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Stability Indicating Method Development and Validation for the Determination of Armodafinil in Pharmaceutical Tablet Dosage Form by RP-HPLC

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Abstract

The present research deals with the development of a stability indicating reverse phase HPLC with PDA detector method for the determination of Armodafinil Agilent XDB-C_{18}, 150×4.6mm, 5µm or Equivalent column. The present research deals with the development of a stability indicating reverse phase HPLC with PDA detector method for the determination of Armodafinil Agilent XDB-C_{18}, 150×4.6mm, 5µm or Equivalent column. The flow rate was kept at 1.0ml/min and the injection volume 10µL and the run time is 8 min and drug Rt is 3.354. The separation was performed at 30°C. Eluents were monitored by PDA detector set at 223nm. The developed method was statistically validated and results for the linearity is 0.999 and for System suitability, theoretical plates are 2500 and its tailing factor is 1.64, Precision is 0.1, LOQ is 1.00µg/ml, LOD is 0.33µg/ml, accuracy is 100.19, Robustness (flow rate, mobile phase) is complied.

Keywords: RP-HPLC; Armodafinil; Forced degradation

Introduction

Armodafinil [1-3] is the Enantiomer pure compound of the euro-genic modafinil (Provigil). It consists of only the (R) (–) enantiomer of the racemic modafinil. Armodafinil is currently FDA-approved to treat excessive daytime sleepiness associated with obstructive sleep apnea, narcolepsy, and shift work disorder. It is commonly used off-label to treat attention deficit hyperactivity disorder, chronic fatigue syndrome, and major depressive disorder. It has been shown to improve vigilance in air traffic controllers.Literature review [4-7] reveals very less works done, it became very interesting to pursue the work and to implement the therapeutic drug monitoring in terms of stability.

Experimental

Drug Profile

IUPAC name : (–)-2-[(R)-(diphenylmethyl)sulfinyl]acetamide
Molecular formula : $C_{15}H_{15}NO_2S$
Molecular weight : 273.35
PK_a value : 8.839
Melting Point : 156-158 °C

HPLC instrumentation & conditions

Instrumentation and analytical conditions: The analysis of the drug was carried out on a HPLC system equipped with a reverse phase HPLC with PDA detector method for the determination of Armodafinil Agilent XDB-C_{18}, 50×4.6mm, 5µm or Equivalent column. A mobile phase consisting of Phosphate Buffer: Acetonitrile (65:35v/v) was employed in this study. The flow rate was kept at 1.0ml/min and the injection volume 10µL and the run time is 8 min. The separation was performed at 30°C. Eluents were monitored by PDA detector detector (Waters 2695 Separation Module Equipped with 2996 PDA) set at 223nm.

Chemicals and reagents:

All the chemicals used were of analytical grade and procured from Qualigens India Ltd., Rankem Chemicals Ltd. The chemicals used for the study were, Potassium di-hydrogen phosphate purchased from Merck, Methanol purchased from Rankem, Water and Acetonitrile from Rankem and other chemicals are Ortho Phosphoric acid, Hydrochloric Acid, Sodium Hydrogen Peroxide, Sodium Hydroxide.

- **Preparation of phosphate buffer:**
  Accurately weighed 2.72gm of potassium dihydrogen phosphate in 1000ml of Volumetric flask add about 900ml of milli-Q water and sonicate and make up to the final volume with milli-Q water, add 1ml of Triethylamine and then PH 5.6 is adjusted with dilute orthophosphoric acid solution.

- **Preparation of mobile phase:**
  Mix 600 ml of phosphate buffer pH 5.6 and 400 ml of Acetonitrile (HPLC grade) in a ratio of (65:35%v/v) degassed in ultra-sonic water bath for 5 minutes. Filtered through 0.45 µ filter under vacuum filtration.

- **Diluent:** Prepare filter and degass the mixture of hplc grade water & methanol in a Ratio of (20:80%v/v).

Preparation of standard solution of armodafinil:

Accurately Weighed and transferred 10mg of Armadofinil working Standards into a 10 ml clean dry volumetric flask, add 7ml of methanol, sonicated for 5 minutes and make up to the final volume with methanol (standard stock 1000µg/ml). From the filtered solution 0.5ml was pipette out into a 10ml volumetric flask made up with diluents.

Assay of Armodafinil 1n Armod 20 Tablets:

Preparation of sample solution of armodafinil:

Twenty tablets were weighed and crushed into powder, in order to calculate the average weight of each tablet. From that powder weight equivalent to 50mg of Armodafinil was transferred into a 100 mL volumetric flask, 70mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluent. The peak areas were measured at 223 nm and concentrations in the samples were determined by interpolation from calibration plot previously obtained.

Estimation of Armodafinil in Tablet Dosage Form:

Assay was performed by using the regression equation ($y = 100769x + 27300$, $R^2=0.9991$) obtained from the standard curve of Temozolomide API.

Forced Degradation Studies:

The drug was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. The various degradation pathways studied are Acid degradation, Alkaline degradation, Oxidative degradation, Thermal degradation, Photo degradation.

Method validation:

Linearity and Calibration Curve

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in samples within a given range or proportional by means of well-defined mathematical transformations. Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) and/or by using separate weight of synthetic mixtures of the test product components, using the proposed procedure [8-10].

Accuracy:

Accuracy was best determined by the standard addition method. Previously analyzed samples of Armodafinil API were added with standard drug solutions and are analyzed by the proposed method. Recovery (%) and RSD (%) were calculated for each concentration

Precision:

Precision was determined as both repeatability and intermediate precision, in accordance with ICH guidelines. Repeatability of sample injection was determined as intra-variation and intermediate variation was determined by measurement of inter day variation. For these determinations, three concentrations of the solutions of Armodafinil API were used.

Robustness:

The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small but deliberate variations in method parameters". The robustness of a method is the ability to remain unaffected by small changes in parameters such as pH of the mobile phase, temperature, % organic solvent strength and buffer concentration etc. To determine the robustness of the method experimental conditions are purposely altered and chromatographic characters are evaluated. Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), temperature (±2^{0}C), wavelength of detection (±2nm) and water content in mobile phase (±2%) were studied to determine the robustness of the method

Limit of detection (LOD):

The limit of detection (LOD) of an analytical method may be defined as the concentration, which gives rise to an instrument signal that is significantly different from the blank. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sa), which may be related to LOD and the slope of the calibration curve, b, by

Limit of quantitation (LOQ):

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ represent the concentration of analyte that would yield a signal-to-noise ratio of 10.

