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Stability Indicating HPTLC Method for Estimation of Modafinil in the Bulk and Tablet Formulation

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Abstract: *A simple, selective, precise and stability-indicating high-performance thin layer chromatographic method for analysis of Modafinil, both as the bulk drug and in a tablet formulation has been developed and validated. Aluminum foil TLC plates precoated with silica gel 60F 254 were used as stationary phase ethyl acetate, acetone and methanol in the volume ratio of (7:2:1 v/v) respectively as mobile phase. A compact band (R_f 0.42±0.02) was obtained for modafinil. Densitometric analysis was performed in absorbance mode at 232 nm. Linear regression analysis revealed a good linear relationship (r^2=0.9995) between peak area and concentration in the range of 80-320 ng /spot. The method was validated for precision, recovery, and robustness. The limits of detection and quantitation were 15 and 50 ng/spot, respectively. Modafinil was subjected to acid and alkaline hydrolysis, oxidation, photochemical and thermal degradation and underwent degradation under all these conditions. Statistical analysis proved the method enables repeatable, selective, and accurate analysis of the drug. It can be used for identification and quantitative analysis of Modafinil in the bulk drug and in tablet formulations.*
Key Words: *Modafinil HPTLC Validation Stability-indicating Degradation*

I. Introduction

Modafinil is chemically 2-[(diphenylmethyl)sulfinyl]acetamide having molecular formula $C_{15}H_{15}NO_2S$ and molecular mass 273.35 gm/mol **(Figure 1)**. Modafinil is an analeptic drug manufactured by Cephalon, and is approved by the U.S. Food and Drug Administration (FDA) for the treatment of narcolepsy, shift work sleep disorder and excessive daytime sleepiness associated with obstructive sleep apnea. Narcolepsy is caused by dysfunction of a family of wakefulness-promoting and sleep-suppressing peptides, the orexins. Orexin neurons are activated by modafinil. Modafinil also affects tuberomammillary nucleus [1-2].

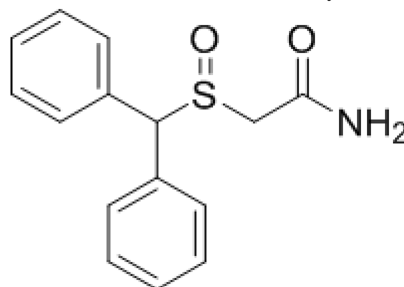

Figure-1 Structure of Modafinil

However extensive research into the interaction of modafinil with a large number of neurotransmitter systems has been done, an exact mechanism or set of mechanisms of action remains uncertain. It seems that modafinil, like other stimulants, increases the release of monoamines [3-4], specifically the catecholamine4, norepinephrine [5-6] and dopamine from the synaptic terminals. Though, modafinil also elevates hypothalamic histamine levels, leading some researchers to consider Modafinil a "wakefulness promoting agent" rather than a classic amphetamine-like stimulant [7-8]. Even though modafinil's histaminergic action, it still partially shares the actions of amphetamine-class stimulants due to its effects on norepinephrine and dopamine [9-11].

Modafinil has been prohibited by World Anti Doping Agency since 2004 so that there are so many bio analytical methods are reported to detect Modafinil in urine and blood plasma by GC-MS[12], LC-MS/MS[12-14],UPLC-MS[15-17] and HPLC[22]. Some methods are also reported for chiral resolution of modafinil enantiomers by HPLC [18-21]. Besides bioanalytical methods few methods are reported to determine Modafinil in tablet dosage form by HPLC [23-25]. Furthermore these methods are not impressionable to achieve the high throughput study which can be possible by optimizing the method in such a way which includes cost effectiveness with optimum selectivity. Hence, it can be maximum utilize for the analysis of formulation development and stability testing as well as at quality control laboratory for routine use.

In recent times, there is an increase tendency towards the development of stability-indicating assay, using the approach of stress testing as mentioned in the ICH guidelines (Q1A). It is also recommended to carry

out stress testing on the drug substance to establish its inherent stability characteristics and to hold up the suitability of the proposed analytical method. In this study our approach is to study degradation behavior of Modafinil and development of a validated stability indicating HPTLC assay method.

II. Experimental

2.1 Chemicals and reagents

The modafinil working standard was provided as a gift sample by Alembic pharmaceutical Ltd, Baroda. All chemicals and reagents used were of AR grade. The formulation, 100 mg and 200 mg is available in market by brand name PROVIGIL.

