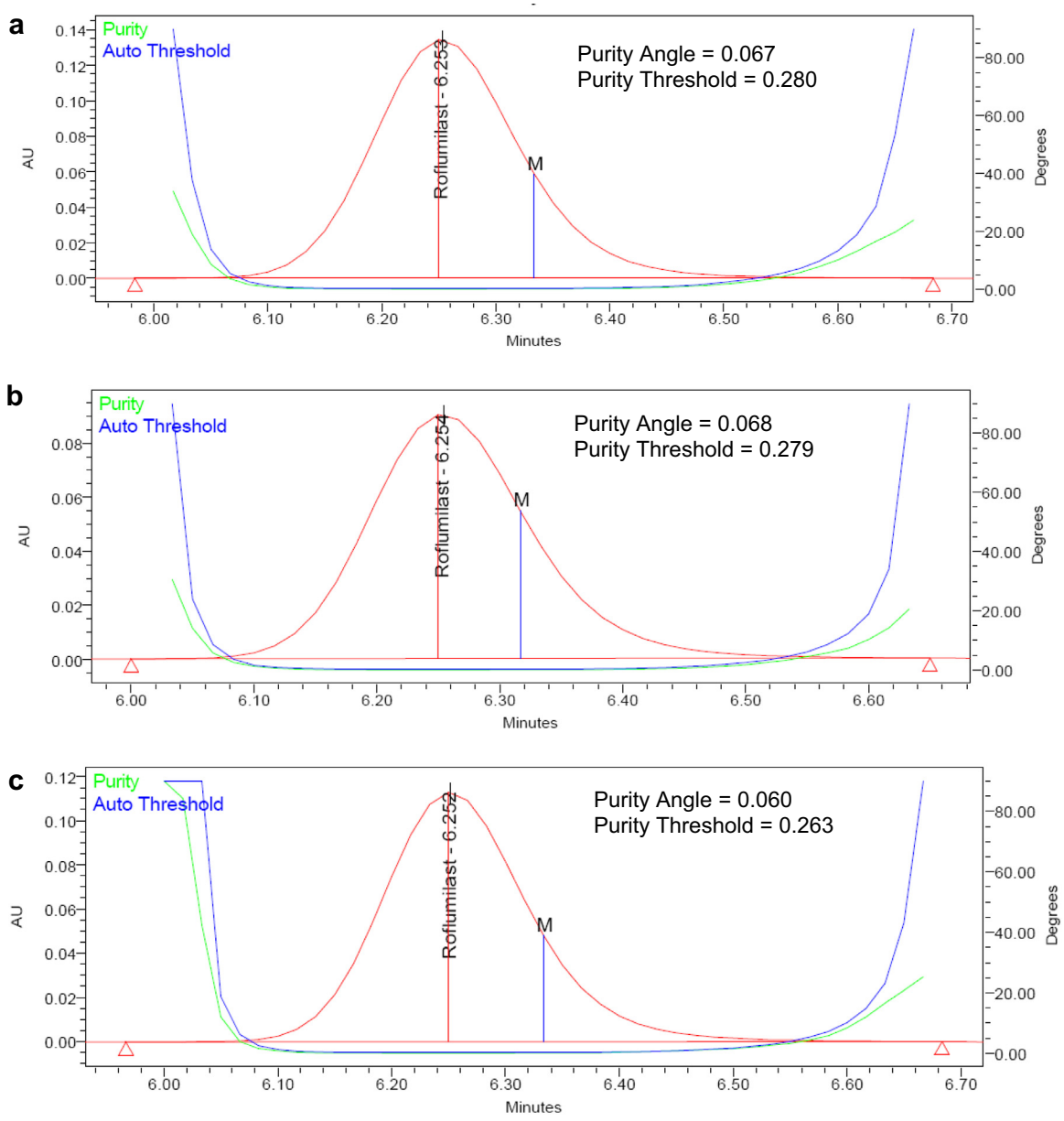

Figure 4 Purity plots of RFL after exposure to neutral (a), acid (b), base (c), oxidative (d), photolytic UV (e) and thermal dry heat degradation (f).

the corresponding regression equation and they were satisfactory. The percentage relative standard deviation (RSD%) and percentage relative error (E_r%) did not exceed 1.1% proving the high precision and accuracy of the developed method for estimation of the analyte in its bulk form (Table 3).

