Analytical Method Development and Validation of Modafinil in Pure and Tablet Dosage Form by UV Spectroscopy

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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 260nm. Linearity range was found to be 2-10 µg/ml. The correlation coefficient was found to be 0.99987. The molar absorptivity was found to be 10409 L mol/cm. The proposed method Sandell's sensitivity was found to be 0.02778 µg cm−2/0.001AU. The limit of detection and limit of quantification were found to be 4.67981and14.18125 µg /ml respectively. The degradation behavior of Modafinil was carried out as per the standard procedures and guidelines. Forced acid hydrolytic degradation, alkali degradation and oxidative degradation of Modafinil was performed in bulk and solid oral formulation using 1N Hydrochloric acid and 0.1M Sodium hydroxide at room temperature in different time intervals. The resulting solutions were analyzed for content by UV spectrophotometry at the maximum absorption of 260 nm. 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.

INTRODUCTION:

Modafinil [1, 4] belongs to the class Narcoeleptics. Chemical name is 2-[(Diphenylmethyl)-sulfinyl] acetamide. Having molecular formula C15H15NO2S and molecular mass 273.35 gm/mol (Figure 1). This is a1-adrenergic agonist. Clinical evaluationin hypersomnia and narcolepsy. It is not official in any of the pharmacopoeia. Modafinil is an analeptic drug manufactured by Cephalon, and is approved by the U.S. Food and Drug Administration (FDA) in December 1998[2] 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 tuberomammillary nucleus [1-2]. 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.

Figure-1 Structure of Modafinil

Modafinil in daily doses of 100–400 mg[3] is clinically used as s in patients with narcolepsy. Controlled studies also demonstrated its effica and fatigue in various diseases such as obstructive sleep apnea, shift v depressive disorder, and multiple sclerosis.

MATERIALS AND METHODS:

Preparation of standard stock solution:

10 mg of Modafinil raw material was weighed and transferred in to 10 mL volumetric flask, then dissolved in methanol and made up to the volume with the same solvent. This solution contains 1000µg/ ml concentration. Take 1 mL of this dilute solution in 10 mL volumetric flask and dilute up to mark with water to get concentration of 100µg/ ml. Again take 1 mL of this dilute solution in 10 mL volumetric flask and dilute up to mark with water to obtained final concentration of 10 µg/ ml and the solution was scanned between 200 and 400 nm using the same solvent as blank. The spectrum was observed in that range and the λ max was found to be 260 nm and was selected as analytical wavelength.

Preparation of linearity studies:

The standard stock solution of Modafinil was transferred into series of 10 ml volumetric flasks and made up to the volume with water [5] . The absorbance of 2,4,6,8,10 µg/ ml solutions were measured at 260 nm. The calibration curve was plotting between concentration vs. absorbance. Modafinil was linear within the concentration range of 2,4 ,6,8,10 µg/ ml at 260 nm.

Validation of developed method:

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Linearity:

A calibration curve was plotted between concentration and absorbance. Modafinil was linear in the concentration range of 2-10 µg/ ml at 260 nm. The linearity was repeated for five times and LOD and LOQ values for calculated**.** [6] . The linearity is shown in **fig.1**

Quantification of formulation:

Twenty tablets of formulation were weighed accurately. The average weight of tablet calculated and the tablets are made into powdered form. The powdered equivalent to 10mg of Modafinil was weighed and transferred into 100 ml volumetric flask. Added a minimum quantity of methanol to dissolve the substance and made up to the volume with the same (100µg/ml). The solution was filtered with Whatmann filter paper. From the clear solution, further dilutions were made by 1ml to 10 ml volumetric flask with water to get 10µg/ml solution theoretically. The absorbance of six replicates was measured and the amount was calculated by using regression equation. This procedure is repeated for six times. [7,8]

Precision:

The repeatability of the developed method was confirmed by the precision analysis. The intermediate precision of the method was confirmed by intraday and interday analysis i.e. the analysis of formulation was repeated three time in the same day and on three successive days. For this process Modafinil 10mg was used. The amount of drugs present was determined and the percentage RSD also calculated.[9]

Accuracy :

Accuracy of the method was confirmed by the recovery studies. To the preanalysed formulation a known quantity of raw material of Modafinil was added in 6 concentration and recovery process are followed as per the quantification process. The amount of recovery was calculated. This procedure is repeated for 6 times and the %RSD was calculated. The results are shown below. [10]

Study of Acid Degradation Modafinil By UV Spectroscopy Method:

Standard Preparation:

Modafinil was transferred to volumetric flask and dissolved methanol to achieve a concentration of 1mg/mL. After 30mins, an aliquot solution was diluted with 1N hydrochloric acid to get a final concentration of 100mcg/mL. [11] .The solution was scanned in the UV region and the maximum absorbance was recorded at 260 nm.

