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Development and validation of reversed phase HPLC method for determination of apremilast in bulk and pharmaceutical dosage form

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***Abstract*---**The present study describes a novel Reversed Phase High-Performance LC method for the determination and estimation of Apremilast (APR) in bulk and pharmaceutical formulation. The chromatographic separation was carried out on Agilent 1120LC Compact system with Binary gradient system connected to UV-Absorbance Detector. Acetonitrile: (30mM) Potassium dihydrogen phosphate buffer pH adjusted to 3.0 with o-phosphoric acid (60:40 v/v), and flow rate 1.0ml/min with an injection volume of 20 µL was selected as it gave sharp symmetric peak for APL with minimal tailing and with desired elution graph.The separation was carried out at a room temperature and the eluents were observed by UV - Vis detector set at 236 nm. The retention time of APR obtained was at 4.46 minutes. The calibration curve for APR was linear (r2= 0.995) over thetested concentration range of 10 - 60 µg/ ml with LOD and LOQ of 0.93 µg/ml and 2.82µg/ml respectively. A recovery of APR in tablet formulation was observed in the range of 98.40 -100.00 %. Thus the proposed method for APR was found to be feasible for the estimation of APR in bulk as well as pharmaceutical dosage form.

***Keywords*---**apremilast, RP-HPLC, validation.

Introduction

The chemical name for APR is N-[2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl) ethyl]-2, 3-dihydro-1,3-dioxo1H-isoindol-4-yl] acetamide.It is utilized for the healing of certain types of Psoriasis andPsonatic arthritis[1]. It also utilized for other immune system associated inflammatory diseases[2,3]. APR is a selective inhibitor of the enzyme phosphodiesterase4 and stops spontaneous production of TNF-alpha from human rheumatoid synovial cells is taken by mouth[4, 5].

An extensive literature search revealed that there exist very few methods on by UVSpectrophotometric method [6], RP-HPLC analysis[7, 8] of Apremilast in Pharmaceutical formulation. Published RP-HPLC method utilizes methanol as solvent. But there is no reported method with acetonitrile solvent[9].So, it is thought worthwhile to develop and validate new RP-HPLC forApremilast.Figure 1 shows the chemical structure of Apremilast[2].

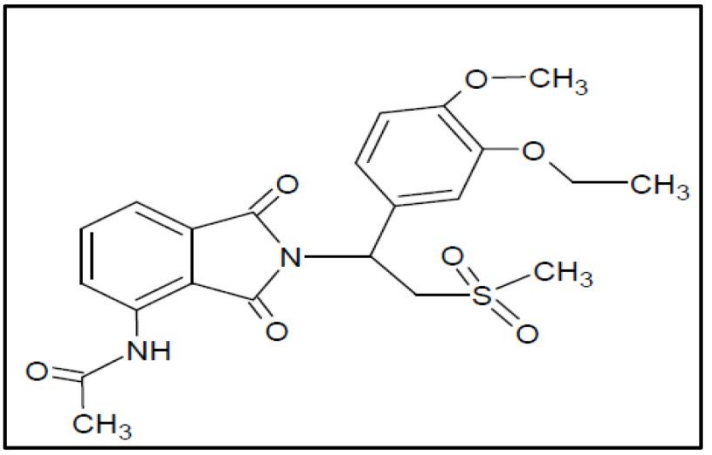

Fig. 1 Structure of Apremilast

Materials

Chemicals and Reagents

Pure drug of APL are received as a gift sample fromMegafinePharma (P) Ltd., Mumbai, India. Otezla (10 mgof Apremilast), is purchased from local market. All solvents used in thechromatography were of AR grade.

Instrument

HPLC system utilized was aAgilent 1120LC Compactsystem withBinary gradient system connected to UV- Absorbance Detector.EZ- chrome Elitesoftware was utilized for data acquisition. A digital balance and a sonicator were utilized in this study.

Methods, Results & Discussions

Selection of mobile phase

Aliquot portion of standard stock solution was appropriately diluted with mobile phase to obtain final concentration of 100µg/ml. After several permutation and combination, it was found that mixture of Acetonitrile and Water pH 3(Adjusted with Formic Acid) gives satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase, Acetonitrile: (30mM) Potassium dihydrogen phosphate buffer pH adjusted to 3.0 with o-phosphoric acid (60:40 v/v), and flow rate 1.0ml/min was selected as it gave sharp symmetric peak for APL with minimal tailing and with desired elution graph. Retention time of APL was found to be 4.46 minutes.

