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NEW UV-SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF MODAFINIL IN PURE AND FORMULATION

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ABSTRACT

The present study deals with development and validation of a simple, rapid, accurate, economical and reproducible UV-Spectrophotometric method was developed for estimation of Modafinil in pure form and tablet dosage form. Modafinil was estimated at 223nm. Linearity range was found to be 2-18 µg/ml. The correlation coefficient was found to be 0.999. The limit of detection and limit of quantification were found to be 0.010µg/ml and 0.032µg /ml respectively. The degradation behavior of Modafinil was carried out as per the standard procedures and guidelines. The assay value of Modafinil in bulk and formulation was calculated at different time intervals for intraday and interday experiments. The proposed method was successfully applied for the determination of Modafinil in bulk and Pharmaceutical formulations (Tablets). The results were demonstrated, that the procedure is accurate, precise and reproducible (relative standard deviation < 2%), Modafinil was found to degrade extensively under alkali conditions. Modafinil has to be stored under such condition where the possibility of alkali hydrolysis does not arise.

Keywords: Modafinil, UV –Spectroscopy, Validation, ICH guidelines, Assay

INTRODUCTION

Modafinil belongs to the class Narcoleptics. Chemical name is 2-[(Diphenyl methyl)-sulfinyl] acetamide. Having molecular formula $C_{15}H1_5NO_2S$ and molecular mass 273.35 gm/mol **[1]**. **(Figure 1)**. This is a1-adrenergic agonist. Clinical evaluationin

hypersomnia and narcolepsy **[2]**. It is not official in any of the pharmacopoeia. Modafinil is an analeptic drug manufactured by Cephalon, and is approved by the U.S **[3]**. Food and Drug Administration (FDA) in December 1998 for the treatment of narcolepsy, shift work sleep disorder and excessive daytime sleepiness associated with obstructive sleep apnea and in adult it is used in attention deficient /hyperactivity disorder (ADHD) 15. 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 tuber mammillary nucleus. Modafinil is BCS class II drug; hence improvement of dissolution will lead to enhancement of bioavailability. It is rapidly absorbed after oral administration with peak plasma concentrations occurring at 2-4 hours.

Modafinil in daily doses of 100–400 mg is clinically used as the first-line treatment for pathological sleepiness in patients with narcolepsy. Controlled studies also demonstrated its efficacy as an adjunct therapy for subjective sleepiness and fatigue in various diseases such as obstructive sleep apnea, shift work sleep disorder, Parkinson's disease, major depressive disorder, and multiple sclerosis.

MATERIALS AND METHODS

Instruments and methods: A gift sample of MODAFINIL with purity of 98.88%w/w was obtained. LAB INDIA (T60) double beam UV/Visible spectrophotometer and ELITE analytical balance were the instruments used. Chemicals and reagents used are of analytical grade. MODAFINIL of 200mg with a brand name PROVIGIL was purchased from the local market.

Preparation of standard stock solution (1000µg/ml): A standard drug solution of MODAFINIL was prepared by adding 100mg of the drug into a 100 mL volumetric flask and made up to the mark with methanol to get a concentration of 1000µg/ml'.

Preparation of working standard solution (100µg/ml): From the above standard stock solution, 10 ml of the sample was transferred to a 100mL volumetric flask and made up to mark with methanol to get a concentration of 100µg/mL. It was then scanned by a UV Spectrophotometer in the range of 200-400nm using methanol as a blank. The absorbance was found to be maximum at 223nm.

Construction of calibration curve: Aliquots ranging from 2-18µg/ml solutions were prepared by using methanol as solvent .The samples were then analyzed at a λmax of 223nm to get respective absorbance. The

values are then plotted to get a calibration curve.

Preparation of the assay solution: The proposed method was applied to analyze the commercially available Modafinil tablets PROVIGIL® (200mg). 10 tablets are weighed and powdered, the amount of powder is equivalent to 100mg of Modafinil was weighed accurately and transferred in to 100ml volumetric flask containing methanol which was further sonicated for 15 mins with vigorous shaking and the volume was bought up to 100ml with methanol. The solution was subjected to filtration through What man filter paper #44. The filtrate was diluted suitably with methanol to get a final solution of 200µg/ml concentration. This was subsequently analyzed using a Double beam UV-VIS spectrophotometer and taking methanol as blank in the UV range 200-400nm. The spectrum was recorded as 223nm. The concentrations of the drug were calculated from the linear regression equation.