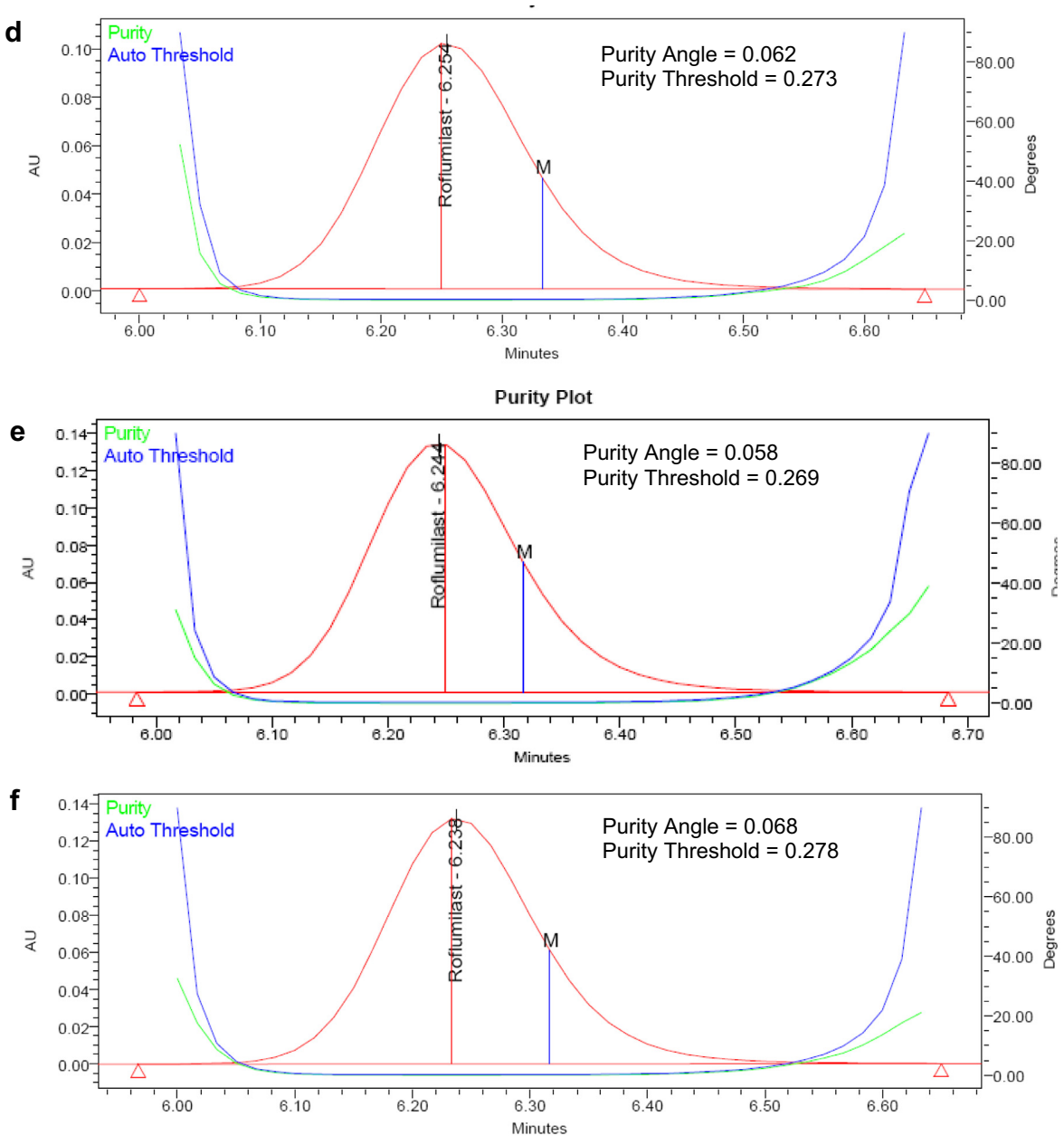

Figure 4 (*continued*)