Selection of analytical wavelength

Aliquot portion of standard stock solution was appropriately diluted with mobile phase to obtain final concentration of 100 µg/ml of APL. The solution was scanned using double beam UV-Visible Spectrophotometer-1700 in the spectrum mode between the wavelength ranges of 400 nm to 200 nm against mobile phase as blank. The wavelength selected was 236 nm as APL showed significant absorbance at this wavelength.

Optimum Chromatographic conditions

HPLC Column : C18 Neosphere, R (25 cm length, 4.6 mm inside diameter, 5
µm particle size)
Column temperature : Ambient temperature
Mobile Phase : Acetonitrile: (30 mM) Potassium dihydrogen phosphate buffer
pH adjusted to 3.0 with o-phosphoric acid (60:40 v/v)
Flow rate : 1.0 ml/min
UV detection : 236 nm
Injection volume : 20 µl
Run time : 10minutes

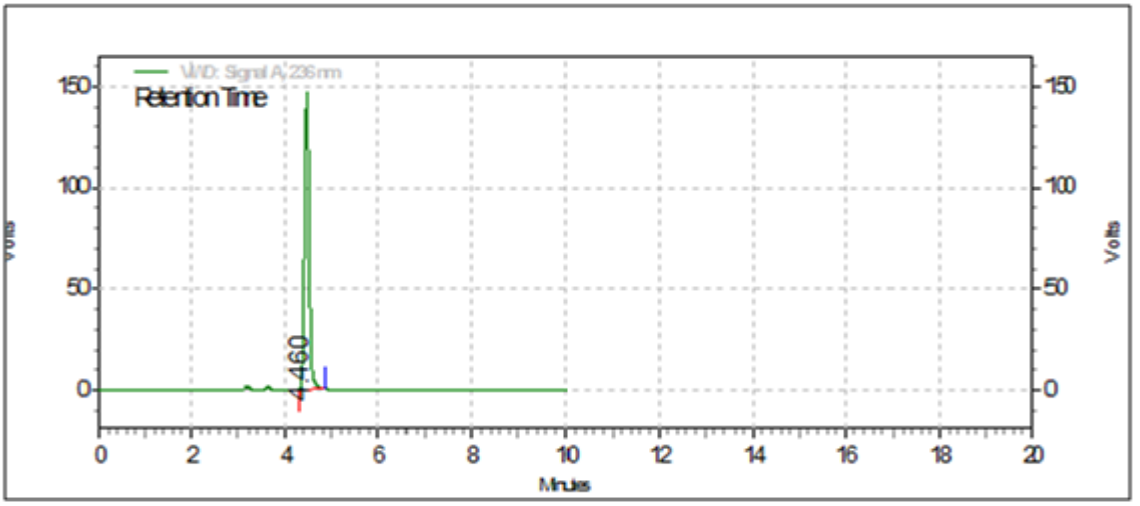

Fig.: 2 Typical chromatogram of Apremilast (RT = 4.460 min).

Table 1: System suitability parameters

Parameter	Standard values	Observed values
Theoretical Plates	NLT 2000	9606
Capacity Factor	2-5	3.46
Tailing factor	NMT 2	1.0

NMT – Not more than NLT – Not less than

Study of linearity range

Six linearity test solutions for APL were prepared as follows:
Accurately weighed quantity (10 mg) of APL, was transferred to 100.0 ml volumetric flask, added 70ml of mobile phase and ultrasonicated for 20 minutes, volume was then made up to the mark with mobile phase. From above solution, 1.0 ml was diluted to 10.0ml with mobile phase. From this solution 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0ml were diluted individually to 10.0 ml with mobile phase (Concentration 10, 20, 30, 40, 50 and 60 µg /ml, respectively). Each solution was then filtered through 0.22 µ membrane filter.The peak area of APL was measured at 236 nm.

Sr. NO	Concentration (µg/ml)	Peak area*
1.	10.0	9141770.0
2.	20.0	17721753.0
3.	30.0	24874329.5
4.	40.0	30566339.0
5.	50.0	41595925.5
6.	60.0	48816289.5

