

Applicability of AQBD Approach in RP-HPLC Method Development of Apremilast

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ABSTRACT

Quality by design is applied for the development of various pharmaceutical processes including analytical methods. By applying AQbD approach chromatographic based analytical method was developed for the estimation of Apremilast (APL) from solid dosage form. Chromatographic separation was performed on RP-C₁₈ column. Mobile phase acetonitrile: water in ratio 72:28 v/v fortified with acetic acid of pH 4.6 ±0.2 was utilised in the method and eluted drug was monitored at 237 nm. Effect of input variables on spectrum and chromatographic characteristics were studied for selection of critical parameters as well as system suitability parameters; and developed method was validated as per ICHQ2 R1 regulatory guidelines. Linearity of the drugs was ascertained over the conc range 1-12 µg/ml. The accuracy was found within 2.9571-0.7505 %; and the precision study was shown acceptable data as RSD was in the range of 2.7761-1.2423 found. The developed method is rigid, robust and efficient for the estimation of apremilast from the solid dosage form. AQbD was applied to build rigid robust method through risk assessment at early stage and defining the design space at the later stage.

KEYWORDS

Analytical QbD, Apremilast, Chromatographic method, ICH, system suitability

I. INTRODUCTION

The quality of the pharmaceutical products cannot solely be controlled by testing; instead it is expected to be built in by design. QbD concepts are mentioned in ICH guidelines Q8 (R1) (Pharmaceutical development), Q9 (Quality risk management), and Q10 (Pharmaceutical quality system) [1-3]. QbD is an expectation from regulatory agencies to increase process and product

understanding and thereby decreasing the risk for patients. ICH guidelines Q8 (R1) defines QbD as a “a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management”[4].

Analytical testing is one of the important aspects of pharmaceutical development. Having the right analytical method is vital in ensuring the quality of the drugs. The key challenge in front of the analytical chemist is to develop a robust and rugged analytical method. Factors to study in analytical quality by design (AQbD) approach may include the type of analytical technique chosen, reagents used and instrument related parameters.

There are similar advantages of applying QbD principles to analytical methods as to manufacturing processes and product [5]. A QbD approach can be beneficial in the development of suitable, robust and low cost (eco-friendly solvent, chemicals) method which is applicable at any stage of the lifecycle of the product. Also some regulatory guidelines have mentioned flexibility of changing analytical method without revalidation if the AQbD approach has been implemented during analytical method development. The first stage of AQbD approach is to fix an analytical target profile (ATP) for the method. ATP defines the goal of the analytical method development process and it is the sign of method performance [6-7]. AQbD based chromatographic method was developed; and AQbD approach was implemented with the study of the effect of method input variables on peak shape, peak area, asymmetry factor, column efficiency, resolution and mobile phase composition were selected for the proposed method and method was validated as per ICH guidelines Q2 (R1). The key definition of QbD is highlighted in Fig No 1.

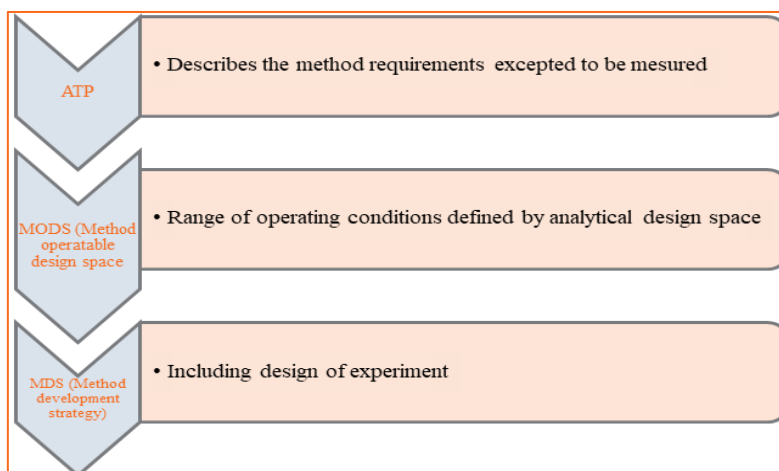


Fig No 1: Key definitions of the QbD

About Drug Molecule

Apremilast (APL) ^[8-9] chemically is *N*-[2-[(1*S*)-1-(3-Ethoxy-4-methoxyphenyl)-2-(methylsulfonyl) ethyl] -2, 3-dihydro-1, 3-dioxo1*H*-isoindol-4-yl] acetamide. Apremilast is a cyclic adenosine monophosphate cAMP specific phosphodiesterase type 4 inhibitor used in the treatment of active psoriatic arthritis and moderate to severe plaque psoriasis. PDE 4 is the main enzyme that degrades cAMP and intra cellular second messenger that mediates various cellular pathways and inflammatory responses. Inhibition of PDE 4 increases intracellular cAMP level and this is through to suppress the synthesis of pro inflammatory mediators ^[10, 11].

