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VALIDATED RP-HPLC METHOD FOR QUANTITATION OF ARMODAFINIL IN BULK DRUG AND ITS PHARMACEUTICAL DOSAGE FORM AND ITS APPLICATION TO STABILITY INDICATING ASSAY

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ABSTRACT

A simple, specific, sensitive, precise stability-indicating high-performance liquid chromatography method for determination of Armodafinil was developed and validated. A Agilent TC C-18 column (250 x 4.6 mm, 5 μ) in isocratic mode, with mobile phase consisting of a mixture of solution Methanol: water (60:40) was used. The quantitation performed at flow rate of 1.0 mL/min at 225 nm and run time was 10 min. The analytical method was validated as per ICH guideline for linearity, accuracy, precision, specificity, limit of detection, limit of quantification, robustness and stability and method can be extended to the analysis of Armodafinil in tablet formulation. The relative standard deviation values for precision was less than 2%, and % recovery was greater than 98% for Armodafinil. The drug undergoes acidic, alkaline, oxidative, photo and thermal degradations.

KEYWORDS

Armodafinil, RP-HPLC, Validation and Stability.

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INTRODUCTION

Armodafinil is a wakefulness-promoting agent for oral administration. Armodafinil is the R-enantiomer of Modafinil which is a mixture of the R and S-enantiomers. Chemically, it is 2-[(R)-(diphenylmethyl) sulfinyl] acetamide with molecular formula C₁₅H₁₅NO₂S. Armodafinil is used for the treatment of narcolepsy and shift work sleep

disorder, and as an adjunctive treatment for obstructive sleep apnea¹. Armodafinil is mostly metabolized by Hydrolytic deamidation, S-oxidation and aromatic ring hydroxylation, subsequent glucuronide conjugation of the hydroxylated products.

Literature survey reveals that few analytical methods have been reported for the estimation of Armodafinil such as UV², HPLC³, Chiral chromatography⁴, LC-MS/MS⁵, Capillary electrophoresis⁶ but Modafinil revealed several methods based on different technique, such as; HPLC with UV detection⁷; LC-MS⁸; GC-MS⁹; HPLC with UV detection assay for its quantification in plasma and serum¹⁰⁻¹⁴ and Chiral Chromatography¹⁵.

Stress testing carried out to elucidate the inherent stability characteristic of the active substances and forms an important part of the API. It suggests that degradation products that are formed under a variety of conditions should be identified and degradation pathways be established. The purpose of stress testing is to provide evidence on how the quality of drug substance varies with time under the effect of varieties of factors such as acidic, alkaline, temperature, light and presence of oxygen. An ideal stability-indicating method is one that quantifies the drug and also resolve its degradation products¹⁶.

The aim of present work is to develop a simple, specific, sensitive, accurate and stability indicating HPLC analytical procedure for the analysis of Armodafinil and validated as per ICH guidelines¹⁷.

MATERIALS AND METHODS

Reagent and Materials

Armodafinil working standard was supplied by Natco Pharma Pvt. Ltd. and sample tablet (Label claim: 150mg; Nuvigil tablet; and manufacturer: Natco Pharma Pvt. Ltd.) were procured from the local market. Methanol and water (HPLC grade) were purchased from Merck, Mumbai, India. Analytical grade sodium hydroxide, hydrochloric acid and hydrogen peroxide were purchased from Fischer scientific, Mumbai, India.

Apparatus

The HPLC system, employed in the method development, forced degradation studies and assay method validation was Agilent 1120 series LC system with variable wavelength detector. The output signal was monitored and processed using EZ chrome elite software.

Methods

Diluting agent preparation

Solvent mixture of methanol and water, in the ratio of 60:40 (v/v) was used as diluting agent.

Stock Solution

Accurately weighed 10 mg of Armodafinil working standard was transferred into 10 ml volumetric flask, dissolved and volume was made up to the mark with diluting agent. The final solution contained 1000 µg/ml of Armodafinil.

Standard Solution

1 ml of Armodafinil stock solution was transferred to a 10 ml clean volumetric flask and the volume was made up with diluting agent and mix well (100 µg/ml). From the above stock solution, 1ml was pipetted out and made to 10ml with diluting agent and then filtered through 0.45 µm ultipor N66 nylon filter. The final solution (10 µg/ml) was injected into the HPLC system.