2.2 HPTLC Instrumentation and conditions

Chromatography was performed on Merck TLC plates pre-coated with silica gel 60 F254 (10 cm ×10 cm with 250 µm layer thicknesses) from E. Merck, Germany. Before use the plates were prewashed with methanol then dried in the current of dry air and activated at 110 °C for 5 min. The samples were applied onto the plates as a band with 4 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). The mobile phase consisted of ethyl acetate-acetone-methanol (7:2:1 v/v/v). Linear ascending development was carried out in a twin trough glass chamber (for 10 x 10 cm) previously saturated with mobile phase vapour for 30 min at room temperature and relative humidity 60 ± 5%. The development distance was approximately 80 mm. After development the plates were dried in current of air by use of an air dryer. Densitometric scanning, at 232 nm, was performed with a Camag TLC scanner III, operated by Wincats Software (V 1.4.2, Camag) in absorbance mode. The selection of wavelength was based on maximum absorbance for optimum sensitivity. The source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range 190–400 nm. The slit dimensions were 5 mm × 0.45 mm.

2.3 Preparation of standard solution

The standard modafinil 10 mg was weighed accurately and transferred to volumetric flask 100 ml. It was dissolved and sonicated for few minutes and diluted up to the mark with methanol to obtain final concentration of 100 µg/ml and the resulting solution was used as working standard stock solution.

2.4 Preparation of sample solution

To determine the content of modafinil in tablets formulations, 20 branded tablets were weighed; their mean weight was calculated and crushed to fine powder. Tablet powder equivalent to 1 tablet of modafinil was weighed and transferred to 100 ml volumetric flask then dissolved with methanol and further diluted with methanol. It was kept for ultrasonication for few min; the solution was then filtered through Whatmann filter paper No. 41 and further dilution was made with methanol to get the final sample solution of 100 µg/ml. The resultant solution was used as such for analysis.

2.5 Method validation

The method was validated in compliance with ICH guidelines. The following parameters were performed and evaluated for the validation of developed method.

2.5.1 Linearity

Appropriate volume of aliquots from standard modafinil stock solutions were prepared and applied on the TLC plate covering the range from 200-800 ng/spot with the help of micro liter syringe using an automatic sample applicator. The plates were developed, dried and scanned densitometrically at 232 nm. Each concentration was applied seven times to the plate and the plate was developed as described above. Peak areas were plotted against corresponding concentrations to furnish the calibration plot

.

Table 1
Regression analysis of calibration curve

Parameters	Result
Linearity Range(ng/Band)	200-800
correlation coefficient	0.998
Slope	82.46
Intercept	120.2

2.5.6 Accuracy

To check the recovery of the drug at different levels in formulations, samples were analysed at 3 different level 50, 100, and 150% of modafinil standard by proposed method and the recovered amount of drugs were calculated. The experiment was conducted in triplicate.

Table 2
Result of recovery studies

Sr. no.	Level of Recovery	Amount of drug taken(mg)	Amount of drug found(mg)	% Recovery[a)]	% RSD
1	50	50	50.11	100.22	1.859
2	100	100	100.31	100.31	1.797
3	150	150	149.97	99.38	1.491

[a)] Mean of three determinations

2.5.3 Precision

Precision of the developed method was measured by repeatability, intra-day and inter-day precision. In repeatability study analyses of six replicates of same concentration (500 ng) were done by developed method. For intra-day and inter-day precision, three sample sets of tablet sample and standard were analyzed in triplicate by developed method on the same day and on three different days, respectively.

2.5.3 Robustness

Small changes in the chromatographic conditions were introduced and the effects on the results were examined. Slight changes in the composition of mobile phase (±0.1ml) were made in the used optimized mobile phase. The time from spotting to chromatography and time from chromatography to scan was varied at ±5 minutes. The chromatographic development distance was varied in the range of ±5 mm. The effects of these changes on both R_F value and peak area were assessed by calculating relative standard deviation (%RSD).

Table 3
Result of robustness studies

Condition	RSD (%)
mobile phase composition	1.34
amount of mobile phase	0.95
chamber saturation time	1.21
time from application to chromatography	1.58
time from chromatography to scanning	0.66
Distance from application to solvent front	0.83

2.5.4 Limits of Detection and Quantification

To determine the limits of detection (LOD) and limit of quantification (LOQ), different dilution of standard solution of drug were applied along with methanol as the blank. The LOD and LOQ were determined on the basis of signal to noise ratio until the average responses of triplicate analysis were obtained approximately 3 and10 times the responses of the blank respectively.

2.5.5 Specificity

The specificity of the method was determined by analysis of drug standards and samples. The band for modafinil in the sample was identified by comparing the R_f value and spectrum of the band with those of the band from a standard. The peak purity of modafinil was assessed by comparing spectra acquired at three different positions on the peak, i.e. the peak start (S), peak apex (M), and peak end (E) positions of the peak.

