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## DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR DETERMINATION OF MODAFINIL IN HUMAN PLASMA

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### Abstract

A simple bioanalytical HPLC method for the estimation of Modafinil in human plasma has been developed and validated. Extracted sample was eluted using C18 (250 x 4.6 mm, 5  $\mu$ m) column. The mobile phase consisted of methanol, water and gl. acetic acid in the ratio of 60:40:0.1 v/v/v which was sonicated to degas and delivered at a flow rate of 0.8 mL/min at ambient temperature.

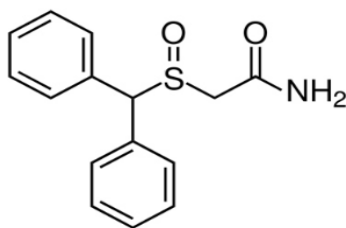
The retention time of Modafinil was 6.792 ( $\pm$  0.1) minutes. Studies were performed using an HPLC system equipped with a UV detector; the response was monitored at 233 nm. The method was validated according to MHLW (Japan) guidelines(2013). The data of linear regression analysis indicated a good linear relationship over the range of 1–10  $\mu$ g/ml concentrations with correlation coefficient value of 0.992. The accuracy of the method is indicated by good recovery in the range of 98.25%-116.17% and precision with RSD less than 7%. The proposed method can be applied to the analysis of Modafinil from human plasma.

**Key Words:** Modafinil, Human Plasma, HPLC, Bioanalytical study, Validation.

### Introduction

Modafinil is chemically 2-[(Diphenyl methyl)-sulfinyl] acetamide. It is a1-adrenergic agonist and is used for clinical evaluation in hypersomnia and narcolepsy<sup>[1]</sup>. It is not official in IP, BP, USP but is listed in the Merck Index and Martindale. When oral dose of 200mg is administered in a day,  $C_{max}$  is reported to be 4.61 $\mu$ g/ml  $\pm$  0.73<sup>[2]</sup>. Literature survey revealed the estimation of Modafinil by several techniques such as simultaneous estimation RP-HPLC techniques<sup>[3-7]</sup>. Determination of related substance in Modafinil and determination of Modafinil by a chiral chromatography<sup>[8]</sup>, LC-MS method for Modafinil in Human Plasma<sup>[9]</sup>. HPLC method for Modafinil in human plasma<sup>[10]</sup>, GC-MS method for Modafinil in Human urine<sup>[11]</sup>. The methods involved either solid phase extraction<sup>[12]</sup> or

Liquid-liquid extraction<sup>[13]</sup>. The focus of present study was to develop and validate a rapid, simple and economic bioanalytical HPLC method with UV detector for the estimation of Modafinil. In the present study, a new bioanalytical HPLC method was developed which shown high reproducibility and sensitivity. The developed method was validated as per MHLW (Japan) guidelines<sup>[14]</sup>.



**Fig. 1 Structure of Modafinil.**

## Materials and Methods

### Instrumentation

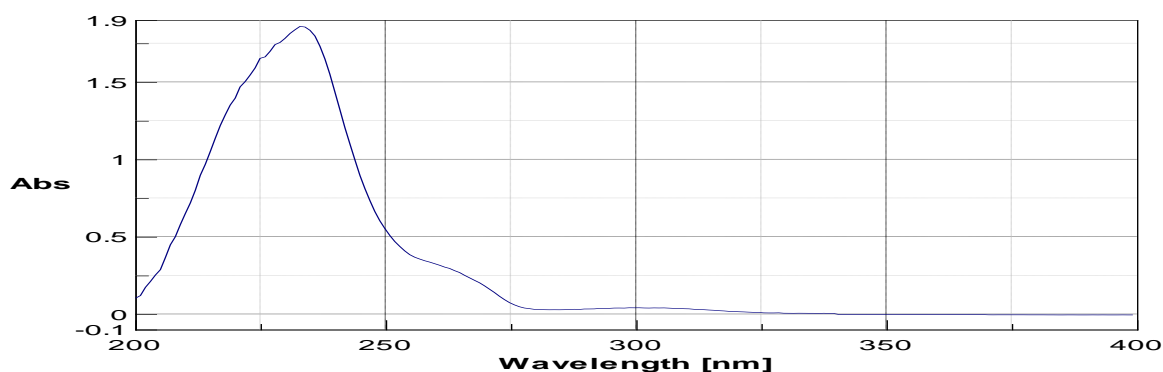
Bioanalytical assay of Modafinil was performed on HPLC(Make-JASCO) equipped with HiQSiL C18 column (250×4.6 mm;5µm particle size), Rheodyne injector and Jasco UV 2075 plus detector. The data acquisition was performed by Borwin chromatography software (version 1.5). Digital Balance Shimadzu make was used for weighing chemicals. Ultra-sonic bath sonicator was used for degassing of the mobile phase.

### Materials

Pure Modafinil used as working standard, was received as gift from Matrix Laboratories Ltd., Secunderabad, India. All chemicals and reagents i.e. Methanol and gl. Acetic Acid employed were of HPLC grade, and purchased from Loba Chemie.

