



ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF APREMILAST USING 2,4-DINITROPHENYLHYDRAZINE (DNPH) IN BULK DOSAGE FORM

Dr. Seema Firdouse*, Bilqees Fatima Jabeen¹, Ayesha Farhath Fatima², Dr. Atla Venkateshwar Reddy³

^{1,2,3}Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad, Telangana-500001

Corresponding author E- mail: seemamiyna@gmail.com

ARTICLE INFO

ABSTRACT

Key words:

Apremilast,
UV-Visible,
Spectrophotometric
Method development.

Access this article online
Website:
<https://www.jgtps.com/>
Quick Response Code:



UV-Visible spectrophotometric method has been developed for the DNHP, determination of Apremilast in bulk dosage form. Method was developed by using chromogenic reagent, DNPH, under oxidative coupling reaction in the presence of methanol, yielding an orange-red colored chromogen that has shown the absorption maximum at around 480nm. Beer's law has been followed in the concentration range from 2µg/ml to 10µg/ml, where the Coefficient of determination (r^2) = 0.998. Limit of Detection 0.057µg/ml and Limit of Quantification 0.173 µg/ml, respectively. The developed method has been validated as per ICH Q2 (R2) guidelines and shows wide applicability for the determination of apremilast in bulk dosage form with good recovery and reproducibility.

INTRODUCTION:

Apremilast, an orally bioavailable small-molecule inhibitor of phosphodiesterase 4 (PDE4), represents a critical therapeutic intervention in the management of chronic inflammatory and autoimmune pathologies. Chemically designated as N-[2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonyl-ethyl]-1,3-dioxoisindol-4-yl]acetamide, the compound functions as a potent immunomodulator by targeting intracellular signaling pathways rather than utilizing the extracellular approach common in biologic therapies.

The pharmacological efficacy of apremilast is rooted in its selective inhibition of the PDE4 enzyme, which is the predominant phosphodiesterase found in inflammatory cells. By preventing the degradation of cyclic adenosine monophosphate (cAMP), apremilast induces an elevation of intracellular cAMP levels. This biochemical cascade subsequently suppresses the production of several key pro-inflammatory mediators, most notably Tumor Necrosis Factor-alpha (TNF-alpha), Interleukin-17 (IL-17), and Interleukin-23 (IL-23). This broad-spectrum modulation allows for the regulation of the inflammatory response

across various systemic pathways, offering a multi-faceted approach to symptom management.

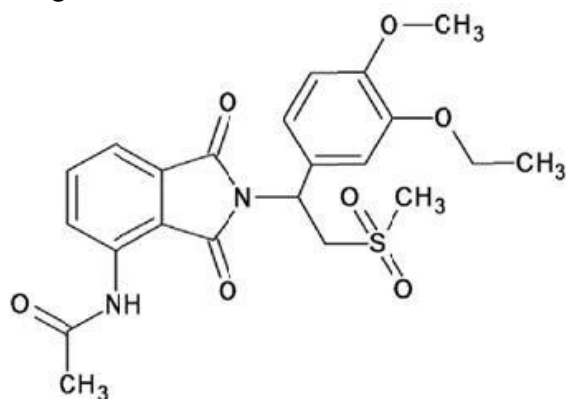


Fig.1: Structure of Apremilast

A survey of the literature indicates that several analytical techniques—including spectrophotometry, high-performance liquid chromatography (HPLC), and liquid chromatography–mass spectrometry (LC-MS)—have been established for the quantification of Apremilast. However, there is a lack of reported methods for the estimation of Apremilast using UV-Visible spectrophotometry coupled with chromogenic reagents. Therefore, the present study focuses on the development and validation of a novel UV-Visible spectrophotometric method utilizing these 2,4 dinitrophenyl hydrazine (DNPH).⁶⁻¹² Spectrophotometry remains a cornerstone of pharmaceutical quality control due to its cost-effectiveness and lack of reliance on complex chromatography or hazardous solvents. To overcome inherent limitations in detection, 2,4-dinitrophenylhydrazine (DNPH) is frequently employed as a derivatizing agent. This chemical modification significantly boosts both analytical sensitivity and selectivity. The present study expands upon previous work to develop and validate streamlined, sensitive spectrophotometric protocols for quantifying specific drugs via DNPH-mediated derivatization.