2.6 Analysis of Marketed Formulation

Assay of marketed tablet was done through developed HPTLC method. The sample solution was made as depicted in section 2.4. Same volume (5 µL) of Standard solution and sample solution were applied on TLC. The experiment was carried out for six sample solutions. The chromatogram obtained is shown in **Figure 2**.

Table 4
Summary of validation parameters

Parameter	Result	%RSD
λ (nm)	232	
R_F	0.42	
Linear Range (ng/Band)	200-800	
Accuracy(at 100% level)	100.31	1.797
Precision		
Intraday precision	99.98	1.579
interday precision	99.57	0.983
Limit Of Detection (ng/Band)	15	
Limit Of Quantitation (ng/Band)	50	

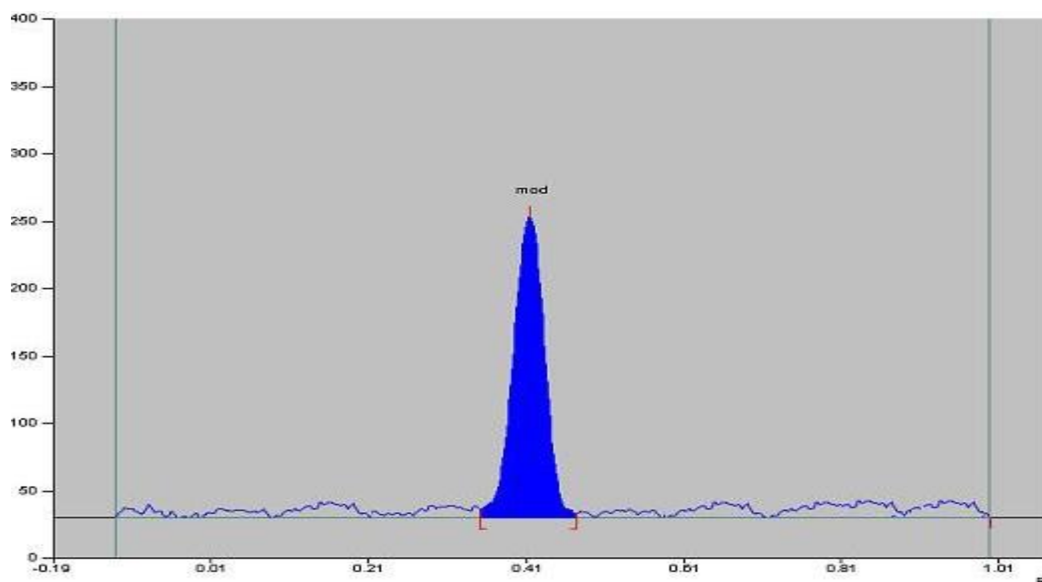

Figure-2 Chromatogram of Modafinil drug sample

Table 5
Analysis of tablet formulation

Method	Drug	Lable claim [mg/tablet]	Amount found [mg/tablet][a)]	Drug Assay [%][a)]	%RSD
TLC-Densitometry	Modafinil	100	99.84	99.84	0.786

[a)] Mean of six determinations

2.7 Forced Degradation studies

A stock solution containing 10 mg modafinil in 10 ml methanol was prepared. This solution was used for forced degradation to provide an indication of the stability indicating property and specificity of the method. In all degradation studies the average peak area of modafinil after application (500 ng/spot) of seven replicates was obtained after development and scanning of the plate as described above.

2.6.1 Acid and Base Degradation

Modafinil (10 mg) was separately dissolved in 10 ml of methanolic solution of 0.2 N HCl and 0.5 N NaOH. These solutions were refluxed at 80ºC for 2 h in the dark in order to exclude the possible degradative effect of light. 1 ml of treated solution were taken and neutralized and then diluted up to 10 ml with methanol. Then this diluted sample was used to apply on TLC plate in triplicate (500 ng/spot). The plates were chromatographed and analysed by proposed method.

2.6.2 Oxidative Degradation

Modafinil (10 mg) was dissolved in 10 ml of methanolic solution of hydrogen peroxide (30%, *v/v*) and the mixture was kept for 48 h at room temperature in the dark, to exclude the possible degradative effect of light. The solutions (1 ml) were diluted to 10 ml with methanol and further proceed as described for acid and base degradation.

2.6.3 Dry Heat Degradation

Dry heat studies were performed by keeping powdered drug in oven (60^0 C) for 6 hrs. Samples were withdrawn, dissolved in methanol, diluted suitably and appropriate volumes of resultant solution were applied on TLC plate and densitograms were developed.