For estimation of APL methods such as DoE based HPLC method ^[12], RP-HPLC method ^[13-15], stability indicating RP-HPLC method ^[16-17], UV spectrophotometric method ^[18-20], Stability indicating UV spectrophotometric method ^[21], Degradation kinetic study and impurity detection method ^[22, 23], review on analytical method ^[24], bio-analytical chromatographic method ^[25] have been reported. Chemical structure of drug is shown in (Fig No 2).

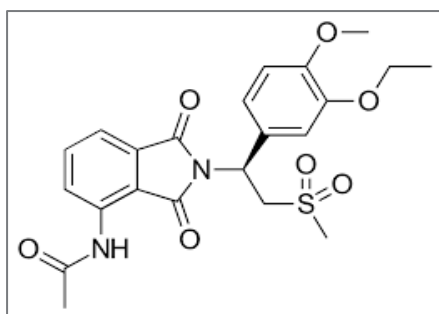


Fig No 2: Chemical structure of Drug molecule

AQbD approach application in method development

For Analytical QbD method development, QbD based approach was applied to study the influence of input variable parameters on chromatographic method performance. Step by step study of each parameter revealed the selection of the stable and suitable mobile phase, retention time selection and symmetrical peak.

II. MATERIALS AND METHODS

Instrumentation

Analysis was performed with a Shimadzu (Japan) prominence chromatograph equipped with an LC-20 AT solvent delivery system, a universal loop injector (Rheodyne 7725) of injection capacity of 20 μ l, and an SPD-20-A UV-Visible detector set at 237 nm. The equipment was controlled by a PC work station with clarity software. The Analyte was separated on a Phenomenex Luna C18 column (250 mm \times 4.6 mm i d, 5 μ m particle size) under reversed phase partition conditions. Wavelength selection was performed on the Shimadzu Double beam UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan) with spectral bandwidth of 2 nm and wavelength accuracy of \pm 1 nm with 10 mm matched Quartz cells was used. Electronic balance Afcoset balance (The Bombay Burmah Trading corpo Ltd) with accuracy \pm 0.1 mg Model No. ER 200A was used for weighing and for degassing the solutions Digital Ultrasonic cleaner 1.8 Ltr (Labman scientific Instruments Chennai) was used; and for filtration a 0.2 μ m filter (Pall corporation, Mumbai) was used.

Reagents and Chemicals

Pharmaceutically pure sample of APL from Glenmark India Ltd was obtained as a gift sample and the commercial formulation was procured from local market. Acetonitrile, methanol, water and acetic acid the entire solvents of HPLC grade were obtained from Merck Life sciences Pvt Ltd and Qualigens India Pvt. Limited, Mumbai, India.

Solvent and Wavelength selection

APL is freely soluble in water, soluble in 0.1 N HCl and methanol. Although the solubility of the procured drug was studied in water, 0.1 N HCl and 0.1 N NaOH separately; and each solution with known conc of analyte were scanned in UV range of 200 nm to 400 nm. The recorded overlain spectra in these solvent is shown in Fig No 3 and 4. It was found that in acidic pH drugs chromophore activates more to absorb UV light at 235nm.

