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STUDY ON VALIDATED RP-HPLC TECHNIQUE FOR STABILITY ANALYSIS OF APREMILAST IN PHARMACEUTICAL APPLICATIONS

Vikas Gupta

Research Scholar, Glocal School of Pharmacy, The Glocal University

Mirzapur Pole, Saharanpur (U.P) India.

Dr. Mohamed Mutahar RK

Research Supervisor, Glocal School of Pharmacy, The Glocal University

Mirzapur Pole, Saharanpur (U.P)

ABSTRACT

This paper describes the newly developed stability-indicating Reversed Phase High-Performance LC method for estimating and determining Apremilast (APR) in bulk and pharmaceutical formulation. An Eclipse XDB model C18 Column (based on 99.999% ultra high purity silica) and an Agilent Technologies model SPD 20A conspicuous UV-Vis detector were used to carry out the chromatographic separation. This study used a sample that measured 250 mm by 4.6 mm and had a particle size of 5 µ. The mobile phase used in the experiment was acetonitrile, which was injected at a volume of 20 µL and flowed at a rate of 1 ml/min. The separation was carried out at room temperature, and the eluents were seen using a laser diode array detector adjusted at 229 nm. The measured APR retention duration was 2.488 minutes. The calibration curve for APR was linear (r2=0.9989) for the concentration range that was examined, with 0.003 µg/ml and 0.001 µg/ml for LOD and LOQ, respectively. In tablet format, APR was found to recover between 99.18 to 101.61%. The bulk medication and APR pills (Otezla) yielded % assay findings of 99.90 ± 0.001 and 99.80 ± 0.002, respectively. The stability of the method was demonstrated by forced degradation trials of the medication under acidic, alkaline, oxidative, photolytic, and thermal stress settings, in accordance with ICH Q1A (R2) requirements. As a result, it was shown that the proposed method for estimating APR could be used to both bulk and pharmaceutical dose form.

Key Words: *Apremilast; RP-HPLC;Validation;Forced degradation.*

INTRODUCTION

The chemical name for APR is N-[2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl) ethyl]-2, 3-

dihydro-1,3-dioxo- 1H-isoindol-4-yl] acetamide. It is soluble in many organic solvents such as acetonitrile and DMSO, but insoluble in aqueous media. It is utilized for the healing of certain types of Psoriasis [1] and Psonatic arthritis. It may also be utilized for other immune system associated inflammatory diseases [3]. APR is a selective inhibitor of the enzyme phosphodiesterase 4 [4-5] andstops spontaneous production of TNF-alpha from human rheumatoid synovial cells is taken by mouth.

Literature Survey shows that the APR has been determined by UV spectrophotometric method [6-7], Ultra HPLC- Mass spectroscopy [UHPLC -MS] [8] in biological fluids like human and rat plasma. However no stability indicating High Performance Liquid Chromatography has been reported for the estimation of APR in bulk and pharmaceutical dosage forms hitherto. Hence the major objective of the present research is to develop and validate a simple, precise, sensitive liquid chromatography method for APR in its bulk and tablet dosage form and stress degradation studies of APR as per International Conference on Harmonization (ICH) Q2 (R2) guidelines. (Figure 1) shows the chemical structure of Apremilast.

METHODOLOGY

Chemicals and Reagents

APR pure drug was supplied as gift sample by Hetero Drugs Ltd., Hyderabad, Telangana, India. The marketed formulation Otezla tablets containing 30 mg of APR tablets were obtained from local market. Acetonitrile was obtained from E. Merck specialties private Ltd., Mumbai, India (Table 1).

Table1: Calibration data of the proposed method for the estimation of Apremilast

S.NO	Concentration (µg/mL)	Peak area
1	2	571428
2	4	982067
3	6	1450540
4	8	1959690
5	10	2463855

Instrument

The HPLC system utilized was a Agilent technologies 1260 infinity system supplied with a gradient pump connected to Photo diode Array DAD VL detector set at 229 nm. Ezchrome elite software was utilized for data acquisition. An digital balance (Essae vibra AJ (0.001g)) and a sonicator (Model no-91250 mode) were utilized in this study.

Method development

Chromatographic conditions:

Accurately weighed 10 mg quantity of APR transferred to a 10 ml volumetric flask, dissolved and filled up to the mark with acetonitrile and was ultra-sonicated for 5 minutes.

Preparation of standard working solution

It was prepared by taking 1 ml of APR stock solution into 10 ml volumetric flask and the final volume was made up with diluent (100 µg/ml). The solution was filtered and then diluted immediately before use to appropriate concentration levels by utilizing mobile phase.

Analytical Method validation

The developed method was validated for different parameters like linearity, precision, accuracy, specificity, ruggedness, robustness, LOD and LOQ as per Q1A(R2) and ICH Q2(R1) guidelines.