LOQ = 10 Sa / b

Where, Sa is the standard deviation of the peak area ratio of analyte to IS (5 injections) of the drugs and b is slope of the corresponding calibration curve.

Specificity:

The specificity of the method was determined by exposing the drug sample to acidic (0.1 N HCl), basic (0.1N NaOH) and oxidizing (3% H_2O_2) stress conditions. The resulting solutions were then analyzed and the analyte peak was evaluated both for peak purity and for resolution from the nearest eluting peak.

Stability:

Stability of pharmaceutical product may be defined as, the capacity of a particular formulation in a specific container or closure system, to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications.

Stability of Armodafinil API was determined after storage of the drug solution for 24 hours at room temperature (25± 2^{0}C).

Results and Discussion:

Optimization of chromatographic conditions:

The chromatographic conditions were optimized by different means i.e. using different column, different mobile phase, different flow rate, different detection wavelength and different diluents for sample preparation etc. and finally the prescribed method is accepted. Chromatographic conditions were listed in Tables 1 and 2 and Figures 1, 2 and 3.

Trial	Column	Flow rate (ml/min)	Temp	Mobile phase	Wave length	Observation	Remark
1	Agilent XDB, C_{18}, 50×4.6mm, 5µm or Equivalent	1.0ml/min	30^{0}C	Phosphate Buffer: Methanol (70:30)	223nm	Broad peak was observed.	Method rejected
2	Agilent XDB, C_{18}, 150×4.6mm, 5µm or Equivalent	1.0ml/min	30^{0}C	Phosphate Buffer: Acetonitrile (90:10)	223nm	No peak was observed	Method rejected
3	Agilent XDB, 150×4.6mm, 5µm or Equivalent	1.0ml/min	30^{0}C	Phosphate Buffer: Acetonitrile (80:20)	223nm	No peak was observed	Method rejected
4	Agilent XDB, C_{18}, 150×4.6mm, 5µm or Equivalent	1.0ml/min	30^{0}C	Phosphate Buffer: Acetonitrile (75:25)	223nm	Poor plate count	Method rejected
5	Agilent XDB, C_{18}, 250×4.6mm, 5µm or Equivalent	1.0ml/min	30^{0}C	Phosphate Buffer: Acetonitrile (70:30)	223nm	Peak with more tailing is observed.	Method rejected
6	Agilent XDB, C_{18}, 150×4.6mm, 5µm or Equivalent	1.0ml/min	30^{0}C	Phosphate Buffer: Acetonitrile (65:35)	223nm	A sharp peak with good plate count is observed.	Method accepted

Table 1: Analytical Method Development Trials

Column	Agilent XDB C18, 150 x 4.6 mm, 5µ.
Detector wavelength	223nm
Column temperature	30°C
Injection volume	10µL
Run time	8 min
Diluent	Water: Methanol (20:80)
Mobile phase	Buffer: Acetonitrile (65:35)
Drug RT	3.354
Elution technique	Isocratic