Chromatographic Condition

Chromatographic separation was achieved at 25°C on a reverse phase C-18 column using mobile phase consisting of methanol and water in the ratio of 60:40 (v/v). The flow rate was kept at 1.0 ml/min and detection was carried out at 225 nm. The sample was injected using 20 µl fixed loop, and the total run time was 10 min.

Calibration of HPLC Method

The standard solutions were prepared by further dilutions of the stock standard solution with the specified mobile phase to reach the concentration range of 1-50 µg/ml.

Triplicate 20 µl injections were made for each concentration and chromatographed under the specific chromatographic conditions described previously. The peak area values were plotted against corresponding concentrations.

Sample Preparation

Ten tablets were weighed accurately; the average weight was determined and then ground to a fine powder. The quantity of powder equivalent to 10 mg of Armodafinil was transferred to 10 ml volumetric flask and dissolve with diluting agent (1000 µg/ml) and solution ultrasonicated to dissolve content for 20 minutes then after volume was made up to mark with same diluting agent, filtered through 0.45 µm nylon filter. This solution was further diluted with same solvent to obtain concentration of 10 µg/ml. Working standard of Armodafinil was also prepared in the same way at that concentration level and 20 µl was injected.

Stress Degradation of Armodafinil

All degradation studies in solution were carried out at a drug concentration at 10 mcg ml⁻¹. Hydrolytic reactions were carried out in 1N HCl and 0.1 N NaOH at room temperature for 24 hrs and 2 hr. Oxidative degradation studies were carried out at room temperature on 0.3% H₂O₂ for 24 hrs. Photo-degradation was carried out in water for 4hr. Pure solid drug (in 1 mm thick layer in petri plate) was also exposed to the same condition for 4hr. Thermal degradation was carried out in water for 4hr at 60°C. Pure solid drug (in 1 mm thick layer in petri plate) was also exposed to the same condition for 4hr at 60°C.

Validation of the Developed Method

Linearity was established by triplicate injections of solutions containing drug in the concentration range 1-50 µg/ml. Intra- day precision was established by making 6 injections of lowest, middle and highest concentration in the above range (5, 10 and 15 µg/ml) on the same day. These studies were also repeated on different days with different weightings to determine inter-day precision. Accuracy was evaluated by fortifying a mixture of decomposed reaction solution with three known concentration of the drug. The recovery of added drug was determined.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

The chromatographic conditions were optimized with a view to develop a stability-indicating assay method for Armodafinil in tablet dosage forms. The final chromatographic system comprising of reverse-phase C-18 column (250 × 4.6 mm, 5µ) with a mobile phase consisting of a mixture of solution methanol and water in a ratio of 60:40 at a flow rate 1.0 ml/min was found optimum (Figure No.2). Detection was performed at 225 nm (Table No.1).

Degradation Behavior

HPLC studies on the Armodafinil under different stress conditions indicated the following degradation behavior (Table No.5).

Acidic Degradation

Armodafinil showed sufficient degradation within 1hr at room temperature in 1 N HCl. The major degradation products were at retention times (RT) 2.953 and 3.183 min (Figure No.3).

Alkali Degradation

The drug underwent alkaline hydrolysis in 0.1 N NaOH for 2hr at room temperature. The major degradation products were at RT 2.907 and 3.190 min (Figure No.4).

Oxidative Degradation

The drug showed sufficient degradation in 0.3% H₂O₂ for 2hr at room temperature. The major degradation products were at RT 2.677 and 3.053 min (Figure No.5).

Photo degradation

The drug was liable on exposure to sun light in neutral condition, showed degradation in sun light for 4hr (Figure No.9). Armodafinil solid drug showed degradation under the same conditions for 3 hrs. No degradation product was observed (Figure No.8).

Thermal degradation

The drug was degraded in both neutral and solid form. Neutral solution showed degradation in temperature 60°C for 3hrs (Figure No.6). The solid drug showed degradation in temperature 60°C for 4hrs. No degradation product was identified (Figure No.7).

VALIDATION OF THE METHOD

Linearity

The linearity could be established for Armodafinil in the concentration range of 1-50 µg/ml (See Table No.2, Figure No.3).

Precision

Table No.2 lists the relative standard deviation (RSD) data obtained on analysis of the samples on the same day (n=3) and on consecutive days (n=3).

A % RSD value was for intra- and inters- day studies, respectively, demonstrates that the method was sufficiently precise.