Method validation: Validation is a process of establishing documented evidence, which provides a high degree of assurance that is a specific activity will consistently produce the desired result, or a product meeting its predetermined specifications and quality characteristics. The method was validated according to ICH guidelines for various parameters like Linearity, Precision, Accuracy, Robustness, Ruggedness, LOD, LOQ, Range and Sensitivity **[5-10]**.

Linearity: The ability of an analytical procedure is to produce test results that are directly proportional to the concentration of an analyte. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration. For estimation of linearity at least 5 concentrations are required.

Accuracy: Accuracy means the expression of closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference true value and the true value and the value found. Accuracy is assessed by using 9 determinations covering a minimum of 3 concentrations.

Precision: The closeness of agreement between the obtained values by analyzing the same sample multiple times under prescribed conditions. There are 3 levels of repeatability, intermediate precision and reproducibility. Repeatability is a measure of the exactness under the same working conditions more than a short in term of time, that is, under ordinary working states of the scientific technique with the same hardware it is also known as intraday precision.

Reproducibility also is known as inter-day precision. Precision is expressed in terms of % Relative Standard Deviation.

% RSD= (Standard deviation)/Meanx100

Standard deviation (SD)

$SD=\sqrt{[\sum(x-\bar{x})^2/(n-1)]}$

Where n=no of entries

Ruggedness: The ruggedness of an analytical procedure is the degree of reproducibility of results by analyzing the same sample under a variety of conditions like laboratories, instruments, analysis, reagents etc.

Robustness: Robustness of an analytical procedure is the capacity to remain unchanged by small but deliberate changes in parameters.

Sensitivity: Limit of detection (LOD) and Limit of quantification (LOQ) of the drug was calculated by using equations according to ICH guidelines.

Limit of Detection: It is the lowest amount of the drug in a sample that can be detected, but not necessarily quantitated.

$$LOD= (3.3x\sigma)/S$$

Where S=standard deviation

Limit of Quantification: It is an amount of analyte that can be quantified with a specified limit of accuracy and precision,

$$LOQ= (10x\sigma)/S$$

Linearity: Different aliquots of Modafinil were prepared from the working standard solution (100µg/mL) in the range of 2-18µg/ml. The solutions were scanned on a Double beam UV-VIS spectrophotometer in the range of 200-400nm using methanol as the blank. The spectrum was recorded at 223 nm. The calibration plot was constructed as concentration Vs absorbance and can be shown in **(Table 1)**.

Precision: The precision of the method was demonstrated by intra-day and inter-day variation studies. In the inter-day variation study, the solutions of the same concentration 10µg/mL were prepared and analyzed six times, for three consecutive days and the absorbance was recorded **(Table 4)**. In the intra-day variation study, six different solutions of the same concentration 10µg/mL were prepared and analyzed thrice a day (Morning, Afternoon and Evening) and the %RSD was calculated and reported **(Table 3)**.

Accuracy: The accuracy of the method was determined by preparing solutions of different concentrations i.e. 80, 100 and 120%, in which the amount of marketed formulation Modafinil was kept constant(10µg/ml)and the amount drug was varied, that is 80µg,100µg,120µg for 80,100, and 120% respectively. The solutions were prepared in triplicate and the accuracy was

indicated by % recovery was calculated and reported in the **(Table 5)**.

Robustness: The Robustness of the method was carried out by analyzing the sample using three different wavelengths (±1 of lambda max) that were and respective absorbance were recorded .The results are indicated in **(Table 6)**.

Ruggedness: The ruggedness of the method was carried out by analyzing the sample using two different analysts and two different cuvettes and respective absorbance were recorded. The results are indicated in **(Table 7 and 8)**.

Sensitivity: Limit of detection (LOD) and limit of quantification (LOQ) of the drug was calculated by using equations according to ICH guidelines. They are calculated by checking absorbance using solvent and calculate using formulae and the results are shown in **(Table 9)**.