MATERIALS AND METHODS¹³⁻¹⁶

Instruments

The UV-Visible spectrophotometer (Shimadzu-1800), interfaced with a computer equipped with Shimadzu UV

Probe 2.0 software was used for all spectrophotometric measurements.

Materials

All chemicals used were of analytical grade. Aprelimast was obtained as a gift sample.

Preparation of reagents

0.1 2,4 Dinitrophenyl hydrazine reagents:

Weigh 0.1g of 2,4 DNPH in a mixture of 10ml methanol and 0.5ml conc. HCL, and dilute the resultant mixture to 100ml with methanol.

Preparation of 0.1N NaOH

4g NaOH was weighed accurately and dissolved in a 100 mL volumetric flask with distilled water and make up the volume to 100ml.

Preparation of standard solution¹⁷⁻²²

Standard Apremilast, 10mg, was weighed and transferred to 10 ml volumetric flask and dissolved in methanol. The content of the flask was shaken for 20mins and made up to the mark with methanol to give 1000µg/ml. From this stock solution 1ml was pipette out in to another 10ml of volumetric flask and the volume was made up to 10ml with methanol to give 100µg/ml. From this solution, 1 mL was pipette into another 10 mL volumetric flask, and the volume was made up with methanol to give 10 µg/mL. The absorbance was measured in the wavelength range from 200 to 300nm against blank as methanol. It shows the maximum absorbance 230nm.

Determination of absorption maximum

1 ml of standard solution was taken into 10ml volumetric flask. To this, add 2ml of 2,4DNPH reagent and 1.5ml of 0.1N NaOH. The mixture was further diluted with methanol to make up to 10ml. The optical absorbance was measured in the wavelength range from 200 to 800nm against a blank as methanol. It shows the maximum absorbance as 480nm.

Method validation²³⁻²⁶

Validation parameters, Linearity, Precision, Accuracy, System suitability and LOD and LOQ were performed according to ICH.