Recovery

Table No.3 shows the recovery of the added drug, obtained from the difference between peak areas of unfortified samples and fortified samples, and was satisfactory at all tested concentrations.

Table No.1: Optimized chromatographic condition of Armodafinil for the proposed method

S.No	Conditions	Results
1	Mobile phase	MeOH:H ₂ O 60:40
2	Column	C18, 250 x4.6 mm, 5 µm
3	Flow rate	1ml/min
4	Detector	VWD
5	Injection Volume	20µl
6	Run time	10min
7	Retention Time of Drug	5.8min

Table No.2: Regression analysis of the calibration curve for the proposed method

S.No	Parameters	Values
1	Calibration range (mg/ml)	1 - 50µg/ml
2	Slope (m)	146986
3	Intercept (c)	42774
4	Correlation coefficient (r ²)	0.9994

Table No.3: Summary of validation parameters for the proposed method

S.No	Parameters	Values
1	Specificity	No interference was found to be w.r.t. Excipients, impurities
2	Linearity range (µg/ml)	1-50
3	Precision (n=6) Intraday precision Intraday precision	<2 <2
4	Accuracy (%)	98.82-99.97
5	LOD (ng/ml)	20
6	LOQ(nags/ml)	60
7	Robustness	Robust#

* % RSD # To the extent of variations applied in analytical conditions

Table No.4: Analysis of marketed formulation

S.No	Brand name	Label claim (mg)	Assay	% RSD
1	Nuvigil	150	101.339	0.263

Table No.5: Stress study data for Armodafinil

S.No	Conditions	Time	% Degradation
1	Acidic (1N HCl)	1hr	10.39
2	Alkaline (0.1N NaOH)	2hr	25.55
3	Oxidative (0.3% H ₂ O ₂)	3hr	16.09
4	Thermal Neutral solution Solid	3hr	6.83
		4hr	11.69
5	Photo Neutral solution Solid	4hr	8.343
		3hr	6.67