3.3.4. *Specificity*

Specificity is defined as the ability to access unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components.[10] Method specificity was already demonstrated by the successful resolution of the intact drug from its forced degradation products. DAD played a crucial role to confirm the purity of RFL peaks in all forced degradation chromatograms (Figs. 3 and 4). Moreover, to judge the specificity of the proposed method, several pharmaceutical compounds of different pharmacological categories were tested to examine their retention properties and their resolution from the target compound. This approach to test the specificity of an HPLC method has been already applied in previous studies.[11,12]

More than 20 compounds were found completely separated from the drug under analysis. Resolution was calculated between RFL and its nearest preceding or successive peaks. Resolution was found not less than 9.53 which implies an adequate baseline separation between the analyte and the added compounds. Table 4 lists the separated compounds along with their retention time values. Fig. 6 shows an example chromatogram for the separation of RFL together with several added pharmaceutical compounds.

3.3.5. *Robustness*

The robustness of an analytical procedure is a measure of its capability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.[10] Robustness was examined

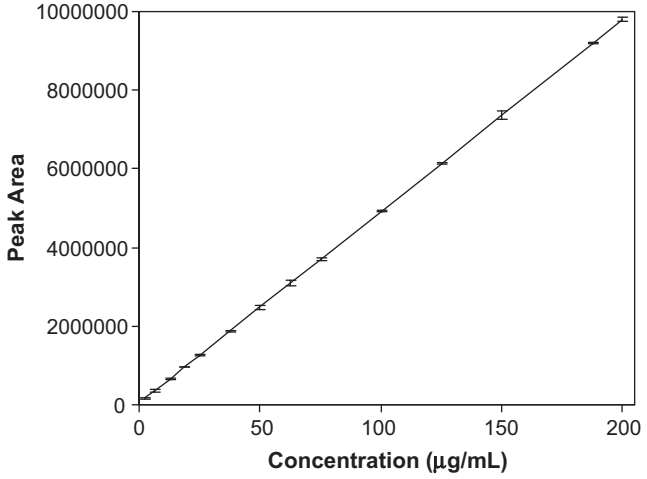

Figure 5 Calibration curve for RFL analysis.

Table 2 Analytical parameters for the determination of RFL using the proposed HPLC-DAD method.

Parameter	Result
Wavelength (nm)	251
Concentration range (µg/mL)	2.5–200
Intercept (a)	39999.1
S_a	20871.6
Slope (b)	48784.9
S_b	210.3
RSD% of the slope	0.43
Correlation coefficient (r)	0.99989
$S_{y/x}$	50982.9
F	53813.1
Significance F	2.77×10^{-23}
LOD (µg/mL)	0.56
LOQ (µg/mL)	1.87

by making small changes in acetonitrile or methanol content in the mobile phase (±2%), flow rate (±0.05 mL/min), column temperature (±5 °C), pH of ammonium acetate solution (±0.3) or working wavelength (±2 nm) and recording the chromatograms of RFL standard solution. These variations did not have significant effects on the measured response (peak area) or retention time of RFL. Recoveries obtained were acceptable (99.4–102%), and RSD% values did not exceed 3.6%. Table 5 shows the effects of the studied variations on the percentage recoveries and retention time of RFL.

Table 4 Retention times of the tested pharmaceutical compounds using the proposed method.

Compound	Retention time (min)
Pheniramine maleate	1.72 and 2.75
Chlorpheniramine maleate	1.72 and 3.52
Dimethindene maleate	1.70 and 3.89
Ketotifen fumarate	1.57 and 4.55
Clemastine maleate	1.65 and 8.80
Salbutamol sulfate	1.97
Terbutaline sulfate	1.97
Dexamethazone sodium phosphate	2.09
Theophylline	2.10
Aminophylline	2.10
Formotrol fumarate	2.32
Guaifenesin	2.38
Fexofenadine HCl	2.65
Prednisone	2.88
Prednisolone	3.09
Cetirizine HCl	3.67
Desloratadine HCl	3.76
Zafirlukast	4.30
Loratadine HCl	14.52
Montelukast sodium	27.27
Ebastine	42.67

3.3.6. Stability of solutions

The stability of RFL working solutions as well as sample solutions in the mobile phase was examined, and no chromatographic changes were observed within 9 h at room temperature. Also, the stock solutions prepared in HPLC-grade methanol were stable for at least 1 week when stored under refrigeration at 4 °C. Retention times and peak areas of the drug remained unchanged and no significant degradation was observed during these periods.