RESULTS AND DISCUSSION

The method was developed and validated as per ICH guidelines. The method was validated in terms of linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. Beers law obeyed over the concentration range of 2-18µg/mL, using regression analysis the linear equation y=0.054x-0.042 with a correlation coefficient if R^2 0.999. The precision results show %RSD less than 2 at each level which indicates clearly that the method is precise enough for the analysis of Modafinil. The accuracy of the method was checked by recovery studies. The high recovery with values indicates the accuracy of the developed method. The robustness and ruggedness studies reveal that the method is more sensitive. There was no interference observed from the excipients present in the formulation, indicated that the method is specific. Determination of Modafinil in tablets formulation showed the content of Modafinil was very close to the label amount. The percentage RSD values in all the parameters were within the acceptable limit (<2%) all the characteristics of the method are represented in the **(Table 10)**.

Table 1: Linearity of working standard solutions

Concentration (µg/mL)	Absorbance
2	0.0566
4	0.1805
6	0.2723
8	0.4039
10	0.5135
12	0.6240
14	0.7157
16	0.8272
18	0.9322

Table 2: Repeatability data

Concentration (µg/mL)	Absorbance	Statistics analysis
10	0.6178	Mean:0.626
10	0.6357	%RSD:1.25
10	0.6281	
10	0.6369	
10	0.6222	
10	0.6206	

Table 3: Intra-day study

Concentration (µg/mL)	% RSD			Average % RSD
	1	2	3	
10	1.72%	1.12%	1.66%	1.5%

Table 4: Inter-day study

Concentration (µg/mL)	%RSD			Average % RSD
	Day 1	Day 2	Day 3	
10	0.94%	1.40%	1.47%	1.27%

Table 5: Accuracy data

Levels of addition (%)	Amount added (µg/ml)	Amount found(µg/ml)	%Recovery	%Mean recovery
80	80	79.17	98.96	99.02%
100	100	99.22	99.22	
120	120	118.16	98.89	

Table 6: Robustness data

Concentration (µg/mL)	Absorbance		
	222 nm	223 nm	224 nm
10	0.6572	0.6835	0.5621
10	0.6360	0.6588	0.5628
10	0.6534	0.6791	0.5657
10	0.6298	0.6667	0.5719
10	0.6390	0.6670	0.5609
10	0.6446	0.6809	0.5572

Table 7: Ruggedness data

Concentration (µg/ML)	Absorbance	
	Analyst 1	Analyst 2
10	0.6065	0.6025
10	0.6085	0.6017
10	0.6052	0.6022
10	0.6036	0.6027
10	0.6043	0.6024
10	0.6037	0.6026

Table 8: Ruggedness data

Concentration (µg/ML)	Absorbance	
	Cuvette 1	Cuvette 2
10	0.5834	0.6290
10	0.5836	0.6178
10	0.5855	0.6035
10	0.5718	0.6248
10	0.5707	0.6042
10	0.5709	0.6043

Table 9: LOD & LOQ

Limit of Detection	Limit of Qualification
0.010 µg/ml	0.032 µg/ml

Table 10: Results of validation parameters

Parameters	Results
Absorption maxima (nm)	223nm
Linearity range(µg/mL)	2-18
Regression equation	Y=0.054x-0.042
Correlation coefficient(R^2)	0.999
Molar extinction coefficient	83513
LOD(µg/ml)	0.010
LOQ(µg/ml)	0.032
Accuracy (%Recovery ± SD)	99.22
Precision	
Intraday precision(%RSD)	1.5
Inter-day precision(%RSD)	1.27
Sand ell's sensitivity (µg/cm²/0.001 absorbance units)	0.018