System suitability

The system suitability test was carried out on freshly prepared Apremilast standard solution (100 %) was used for the evaluation of the system suitability parameters such as Area, retention time, unique selling proposition peak tailing, and the number of theoretical plates, asymmetry factor, LOD and LOQ. The system suitability data and the optimum chromatographic conditions are reported in (Table 2).

Table 2: Optimum chromatographic conditions and system suitability data

Parameter	Chromatographic conditions
Instrument	Agilent Technologies.
Column	eclipse XDB model C_{18} Column (4.6 mm i.d. X 250 mm, 5 µm particle size)
Detector	1260 DAD VL detector
Flow rate	1 mL/min.
Detection wave length	229 nm
Run time	Five minutes
Temperature	Ambient temperature (30 ^{o}C)
Volume of injection loop	20 µL

Retention time (Rt)	2.488 minutes
Theoretical plates	8191
Asymmetry factor	1.231

Linearity

Under developed experimental conditions the relationship between the peak area and concentration of APR was studied. The calibration curve was plotted against concentration vs peak area by the prepared different aliquots i,e., (2-10 µg/ml at 229 nm) ofstock solution, and r^2 value was determined. Five replicate of prepared 10 µg/ml solution of APR taken from different stock solution and measured area. The relative standard deviation was determined. **(**Figure 2) shows the linearity curve of Apremilastand overlain spectra of is shown in (Figure 3).

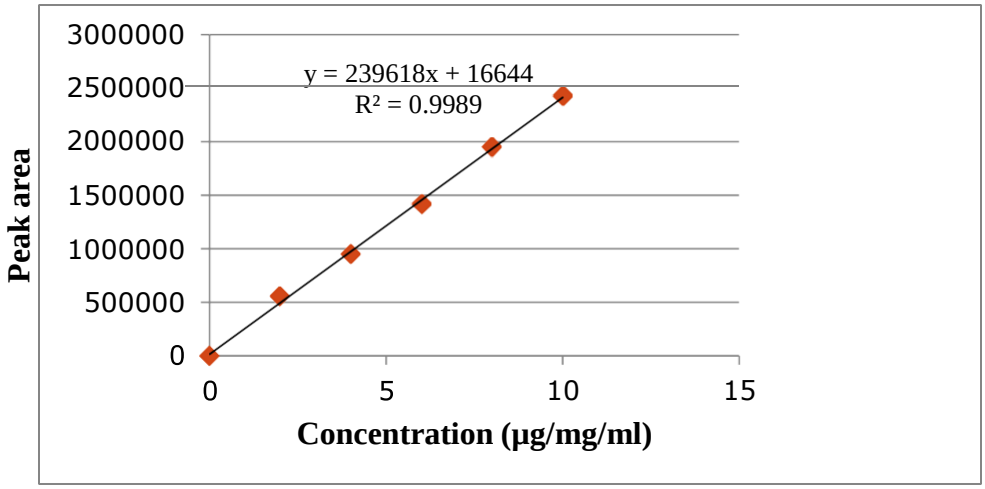

Figure 2: Linearity curve of Apremilast

Accuracy

Accuracy of the method was resolved by standard addition method in which standard addition of pure APR at three different concentration levels of 50 %, 100 % and 150 % was performed in triplicate. Accuracy of the method is calculated in the terms of % recovery of the APR. **Robustness**

Robustness of the method was determined by varying the method parameters such as change in flow rate (± 0.2 mL/min), temperature (± 2 %) and wavelength (± 1 nm).

Precision

Precision of the method was determined by evaluating repeatability, intraday and interday precision.

Repeatability was confirmed by injecting same concentration in six replicates and corresponding areas were calculated. Intra-day and Inter-day variation APR was analyzed by selecting three concentrations which were 4, 6 and 8 µg/ml from linearity range. Intraday analysis was carried on same day whereas interday analysis was carried on three different days in replicates of three. The respective peak areas for different concentrations were reported.

Ruggedness

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective peak areas were noted.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

A limit of detection (LOD) and a limit of quantification (LOQ) were calculated according to the formula: LOD = 3.3 σ/s

LOQ = 10 σ/s

Where, 'σ' is the standard deviation of 'y' intercept of regression line and 's' is the slope of the calibration curve.

Force degradation studies

To conduct the force degradation study, 10 mg APR was subjected to acidic, alkaline, oxidising, thermal, and UV light conditions. For acidic degradation, 10 mg APR was dissolved in 5 ml of acetonitrile to which 5 ml of 0.1 N HCl was added and heated under reflux at 70 ^{0}C for eight hours. The mixture was neutralized by the addition of 1 M NaOH. For alkaline degradation, 10 mg drug was dissolved in 5 ml of Acetonitrile to which 5 ml of 1 M NaOH was added and heated under reflux at 70 ^{0}C for eight hours before the mixture was neutralized by the addition of 0.1 N HCl.