3.4. Assay of RFL in tablets

The developed method was applied for the assay of the drug in its commercial pharmaceutical formulation (Dalivent® tablets). An accurate amount of the tablets powder was extracted into the mobile phase and an aliquot of the tablet extract was directly injected to the column. The active ingredient eluted at its specific retention time, and no interfering peaks were observed from any of the excipients of the assayed tablets. The diode array detection enables peak purity verification where no signs of co-elution from any of the excipients were detected (Fig. 7). Recoveries were calculated using both external standard and standard addition methods. Assay results

Table 3 Precision and accuracy for the determination of RFL in bulk form using the proposed method.

Nominal value (µg/mL)	Within-day			Between-day		
	Found ± SD[a] (µg/mL)	RSD (%)	E_r (%)	Found ± SD[a] (µg/mL)	RSD (%)	E_r (%)
12.5	12.51 ± 0.03	0.24	0.08	12.53 ± 0.09	0.72	0.24
25	25.19 ± 0.09	0.36	0.76	25.15 ± 0.19	0.76	0.60
50	49.62 ± 0.24	0.48	−0.76	49.62 ± 0.41	0.83	−0.76
150	150.49 ± 0.48	0.32	0.33	150.15 ± 1.64	1.09	0.10

[a] Mean ± standard deviation for three determinations.

Table 5 Robustness evaluation for the determination of RFL using the proposed HPLC-DAD method.

Parameter	% Recovery ± SD[a]	RSD%	Retention time ± SD
Methanol ratio in mobile phase ±2%	99.42 ± 0.74	0.74	6.264 ± 0.64
Acetonitrile ratio in mobile phase ±2%	99.45 ± 0.73	0.73	6.289 ± 0.73
Column temperature 30 ± 5 °C	100.47 ± 0.70	0.70	6.235 ± 0.25
Flow rate 1.3 ± 0.05 mL/min	100.74 ± 3.58	3.55	6.238 ± 0.19
Wavelength 251 ± 2 nm	99.45 ± 0.69	0.69	6.243 ± 0.002
pH 6.3 ± 0.3	101.94 ± 1.26	1.24	6.237 ± 0.005

[a] Mean ± standard deviation for 3 determinations.

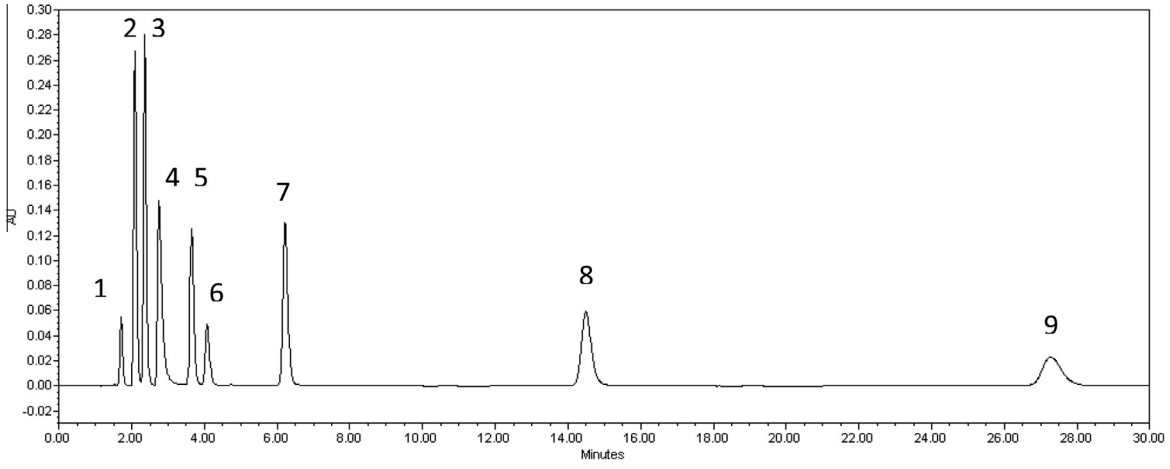

Figure 6 Representative chromatogram of a mixture containing (1) maleate, (2) theophylline, (3) guaifenesin, (4) pheniramine, (5) cetirizine HCl, (6) zafirlukast, (7) RFL, (8) loratadine HCl and (9) montelukast.