Table 2: Optimized Chromatographic Conditions

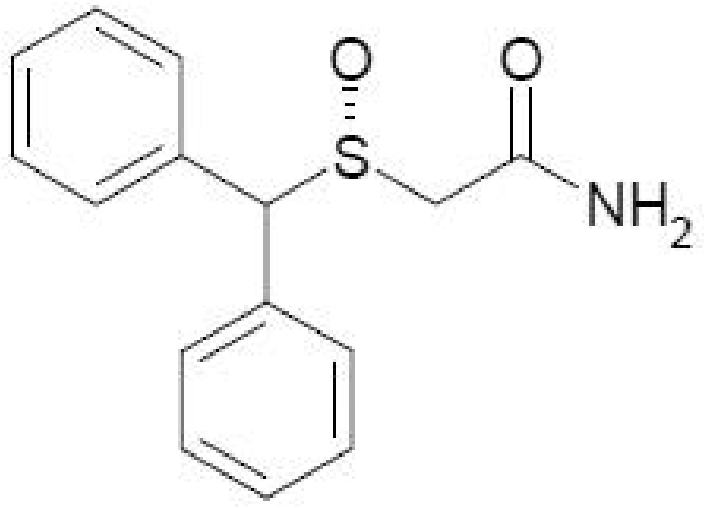


Figure 1: Chemical structure of Armodafinil

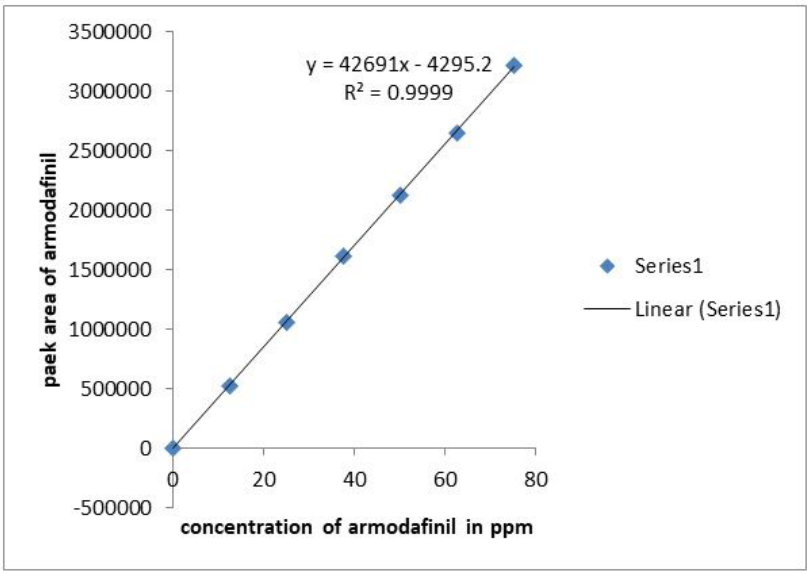


Figure 2: Calibration curve of Armodafinil API

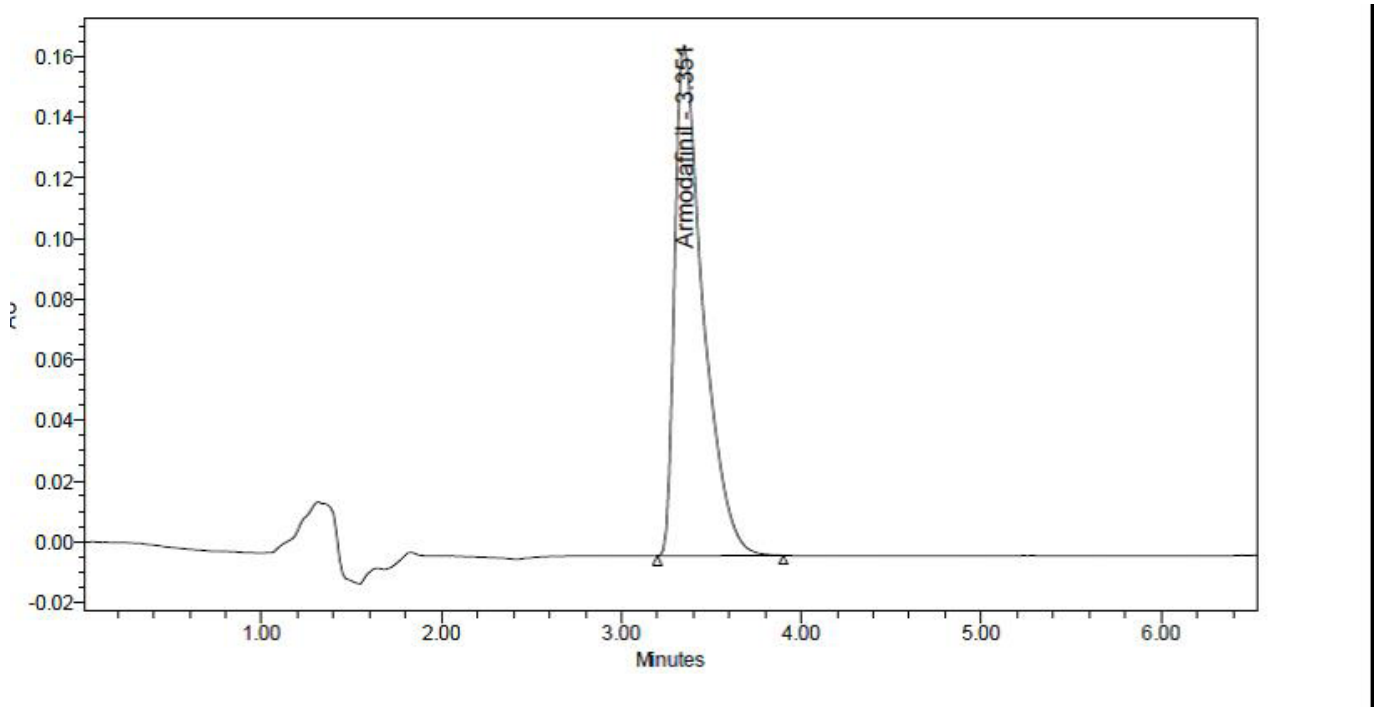


Figure 3: Chromatograph of optimized trial

Accuracy -Recover study:

The recovery of the method, determined by adding a previously analyzed test solution with additional drug standard solution, was 100.19 %. The values of recovery (%) and RSD (%) listed in Table 5 indicate the method is accurate-chromatogram was shown in Figure 5.

Precision: System precision and method precision:

This method was carried out and the high values of mean assay and low values of standard deviation and % RSD (RSD NMT 2.0%) within a day and day to day variations for armodafinil revealed that the proposed method is precise and the final result obtained % RSD is 0.1.Results obtained are shown in Figures 6 and 7and Tables 6 and 7.

	Retention Times	Peak Area	Tailing Factor	Theoretical Plates
1	3.301	1822152	1.65	2447
2	3.307	1825225	1.68	2408
3	3.31	1810134	1.67	2482
4	3.325	1810210	1.63	2483
5	3.326	1810976	1.61	2363
6	3.35	1815203	1.61	2456
Mean		1815650		
SD		6573		
%RSD		0.3		

Table 3: System Suitability Parameters

Linearity Level (%)	Concentration (ppm)	Area
20	12.5	524067
50	25	1056889
70	37.5	1616960
100	50	2120262
120	62.5	2647995
150	75	3210254
	Linearity concentration	12.5-75PPM
	Slope	42691
	Intercept	4295
	Correlation coefficient	0.999

Table 4: Calibration of the HPLC method for armodafinil

Accuracy	Area	% Recovery	Mean Recovery
S1: 50%	919713	101.11	Mean=100.72%
S2: 50%	913107	100.38	S.D = 0.365
S3: 50%	915759	100.67	%RSD = 0.36
S4: 100%	1810501	99.52	Mean = 99.88%
S5: 100%	1824997	100.31	S.D = 0.404
S6: 100%	1815611	99.80	%RSD = 0.40
S7: 150%	2725468	99.87	Mean =99.98%
S8: 150%	2735077	100.23	S.D = 0.2119
S9: 150%	2734699	99.84	%RSD = 0.21

Table 5: Results of accuracy

Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & buffer in mobile phase (±2%) studied to determine the robustness of the method are also in favor of (Table 4, % RSD < 2%) the developed RP-HPLC method for the analysis of Armodafinil API. Results obtained are shown in Figure 10 and Table 8.