Sample Preparation:

Modafinil tablets were powdered and weighed and then transferred into volumetric flask and dissolved methanol to achieve a concentration of 1mg/mL. After 30mins, an aliquot solution was diluted with 1N hydrochloric acid to get a final concentration of 100mcg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 260 nm.

Blank Preparation:

A blank preparation of Hydrochloric acid (1N) solution was prepared in a similar manner.. After the stipulated time, the absorption of the resulting solution showed maxima 260nm against reagent blank treated in the same way. The determinations were made and the assay value was estimated the obtained values are concurrent.

For Inter day study Standard preparation The standard preparation was prepared in a similar manner which was mentioned in an intraday preparation Standard preparation Same method was followed, but the final solution was scanned and absorption was recorded at the following time intervals 1st, 3rd, and 5th day

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Study of alkali degradation modafinil by UV spectrophotom

Standard Preparation:

Modafinil 10mg was transferred to volumetric flask and dissolved methanol to achieve a concentration of 1mg/mL. After 30mins, an aliquot solution was diluted with distilled with 0.1M Sodium hydroxide to get a final concentration of 100mcg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 260nm.

Sample Preparation:

Modafinil granules were weighed and transferred to volumetric flask and dissolved methanol to achieve a concentration of 1mg/mL. The solution was diluted with 0.1M Sodium hydroxide to get a final concentration of 100mcg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 260nm.

Blank Preparation:

A blank solution of Sodium hydroxide (0.1M) solution was prepared in a similar manner. After the stipulated time, the absorption of the resulting solution showed maxima 260nm against reagent blank treated in the same way. The determinations were made and the assay value was estimated the obtained values were concurrent.

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Sample Preparation:
Same method was followed, but the final solution was scanned and absorption was recorded at the following time intervals 1st, 3rd, and 5 th day. Blank preparation is similar to intraday preparation. After the stipulated time, the absorption of the resulting solution showed maxima 260nm against reagent blank treated in the same way. The determinations were made and the assay value was estimated and concurrent values were obtained.

Study of oxidative degradation modafinil by UV spectrophotometry method:
Standard Preparation:
Modafinil 10mg was transferred to volumetric flask and dissolved methanol to achieve a concentration of 1mg/mL. After 30mins, an aliquot solution was diluted with distilled with 10% hydrogen peroxide to get a final concentration of 100mcg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 260nm.

Sample Preparation:
Modafinil granules were weighed and transferred to volumetric flask and dissolved methanol to achieve a concentration of 1mg/mL. After 30mins, an aliquot solution was diluted with 10% hydrogen peroxide to get a final concentration of 100mcg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 260nm.

Blank Preparation:
A blank solution of hydrogen peroxide(10%) solution was prepared in a similar manner. The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 260nm against reagent blank treated in the same way. The determinations were made and the assay value was estimated the obtained values were concurrent.

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Sample Preparation:
Same method was followed, but the final solution was scanned and absorption was recorded at the following time intervals 1st, 3rd, and 5 th day. Blank preparation is similar to intraday preparation. The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 260nm against reagent blank treated in the same way. The determinations were made and the assay value was estimated and concurrent values were obtained.

RESULT AND DISCUSSION:

The new, simple and cost effective Spectrophotometric method was developed for the estimation of Modafinil in bulk and pharmaceutical formulations and study of acid, alkali and oxidative degradation.
Modafinil was estimated at 260 nm by using water. The drug was soluble in aqueous solvent but it is not produce stablemax and absorbance. So we tried with water Linearity range was found to be 2−10 µg/ml.