Table 2: Standard calibration data of Apremilast

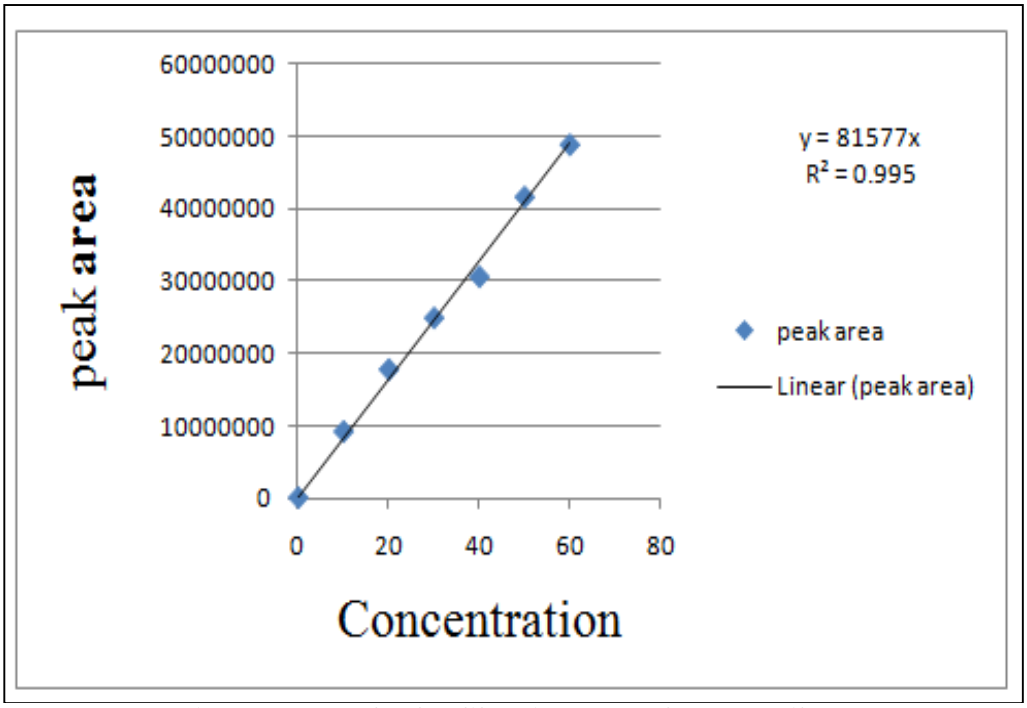

Figure 3: Standard calibration curve for Apremilast

Analysis of marketed formulation

- Preparation of standard solution:
  From standard stock solution, 2.0 ml solution was diluted to 10.0 ml with mobile phase (concentration 20 µg/ml). The solution was then filtered through 0.22 µ membrane filter.
- Preparation of sample solution:
  Twenty tablets were weighed; average weight was calculated and crushed to obtain fine powder.Accurately weighed quantity of tablet powder equivalent to about 10mg APLwas transferred to 100.0 ml volumetric flask, added 70.0 ml of mobile phase and ultrasonicated for 20 min, volume was then made upto the mark with mobile phase. The solution was then mixed and filter through Whatmann filter paper no. 42. From the filtrate, 1.0 ml solution was diluted to 10.0 ml with mobile phase. The solution was filtered through 0.22 µ membrane filter.
  Equal volume (20 µL) of standard and sample solution was injected into the HPLC system and chromatographed using optimum chromatographic conditions. Each solution was injected and chromatographed in triplicate. The chromatograms were recorded and peak area of APL was measured at 236 nm.

$$\% \text{ Estimation} = \frac{\text{Amount of Drug estimated (mg)}}{\text{Amount of Pure drug taken (mg)}} \times 100 \quad \text{---(15)}$$

---(1)

Otezla Avg. wt. 103.2 mg			Label Claim: 10 mg	
Sr. No.	Weight of tablet powder (mg)	Peak area*	Amount Found (mg/tab)	% label claim
1.	103.2	16159518	9.809	98.09
2.	103.2	16351182	9.926	99.26
3.	103.3	16535735	10.03	100.3
4.	103.3	16537510	10.03	100.3
5.	103.3	16582842	10.06	100.6
6.	103.4	16671456	10.12	101.2

Table 3: Results of analysis of marketed formulation

Amount Found (mg/tab)*	% Label claim*	S.D. (±)	R.S.D.
9.99	99.973	0.61	0.610

* Mean of six determinations

Table 4: Statistical validation for analysis of marketed formulation

Method validation

The proposed method was validated by studying several parameters such as accuracy, precision, linearity, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at 80, 100 and 120 % of the test concentration as per ICH guidelines.

Preparation of standard solution

Standard solution was prepared in similar manner as discussed under analysis of marketed formulation.