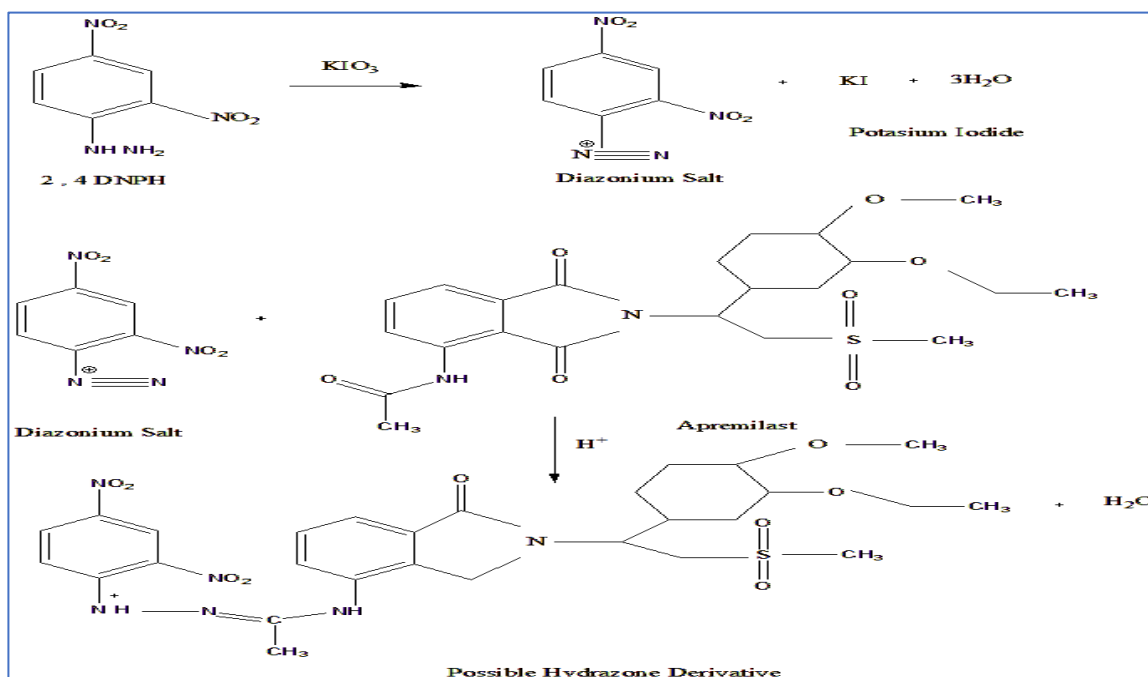


Fig. 2: Possible reaction of Apremilast with DNPH

RESULTS

Linearity:

S.No	Volume of Solution	Concentration ($\mu\text{g/ml}$)	Absorbance
1	0	0	0
2	0.2	2	0.023
3	0.4	4	0.045
4	0.6	6	0.066
5	0.8	8	0.087
6	1	10	0.104

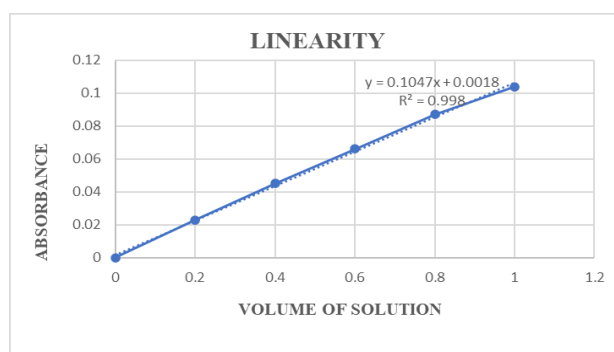


Fig.3: Calibration curve of Apremilast with DNHP reagent

Table No.1: Calibration curve of Apremilast

Precision:

Repeatability

Table No.2: Repeatability data of apremilast using DNHP

S.No.	Concentration($\mu\text{g/ml}$)	Absorbance	Mean $\pm$ SD	%RSD
1	3	0.032	0.031 $\pm$ 0.005	1.3
2	3	0.031		
3	3	0.032		
4	3	0.032		
5	3	0.031		
6	3	0.032		

Precision -Intra Day**Table No.3: Intra-day precision data of Apremilast using DNHP reagent**

S.No.	Concentration($\mu\text{g/ml}$)	Absorbance		Mean $\pm$ SD	%RSD
1	2	Day -1	0.023	0.022 $\pm$ 0.005	1.9
	2		0.022		
	2		0.023		
2	3		0.031	0.031 $\pm$ 0.005	1.8
	3		0.032		
	3		0.032		
3	4		0.044	0.044 $\pm$ 0.005	1.2
	4		0.045		
	4		0.045		

Precision -Inter Day**Table No.4: Inter –day precision data of Apremilast using DNHP reagent**

S.No.	Concentration($\mu\text{g/ml}$)	Absorbance			Mean $\pm$ SD	%RSD
		Day -1	Day -2	Day -3		
1	2	0.023	0.023	0.024	0.022 $\pm$ 0.005	1.9
2	3	0.031	0.032	0.032	0.031 $\pm$ 0.005	1.8
3	4	0.044	0.044	0.045	0.04 $\pm$ 0.005	1.3

Accuracy**Table No.5: Accuracy results of Apremilast using DNHP reagent**

S.No	Levels	Concentration	Amount added	Amount Recovered	% Recovery $\pm$ SD
1	50%	100	5	4.92	98.4 $\pm$ 0.5
2	100%	100	10	9.98	99.8 $\pm$ 0.5
2	150%	100	15	14.92	99.4 $\pm$ 0.5

LOD & LOQ

$$\text{LOD} = 3.3 * \text{SD/S}$$

SD = Standard deviation of slope

S= Slope of calibration

$$\text{LOD} = 3.3 * 0.0018/0.104 = 0.057$$

$$\text{LOQ} = 10 * \text{SD/S}$$

$$\text{LOQ} = 10 * 0.0018/0.104 = 0.173$$

Table No.6: Statistical data of Aprelimast

Parameter	Result
λ_{max} (nm) of drug	230
λ_{max} (nm) of drug+DNPH	480
Beer's law Limits ($\mu\text{g/ml}$)	2-10
Regression equation	$0.1047x + 0.0018$
Slope	0.104
Intercept	0.0018
Regression coefficient (r ²)	0.998
LOD	0.057
LOQ	0.173
% Recovery	98.4-99.4