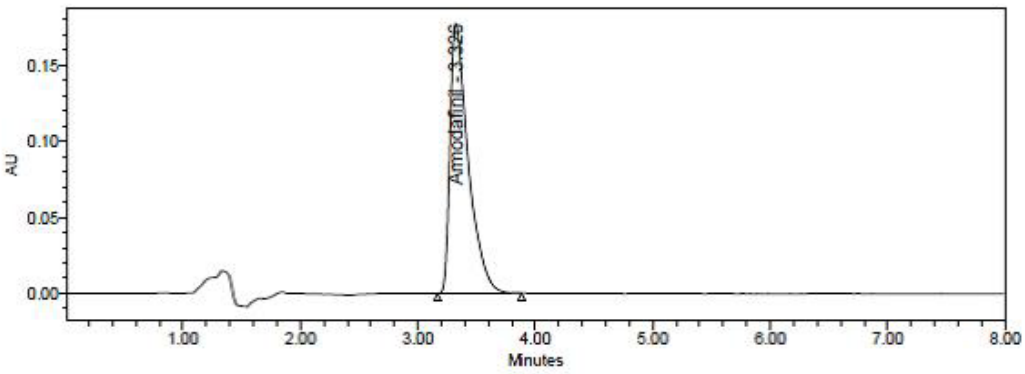


Figure 4: Chromatogram of System suitability standard

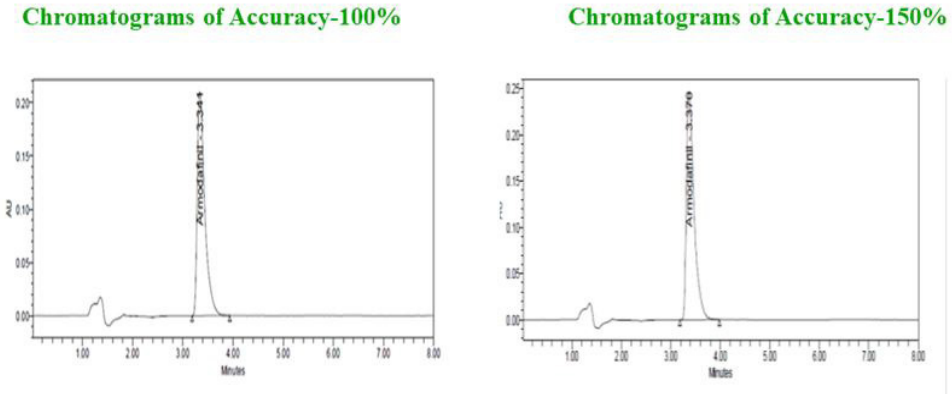


Figure 5: Chromatogram of Accuracy

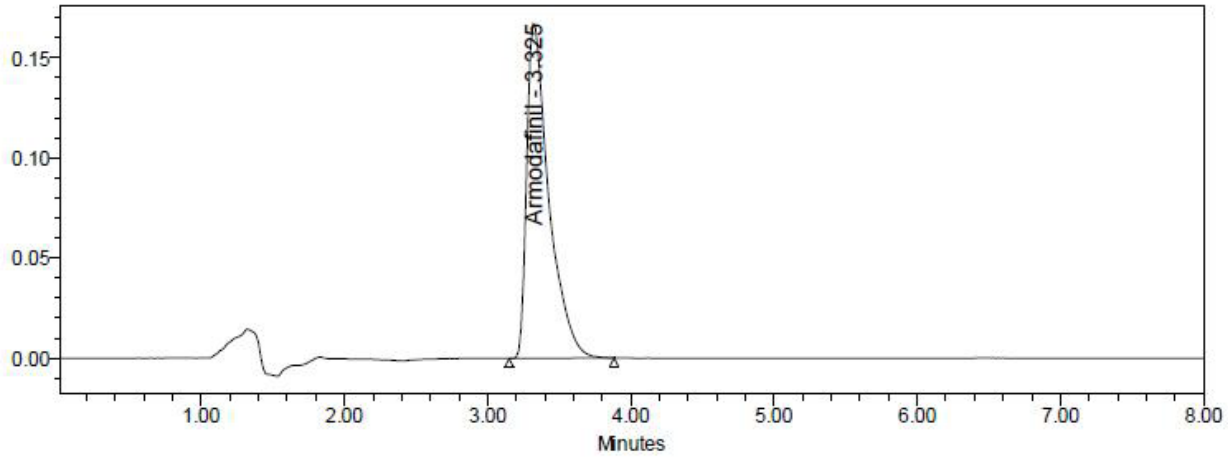


Figure 6: Chromatogram of System precision

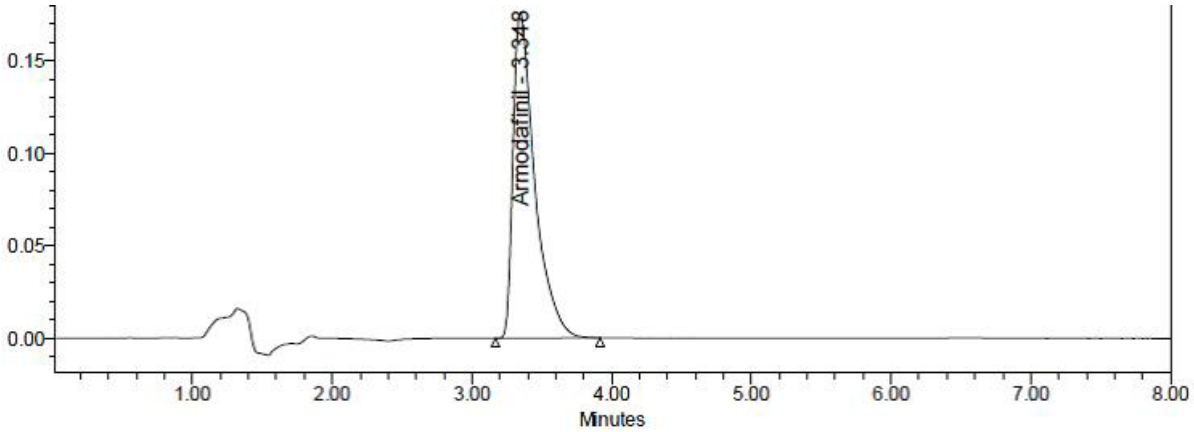


Figure 7: Chromatogram of Method precision

SYSTEM SUITABILITY	AREAS
1	1822152
2	1825225
3	1810134
4	1810210
5	1810976
6	1815203
AVG	1815650
SD	6573.01
%RSD	0.36

Table 6: Results of system precision

Sample No	Sample Areas	%Assay
1.	1815404	99.79
2.	1811942	99.60
3	1819899	100.03
4	1816055	99.82
5	1815040	99.77
6	1816870	99.87
AVG	1815868	99.81
STANDARD DEVIATION	2592.30	0.1425
RELATISTANDARD DEVIATION(%RSD)	0.10	

Table 7: Results of method precision

Inj.Sample	Change parameter	modification	Peak area 1	Peak area 2	mean	%RSD
Armodafinil	Flow rate	0.8 ml/min	2018950	2008764	2008857	0.1
		1 .1ml/min	1620562	1615142	1617852	0.23
	Mobile phase	60:40	1805335	1811345	1808340	0.23
		70:30	1816046	1815471	1816258	0.1
	temperature	25°c	1803514	1807444	1805479	0.2
		35°c	1799955	1798639	1799297	0.1

S.NO	PARAMETERS	LIMIT	OBSERVATION
1	System suitability	Theoretical plates should not less than 2000 Tailing factor should not more than 2.0	Theoretical plates 2500 Tailing factor 1.64
2	Precision	RSD NMT 2.0%	0.1
3	Linearity	Correlation coefficient NLT0.99	0.999
4	Accuracy	%Recovery range 98-102	100.19
5	Robustness (flow, mobile phase)	System suitability parameters should comply	Complies