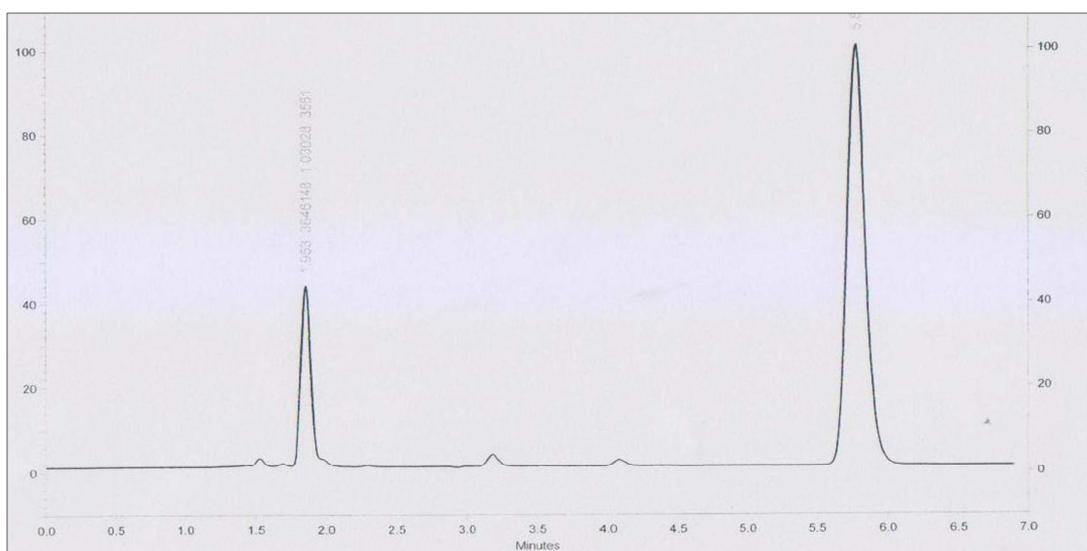


Figure No.1: Optimized Chromatographic Condition

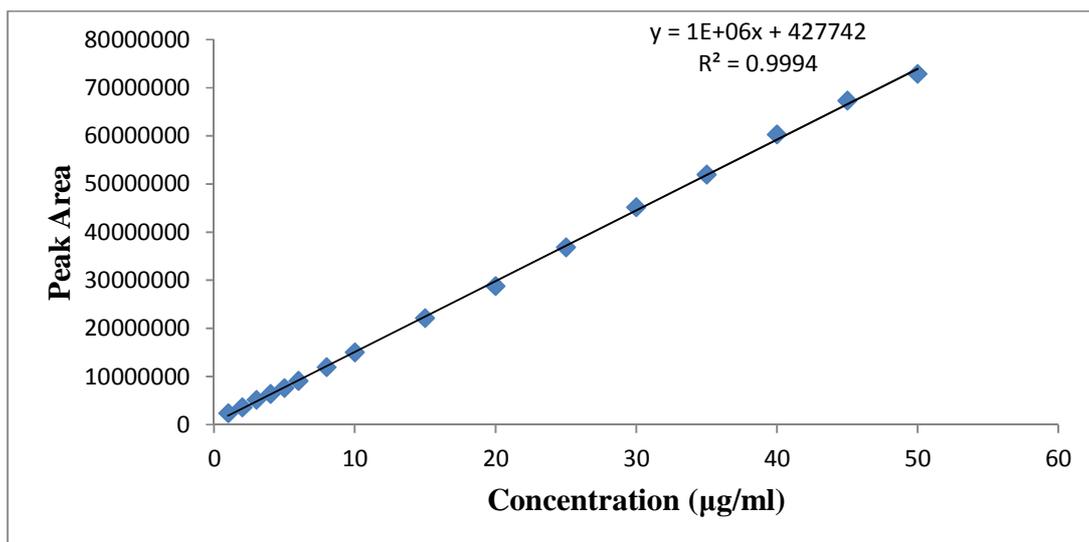


Figure No.2: Calibration Curve of Armodafinil



Figure No.3: Degradation in 1N HCl

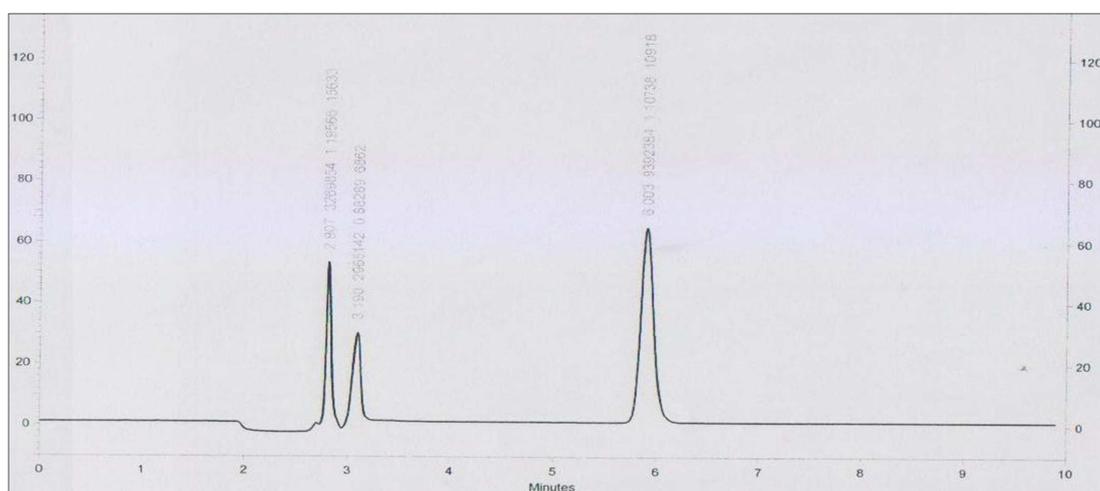


