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Article

Novel Validated Stability-indicating Ultra-performance Liquid Chromatography Method for the Determination of Roflumilast and its Degradation Products in Active Pharmaceutical Ingredient and in Pharmaceutical Dosage Forms

Varaprasad Jagadabi[1,2], P. Venkata Nagendra Kumar[1]*, Srinivasu Pamidi[2], L. A. Ramaprasad[2], R. Ramakrishna[2]

[1]Department of Chemistry, School of Technology, GITAM University, Hyderabad – 502 329, Telangana, India, [2]Analytical R&D, Hetero Labs Ltd., Hyderabad – 550 055, Telangana, India

ABSTRACT

A novel stability-indicating reverse-phase ultra-performance liquid chromatographic method was developed for quantitative determination of roflumilast and its related impurities and degradation products. Chromatographic separation was achieved using a waters acquity ultra-performance liquid chromatography BEH C18 100 mm × 2.1 mm, 1.7 µ column with mobile phase containing a gradient mixture of mobile phase A and B at 60°C with a flow rate of 0.4 mL/min. The related compounds were monitored at 220 nm. The run time was 15 min within which roflumilast and its seven related impurities were well resolved. The developed method was validated as per ICH guidelines with respect to specificity, linearity, limit of detection, limit of quantification, accuracy, precision, and robustness. The calibration curves obtained for the seven impurities were linear over the range of 0.202–3.880 µg/mL. The relative standard deviations of intra- and inter-day experiments were <3.0%. The detection limits ranged from 0.070 to 0.085 µg/mL depending on the impurity.

Key words: Roflumilast, Method validation, Potential degradation products, Impurities, Stability indicating, Ultra-performance liquid chromatography.

1. INTRODUCTION

Acquity ultra-performance liquid chromatography (UPLC) systems take advantage of technological strides made in particle chemistry performance, system optimization, detector design, and data processing and control. When taken together, these achievements have created a step-function improvement in chromatographic performance. Defined as UPLC [1], this new category of analytical separation science retains the practicality and principles of high-performance LC (HPLC) while increasing the overall interlaced attributes of speed, sensitivity, and resolution.

Roflumilast is a novel phosphodiesterase-4 (PDE-4) inhibitor [2-4]. The chemical name of roflumilast is N-(3,5-dichloropyridin-4-yl)-3-cyclopropylmethoxy-4-difluoromethoxy-benzamide**.** Roflumilast and its active metabolite (roflumilast N-oxide) are selective PDE-4 inhibitors. Due to its selective inhibition of the PDE-4 isoenzyme in lung cells, roflumilast is indicated for the management of chronic obstructive pulmonary disease exacerbations. Treatment with roflumilast is associated with an increase in psychiatric adverse reactions including suicide and suicidal attempts. It is a weak acid with a pKa of 8.74. It is practically insoluble in water (0.52–0.56 ml/L) and hexane. The solubility n aqueous solvents increase from about 0.8 mg/L under neutral conditions to 35.8 g/L at pH 10. It is sparingly soluble in ethanol and soluble in acetone. Its melting point is 160°C. Its molecular formula is $C_{17}H_{14}Cl_2F_2N_2O_3$ and the molecular weight is 403.22.

A few methods were available for the determination of roflumilast by HPLC [5,6,7,8], by ultraviolet (UV) [9] and by LC with tandem mass spectrometry (LC-MS/MS) [10]. No method has been reported till now for the quantitative determination of roflumilast and its related impurities (Figure 1) by UPLC, in its formulation as well as for active pharmaceutical ingredient. Hence, a reproducible and a stability-indicating method was developed for the quantification of roflumilast and its seven related impurities, namely RC.01, RC.02, RC.03, RC.04, RC.05, RC.06, and RC.07 (Figure 1) as well as its degradation impurities using UPLC, which can reduce the analysis run time without compromising the resolution and sensitivity. This method was validated accordingly ICH guidelines [11].