2.6.4 Photo Degradation

Photo degradation studies were carried out by keeping modafinil drug sample in sunlight for 4 days. Sample solution was taken and diluted in methanol, and appropriate volume of the resultant solution were applied and chromatographed as described above.

III. Results And Discussion

3.1 Development of the optimum mobile phase

TLC procedure was optimized with a view to develop a stability-indicating assay method. The drug reference standards were spotted on the TLC plates and developed in different solvent systems. Different mobile phases were tried to resolve modafinil and degradation products. Best suited mobile phase was found to ethyl acetate, acetone and methanol in the volume ratio of (7:2:1 v/v) respectively. Developed mobile phase enabled good resolution, and a sharp and symmetrical peak of R_F 0.42 for modafinil (Fig. 2) form a compact and non-diffused band. It was observed that prewashing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 30 min (the optimum saturation time) ensured good reproducibility and peak shape.

3.2 Validation of the developed stability-indicating method

The response for the drug was found to be linear in the concentration range 200-800 ng/band for modafinil. The results of regression analysis are depicted in **Table 1**. The accuracy of the method was determined at 50, 100 and 150% level. Results of accuracy study are expressed in terms of % recovery **(Table 2**). According to results of robustness studies shown in **Table 3.** %RSD value does not exceed more than 2. So the developed method is robust. The %RSD value for precision study was found to be not more than 2 % for modafinil, thus confirming precision of the method. The Limit of Detection and Limit of Quantitation were found to be 15 ng/band and 50 ng/band respectively for analyte. The results are shown in **Table 4**. The specificity of the method was ascertained by peak purity profiling studies. In analysis of marketed formulation the mean assay result was found to be 99.84% **(Table 5).** From the result obtained we can conclude that there is no interference of the excipients present in the formulation. The low %RSD suggests the suitability of developed method for routine analysis.

3.3 Degradation observed

After applying different stress conditions upon modafinil, following results were observed by analyzing samples using the developed method. About 21.50% drug was degraded **(Figure 3)** under oxidative condition and major degradation peaks were observed at 0.18, 0.20 and 0.23 R_F values. In acid hydrolysis modafinil was degraded **(Figure 4)** about 17.35% and degraded product was observed at 0.14 and 0.19 R_F value, however in alkali hydrolysis some harsh condition was required compared to that in acid degradation. Initially, 0.1 N NaOH was used and refluxed at 80^0C for 2 hours, yet no degradation was observed; then strength of alkali was increased to 0.5 N and refluxed at 80^0C for 2 hours resulting in 8.20% degradation **(Figure 5)** and degradation peaks were observed at 0.23, 0.27 and 0.32 R_F values. Under dry heat (Oven, 80 0C, 6 hr), drug was found to be degraded up to 9.25% with decrease in area only, whereas under Photolytic studies, no additional peaks were observed and drug peak area remained constant. This indicates stability of modafinil in UV light. In all forced degradation condition the drug peak was well resolved from degradation products.