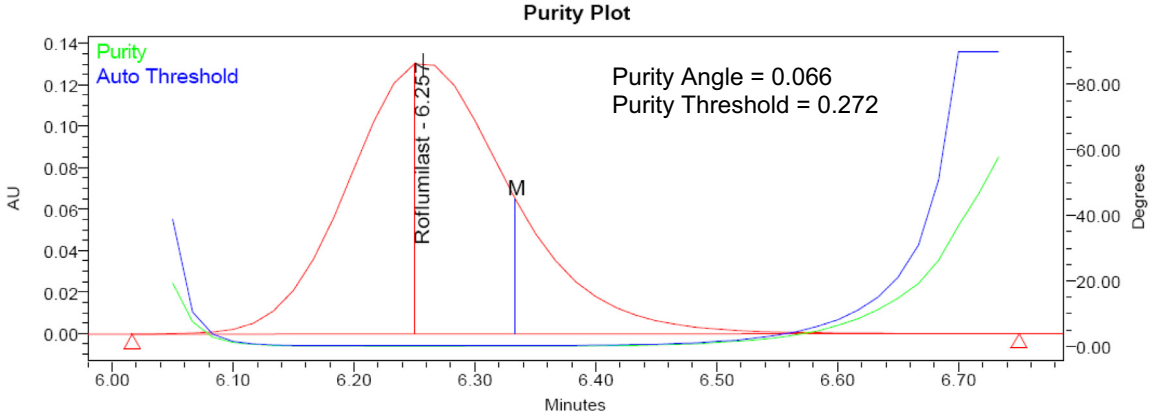

Figure 7 Purity plot of RFL in its commercial dosage form (Dalivent® tablets).

revealed satisfactory accuracy and precision as indicated from % recovery, SD and RSD% values (Table 6). Furthermore, a previously published UV spectrophotometric method was applied as a comparison method for estimation of the drug in its formulation.[9] This reported method depends on measurement of RFL at its λ_{max} 248 nm in methanol. Recovery data obtained from the developed HPLC-DAD method were statistically compared with those of the comparison method using Student's *t*- and the variance ratio *F*-tests. In both tests, the calculated values did not exceed the theoretical

Table 6 Application of the proposed HPLC-DAD method to the analysis of RFL in its dosage form (Dalivent® tablets).

	External standard	Reference method[b]	Standard addition
% Recovery ± SD[a]	100.51 ± 0.44	99.75 ± 0.83	99.01 ± 0.47
RSD%	0.44	0.83	0.48
t	1.81		
F	3.56		

Theoretical values for *t* and *F* at $P = 0.05$ are 2.31 and 6.39, respectively.
[a] Mean ± standard deviation for five determinations.
[b] Ref. 9.

ones at the 95% confidence level which indicated that there were no significant differences between the recoveries obtained from both methods (Table 6). It is evident from these results that the proposed method is applicable to the assay of RFL in tablets with minimum sample preparation and satisfactory level of accuracy and precision.

4. Conclusion

This study described a simple, selective and reliable HPLC-DAD procedure for the assay of roflumilast in tablet dosage form. To the best of our knowledge, this is the first HPLC report describing the quantification of RFL in its tablet dosage form. The described method is superior to the previously reported non-selective UV spectrophotometric method for tablets assay.[9] A significant advantage in the study is the separation of RFL from several degradation peaks obtained by hydrolytic, oxidative, photolytic and thermal forced degradation experiments. In addition, specificity was further demonstrated by separation of the analyte from several pharmaceutical compounds of different pharmacological categories. The described method is advantageous to the previously reported HPLC methods[7,8] since the proposed method involves the use of simple isocratic elution compared to the multi-step gradient elution of these previous methods.[7,8] In addition the proposed method was able to resolve roflumilast in about 6 min compared to the published methods that required about 13 min runtime.[7,8] The previous stability-indicating study reported obvious degradation of RFL in only alkaline medium,[7] while this current study demonstrates the significant degradation of the drug in acidic, alkaline and oxidative conditions. Reliability was guaranteed by testing various validation parameters of the method and the successful application to commercial tablet dosage form. The method can thus be used for routine analysis, quality control, and for checking quality during stability studies of pharmaceutical preparations containing the drug.

5. Conflict of interest

We have no conflict of interest to declare.

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