1.1. Determination of Roflumilast and Related Compounds

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2. EXPERIMENTAL

2.1. Materials and Reagents

Roflumilast working standard, standard materials of both degradation impurities and process Impurities were obtained from Hetero Labs, Hyderabad, India. Roflumilast tablets 500 mcg were made the in-house laboratory of Hetero Labs, Hyderabad, India. Monobasic potassium phosphate (analytical reagent [AR] grade), potassium hydroxide (AR

***Corresponding author:**
E-mail: pv.nagendrakumar@yahoo.com

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grade), methanol (HPLC grade), and acetonitrile (HPLC grade) were purchased from Merck Chemicals, India. Water was purified by milli-Q-water purification system (Siemens water purification by Elga), used for the preparation of mobile phase.

2.2. Preparation of Standard Solution

A standard stock solution of roflumilast (250 µg/mL) was prepared an appropriate proportion of roflumilast working standard in diluent (methanol and acetonitrile in [30:90%v/v]). A standard solution containing 2.5 µg/mL was prepared from standard stock solution.

2.3. Preparation of Sample Preparation

A test solution containing of 500 µg/mL of roflumilast was prepared by taking tablet powder equivalent to 25 mg of roflumilast into a 50 mL volumetric flask, added 30 mL diluent and sonicated for 30 min, cooled to room temperature, diluted to volume with diluent, and filtered the test solution through 0.22 µ PVDF (millipore) filter.

Another sample was prepared with spiked with related impurities (RC-01, RC-02, RC-03, RC-04, RC-05, RC-06, and RC-07) at 0.5% of sample concentration (500 µg/mL), i.e. 2.5 µg/mL (Figure 2).

2.4. Chromatographic Conditions and Equipment

The analysis was performed on waters acquity system H-class equipped with quaternary solvent delivery pump and photodiode array (PDA) detector. Data acquisition and processing were done using EMPOWER-3 software (Waters Corporation USA). The chromatographic separation was performed using acquity UPLC BEH C18 100 mm × 2.1 mm, 1.7 µ column. Mobile phase A was monobasic potassium phosphate (2.72 g/L) adjusted pH 6.0 with 5% potassium hydroxide solution and methanol in the ratio of 90:10% v/v and mobile phase B was monobasic potassium phosphate (2.72 g/L) adjusted pH 6.0 with 5% potassium hydroxide solution and methanol in the ratio of 10:90% v/v. The gradient programme T (min) =% mobile phase B: 0=45, 3=60, 10=70, 12=70, 12.1=45, and 15=45 with flow rate of 0.4 mL/min. The injection volume was 1 µL and detection wavelength was set at 220 nm. The column temperature was maintained at 60°C. Sample cooler was maintained at 5°C.

2.5. LC–MS/MS Conditions

An LC–MS/MS system (WATERS QUATTRO MICRO MASS with Empower software) was used for the known compounds formed during forced degradation studies. A Hypersil BDS C18, 250 mm–4.6 mm, 5 mm column (Thermo) was used as the stationary phase. A 0.01 M solution of ammonium formate (Merck, Germany) in water, pH adjusted 6.0 with formic acid was used as a buffer. The ammonium formate buffer and acetonitrile in a ratio of 90:10 (v/v) were used for solvent A, and ammonium formate buffer and acetonitrile in a ratio of 10:90 (v/v) were used for solvent B. The gradient program (time/%B) was set as 0.01/35, 10/55, 25/55, 30/60, 40/60, 50/35, and 60/35. Methanol and acetonitrile

