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Development and Validation of Stability Indicating Rp-Hplc Method for Modafinil in Bulk and Tablet Formulation

Dr. Krupa K. Patel *[1], Dr. Arun Kumar Gupta[2]

1. *Professor, Chameli Devi Institute of Pharmacy, Indore, Madhya Pradesh, India.*
2. *Principal, Chameli Devi Institute of Pharmacy, Indore, Madhya Pradesh, India.*

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ABSTRACT:

A simple, precise, rapid and accurate stability indicating RP HPLC method was developed for the assay of Modafinil in tablet formulation. The solvent system and wavelength were optimized in order to maximize the sensitivity of the proposed method. The separation was carried out on a hypersil BDS c18 (25cm*0.46cm) 5 µ particle sizes with mobile phase Acetonitrile: Water (80: 20 v/v) at flow rate of 1.0 ml/min.The detection is carried out at 223.6nm. The retention time of the drug was 4.2 min. The Linearity was observed in the concentration range of 7.5 -22.5 µg/ml with correlation coefficient 0.9993. The method was validated for accuracy, precision, linearity, LOD & LOQ of sample solution. Modafinil stock solutions were subjected to acid, base, oxidation, Photo and thermal degradation. The degraded peaks were well resolved from the pure drug peak with significant difference in their retention time values.

KEYWORDS: Stability indicating, RP-HPLC, Modafinil, validation, tablet formulation

I. INTRODUCTION:

PROVIGIL (Modafinil) is a wakefulness-promoting agent for oral administration. Modafinil is a racemic compound. The chemical name for Modafinil is 2-[(diphenylmethyl) sulfinyl] acetamide. It belongs to CNS stimulant category. The molecular formula is $C_{15}H_{15}NO_2S$ and the molecular weight is 273.35 gm/mol. Modafinil is a solid, white to off white crystalline powder. It is practically insoluble in water and cyclohexane, sparingly to slightly soluble in methanol and acetone. Modafinil is a stimulant drug marketed as a 'wakefulness promoting agent' and is one of the stimulants used in the treatment of narcolepsy shift work sleep disorder and excessive daytime steepness associated with obstructive sleep apnea. The exact mechanism of action is unclear, although in vitro studies have shown it to inhibit the reuptake of dopamine by binding to the dopamine reuptake pump, and lead to an increase in extracellular dopamine[1,2,].

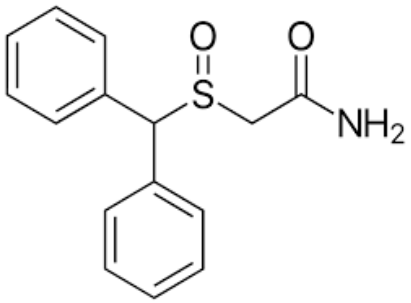

FIG.1: **CHEMICAL STRUCTURE OF MODAFINIL**

II. LITERATURE SURVEY.

Literature survey revealed the estimation of Modafinil by several techniques such as UV spectroscopy [3, 4, 5], RP-HPLC techniques[6-10], stability indicating HPTLC[11], simultaneous determination by RP-HPLC[12], chiral chromatography[13] and GC-MS[14] . The focus of present study was to develop and validate a rapid accurate stability indicating RP-HPLC method for the estimation of Modafinil in bulk and its formulation.

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III. MATERIALS AND METHODS:

Instrumentation:

HPLC analysis was performed on Shimadzu LC -20 AT equipped with SPD 20 detector with c18 column. Aux 200 shimadzu analytical balance was used for weighing standard and samples. All the glasswares were rinse thoroughly with double distilled water and dried in hot air oven.

Chemicals and reagents:

Modafinil pure drug was obtained from Rivan Pharmaceutical Pvt. Ltd. Ahmedabad, India. **Modalert** (100mg) tablets were purchased from the local market. Reagents in this assay were of HPLC grade.

CHROMATOGRAPHIC CONDITIONS:

The mobile phase was a mixture of Acetonitrile and water (80:20 % (v/v)). The flow rate of the mobile phase was adjusted to 1.0 ml /min. The detection was carried out at wavelength 223.6 nm. (Fig. 2) The injection volume of the standard and sample solution was set at 20 µl.