For degradation under oxidising conditions the drug was heated under reflux with 3 % H_2O_2 (v/v) at 40 ^{0}C for 2 days. For thermal degradation the powdered drug was exposed at 80 ^{0}C for five days. Regarding UV light degradation, powdered APR was exposed to UV light for five days. Pharmaceutical APR dosage forms were also subjected to the same stress conditions to determine whether any peaks arose from the degraded excipients. After completing the treatments, the APR solutions were left to return to room temperature diluted with solvent mixture to obtain 10 µg/ml solutions.

Assay of marketed formulation

Twenty OTEZLA® (APR) tablets were weighed, average weight was calculated, and was made to fine powder.

APR powder proportionate to 10mg was taken in a 10 ml volumetric flask to which small amount of acetonitrile (ACN) was added. The flask is then ultra-sonicated for fifteen minutes and volume is made up with ACN. The tablet APR solution is then filtered through whatman filter paper (No. 42) to get rid of insoluble materials. From the above solution 10 mL is added to 100 mL with diluent so as to attain concentration of 100 µg/mL for the assay. It was further diluted according to the need and then analyzed following the proposed procedure. The content of the Otezla was calculated either form the previously plotted calibration graph or utilizing regression equation.

Determination of APR in Bulk drug

For the analysis of bulk drug accurately weighed 10 mg APR was taken in a 100 ml volumetric flask and the volume was filled up to the mark with mobile phase to get 100 µg/ml concentration. From this 1ml was taken and transferred to a 10 ml volumetric flask and the volume was made up to the mark with mobile phase to get 10 µg/ml concentration. The concentration of the bulk drug was calculated from the linear regression equation.

RESULTS AND DISCUSSION

Several mobile phases of different compositions were tested so as to develop an optimization of chromatographic conditions such as tailing factor, decorous peak shape, and theoretical plates. For the selection of the mobile phase primarily methanol, acetonitrile, CH3OH: water, ACN: water has been tried in different compositions. Eventually only acetonitrile used at a flow rate of 1 ml/min was found to be satisfactorily and decorous system suitability parameters. The average retention time (R_t) got for Apremilast was at 2.488 min. The tailing factor and theoretical plates for APR were found to be 1.231and 8191 respectively. Accuracy of APR was determined by calculating the % recovery. The method was found to be accurate with % recovery between 99.18 -101.61 %. Accuracy is shown in (Table 3). Intra and interday precision was calculated. Infact the method was precise with percentage RSD > 2%. Intra and interday precision are shown in (Table 4 and 5) respectively. The % RSD value of robustness which is less than 2% for Apremilast reveals that the proposed method is robust (Table 6). (Change in flow rate, temperature and wavelength). The % RSD values of ruggedness for Apremilast reveal that the proposed method is quite rugged which is shown in (Table 7). The LOD and LOQ of Apremilast were found 0.003 µg/ml and 0.001 µg/ml respectively. The % assay of the bulk was found to be 99.90 ± 0.001. The average content of APR was 99.80 ± 0.002, which was in good agreement with labelled claim. (Table 8 and 9) displays the assay of the APR tablets and Bulk drug. The method was specific and has no interference observed when the APR were estimated in presence of excipients.

Table 3: Accuracy studies of Apremilast

Level (%)	Sample concentration (µg/ml)	Amount of standard added (µg/ml)	Total concentration (µg/ml)	Found concentration (µg/ml)	% RSD*	% recovery
50	6	3	9	8.98	0.329	99.81
	6	3	9	8.99		
	6	3	9	8.98		
100	6	6	12	11.89	0.563	99.05
	6	6	12	11.88		
	6	6	12	11.89		
150	6	9	16	15.96	0.716	99.85
	6	9	16	15.98		
	6	9	16	15.99		
Note: *Average of three determinations (n=3).						

Table 4: Intra-day precision studies of Apremilast

Amount of Standard taken (µg/ml)	Area (mAU)	Retention Time (Rt) (minutes)	Asymmetry factor	Theoretical plates	% Relative Standard Deviation (n =3)
Day-1 (Morning)					
4	682056	2.48	1.19993	8171	0.0031
6	1450532	2.48	1.12340	8084	0.0015
8	1959693	2.48	1.16215	8200	0.0009
Day-1 (Afternoon)					
4	682074	2.48	1.19991	8170	0.0026
6	1450556	2.50	1.12343	8086	0.0012
8	1959674	2.48	1.16216	8204	0.0006
Day-1 (Evening)					
4	682099	2.48	1.19995	8168	0.0021
6	1450576	2.48	1.12341	8082	0.0009
8	1959656	2.48	1.16212	8205	0.0002