Figure No.4: Degradation in 0.1N NaOH

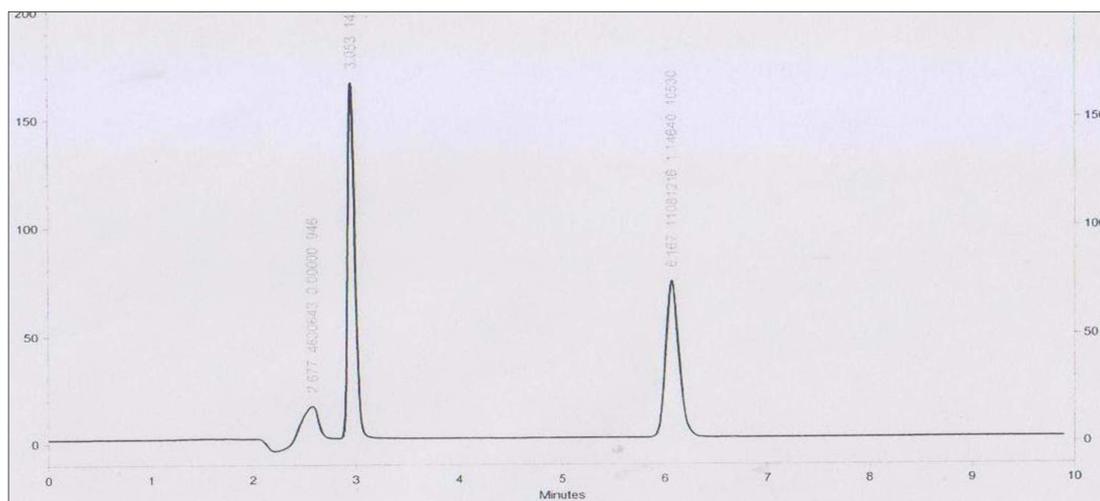


Figure No.5: Degradation in 0.3% H₂O₂

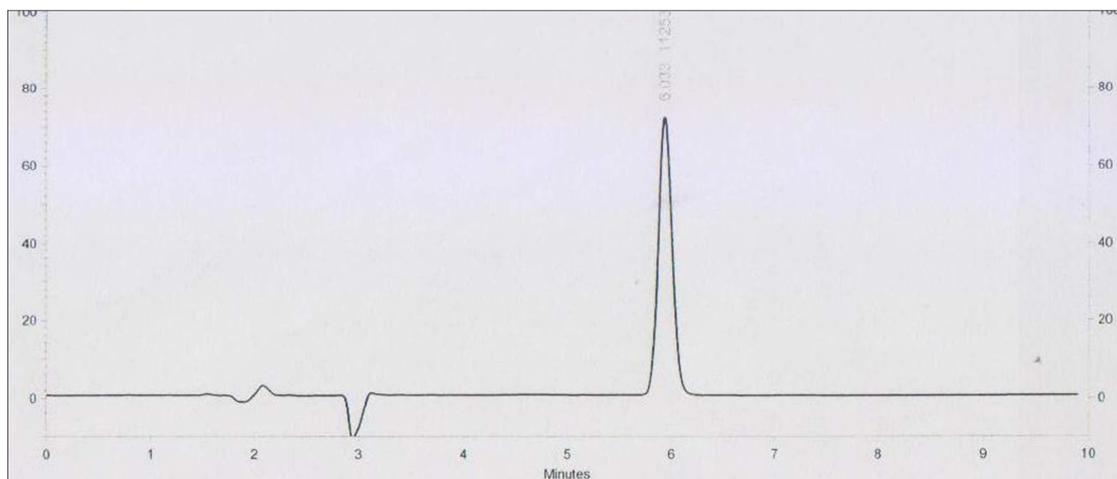


Figure No.6: Degradation in Thermal (solution)

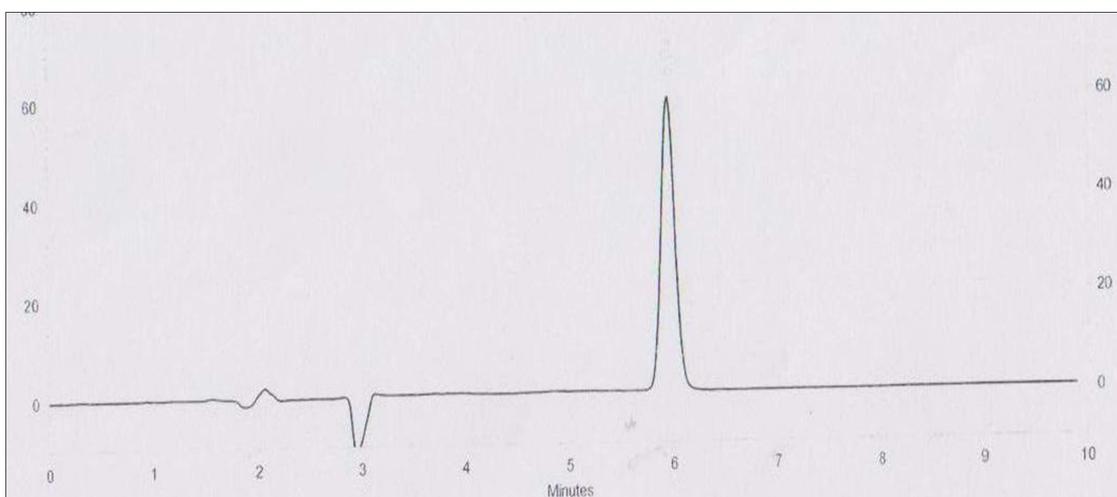


Figure No.7: Degradation in Thermal (solid)