Table 9: Summary of Method Validation Parameter

Mode of degradation	Conditions	Armodafinil		
		%Degradation w.r.t. control	Purity angle	Purity Threshould
Control	No treatment	-	-	-
Acid degradation 1N HCl	60°C/30min	4.04	0.350	0.495
Alkali degradation 0.1N NaOH	60°C/30min	7.97	0.12	0.30
Peroxide degradation 10%W/V H_2O_2	60°C/30min	10.02	0.131	0.353
Thermal degradation	105°C/6hr	7.4	0.271	0.456
Photolytic	UV/7days	6.4	0.252	0.492

Table 10: Summary of Forced Degradation Studies

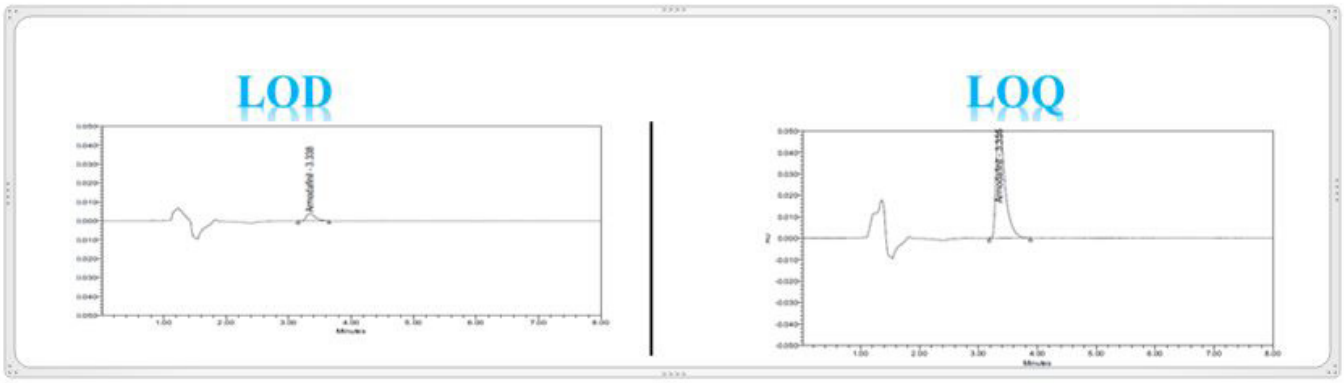


Figure 8: Chromatogram of LOD AND LOQ

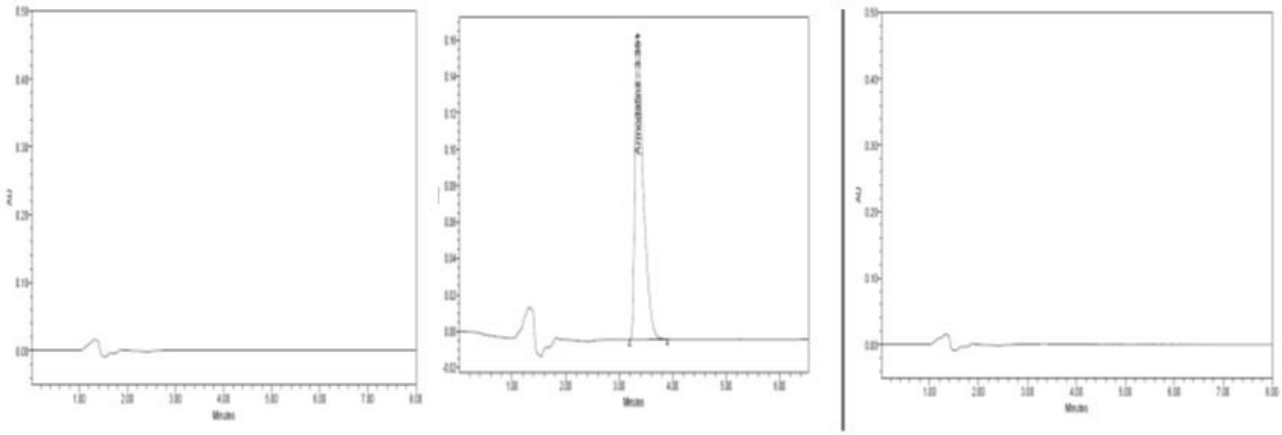


Figure 9: (A) Chromatography of Mobile phase; **(B)** Chromatography of placebo; **(C)** Chromatography of drug peak

(specificity) (specificity) (specificity)