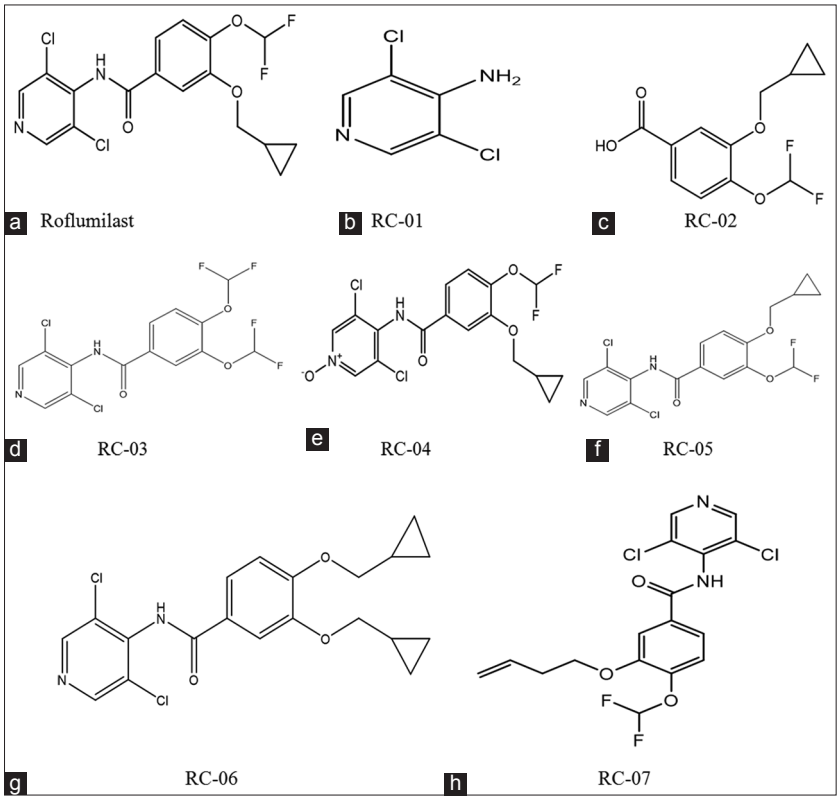

Figure 1: (a) Roflumilast molecular formula: $C_{17}H_{14}Cl_2F_2N_2O_3$ Mol. Wt: 403.22 3-(cyclopropylmethoxy)-N-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy)benzamide; (b) RC-01 Molecular formula: $C_5H_4Cl_2N_2$ Mol.Wt:163.00 3,5-dichloro pyridine-4-amine; (c) RC-02 molecular formula: $C_{12}H_{12}F_2O_4$ Mol. Wt: 258.22 3-(Cyclopropylmethoxy)-4-(difluoromethoxy)benzoic acid; (d) RC-03 molecular formula: $C_{14}H_8Cl_2F_4N_2O_3$ Mol.Wt:399.12 N-(3,5-dichloro pyridine-4-yl)-3,4-bis(difluoromethoxy)benzamide; (e) RC-04 Molecular formula: $C_{17}H_{14}Cl_2F_2N_2O_4$ Mol. Wt: 419.21 3-(Cyclopropylmethoxy)-N-(3,5-dichloro-4-pyridinyl-1-oxide)-4-(difluoromethoxy) benzamide; (f) RC-05 molecular formula: $C_{17}H_{14}Cl_2F_2N_2O_3$ Mol.Wt:403.21 4-(Cyclopropylmethoxy)-N-(3,5-dichloro-4-pyridinyl)-3-(difluoromethoxy)benzamide; (g) RC-06 molecular formula: $C_{20}H_{20}Cl_2N_2O_3$ Mol. Wt: 407.29 3,4-bis(Cyclopropylmethoxy)-N-(3,5-dichloropyridin-4-yl)benzamide; (h) RC-07 molecular formula: $C_{17}H_{14}Cl_2F_2N_2O_3$ Mol. Wt: 403.21 3-(But-3-en-1-yloxy)-N-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy)benzamide.