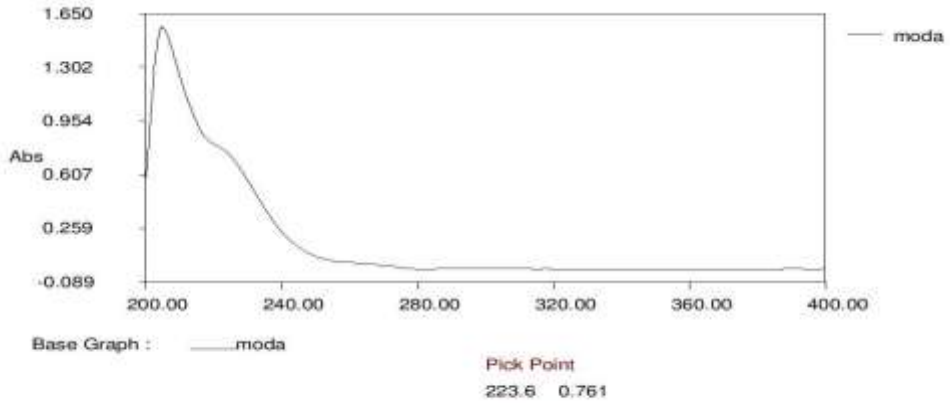

FIG. 2: UV SPECTRUM OF MODAFINIL

PREPARATION OF STANDARD STOCK SOLUTION:

15mg of Modafinil was weigh accurately and transferred to volumetric flask of 100 ml capacity. Small amount of mobile phase was added to dissolve the sample and further the volume made using mobile phase. This solution was filtered using whatman filter paper to remove the undissolved matter. From this solution 0.5, 0.75, 1, 1.25 and 1.5 ml solution was pipette out and placed into 10 ml of volumetric flask. The volume was made upto mark with mobile phase to give a solution containing 7.5, 11.25, 15, 18.75 and 22.5µg/ml respectively. The absorbance of resulting solution was determined at 226.4.6nm. A typical chromatogram of Modafinil standard was shown in (Fig. 3). The calibration curve is shown (Fig. 4). Linear regression data from calibration curve are shown in table no 1.

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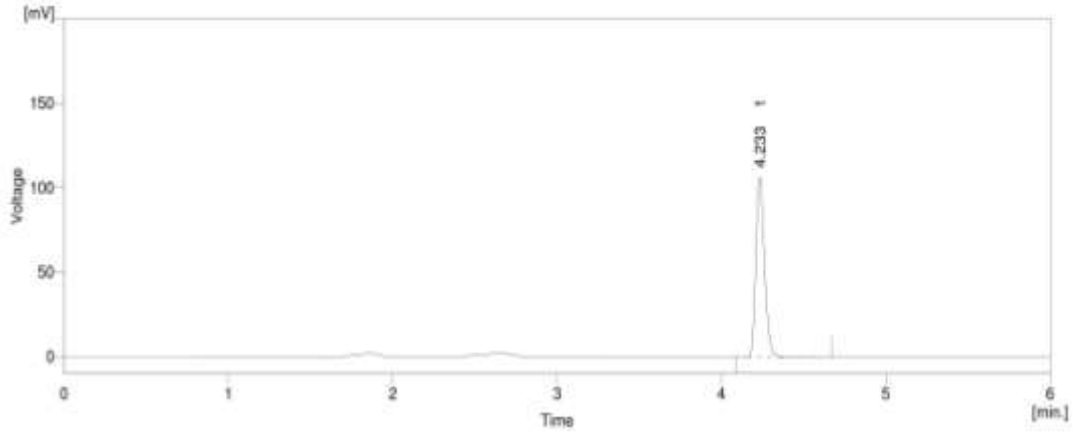

FIG. 3: TYPICAL CHROMATOGRAM OF MODAFINIL (STANDARD)