DISCUSSION

The study aimed to enhance sensitivity by performing a derivatization reaction of Apremilast using 2,4-dinitrophenyl hydrazine (DNPH). Optimization of reagent volume to Aprelimast, yielding a prominent bathochromic shift and a stable chromophore with maximum absorbance at 480 nm. The method demonstrated excellent linearity over the range of 2–10 µg/mL ($R^2 = 0.998$), with a reliable regression model suitable for routine quantification. Precision was confirmed by intra- and inter-day %RSD values below 2%, and accuracy by recovery in the range of 98.4–99.4%, indicating minimal matrix effect and strong method trueness. Sensitivity was high, with LOD and LOQ of 0.057 µg/mL and 0.173 µg/mL, respectively, supporting trace-level detection. System suitability was consistently met, underscoring the method's robustness.

CONCLUSION

The study successfully established and validated a simple, sensitive, and precise spectrophotometric method for quantifying apremilast via derivatization with 2,4-dinitrophenyl hydrazine (DNPH). In conclusion, the validated DNPH-derivatized spectrophotometric method shows reproducible performance characteristics that meet requirements for routine pharmaceutical analysis and provide a strong foundation for broader applications and method enhancements.

Acknowledgement

We sincerely thank our Principal and Management, AUCOP, Hyderabad, India, for their invaluable motivation and kind support.

Conflict of Interest:

The authors declare no conflict of interest.

References:

1. Boggula N. Development and Validation for Determination of Apremilast in Bulk and in Tablets by UV Spectrophotometer. *Asian Journal of Pharmaceutics (AJP)*. 2024 Sep 15;18(3).
2. Shakeel F, Alam P, Alqarni MH, Iqbal M, Anwer MK, Alshehri S. A greener RP-HPTLC-densitometry method for the quantification of apremilast in nanoformulations and commercial tablets: Greenness assessment by analytical eco-scale, ChlorTox, and AGREE methods. *Arabian Journal of Chemistry*. 2024 Feb 1;17(2):105571.
3. Sutar KP, Jalalpur SS, Kurangi B. Novel Stability-Indicating Reverse-Phase High-Performance Liquid Chromatography Technique for Apremilast Quantification in Pharmaceuticals and Nanovesicular Systems: A Design of Experiment Approach. *Separation Science Plus*. 2024 Aug;7(8):e202400070.
4. Pawar A. Recent Innovations in High-Performance Liquid Chromatography (HPLC): Method Development and Validation Strategies. *Journal of Drug Delivery and Biotherapeutics*. 2024 Aug 25;1(01):55-61.
5. Tamilselvi N, Nishanth R, Gopala satheeskumar K. Bio Analytical Method Development for Analysis of Apremilast in Rat Plasma Using Reverse Phase HPLC-UV for Bioequivalence and Pharmacokinetic Application. *Analytical Chemistry Letters*. 2020 Nov 1;10(6):732-9.
6. Kulkarni P, Deshpande A. Analytical methods for determination of apremilast from bulk, dosage form and biological fluids: A critical review. *Critical Reviews in Analytical Chemistry*. 2021 May 7;51(3):258-67.
7. Shelke A, Mankar S, Kolhe M. A review on analytical methods for estimation of apremilast in bulk, pharmaceutical formulation and in biological samples. *Research Journal of Science and Technology*. 2021;13(2):142-6.
8. Kolsure, A., Soni, R., & Bhat, M. (2022). Development and validation of reversed phase HPLC method for determination of apremilast in bulk and pharmaceutical dosage form. *International Journal of Health Sciences*, 6(S2), 9484–9493. <https://doi.org/10.53730/ijhs.v6nS2.749>.
9. Patel N, Patel S, Surati J, Akbari A, Shah D. Apremilast-a review of analytical methods developed for API with its impurities, pharmaceutical formulations and

biological matrices. *Int J Pharm Res Appl*. 2021 May(6):735-55.