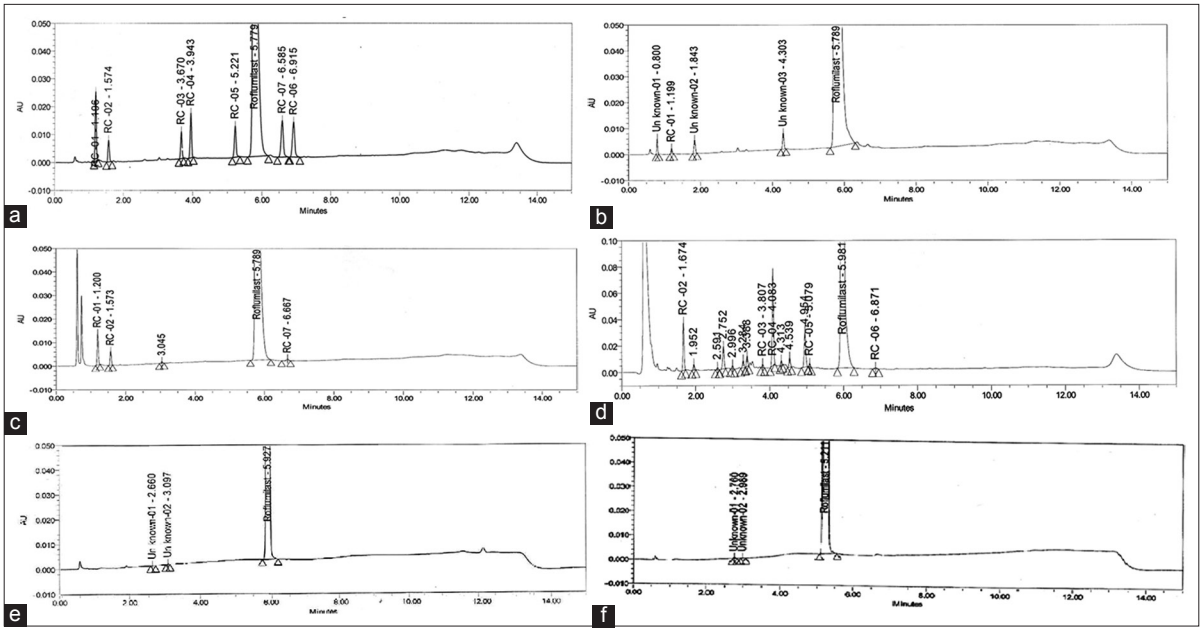

Figure 2: (a) Impurities spiked chromatogram; (b) acid degradation chromatogram; (c) base degradation chromatogram; (d) peroxide degradation chromatogram; (e) photodegradation chromatogram; (f) thermal degradation chromatogram.

in a ratio of 30:70 (v/v) was used as a diluent. The flow rate was 1.0 mL/min. The analysis was performed in positive electrospray/positive ionization mode, the ion source voltage (capillary) was 3.50 KV, and the source temperature was 120°C. Dissolvation temperature was 350°C, cone gas flow was 100 L/h, and dissolvation gas flow was 950 L/h.

3. STRESS STUDIES

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities [10]. The specificity of the developed LC method for roflumilast was carried out in the presence of its seven impurities. Stress studies were performed at an initial concentration of 500 µg/mL of roflumilast to provide an indication of the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted to stress condition of UV light (254 nm), heat (105°C), acid (5 N HCl at 80°C), base (1 N NaOH at 80°C), oxidation (10.0% H2O2 at 80°C), and a photolytic degradation at which the sample is exposed to UV light at 254 nm for 7 days to evaluate the ability of the proposed method to separate roflumilast from its degradation products. For heat, the study period was 24 h; for acid and base studies, the period was 2 h; and for oxidation studies, the period was 2 h. The purity of peaks obtained from stressed samples was checked by the use of the PDA detector. The purity angle was within the purity threshold limit obtained in all stressed samples and demonstrates the analyte peak homogeneity.

4. METHOD VALIDATION

The described method has been validated for the related compounds by UPLC determination. According to the FDA and ICH, the key analytical parameters that are required for validation are precision, accuracy, linearity, limit of detection (LOD), limit of quantification (LOQ), and ruggedness.

4.1. Precision

The precision of the related substance method was checked by injecting six individual sample preparations of (500 µg/mL) roflumilast spiked with seven impurities (Figure 2) with respect to analyte concentration. Percentage relative standard deviation (%RSD) of area for all the impurities calculated. The intermediate precision of the method was also evaluated using different analyst, different day, and different make instrument in the same laboratory. The %RSD for both the results was found to be within 4%.