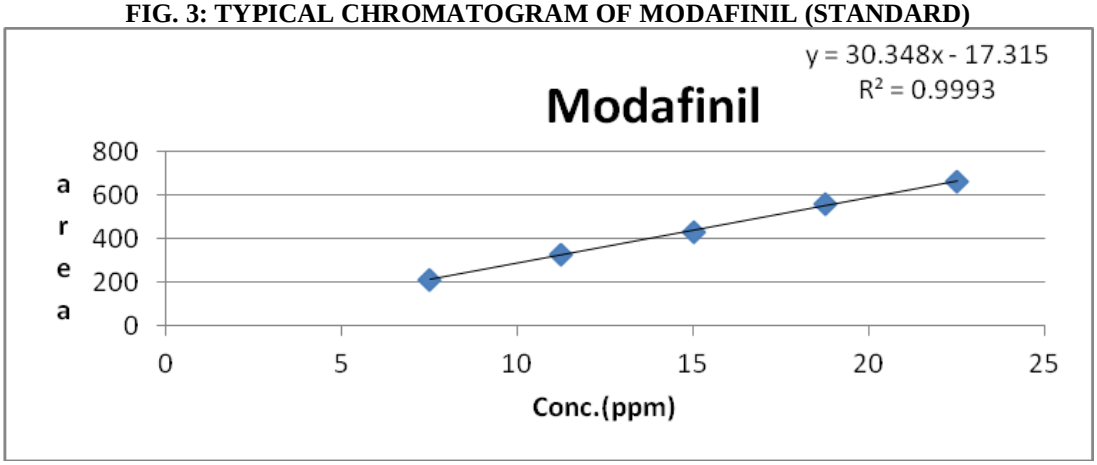

FIG. 4: CALIBRATION CURVE FOR LINEARITY

TABLE 1: LINEAR REGRESSION DATA FROM CALIBRATION CURVE

Parameter	Observation
λmax(nm)	223.6nm
Linearity range(µg/ml)	7.5-22.5 µg/ml
Slope(m)	30.348
Intercept(c)	17.315
Correlation coefficient	0.999

METHOD VALIDATION:

The method was validated for accuracy, precision and limit of detection, and limit and quantitation as per ICH guideline[15]. Summary of validation parameters are given in table no. 2

Accuracy:

The accuracy of the method was assessed by determination of the recovery of the method at 3 different concentrations (80%, 100% and 120% concentration) by addition of known amount of standard to the placebo. For each concentration three sets were prepared.

Precision:

Variation of results within the same day (intraday), variation of results between days (interday) was analyzed. Intraday precision was determined by analyzing Modafinil for three times in the same day at 223.6 nm. Inter day precision was determined by analyzing Modafinil daily for three days at 223.6 nm. Precision was calculated as repeatability and intra and inter day variation for the drug.

Limit of Detection &Quantitation:

LOD and LOQ were calculated using following equation as per ICH guidelines. LOD = 3.3 × σ/ S and LOQ = 10×σ /S, where σ is the standard

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deviation of response and S is the slope of the calibration curve.

TABLE 2: SUMMARY OF VALIDATION PARAMETERS

Parameter	Values
Accuracy (%)	99.51-101.0
Precision(CV)	
Intra-day (n=3)	0.376-0.768% RSD
Inter-day(n=3)	0.83-0.99% RSD
Limit of detection(µg/ml)*	0.168411478 (µg/ml)
Limit of quantitation(µg/ml)*	0.510337812(µg/ml)

*Based on S.D. of response and slope of Regression curve

ASSAY OF MODAFINIL TABLETS:

Twenty tablets were weighed and finely powdered. The powder equivalent to 100mg of Modafinil was accurately weighed and transferred to volumetric flask of 100 ml capacity. Some amount of mobile phase was transferred to volumetric flask and sonicated for 10 minutes. The flask was shaken and volume was made up to the mark with mobile phase. The above solution was filtered through whatman filter paper (0.45µ). 1 ml sample solution transferred to 10 ml volumetric flask and Volume was made up to the mark with mobile phase to give concentration 15 µg/ml. The resulting solution was analyzed by proposed method. A typical sample chromatogram is shown in (Fig.5). Assay result of market formulation is given in table no.3.