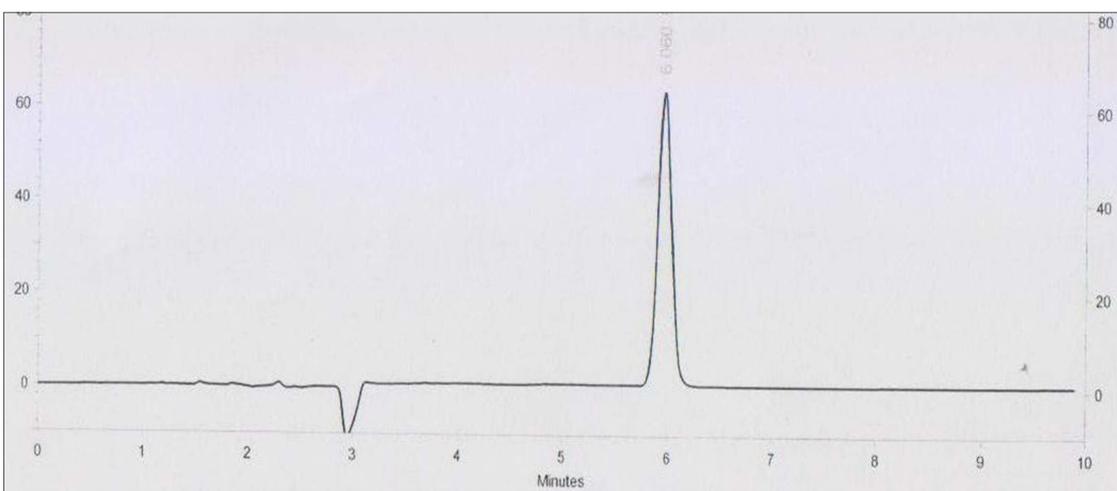


Figure No.8: Degradation in Photo (solid)

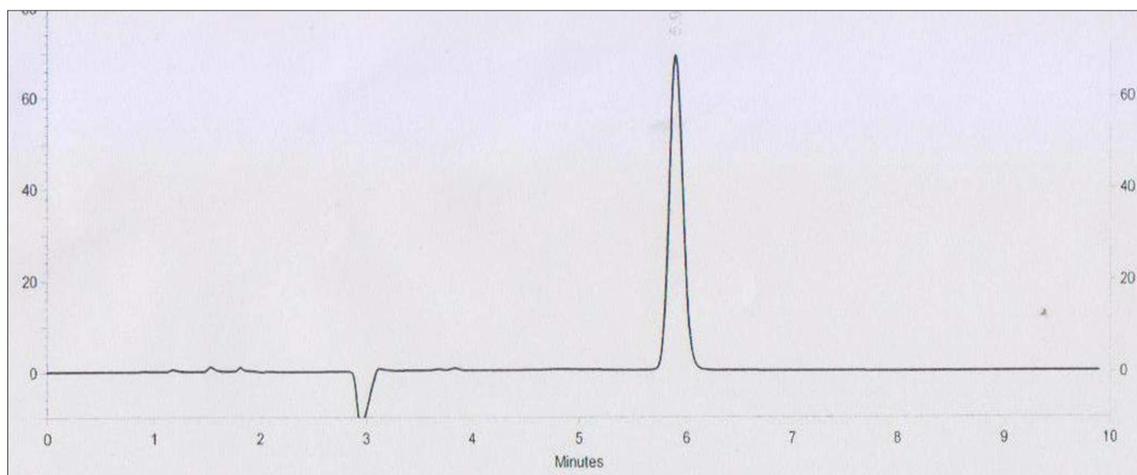


Figure No.9: Degradation in Photo (solution)

CONCLUSION

The developed HPLC method is specific, accurate and stability indicating. Statistical analysis proves that method is precise, robust and selective for analysis of Armodafinil in tablet dosage form. The developed method is suitable for the quality control analysis of Armodafinil in tablet dosage form.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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