4.2. LOD and LOQ

The LOD and LOQ for seven impurities as well as for roflumilast were estimated at a signal-to-noise ratio method. The LOD of roflumilast and its impurities were found to be 0.01–0.03 µg/mL (of analyte concentration of 500 µg/mL). The LOQ of roflumilast and its impurities were found to 0.03–0.05 µg/mL. Precision study was also carried at the LOQ level by injecting six individual preparations impurities and was found to be <3.0%.

4.3. Accuracy

The accuracy for all the seven impurities in formulation samples was studied. The study was carried out in triplicate at LOQ, 0.1%, 0.5%, and 0.75% of the analyte concentration (500 µg/mL). The percentage recoveries for impurities were ranged from 95% to 105%.

4.4. Linearity of Response

The linearity of the method was tested to demonstrate proportional relationship of response versus analyte concentration over the working range. It is usual practice to perform linearity experiments over a wide range of analyte. This gives confidence that the response and concentration are proportional and consequently ensures that calculations can be performed using a single reference standard/working standard, rather than the equation of a calibration line. The linearity of detector response to different concentrations of impurities was studied by preparing a series of solutions using roflumilast and its related impurities at five different concentrations levels ranging from LOQ to 0.75% w/w of test concentration (500 µg/mL). The correlation coefficients, slopes, and Y-intercepts of the calibration curve were determined.

4.5. Robustness

To determine the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between roflumilast and its all seven related impurities was

evaluated. To study the effect of flow rate on the resolution, the same was altered by 0.1 units, i.e. from 0.30 to 0.50 mL/min. The effect of pH on resolution of impurities was studied by varying ±0.2 pH units (at 5.8 and 6.2 buffer pH). The effect of column temperature on resolution was studied at 55°C and 65°C instead of 60°C. All the other mobile phase components were held constant. In all the variations, the resolution was found to be more than 2.0.

4.5. Solution Stability and Mobile Phase Stability

The diluent used for the preparations of standard and sample preparation was a mixture of acetonitrile and methanol, i.e. 100% organic solvent, hence, to avoid volatility of sample and standard preparation, cooler temperature was used for solution stability. The stability of standard and spiked sample solutions was tested at regular intervals. The stability of solutions was determined by comparing results with freshly prepared standard solution and sample solutions. The differences in values were within 0.05% for identified and 0.01% for unidentified impurities up to 48 h. No significance change observed in the content of impurities during solution stability and mobile phase stability experiments and this confirms that sample solution and mobile phase used during the study was stable up to 48 h at 5°C.