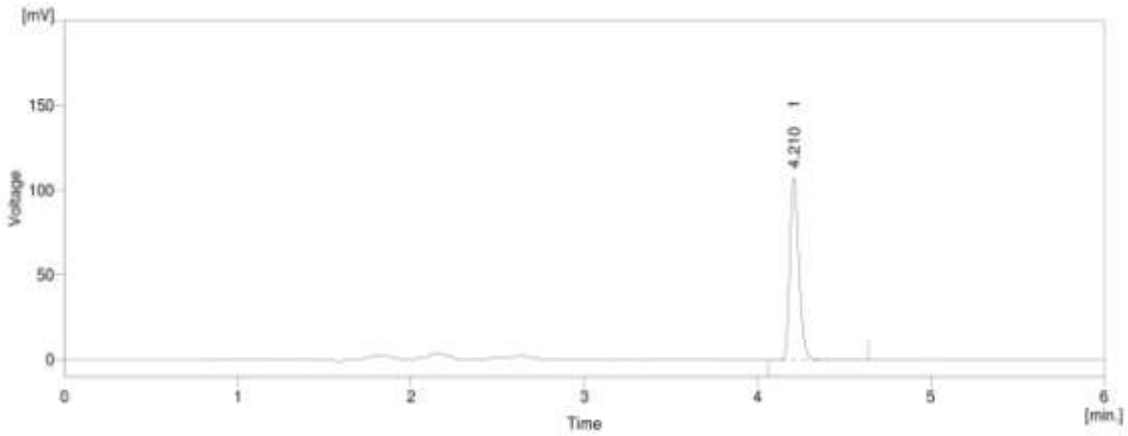

FIG. 5: TYPICAL CHROMATOGRAM OF MODAFINIL (SAMPLE)

TABLE 3: ASSAY RESULTS OF MARKETED FORMULATION (MODALERT)

Formulation	Actual concentration (µg/ml)	% Modafinil
Tablet	15	98.33

FORCED DEGRADATION STUDIES:

Force degradation studies were carried under the condition of acid, base, peroxide, thermal and photolytic .The sample was exposed to these conditions and the API peak was studied for the peak purity, which will indicate the method effectively separated from the degradation products. Summary of forced degradation studies is given in table no.4

Degradation in Acidic Condition:

1 ml from sample solution (150 µg/ml) was transferred to 10 ml volumetric flask and was treated with 2 ml 0.1 N HCl. Volume was made up with mobile phase. Sample was allowed to stand for 2 hours. Then the treated sample was injected into the HPLC system. Acid treated sample chromatogram is shown in (Fig.6).

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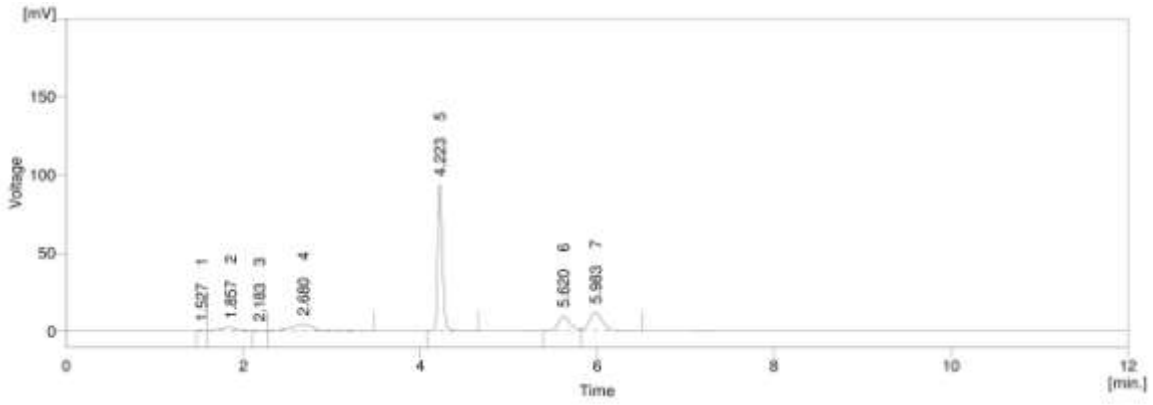

FIG. 6: ACID TREATED SAMPLE CHROMATOGRAM

Degradation in Basic Condition:

1 ml from sample solution (150 µg/ml) was transferred to 10 ml volumetric flask and was treated with 2 ml 0.1 N NaOH. Volume was made up with mobile phase. Sample was allowed to stand for 2 hours. Then the treated sample was injected into the HPLC system. Base treated sample chromatogram is shown in (Fig.7).