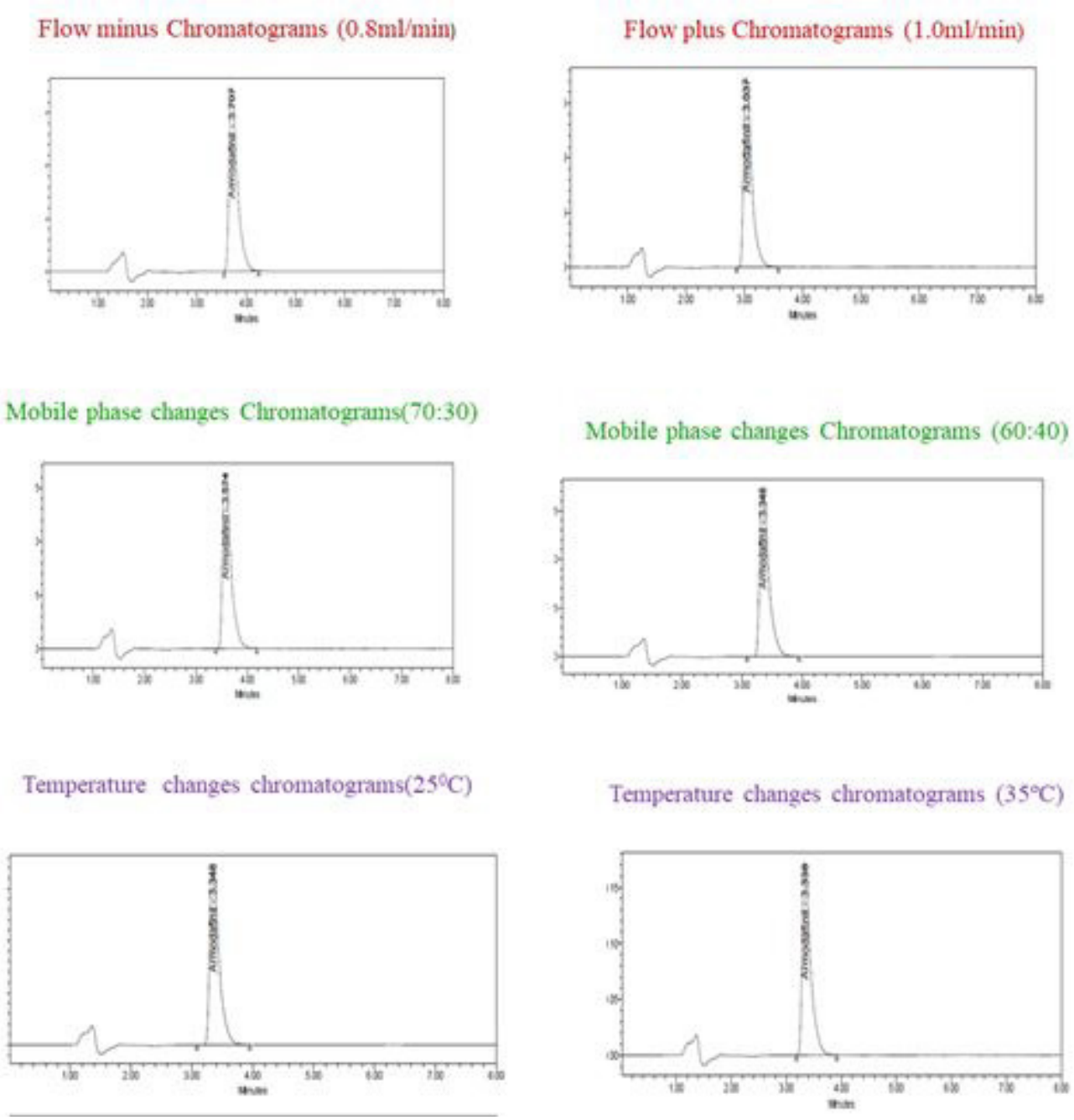


Figure 10: Chromatograms of Robustness

Linearity:

In the linearity, correlation coefficient obtained is 0.999. Chromatograph of calibration curve is shown in Figure 2 and observe Table 4.

LOD & LOQ:

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.33 µg/ml and 1.006 µg/ml respectively. Chromatograms obtained are shown in Figure 8.

Specificity: No peak was found at the retention time of armodafinil peak. Observe the peaks in Figures 9A, B and C.

System suitability: Theoretical plates are about 2500 and tailing factor obtained was 1.64. Observe the Table 3 and chromatograms obtained is shown in Figure 4.

Summary: Method validation parameters is shown in Table 9.

Stability in analytical solution:

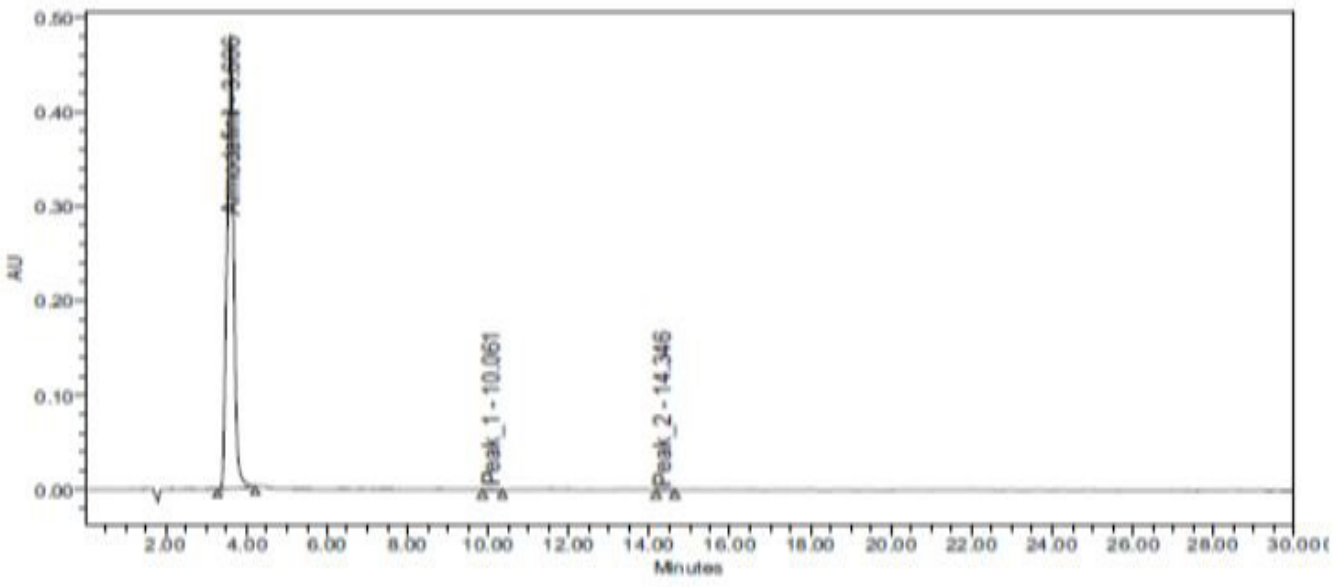
It is important to mention here that the armodafinil API was stable in solution form up to 72 hours at 25^0C.

Forced degradation studies:

Forced degradation studies Based on peak purity results, obtained from the analysis of force degradation samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of armodafinil indicated that the developed method is specific for the estimation of armodafinil in presence of degradation products. The results of the forced degradation studies were given in Table 10 and Figures 11,12,13,14 and 15.