5. RESULTS AND DISCUSSION

5.1. Method Development and Optimization

From the literature, it was found that pKa value for roflumilast is 8.74. The main objective of the chromatographic method is to achieve the separation of impurities (key RAW materials, intermediates, byproducts from the synthesis of roflumilast, stability impurities, and degradation products) and the main component roflumilast. Roflumilast exhibits maximum absorbance at 212 nm and at 254 nm, but based on the UV absorption spectra and response of roflumilast and its impurities, 220 nm was selected as detection wavelength for the method. The blended solution containing roflumilast 500 µg/mL and 2.5 µg/mL each of the impurity was prepared in the diluent. Different buffers such as potassium phosphate, sodium phosphate, and ammonium acetate were evaluated for spiked sample and overall chromatographic performance. Initially, ammonium acetate was used to optimize the buffer as it can be suitable LC/MS method, but baseline was observed to be not good. Potassium phosphate was found to be suitable for better separation of impurities peak tailing. Potassium phosphate buffer ranging from 10 mM to 50 mM was tried, and it was observed that change in buffer concentration did not offer significant changes in elution patterns as well as resolution. However, 20 mM concentration increased the sensitivity of method. Phosphate buffers only choose because its detection at 220 nm. From the preparation of blend sample, it is clear that resolution is critical between all impurities. Hence, different columns with different stationary phases such as C8, C18, and phenyl with different technologies such as BEH C18, BEH phenyl, HSS, and CSH were used for optimization and finally resolution between all impurities was found to be good in acquity UPLC BEH C18 with 100 mm × 2.1 mm, 1.7 µ column. The pH values optimized were 3.0, 5.0, 6.0, 6.5, and 7.0. Finally, the best results were obtained at pH 6.0 ± 0.05 by adjusting with 5% potassium hydroxide. Choice of mobile phase and its pH is justified by the excellent symmetry of peaks and adequate retention times of roflumilast, known impurities, and its degradants. After a series of experiments, the method has been finalized on acquity UPLC BEH C18 100 mm × 2.1 mm, 1.7 µ column using mobile phase A with monobasic potassium phosphate (2.72 g/L) adjusted pH 6.0 with 5% potassium hydroxide solution and methanol in the ratio of 90:10% v/v and mobile phase B with monobasic potassium phosphate (2.72 g/L) adjusted pH 6.0 with 5% potassium hydroxide solution and methanol in the ratio of 10:90% v/v. The UPLC gradient programme T (min) =% mobile phase B: 0=45, 3=60, 10=70, 12=70, 12.1=45, and 15=45. The flow rate of mobile phase was 0.4 mL/min. Different column oven temperatures were tried with 40°C, 50°C, 55°C, 60°C, and 65°C for better peak shape, baseline, and resolution. Better baseline and resolution between impurities were observed at a column oven temperature 60°C. Interference with the excipients (placebo) was also checked, and no interference was observed between the impurity peaks and the roflumilast peak. Several preliminary chromatographic runs were performed to investigate the suitability for drug content estimation and cost because of the increasing importance of rapid economic analysis in pharmaceutical analysis to increase the throughput. The system suitability parameters were evaluated for roflumilast and its seven impurities. The United States Pharmacopeia (USP) tailing factor for all impurities, and roflumilast was found to be <1.5. The USP resolution (Rs) of roflumilast and the potential impurities were >2.0 between all impurities.

5.2. Validation of the Method

5.2.1. Precision

The RSD (%) of peak area for the seven impurities, namely RC-01, RC-02, RC-03, RC-04, RC-05, RC-06, and RC-07 in the study of the repeatability is shown in Table 1. RSD (%) results of roflumilast and its impurities for intermediate precision (intra- and inter-day repeatability) are within 3.0%. These results confirmed that the method was highly precise.

5.2.2.Limits of detection and quantification

The determined LOD, LOQ, and precision at LOQ values for roflumilast and its impurities are reported in Table 1.

Table 1: LOD, LOQ, linearity, and precision data results for roflumilast and its impurities

Parameter	Roflumilast[e]	RC-01	RC-02	RC-03	RC-04	RC-05	RC-06	RC-07
LOD (µg/mL) [a]	0.003	0.085	0.085	0.080	0.075	0.070	0.085	0.070
LOQ (µg/mL) [a]	0.25	0.25	0.26	0.24	0.22	0.21	0.25	0.21
Range (µg/mL) [a]	0.25-3.85	0.25-3.85	0.26-3.88	0.24-3.85	0.22-3.85	0.21-3.87	0.25-3.85	0.21-3.85
Linearity[b]	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999
Repeatability[c]	NA	0.537	0.568	0.535	0.525	0.467	0.571	0.494
[d]RSD (%)	NA	2.1	2.6	2.2	1.6	1.4	1.6	1.2
Intermediate precision[c]	NA	0.541	0.565	0.532	0.528	0.471	0.568	0.490
[d]RSD (%)	NA	2.6	2.0	1.9	2.0	2.1	2.1	1.8

[a]Based on signal-to-noise (S=N) ratio. [b]Determined on five levels. [d]Determined on six values. [e]Roflumilast. LOD: Limit of detection, LOQ: Limit of quantification. RSD: Relative standard deviation

5.2.3. Accuracy

The recovery of roflumilast from pharmaceutical dosage forms ranged from 98.2 to 101.5%. The recovery of the impurities from pharmaceutical dosage forms ranged from 95.7 to 106.4% (Table 2).