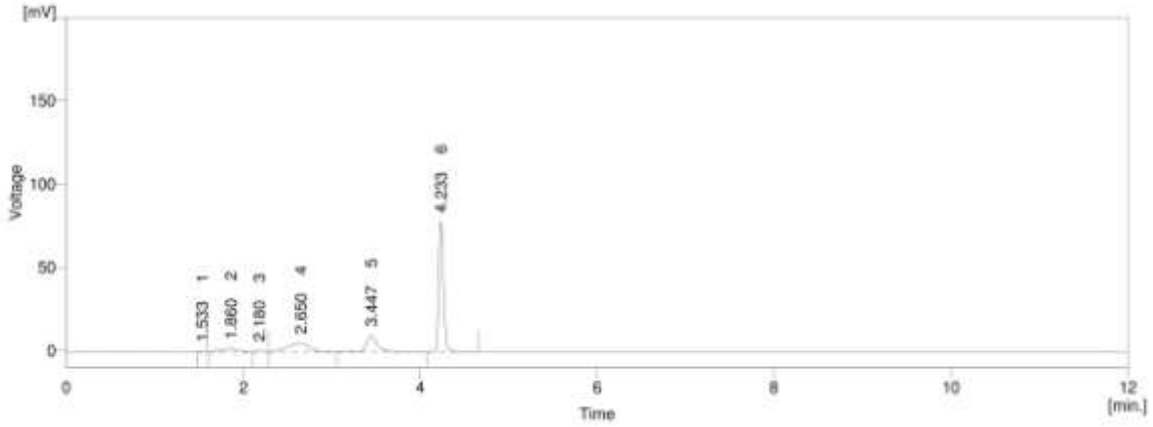

FIG. 7: BASE TREATED SAMPLE CHROMATOGRAM

Oxidation Degradation:

1 ml from sample solution (150 µg/ml) was transferred to 10 ml volumetric flask and was treated with 2 ml 3% H_2O_2. Volume was made up with mobile phase. Sample was allowed to stand for 2 hours. Then the treated sample was injected into the HPLC system. Peroxide treated sample chromatogram is shown in (Fig.8).

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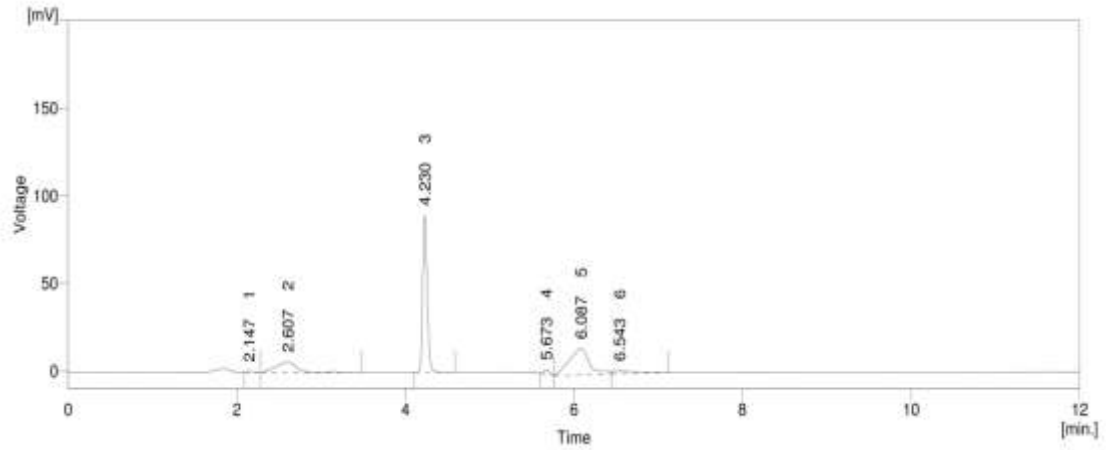

FIG. 8: PEROXIDE TREATED SAMPLE CHROMATOGRAM

Photolytic Degradation:

1 ml from sample solution (150 µg/ml) was transferred to 10 ml volumetric flask and volume was made up with mobile phase. Sample was allowed to stand for 3-4hours in UV chamber. Then the treated sample was injected into the HPLC system. Photolytic treated sample chromatogram is shown in (Fig.9).