The correlation coefficient was found to be 0.99987 and the molar absorptivity was found to be 10409 L mol^{-1} cm^{-1} in water. The proposed method Sandell's sensitivity was found to be about 0.02778 µg cm^{-2}/0.001AU.

The limit of detection and the limit of quantification were determined by the linearity studies, the process was repeated for six times and the limit of detection (4.679) and the limit of quantification (14.181) were calculated.

Fig.1 Calibration curve of Modafinil
And the concentration with the absorbance value are given in table.1

S.No.	Concentration (µg /ml)	Absorbance
1.	2	0.092
2.	4	0.164
3.	6	0.240
4.	8	0.308
5.	10	0.380

Table no:2. OPTICAL CHARACTERISTICS OF MODAFINIL

PARAMETERS	VALUES*
max (nm)	260
Beer's law limit (µg/ ml)	2-10
Sandell's sensitivity (g/cm^2/0.001 A.U)	0.02778
Molar absorptivity (L mol^{-1} cm^{-1})	10409
Correlation coefficient (r)	0.99987
Regression equation (y=mx+c)	Y = 0.036x + 0.0208
Slope(m)	0.036
Intercept(c)	0.0208
LOD (µg/ ml)	4.679
LOQ (µg/ ml)	14.181
Standard error	0.749

From the linearity curve, the mean concentration of 6µg/ml was selected and quantification in tablets was performed. The 100 mg tablets were selected for analysis. The amount present was determined by average of six replicate analysis and the amount present were found to be 5.83, 6.06, 5.95, 6.14, 5.61, 6.08 mg respectively. The results were shown in Table no.3.

The accuracy of the developed method was carried out by standard addition method. The known amount of pure drug was added to the previously analyzed solution containing tablets and the mixture was analyzed by the proposed method and the recoveries were calculated. The percentage recovery of formulation was found to be 97.16, 100, 101, 99.16, 100.5, and 97.66. The results were shown in Table no. 4.

Precision of the method has done by making repeated analysis of the same sample and it was carried out three times in a day for 3 days. The percentage standard deviation for inter day and intraday analysis of Modafinil was found to be 1.69and 1.97 respectively and shown in table .5 and 6 .

Table no.3: Quantification of formulation.

Drug	Sample no.	Amount added (µg/ ml)	Amount present (µg/ ml)	Percentage obtained	Average%	S.D	%RSD	S.E
	1.	6	5.83	97.16				
	2.	6	6.06	101.01				
Modfinil	3.	6	5.95	99.16	99.08	1.83	1.83	0.749
	4.	6	5.86	97.6				
	5.	6	5.64	94.07				

Table no.4: Recovery analysis of Modafinil

Drug	Sample no.	Amount present µg/ ml	Amount added µg/ ml	Amount found µg/ ml	Amount recovered µg/ ml	% recovered	S.D	%RSD	S.E
	1.	3	3	5.83	2.83	97.16			
	2.	3	3	6.00	3.00	100			
Modafinil	3.	3	3	6.06	3.06	101	1.55	1.57	0.634
	4.	3	3	5.95	2.95	99.16			
	5.	3	3	6.03	3.03	100.5			
	6.	3	3	5.86	2.86	97.66			

Table no.5: Intraday analysis of Modafinil

Drug	Sample no.	Amount Present (µg/ml)	Amount found (µg/ml)	Percentage obtained	Average %	S.D	%RSD	S.E
	1.	6	6.06	101				
Modafinil	2.	6	5.95	99.16	99.27	1.67	1.69	0.965
	3.	6	5.61	97.66				

Table no.6: Interday analysis of Modafinil

Drug	Sample no.	Amount present (mg)	Amount found (mg)	Percentage obtained	Average %	S.D	%RSD	S.E
	1.	6	5.83	97.16				
Modafinil	2.	6	5.95	99.16	97.21	1.91	1.97	1.105
	3.	6	5.72	95.33				

Ruggedness:

The ruggedness of the developed method was confirmed by using different instruments and different analysts. The % RSD was calculated by using different analyst 1.39% and 1.57%. The results are within the limit. So it indicates the developed method was more rugged. The results were shown in Table 7.