Table 5: Inter-day precision studies of Apremilast

Amount of Standard taken (µg/ml)	Area (mAU)	Retention Time (Rt) (in minutes)	Asymmetry	Theoretical plates	% Relative Standard Deviation (n =3)

Day-1 (Morning)					
4	682054	2.48	1.19993	8166	0.002
6	1450543	2.48	1.12340	8082	0.001
8	1959692	2.50	1.16214	8201	0.0008
Day-1 (Afternoon)					
4	682066	2.48	1.19997	8178	0.001
6	1450567	2.48	1.12343	8084	0.01
8	1959667	2.51	1.16215	8206	0.0004
Day-1 (Evening)					
4	682082	2.48	1.19994	8175	0.002
6	1450 587	2.48	1.12346	8080	0.02
8	1959673	2.50	1.16218	8203	0.001

Table 6: Robustness studies of Apremilast

S. No	Parameter	Optimized	Used	Retention time (Rt), min	Plate count	Peak asymmetry	% RSD
1	Flow rate (±0.2 mL/min)	1.0 mL/min	0.8 mL/min	2.48	8120	1.135980	0.011
			1.0 mL/min	2.50	8111	1.135987	0.010
			1.2 mL/min	2.50	8118	1.135982	0.012
2	Detection wavelength (±1 nm)	229 nm	228 nm	2.47	8079	1.19997	0.010
			229 nm	2.48	8084	1.19992	0.010
			230 nm	2.50	8080	1.19995	0.099
3	Change in Temperature (±2°C)	30 ^{0}C	28 ^{0}C	2.45	8382	1.19989	0.012
			30 ^{0}C	2.48	8388	1.19990	0.010
			32 ^{0}C	2.50	8174	1.19982	0.011

Table 7: Ruggedness studies of Apremilast

Analyst	Area (mAU)	Retention Time (Rt) (minutes)	Asymmetry factor	Theoretical plate	% Relative Standard Deviation
Analyst 1	1959693	2.48	1.16210	8082	0.010
Analyst 2	1959689	2.50	1.16214	8080	0.011

Table 8: Stability studies of Apremilast

Stress condition	Mean peak area	Drug recovered (%)	Drug decomposed (%)	Theoretical plates	Asymmetry factor
Standard drug	583239	100	Nil	8111	1.35987
Acidic degradation	195198	33.46	66.54	7306	1.11105
Alkaline degradation	203454	34.83	65.17	3718	1.22746
Oxidative degradation	531910	91.19	9.01	3114	1.56435
Thermal degradation	473260	81.14	18.86	3477	1.55941
UV light degradation	220502	37.80	62.20	3253	1.29968

Table 9: Assay of Apremilast tablets

Name of the Formulation	Concentration taken	Amount obtained	% assay ± S.D.	% RSD (n=6)
OTEZLA 30 mg	10 µg/ml	9.98 µg/ml	99.80 ± 0.002	0.010
Bulk drug	10 µg/ml	9.99 µg/ml	99.90 ± 0.001	0.012

Degradation behaviour of APR under various stress conditions is studied. The percent of the degradation products of APR were calculated and found to be 66.54 %, 65.17 %, 9.01 %, 18.86 %, 62.20 % in case of acid hydrolysis, alkaline hydrolysis, oxidation, UV light and thermal stability respectively. In acidic conditions for eight hours (0.1 N HCl) 66.54 % of APR drug was degraded with generation of one novel peak in addition to the peak of the APR. (Figure 4) More degradation of APR observed when conducted different stress conditions such as acidic, alkaline, UV light and thermal degradation so these stress conditions particularly interfere with detection of APR. Degradation behaviour of APR under various stress conditions are shown in (Table 8) and degradation figures are shown in (Figure 4- 8). The assay method of APR in pharmaceutical formulation

was successfully developed and validated for its intended purpose. Infact there was no particular precaution necessary during manufacturing and storage of APR formulation because there was no degradation studied at room temperature.

CONCLUSION

The creation of a stability-indicating RP-HPLC method for determining APR in pharmaceutical dosage form and bulk is the focus of the current study. The LOD, LOQ, robustness, ruggedness, accuracy, and precision values were all within the acceptable ranges. APR is unstable in alkaline, acidic, oxidative, thermal, and UV radiation conditions due to its high sensitivity. The statistical analysis of the data shows unequivocally that the approach is appropriate for determining APR in tablet and bulk forms without interference, and that no extra care is required when storing and formulating the material at room temperature. This investigation has led to the conclusion that the new RP-HPLC method for determining APR in a bulk and tablet formulation was effectively created and proven to work as planned.

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