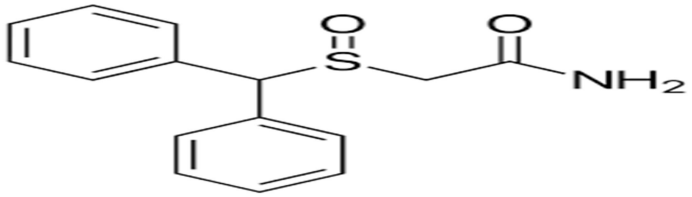

Figure 1: Structure of Modafinil

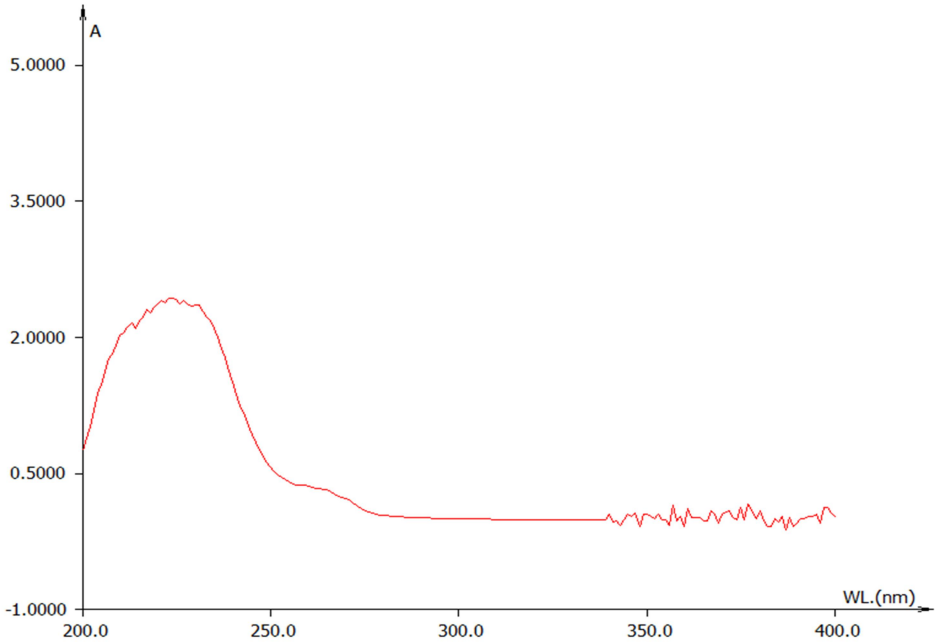

Figure 2: Determination of λmax of Modafinil

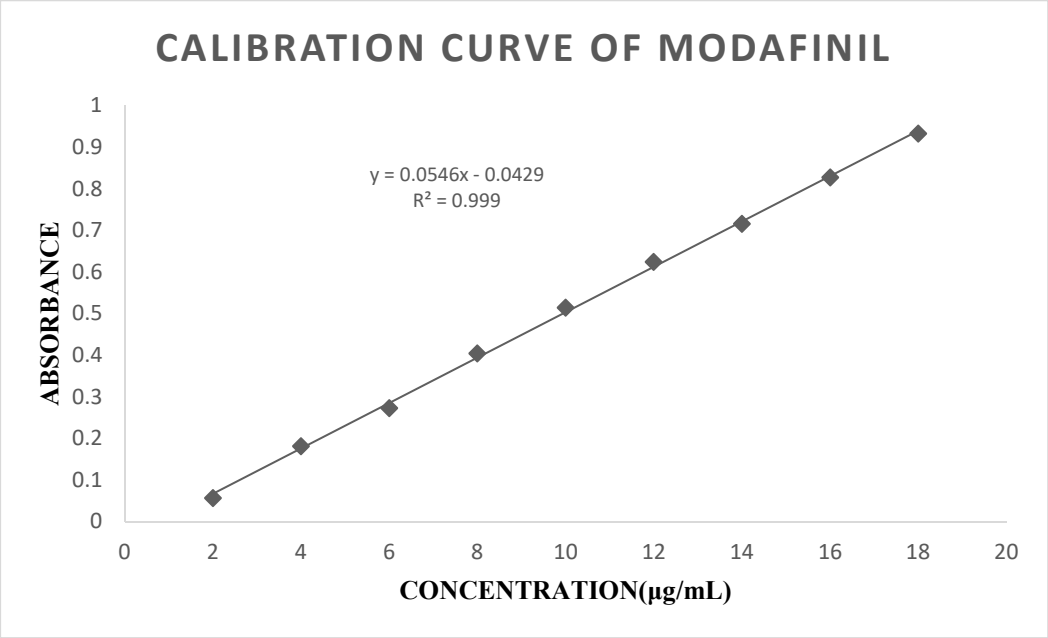

Figure 3: Standard calibration curve of Modafinil

CONCLUSION

A UV spectrophotometric method has been validated for the estimation of Modafinil in bulk as well as the pharmaceutical dosage form. The developed method was found to be simple, accurate, precise, specific, reproducible and linear over the concentration range studied. The proposed method can be used for the routine analysis of Modafinilin bulk as well as pharmaceutical formulations.

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