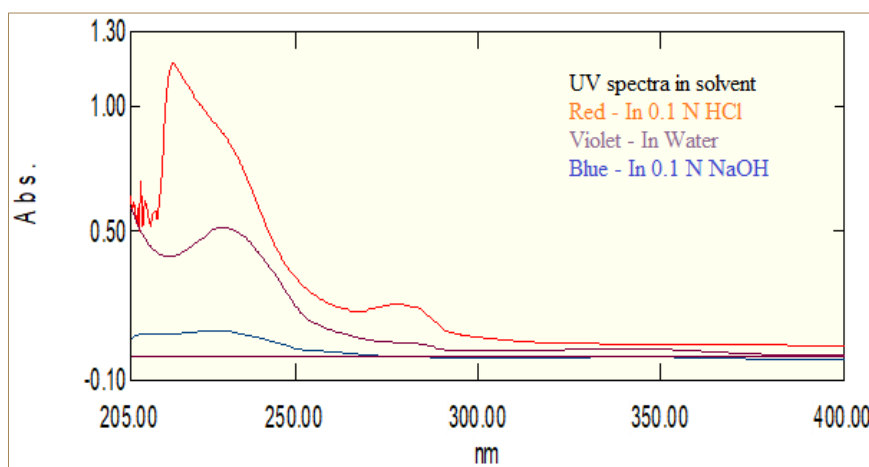


Fig No 3: UV overlaid spectra of Apremilast in selected solvent

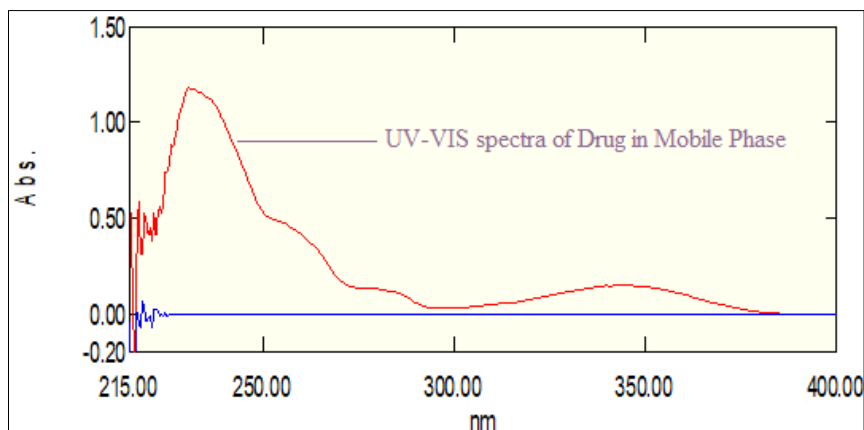


Fig No 4: UV overlaid spectra of Apremilast in mobile phase

Optimization of Mobile phase

Optimization of mobile phase in HPLC involves adjusting solvent type, composition and pH to achieve maximum resolution, selectivity and peak shape. For efficient separation proper composition of solvents in mobile phase is critical factor. As apremilast is water soluble so optimization was progressed with more proportion of water in the mobile phase. Initially trial with mobile phase acetonitrile and water in the ratio 40: 60 fortified

with acid made of pH 4.9 was performed and subsequently with A: W in the ratio 60: 40 and by increasing the proportion of acetonitrile to 78: 28 trials was carried out.

Preparation of stock solutions and standard solutions

10 mg APL drug was accurately weighed; and transferred into 10 ml volumetric flask. Dissolved into mobile phase and volume was made to 10 ml. Subsequent standard solution with conc 100µg/ml of

drug was prepared by diluting aliquot 1 ml of stock solution to 10 ml into 10 ml capacity volumetric flask.

Preparation of mobile phase

The mobile phase was a 72: 28 % (v/v) mixture of Acetonitrile: Water (pH 4.6 ± 0.2 , adjusted with Acetic acid). The flow rate was set at 0.8 ml/min and the run time was 6 min. Before analysis both the mobile phase was filtered through 0.45 μ membrane filter and sonicated the solution for degassing.

Selection of critical parameters viz. wavelength and conc range

From UV spectra it was found that APL has maximum absorbance at 237 nm. A reasonable and rational UV spectrum is the prime need for proper selection of wavelength. Hence UV spectrum was obtained in prepared mobile phase which was rational approach for selection of proper wavelength. Also this method guided approximate working conc range for method development. The critical parameters selected for the method is shown in Table No 1.

Table No 1: Selected critical parameter for analytical method of APL

Parameter	Selected variables of method
Detector set at	237 nm
Optimized mobile phase	Acetonitrile: Water 72:28
Flow rate	0.8 ml/min
Conc range	1 to 12 mcg/ml

The method was validated as per ICH guidelines. To attain analytical target profile of the method, selected critical parameters were studied to meet the performance characteristics of the analytical method. An ICH guideline Q2 R1 was referred to study methods performance with critical parameters in order to implement AQBd approach.