10. Chaudhari SR, Shirkhedkar AA. Design of experiment avenue for development and validation of RP-HPLC-PDA method for determination of apremilast in bulk and in in-house tablet formulation. *Journal of Analytical Science and Technology*. 2019 Feb 27;10(1):10.
11. Gaikwad MB, Dumbare RK, Gowekar NM. Development and validation of rp-hplc method for estimation of apremilast in tablet dosage form.
12. Bhole RP, Naksakhare SR, Bonde CG. A stability indicating HPTLC method for apremilast and identification of degradation products using MS/MS. *Journal of Pharmaceutical Sciences and Research*. 2019 May 1;11(5):1861-9.
13. Anumolu PD, Gurralla S, Gellaboina A, Mangipudi DG, Menkana S, Chakka R. Spectrophotometric quantification of anti-inflammatory drugs by application of chromogenic reagents. *Turkish journal of pharmaceutical sciences*. 2019 Nov 11;16(4):410.
14. Chaudhari SR, Shirkhedkar AA. Design of experiment avenue for development and validation of RP-HPLC-PDA method for determination of apremilast in bulk and in in-house tablet formulation. *Journal of Analytical Science and Technology*. 2019 Feb 27;10(1):10.
15. Gummadi S, Kommoju M. Colorimetric approaches to drug analysis and applications. A Review,” *AJPTR*. 2019 Feb;9(1):14-37.
16. Chaudhari SR, Patil AS, Shirkhedkar AA. Studies on derivative spectroscopy and area under curve UV-spectrophotometric methods for estimation of Apremilast in bulk and in-house Tablets. *Asian Journal of Pharmaceutical Research*. 2018;8(1):11-6.
17. Foroughbakhshfasaei M, Szabó ZI, Tóth G. Validated LC method for determination of enantiomeric purity of apremilast using polysaccharide-type stationary phases in polar organic mode. *Chromatographia*. 2018 Dec;81(12):1613-21.
18. Ravisankar P, Sulthana MS, Babu PS. Development and validation of stability-indicating UV spectrophotometric method

for determination of Apremilast in bulk and pharmaceutical dosage form. *Indian Journal of Research in Pharmacy and Biotechnology*. 2017 Jan 5;5(1):47-53.

19. Landge SB, Dahale SB, Jadhav SA, Solanki PV, Bembalkar SR, Mathad VT. Development and Validation of stability indicating rapid RP-LC method for determination of process and degradation related impurities of apremilast, an anti-inflammatory drug. *American Journal of Analytical Chemistry*. 2017;8(06):380.
20. Intwala JK, Doshi DB. Development and validation of sophisticated analytical method for the estimation of Apremilast. *Pharma Science Monitor*. 2017 Apr 1;8(2):267-76.
21. Xiong K, Ma X, Cao N, Liu L, Sun L, Zou Q, Wei P. Identification, characterization and HPLC quantification of impurities in apremilast. *Analytical Methods*. 2016;8(8):1889-97.
22. Gurupadayya BM, Sama NS, Kumar CA. Spectrophotometric determination of mesalamine by PDAC and NQS reagents in bulk and tablet dosage form. *J. Pharmacy Research*. 2011;4(1):39-41.
23. Annapurna MM, Pradhan DP, Sushmitha M. A new Stability indicating RP-HPLC method for the determination of Apremilast-An Antirheumatic drug. *Research Journal of Pharmacy and Technology*. 2017;10(4):1160-4.
24. Intwala JK, Doshi DB. Development and validation of sophisticated analytical method for the estimation of Apremilast. *Pharma Science Monitor*. 2017 Apr 1;8(2):267-76.
25. Xiong K, Ma X, Cao N, Liu L, Sun L, Zou Q, Wei P. Identification, characterization and HPLC quantification of impurities in apremilast. *Analytical Methods*. 2016;8(8):1889-97.
26. Gurupadayya BM, Sama NS, Kumar CA. Spectrophotometric determination of mesalamine by PDAC and NQS reagents in bulk and tablet dosage form. *J. Pharmacy Research*. 2011;4(1):39-41.