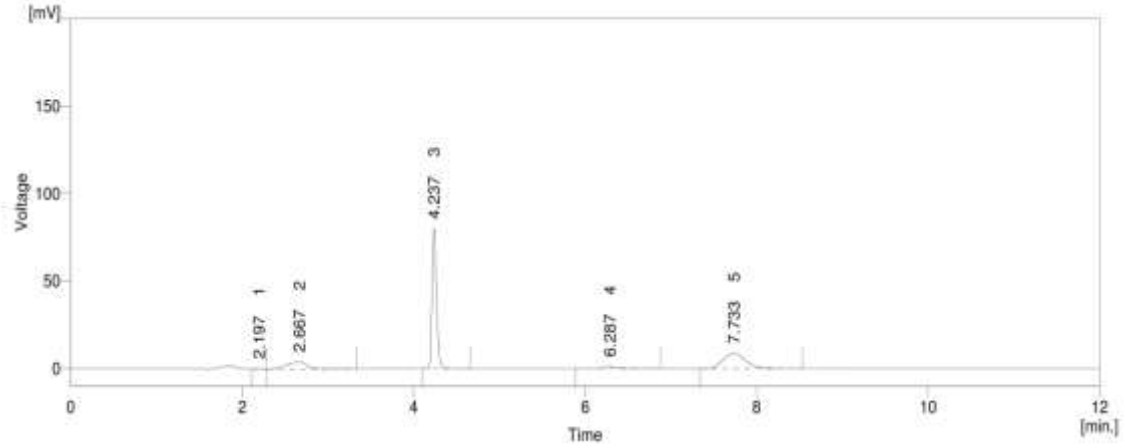

FIG. 9: PHOTOLYTIC TREATED SAMPLE CHROMATOGRAM

Thermal Degradation:

1 ml from sample solution (150 µg/ml) was transferred to 10 ml volumetric flask and volume was made up with mobile phase. Sample was allowed to stand for 3-4hours in oven at 80^0c . Then the treated sample was injected into the HPLC system. Thermal treated sample chromatogram is shown in (Fig.10).

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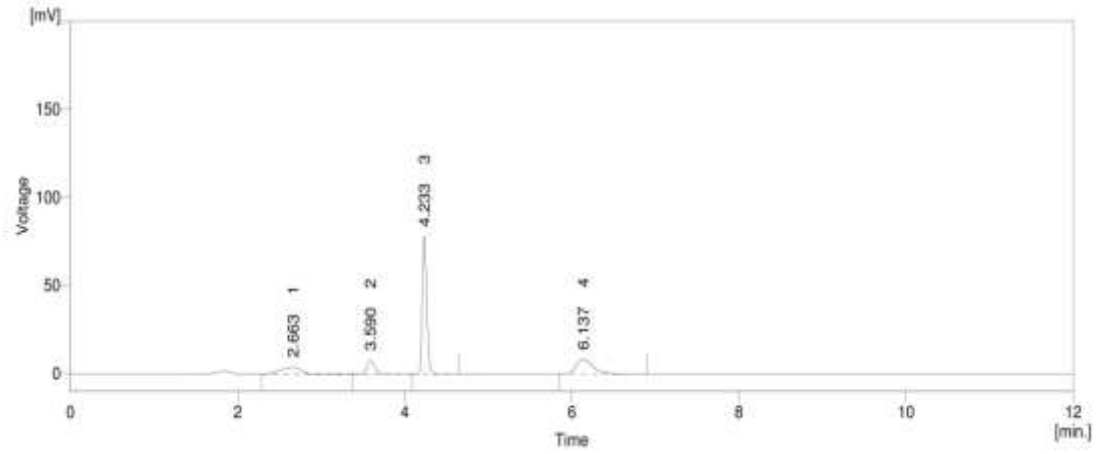

FIG. 10: THERMAL TREATED SAMPLE CHROMATOGRAM

TABLE 4: SUMMARY OF FORCED DEGREDATION OF MODAFINIL

Degradation condition	%Degradation	%Assay
Acid	14.26	85.73
Base	27.87	72.13
Oxidation	17.08	82.91
Photolytic	25.69	74.31
Thermal	27.46	72.53

IV. RESULT & DISCUSSION:

In this work stability indicating RP-HPLC method for assay in tablet formulation was developed and validated. The chromatographic conditions were optimized and separation was performed on a BDS Hypersil C18 (Thermo) column using a mobile phase consisting of Acetonitrile: water (80:20 v/v). Under the chromatographic conditions described, Modafinil was eluted about 4.210 min. Calibration curve was constructed using standard Modafinil solutions in the range of 7.5-22.5 µg/ml. The linearity of the calibration curve was validated by high value of correlation coefficient (r^2 = 0.999).

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