Preparation of sample solution

Nine samples were prepared and analysed in following manner:
Accurately weighed quantity of pre-analysed tablet powder equivalent to about 10 mg of APL was transferred to nine different 100.0 ml volumetric flasks. To each of the flask following quantities of APL was added:

Flask no.	1	2	3	4	5	6	7	8	9
Level of Recovery	80	80	80	100	100	100	120	120	120
Amount of APLadded (mg)	8.0	8.0	8.0	10.0	10.0	10.02	12.01	12.00	12.01

Then, 50ml mobile phase was added to each flask and content of the flask were ultrasonicated for 20 minutes, volume was then made upto the mark with mobile phase. The solution was individually mixed and filtered through Whatmann filter paper no. 42. From the filtrate, 5.0 mlsolution was diluted to 10.0 ml with mobile phase.Further diluted 1ml of above solution to 10.0 ml with mobile phase. The diluted solution was filtered through 0.22 µ membrane filter. Equal volume of (20µL) standard and sample solution was injected into the column and chromatographed using optimized chromatographic conditions. Each solution was injected and chromatographed in triplicate. The corresponding chromatograms were recorded and area of each peak for APL was measured at 236.0 nm.

Total amount of drug estimated in sample was obtained by comparing the peak area of sample with that of the standard using equation no. 2

Total amount of drug estimated in sample = Peak Area of sample / peak Area of Std. x conc. of Std (mg/mL) x DF

- (16) --- (2)

Amount of the drug recovered (mg) was calculated by using equation 3, where percent recovery was calculated using equation no.4.

Amount of drug recovered = Total amount of drug estimated in sample - Amt of drug contributed by tab powder

-----(3)

$$\% \text{ Recovery} = \frac{\text{Amount of Drug Recovered}}{\text{Total Amount of Pure Drug Taken}} \times 100$$

Results of recovery studies and its statistical evaluation are shown in Table 5 and 6, respectively.

Sr. No.	Level of recovery	Weight of tablet powder taken (mg)	Amount of drug added (mg)	Amount of drug recovered (mg)	% Recovery
1.	80 %	103.2	8.0	7.96	99.60
		103.1	8.0	7.90	98.75

		103.3	8.0	7.95	99.52
2.	100 %	103.1	10.0	9.84	98.4
		103.2	10.0	10.0	100.0
		103.2	10.0	9.96	99.64
3.	120 %	103.1	12.0	11.92	99.33
		103.2	12.0	11.93	99.41
		103.2	12.0	12.0	100.0

Table 5: Results of recovery studies

Level of recovery	% Recovery*	S.D. (±)	R.S.D.
80%	99.29	0.251	0.251
100%	99.34	0.442	0.442
120%	99.58	0.190	0.190

*****Mean of three determinations

Table 6: Statistical validation for recovery study

Precision

Intermediate precision (Intra-day and Inter-day precision)

Intraday and interday precision was determined by analyzing tablet sample solutions at different time intervals on the same day and on three different days, respectively. Tablet sample solution was prepared and analyzed in the similar manner as described in analysis of the marketed formulation.

* Mean of sixdeterminations

Drug	Intra-day Precision			Inter-day Precision		
	% Label claim*	S.D. (±)	R.S.D.	% Label claim*	S.D. (±)	R.S.D.
APL	99.98	0.2254	0.2254	98.31	0.3221	0.3221

Table 7: Result of Intra-day and Inter-day precision of RP– HPLC method

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of y- intercept and slope of the calibration curves were used to calculate the LOD and LOQ.

Parameters	APL
LOD (µg/ml)	0.93
LOQ (µg/ml)	2.82

Table 8: Result of LOD and LOQ

Robustness of method

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate on retention time and tailing factor of drug peak was studied. The mobile phase composition was changed in ± 2ml proportion and the flow rate was varied by ± 0.1 ml/min, of optimized chromatographic condition. The solution containing APLwas injected into the HPLC system and chromatographed under varied conditions.

Chromatographic Changes			
Factor	Level	Retention time	Tailing factor
Flow Rate (ml/min)	(±0.1)		
0.9	– 0.1	4.53	1.04
1	0	4.46	1.00
1.1	+ 0.1	4.07	1.05
	Mean	4.35	1.37
	S.D. (±)	0.13	0.015
Mobile Phase (v/v)	(±2)		
58:42	-2	4.73	1.07
60:40	0	4.46	1.00
62:38	+2	4.18	1.06
	Mean	4.45	1.04
	S.D. (±)	0.14	0.017

Table 9: Result of robustness study

Conclusion

The current research deals with the development of a RP-HPLC method for determination of APR in bulk as well as pharmaceutical dosage form. The values of accuracy, precision, robustness, ruggedness, LOD and LOQ were within the limits.. From this study it is concluded that this novel RP-HPLC method for the determination of APR in a bulk and tablet formulation was successfully developed and validated for its intended purpose.

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