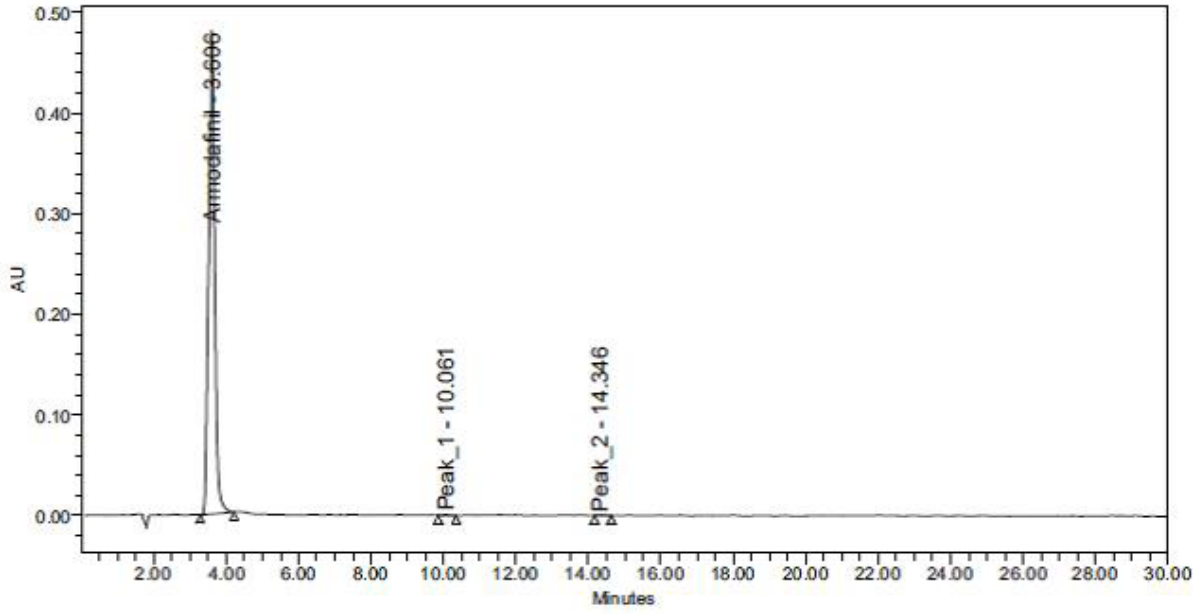
Estimation of Armodafinil in Tablet Dosage Form:

Assay was performed by using the regression equation (y = 100769x + 27300, R^2=0.9991) obtained from the standard curve of Temozolomide API. Results obtained are given in Table 5. The assay of containing armodafinil was found to be 99.8 % as per the method.



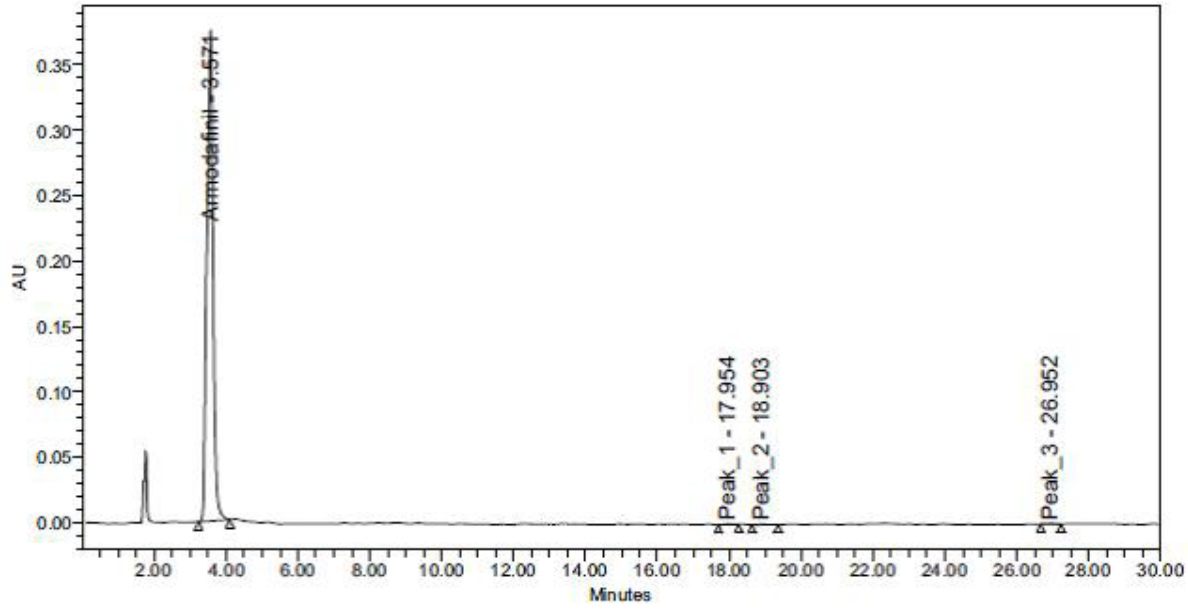
	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	Armodafinil	3.606	1678315	98.18	1875	1.03	0.121	0.304
2	Peak_1	10.061	10804	0.92	6766	1.16	0.008	0.183
3	Peak_2	14.346	10000	0.88	19669	1.29	0.062	0.161

Figure 11: Chromatogram of Acid Degradation (1N HCL)



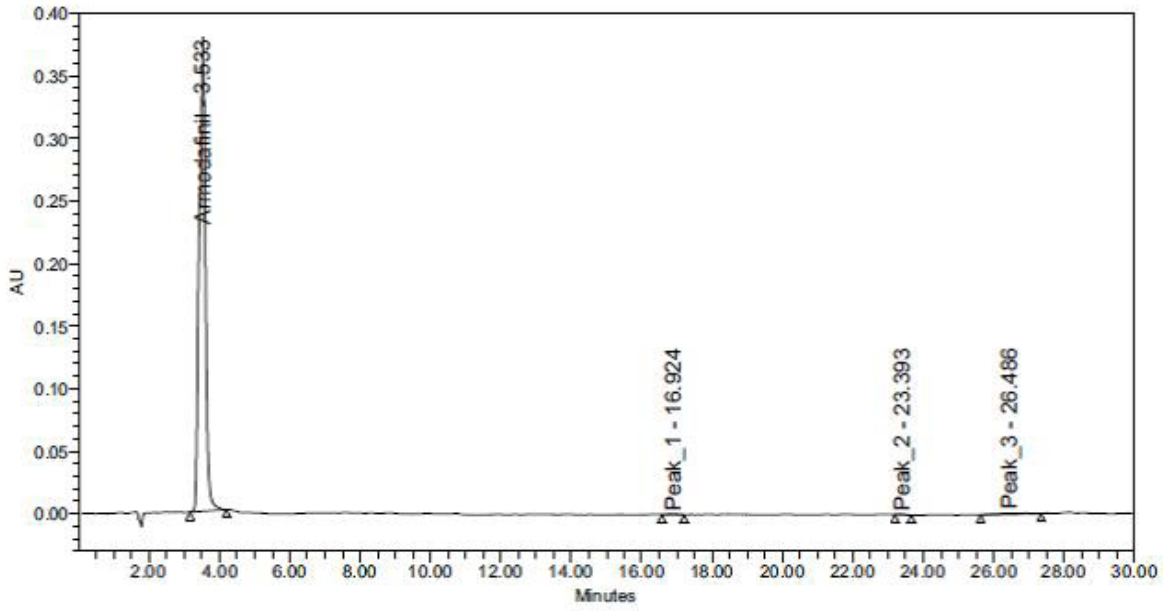
	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	Armodafinil	3.606	1678315	98.18	1875	1.03	0.121	0.304
2	Peak_1	10.061	10804	0.92	6766	1.16	0.008	0.183
3	Peak_2	14.346	10000	0.88	19669	1.29	0.062	0.161