System suitability

System suitability is studied to demonstrate the suitability of the developed procedure under consideration for the analytical method. Six replicates of working standard solutions having conc 6 μ g/ml of APL were injected in sequence order and peak area was recorded; results are validated by calculating SD and % RSD of the obtained response.

Validation of the analytical Method

Linearity

The linearity of an analytical method is its ability to obtain response i.e. peak area which is directly

proportional to the conc of analyte. Series of working standard solutions were prepared in conc. range of 0 -12 μ g/ml for APL and injected in the column and detector was set at 237 nm to obtain peak area. Microsoft office excel software tool was used to obtain the standard regression curve and its analysis as slope, intercept, and correlation coefficient.

Assay of formulation

Assay was carried out by proposed methods. The Tablet powder equivalent to 10 mg APL was weighed accurately and transferred into 10 ml volumetric flask. Dissolved into mobile phase and volume was made to 10 ml with same solvent. Solution was filtered through 0.22 μ syringe filter and aliquots of solution were further diluted to obtain tablet solution. Solution was injected to obtain chromatograph and interpreted. Obtained peak area was utilised to estimate unknown conc of formulation; and results are statistically validated to obtain % of nominal conc, standard deviation and % of RSD.

Accuracy and Precision

The accuracy of an analytical method expresses the closeness of an agreement between test result and true result. Accuracy study was performed by recovery study i.e. standard addition method; diluted standard solutions of APL were prepared and standard solutions added in 80,100 and 120% proportionate to the tablet solution. Three replicates at each of these three levels were prepared and measured and % of conc, SD and RSD of replicates were calculated. The precision study was carried out by performing assay of tablet six times; also the reproducibility in result was studied by interday and intraday precision.

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

The LOD and LOQ of APL by the proposed method were determined using calibration graph method and calculated as $3.3\sigma/s$ and $10\sigma/s$ for LOD and LOQ respectively, σ is the standard deviation of calibration curve and s is the slope of regression line.

Robustness and Ruggedness

It is measure of capacity of analytical procedure to remain unaffected by small but deliberate variations in method parameter.

III. RESULTS AND DISCUSSION

Method development comprises numerous steps of which optimization of mobile phase, appropriate method selection for measurement etc. are significant one. Drugs underlying analysis must

have appreciable solubility in the optimized mobile phase. Chemical structure of the drug and physico-chemical properties available in the literature guides about use of appropriate solvent in the method.

Optimization of Mobile phase

Initial trial with mobile phase acetonitrile and water in the ratio 40: 60 fortified with acid

made pH 4.9 was resulted into retention time more than 10 min and in the next trial with A: W in the ratio 60: 40 RT was 6.94 min obtained; further by increasing proportion of acetonitrile to 78: 28 RT marginally reduced to 4.7 min in the reasonable range for the method shown in Fig No 5.