5.2.4. Linearity

For all seven impurities and roflumilast, linear calibration curve was obtained ranging from 0.0416% to 0.75% (0.05%, 50%, 75%, 100%, and 150%). The correlation coefficient obtained was >0.999 (Table 1). The results indicate excellent linearity.

5.2.5. Robustness

In all the deliberately varied chromatographic conditions (flow rate, column temperature, mobile phase composition, and pH variation), all of the analytes were adequately resolved, and the order of elution remained unchanged. The resolution between roflumilast and all impurities was >2.0 (Table 3).

5.3. Stability in Solution and in Mobile Phase

The diluent used for the preparations of standard and sample preparation was a mixture of acetonitrile and methanol, i.e. 100% organic solvent, hence, to avoid volatility of sample and standard preparation, cooler temperature was used for solution stability. The stability of standard and spiked sample solutions was tested at regular intervals. The stability of solutions was determined by comparing results with freshly prepared standard solution and sample solutions. The differences in values were within 0.05% for identified and 0.01% for unidentified impurities up

Table 2: Accuracy of impurities

Compound	Level	Amount added	Amount recovered	% recovery
RC-01	LOQ[a]	0.0498	0.0512	102.9
RC-02		0.0512	0.053	104.6
RC-03		0.0486	0.0474	96.0
RC-04		0.0446	0.0467	104.9
RC-05		0.0416	0.0421	101.2
RC-06		0.0500	0.0482	96.4
RC-07		0.0422	0.0434	102.8
RC-01	50%[a]	0.2725	0.2735	99.8
RC-02		0.2740	0.2745	100.2
RC-03		0.2813	0.2842	101.0
RC-04		0.2675	0.2742	102.5
RC-05		0.2456	0.2603	106.0
RC-06		0.2283	0.2112	92.5
RC-07		0.2754	0.2658	96.5
RC-01	75%[a]	0.4410	0.4552	103.2
RC-02		0.4220	0.4405	104.4
RC-03		0.4013	0.3805	94.8
RC-04		0.3684	0.3757	102.0
RC-05		0.3424	0.3565	104.1
RC-06		0.4132	0.4015	97.2
RC-07		0.3479	0.3358	96.5
RC-01	100%[a]	0.5480	0.5470	99.8
RC-02		0.5627	0.5879	104.5
RC-03		0.5350	0.5526	103.3
RC-04		0.4912	0.5215	105.8
RC-05		0.4565	0.4677	102.5
RC-06		0.5509	0.5905	107.2
RC-07		0.4639	0.4747	102.3
RC-01	150%[a]	0.7973	0.7781	99.8
RC-02		0.8184	0.8397	102.6
RC-03		0.7782	0.7968	102.4
RC-04		0.7145	0.7479	0.7479
RC-05		0.6639	0.6871	103.5
RC-06		0.8011	0.8308	103.7
RC-07		0.6748	0.6993	103.6

[a]Determined with triplicate at each level. LOQ: Limit of quantification

Table 3: Robustness data of roflumilast impurities

System suitability parameter	Robustness Parameter	RC-01	RC-02	RC-03	RC-04	RC-05	Rof[a]	RC-07	RC-06
Resolution	Repeatability	-	12.64	-	4.27	-	5.86	-	2.50
	pH (+0.2units)	-	5.31	-	3.1	-	5.58	-	2.92
	pH (−0.2 units)	-	5.73	-	3.77	-	5.74	-	2.65
	Column Temp (+5°C)	-	7.42	-	3.23	-	5.05	-	1.95
	Column Temp (−5°C)	-	12.78	-	4.35	-	5.80	-	2.70
	Flow (0.3 mL/min)	-	12.14	-	4.18	-	6.17	-	2.61
	Flow (0.5 mL/min)	-	12.49	-	4.38	-	5.59	-	2.74
	Organic (+10%)	-	11.54	-	4.02	-	5.03	-	2.45
	Organic (−%)	-	12.55	-	4.43	-	5.88	-	2.5