Table no.7: Ruggedness study by different analysts

Drug	Condition	Sample No	Amount present (µg/ml	Amount Found (µg/ml)					
Modafinil	Analyst 1	1	6	6.06	101				
		2	6	6.2	103.33				
		3	6	6.06	101				
		4	6	5.95	99.16	101.91	1.39	1.39	0.570
		5	6	6.00	100				
		6	6	6.06	101				
Modafinil	Analyst 2	1	6	5.83	97.16				
		2	6	6.06	101				
		3	6	6.03	100.5				
		4	6	5.86	97.66	99.24	1.55	1.57	0.634
		5	6	5.95	99.16				
		6	6	6.00	100				

Table no.8: Degradation study of modafinil

Conditions	Conc (µg/ml)	Time	% Degraded	
			Standard*	Sample*
Acidic Degradation	100	30 min	31.34	29.8
Alkaline Degradation	100	30 min	25.46	21.5
Oxidative Degradation	100	2 hours	30.28	28.2

CONCLUSION: In this study a simple, precise, accurate and sensitive UV-spectroscopy methods were developed for the simultaneous estimation of Modafinil in bulk and in tablet dosage form. The Correlation coefficient (γ) values of the proposed method was close to 2, it indicate that the concentration used for plotting calibration curve were obeying Beer's law strictly. Additives and impurities commonly present in the dosage forms but did not show any interference in the proposed method. Statistical validation was done it shows that the method was reproducible and accurate. Also the various parameters were calculated such as standard deviation and percentage relative standard deviation. The values are complies the entire limit as per ICH guidelines. The forced acid, alkali and oxidative degradation study of Modafinil was studied by UV spectroscopy at various time interval (1st, 3rd, 5th day ;) it is observed that the drug Modafinil is degrading. Therefore the drug Modafinil has to be stored under such condition where the possibility of acid, alkali and oxidative hydrolysis does not arise.

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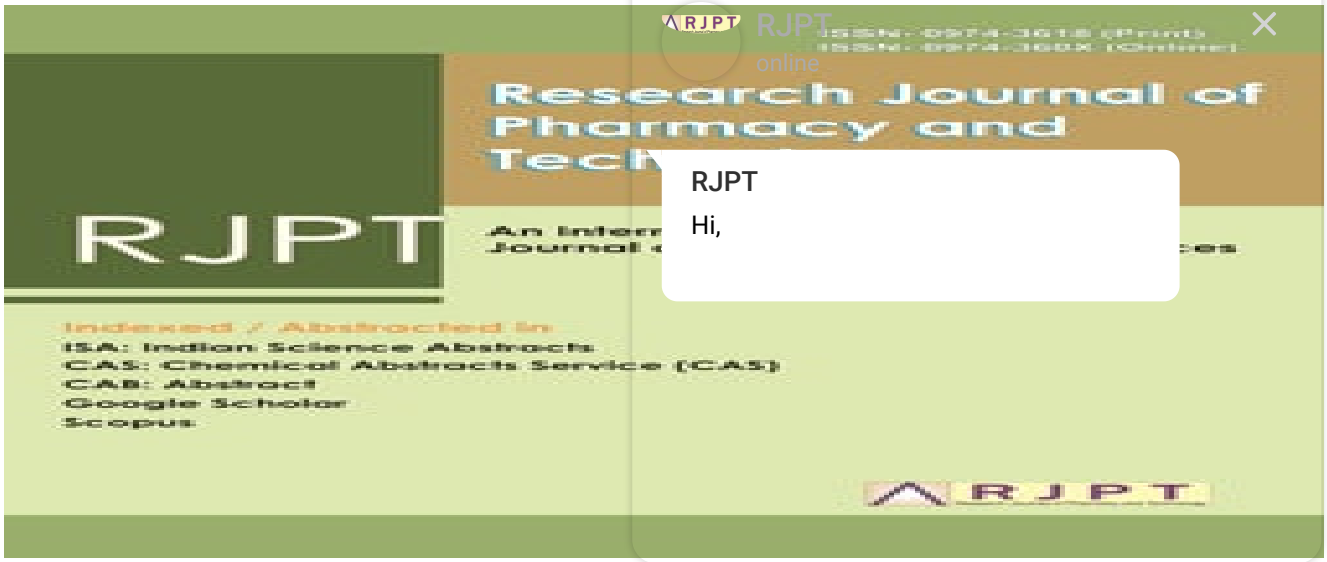

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