Figure 12: Chromatogram of Base Degradation (0.1 N NaOH)



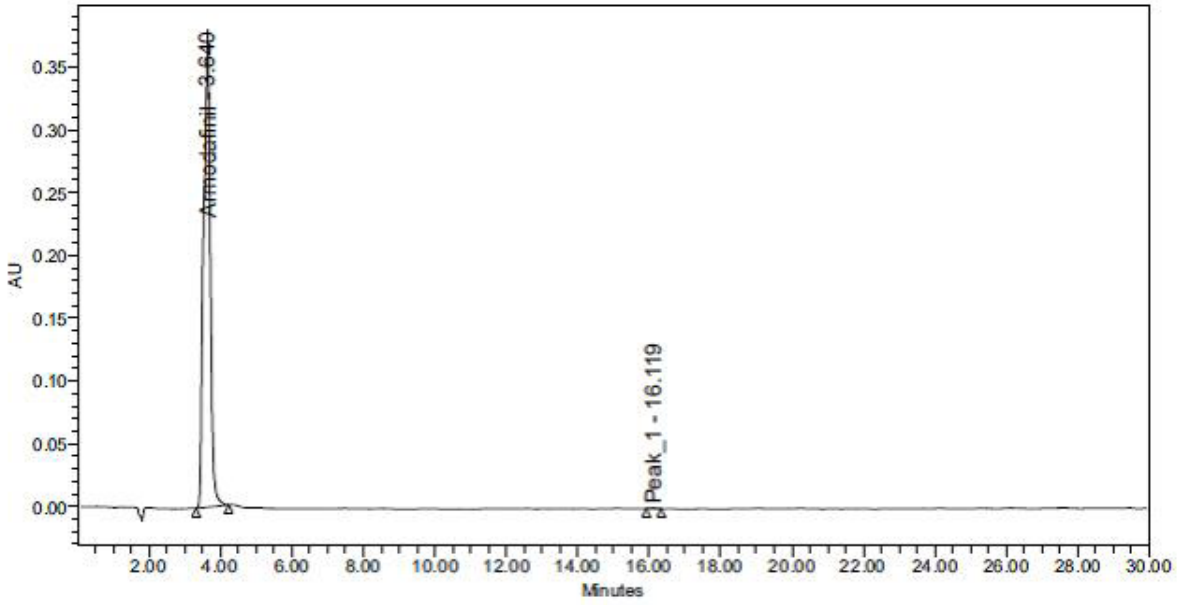
	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	Armodafinil	3.571	1637637	97.21	1039	0.97	0.131	0.353
2	Peak_1	17.954	10188	0.64	44121	1.13	0.120	0.202
3	Peak_2	18.903	12466	1.08	19775	1.41	0.124	0.407
4	Peak_3	26.952	12054	1.07	36571	0.96	0.010	0.212

Figure 13: Chromatogram of Oxidation Degradation (10% H2O2)



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	Armodafinil	3.533	1689303	98.38	2896	0.95	0.271	0.456
2	Peak_1	16.924	4674	0.84	14141	0.91	0.220	0.449
3	Peak_2	23.393	4071	0.72	34674	1.18	0.228	0.642
4	Peak_3	26.486	4816	2.95	7337	1.02	0.200	0.303

Figure 14: Chromatogram of Thermal Degradation



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing	Purity1 Angle	Purity1 Threshold
1	Armodafinil	3.640	1706934	98.91	1005	0.96	0.252	0.492
2	Peak_1	16.119	7870	1.09	30593	0.98	0.052	0.133

Figure 15: Chromatogram of Photolytic Degradation

Conclusion

In conclusion, the proposed HPLC method was found to be simple, precise, accurate and sensitive for the determination of armodafinil in pharmaceutical dosage form. These are within short analysis time and the low value of RSD indicate that the proposed methods are highly precise. High percentage of recoveries suggests that the proposed methods are accurate. Forced degradation studies based on peak purity results, obtained from the analysis of force degradation samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of armodafinil indicated that the developed method is specific for the estimation of armodafinil in the presence of degradation products.

References

1. Rxlist (2020) Nuvigil-drug side-effects-interactions, USA.

2. Drug bank, USA.

3. RXList-nuvigil, USA.

4. Nageswara Rao R, Shinde DD, Kumar Talluri MV (2008) Enantioselective HPLC resolution of synthetic intermediates of armodafinil and related substances. J Sep Sci 31: 981-9.

5. Devi R, Singirikonda R, Habibuddin M (2012) Development and Validation of New LC-MS/MS Method for the Determination of armodafinil in Human Plasma. Current Pharmaceutical Analysis 8: 295-305.

6. Vivek Sagar P, Bagum N, Rani SS (2014) Stability Indicating RP HPLC Method for the estimation of Armodafinil In Tablet Dosage Form. International journal of pharmacy and pharmaceutical sciences 6: 604-9.

7. Nagappan KV, Sungroya N, Devi D, Yamjala K, Byaran G, et al. (2017) Development and Validation of Stability Indicating RP HPLC method for the estimation of armodafinil and Characterization of its base Degradation Product by LC-MS/MS, GSTF. Journal of Advances in Medical Research 2: 1-8.

8. ICH (1996) Validation of analytical procedure: Methodology Q2B, Tripartite Guidelines. J Pharm Biomed Anal 1996.

9. ICH (2006) hHarmonized tripartite guideline. Impurities in New Drug products Q3B R2 current step 4 versions dated 2 June 2006.

10. ICH (2005) Guideline, Validation of analytical procedures: Text and Methodology Q2 (R1);November. J Pharm Biomed Anal 2005.