[a]Roflumilast

Table 4: Forced degradation results

Degradation condition	Time	RS by UPLC % degradation	Remarks/observations
Acid hydrolysis (1 N HCl/80°C)	2 h	0.6%	Impurity RC-01 degradation product formed
Base hydrolysis (1 N NaOH/80°C)	2 h	1.0%	Impurity RC-01 and RC-02 degradation products formed
Oxidation (10% H2O2/80°C)	2 h	11.5%	Impurity RC-02, RC-03, and RC-04 degradation products formed
Photolytic (UV at 254)	7d	0.2%	No degradation observed
Thermal (105°C)	24 h	0.3%	No degradation observed

RS: Related substance, UPLC: Ultra-performance liquid chromatography, UV: Ultraviolet

to 48 h. No significance change observed in the content of impurities during solution stability and mobile phase stability experiments, and this confirms that sample solution and mobile phase used during the study was stable up to 48 h at 5°C.

5.4. Results from Forced Degradation Studies

Forced degradation studies were performed on roflumilast tablets to demonstrate the selectivity and stability-indicating capability of the proposed reverse-phase UPLC method. Accordingly degradation stress studies were conducted by stressing with acid, base, peroxide, photolytic, and thermal conditions.

Degradation was not observed in roflumilast sample during thermal and photolytic stress. About 0.6%, 1.0%, 11.5%,0.2%, and 0.3% degradations were observed in acid (5 N HCl at 80°C for 2 h) (Figure 2b), base (1 N NaOH at 80°C for 2 h) (Figure 2c), peroxide (10% H2O2 at 80°C for 2 h) (Figure 2d), thermal stress at 105°C for 24 h (Figure 2e), and photolytic (7 days at UV 254 nm) (Figure 2f) stress conditions. RC-04 (N-oxide) formed in peroxide degradation was reported as metabolite, and it placed an important role in the bioavailability.

Peak purity test results from the PDA detector confirmed that roflumilast peak obtained from all of the stress conditions were homogeneous and pure. The mass balance results were calculated for all stressed samples were found to be more than 95% (Table 4). The purity and assay of roflumilast were unaffected by its impurities and degradation products, which confirms the stability-indicating power of the developed method. From the observations of results, it can be concluded that RC-01, RC-02, and RC-04 (N-oxide) were found to be degradants and remaining impurities were process related impurities. The formation of known impurities was confirmed by LC–MS/MS analysis as per the experimental conditions section (Figure 1). The unknown impurities formed were not identified because these were not observed during stability studies.

6. CONCLUSIONS

The validated stability-indicating UPLC method has proved to be rapid, simple, accurate, precise, and reliable. The proposed method provides a good resolution between all the related compounds and potential degradants. The behavior of roflumilast under various stress conditions was studied and presented for the 1st time. The information presented herein could be very useful for quality monitoring as well as impurity profiling of formulation samples during stability studies. The developed method is stability indicating and can be used for routine analysis of samples.

7. ACKNOWLEDGMENTS

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****Bibliographical Sketch***

Dr Venkata Nagendra Kumar Putta has joined GITAM University, Hyderabad in June 2010 as Assistant Professor in the department of chemistry. He served as Head of the Department of Chemistry from year 2012 to 2015 for a period of three years and he is instrumental in establishing the department of Chemistry at the new Campus. Earlier he worked at Sri Venkateswara University and Yogi Vemana University. He has 22 years of teaching and research experience. He has presented many talks on chemistry and promoting science in various schools and colleges of Telangana and Andhra Pradesh states. Dr Venkata has also acting as resource person for BARC works shops as IANCAS life member. He is the life member of five national and one international professional bodies. Dr Venkata has got research award as SRF-2013 by Indian national science academy. He has 15 international journal publications and 25 conference papers for his credit.