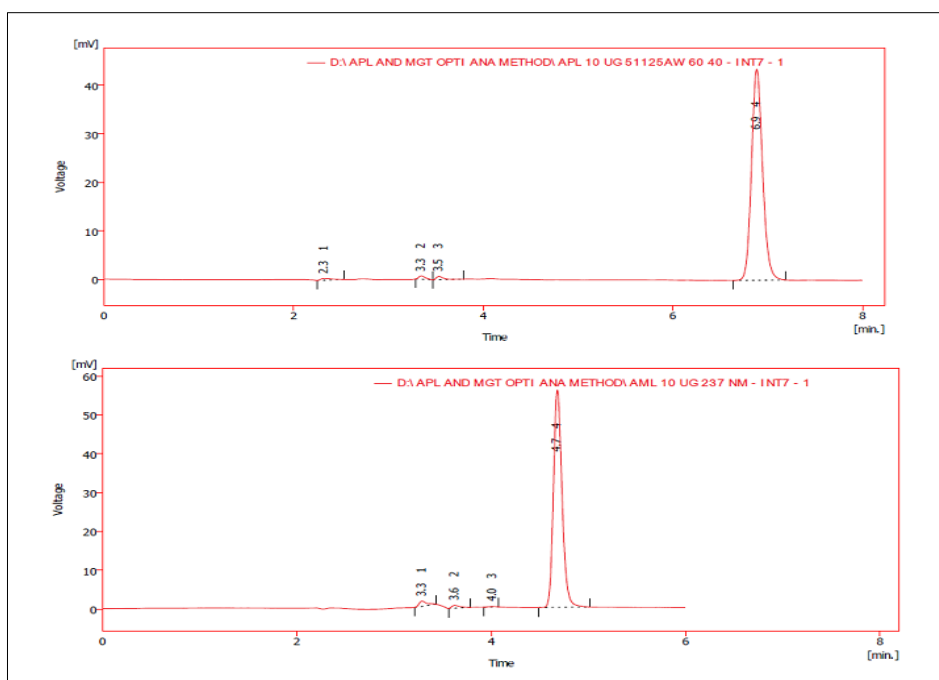


Fig No 5: Chromatogram of drug in optimization study

System Suitability

The system suitability test (SST) parameters of six replicates of standard solutions are reported in Table No 2. The SD and % RSD was found and meets the system suitability requirements

indicates suitability of the method for analysis. The stock solution was stable during method development and calculated SD was shown consistency in the SST tests shown in Table No 2.

Table No 2: System suitability study of APL

Parameters	Apremilast	SD	RSD
Retention time*	4.685	0.01026	0.2189
Tailing factor*	1.1911	0.00584	0.4907
Asymmetrical factor*	1.2993	0.02630	2.0243
Number of Theoretical plates*	270868	1.95482	2.3698
Resolution*	5.164	1.45876	2.1785
Flow rate ml/min	0.8 ml/min	-	-
Mobile phase Acetonitrile: Water	72: 28	-	-

*Mean of 6 readings

Linearity

The linear relationship between peak area and conc was obtained for the drug is shown in Fig No 6 and 7. Further it was validated by obtaining the calibration curve for the drug and found linear in the

conc range of 2-12 µg/ml as shown in Fig No 8. The regression equation of line and its parameters slope, r² value and intercept are tabulated in Table No 3, which proved the linear relationship between conc and obtained response.