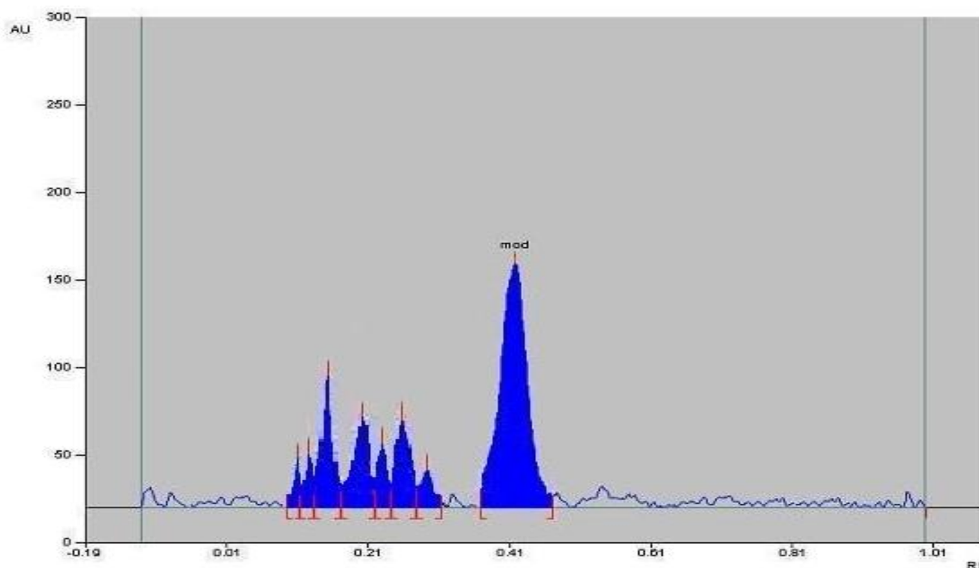

Figure-3 Chromatogram of Modafinil after Oxidative degradation

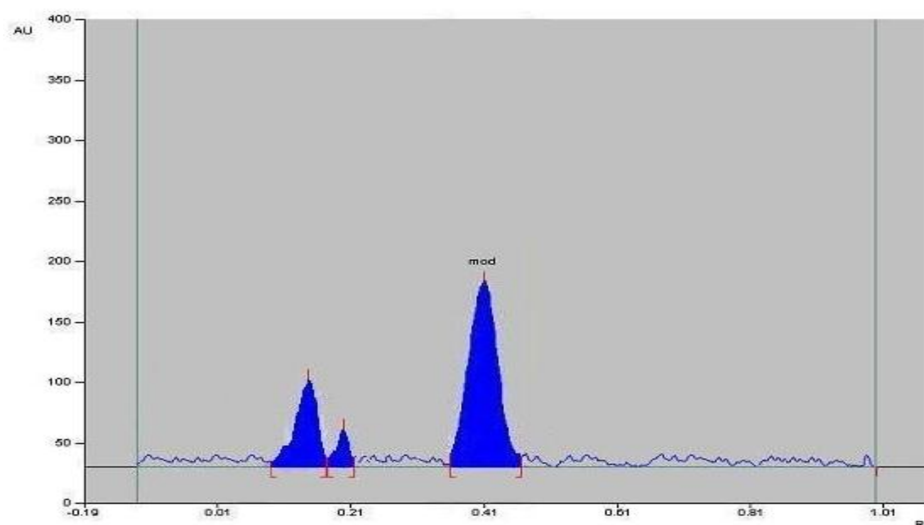

Figure-4 Chromatogram of Modafinil after Acid degradation

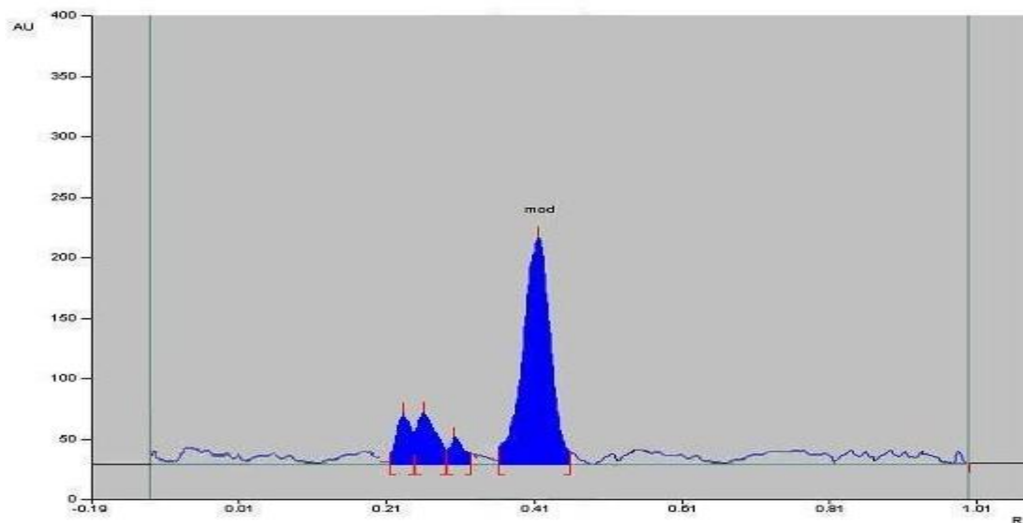

Figure-5 Chromatogram of Modafinil after Alkali degradation

IV. Conclusion

Based on the above study and result, it can be concluded that the developed HPTLC method is simple, precise, accurate, rapid, selective and sensitive for the determination of modafinil in tablet formulation as well as in bulk drugs. The products formed after forced decomposition studies were resolved from the bulk drug response. From the peak purity profile studies, it was confirmed that the peak of the degradation product was not interfering with the peak of drugs. It confirms that peak for degradation product of drug can be resolved from the drug peak by this method; hence it can be used as stability indicating method. The method can minimize the time and cost of analysis. It has also utilized the merit of applying several samples on TLC plates which can be advantageous for regulatory quality control laboratories. The method does not require expensive chemical and solvents, thus it can stand for a good alternative for already existing hplc methods. It is proposed for routine analysis of these drugs in presence of degradation products in stability study.

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