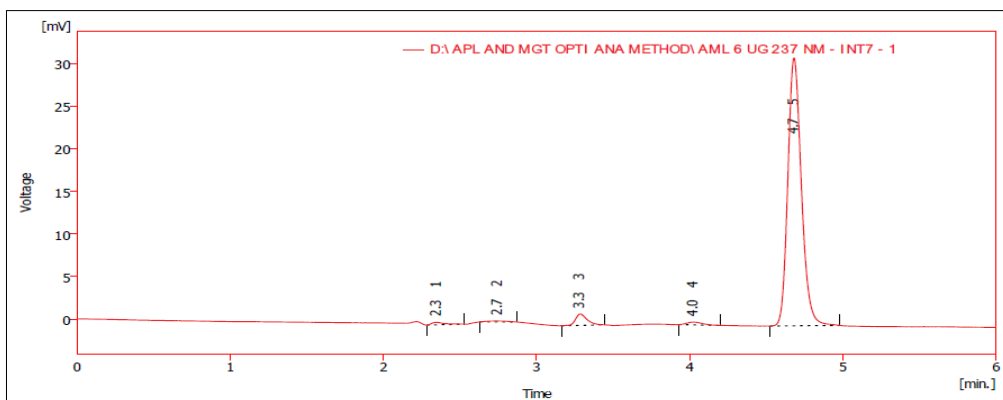


Fig No 6: Chromatogram of drug in linearity study

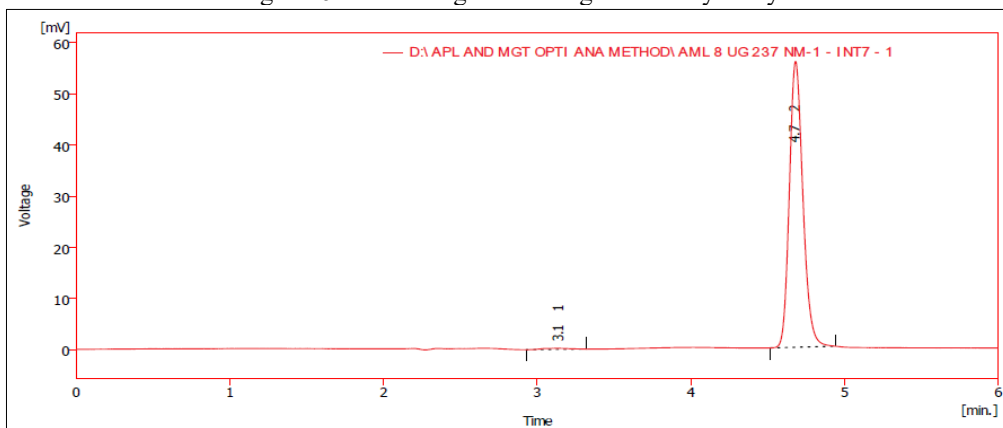


Fig No 7: Chromatogram of drug in linearity study

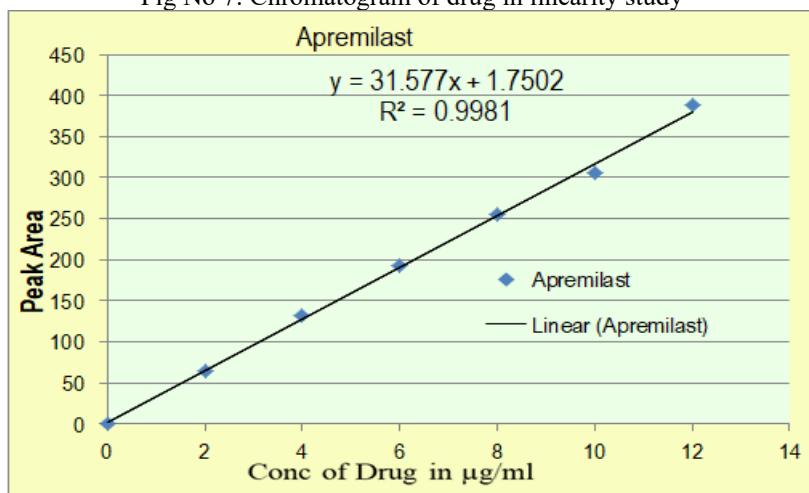


Fig No 8: Calibration curve of drug in linearity study

Table No 3: Parameters of regression equation obtained in Microsoft excel

Parameters	APL
Detection wavelength	237
Linearity range (µg/ml)	2-12 µg/ml
Correlation coefficient (r ²)	0.9981
Regression equation (y = mx + c)	Y = 31.577 X + 1.7502

Assay

The assay was carried out by the proposed method. The spectra of formulation was obtained is shown in Fig No 9. Calculated % of nominal conc and RSD was found within acceptable limits are summarized in Table No 4. The results indicated applicability of the method for estimation of formulation.

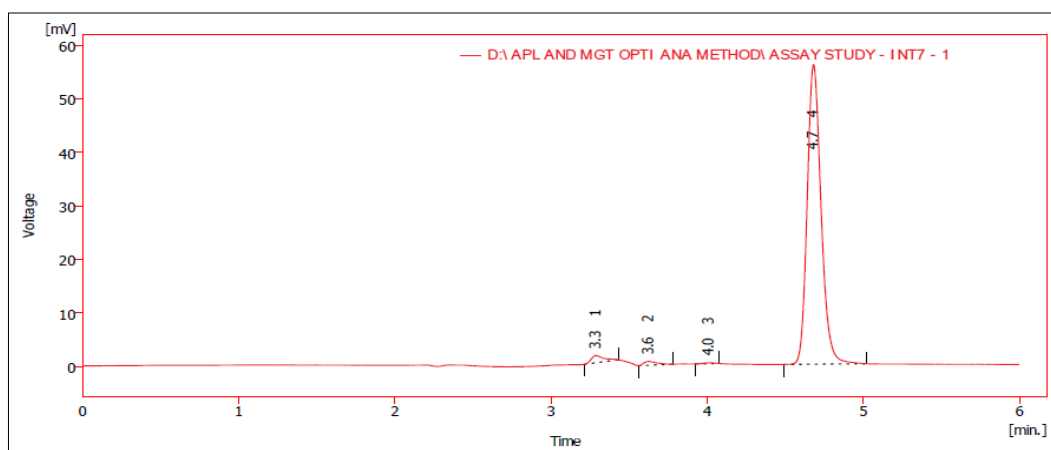


Fig No 9: Chromatogram of formulation in assay

Table No 4: Results of assay of formulation by proposed method

Formulation	Drug	Label Claim (mg/Tablet; n=6)	Amount found / mg	Drug Content	Std Deviation	% RSD
Formulation Apraize	Apremilast	10 mg	10.75	107.5 %	2.82204	0.82605

Accuracy and Precision

The results of accuracy (chromatograph shown in Fig No 10) are summarised in Table No 5, the obtained results were within acceptable limit; and methods accuracy was justified by calculating % drug content.

The precision study was carried out by performing assay of solutions; further the reproducibility in result was studied by inter day and intraday precision. The values obtained SD and % RSD was shown methods precision and are summarised in Table No 5.

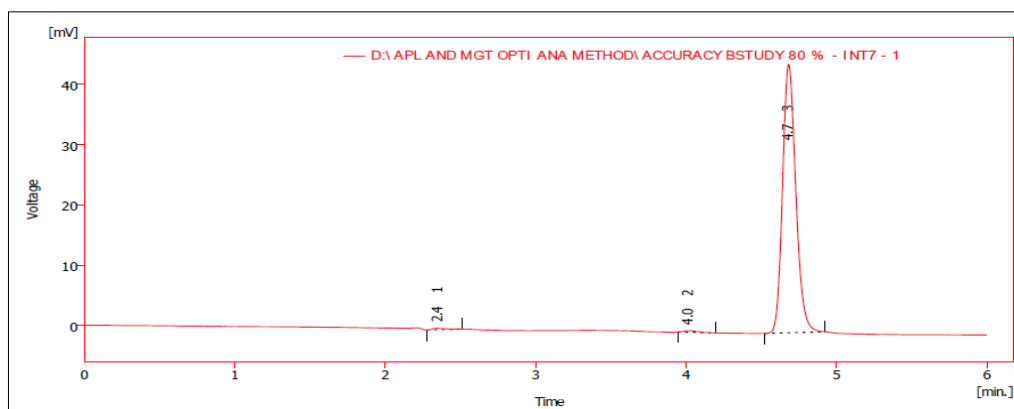


Fig No 10: Chromatograph of apremilast in accuracy study

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

The LOD and LOQ of APL by the proposed method were shown in Table No 5. The standard deviation of the calibration curve was obtained in Microsoft excel word and found 0.06087 for APL.

Robustness and Ruggedness

Robustness was studied and capacity of analytical procedure to measure analyte was remain unaffected

by small but deliberate variations in method parameter shown in Table No 5. The analytical method was found rugged during development; similarity the result was produced by performing the analysis by different analyst.

Specificity

Columns performance was tested by injecting blank solution shown in Fig No 11; no sign of any abnormal peak indicated specificity of the method.

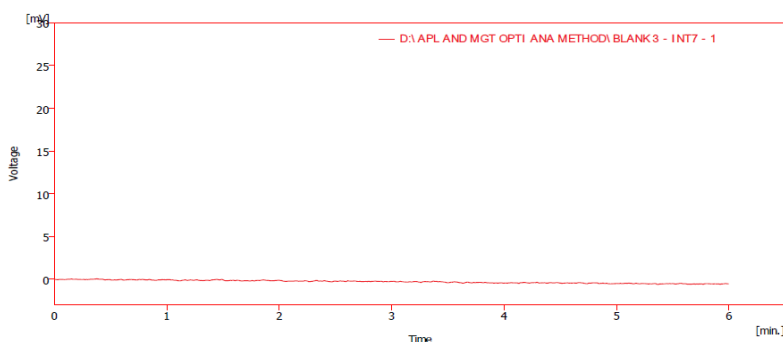


Fig No 11: Chromatograph of blank mobile phase

Table No 5: Results of accuracy and precision, LOD and LOQ

Sr. No.	Parameter	Level of study	Drug Name	S.D.	RSD
1	Precision study of APL*	Intraday Precision	Mean % - 103.23	2.7761	2.6892
		Interday precision	Mean % - 102.63	1.2751	1.2423
2	Accuracy study of APL*	80%	APL Percentage purity*	2.4379	0.8946
		100%		2.5707	0.7505
		120%		2.9571	0.7692
3	LOD	0.0636	LOQ	0.1927	-
4	Robustness± 2 nm	SD- 2.6481	Ruggedness	Analyst I	2.6891
				Analyst II	1.8427

*Mean of three

IV. CONCLUSION

The drug was estimated from the marketed formulation by the proposed method. Results obtained in the proposed and validated method were within acceptable limits given in the regulatory guidelines and official books. The chromatographic technique is simple, rapid, precise, accurate, robust and reproducible hence can be routinely applied for dosage form estimation.

CONFLICT OF INTEREST

All Authors declared that there is no conflict of interest

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