### Selection of Wavelength

From Standard stock solution further dilutions were done using Methanol and it was scanned over the range 200-400 nm. It was observed that drug show considerable absorbance at 233 nm.(fig. 2 )



**Fig.2: UV Spectrum of Modafinil(10µg/mL).**

### Mobile Phase Preparation

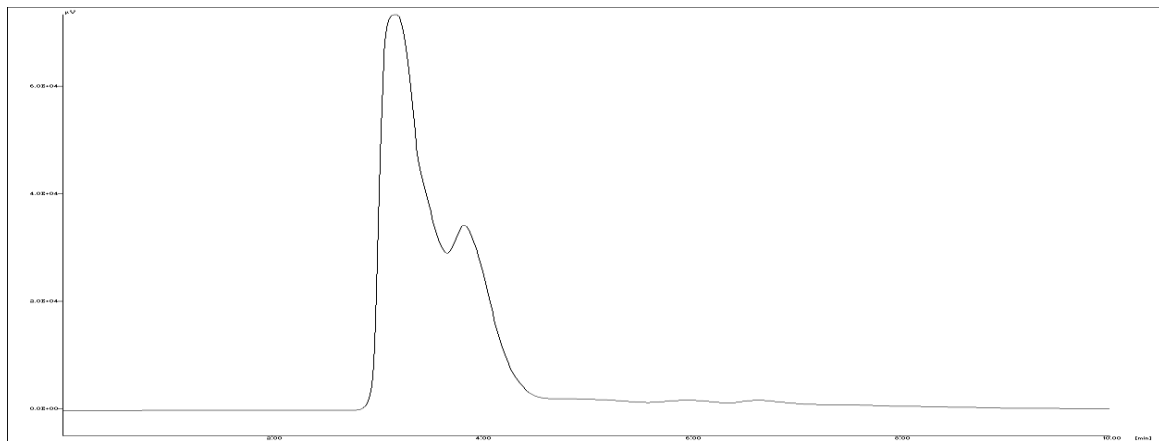
Prepare a filtered and degassed mixture of Methanol: Water: gl. Acetic acid in ratio 60:40:0.1 v/v/v.

### Preparation of solutions

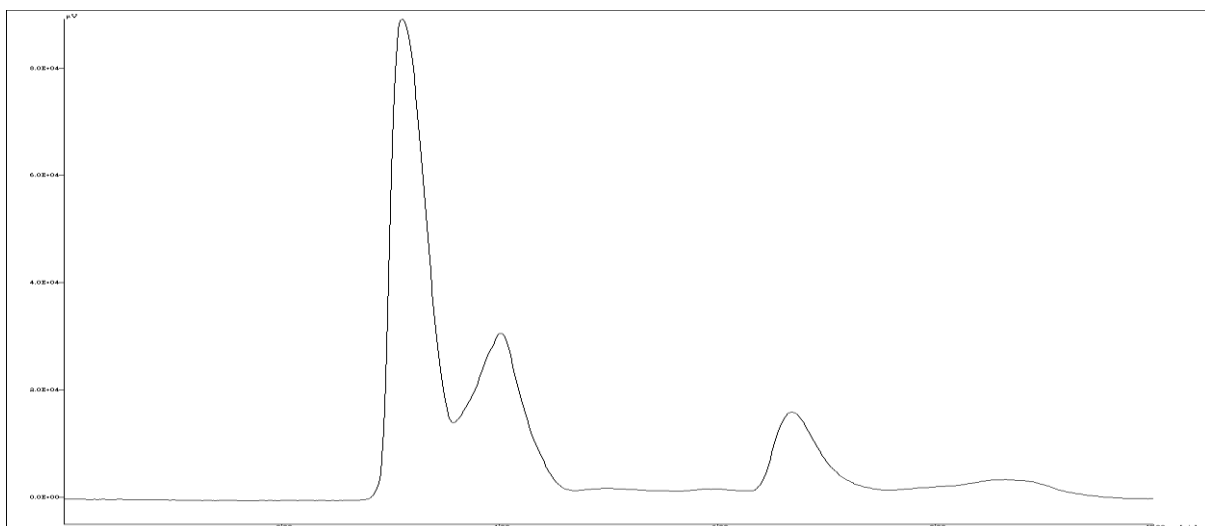
Stock solution of Modafinil was prepared by transferring accurately weighed 10 mg of Modafinil into a 10mL volumetric flask and making up volume with Methanol.

### Preparation of Spiked plasma sample

The reported plasma peak concentration values for Modafinil is 4.61 mg /Lit. i.e. 4.61  $\mu\text{g/mL}$ . On this basis, the linearity range was chosen as 1-10  $\mu\text{g/mL}$ . Spiked plasma was prepared by spiking 4.5 mL plasma with 0.5mL of stock solution (10, 20 ,30, 40, 50, 75, 100  $\mu\text{g/mL}$  ).The content were mixed by Vortex mixer for 5 min. 1 mL of this solution was pipetted into separate test tube to which 2mL Methanol was added. These solutions were thoroughly mixed by Vortex Mixer and then were centrifuged for 30 min. The clear supernatant was injected. A blank plasma sample was treated similarly.



**Fig 3 – Chromatogram of blank human plasma.**



**Fig. 4 – Typical Chromatogram of blank human plasma spiked with Modafinil 7.5 $\mu\text{g/mL}$  ( $R_t$ – 6.792 min).**

## **Method Validation**

The method was validated in accordance with MHLW guidelines, Japan. According to this guidelines there are parameters for bioanalytical method validation are

1. Selectivity- The selectivity of the method was evaluated by analysing pooled plasma samples spiked at LLOQ.
2. Calibration Curve-Linearity was tested for the range concentration 1-10  $\mu\text{g/mL}$ . Each sample in 5 replicates was analysed and peak area were recorded.
3. Accuracy and Precision –Accuracy was measured by using minimum 5 determination per 4 concentration i.e. at LLOQ, LQC, MQC, HQC. The precision of this method was evaluated by % CV at different concentration levels.
4. Recovery-It was evaluated by replicate analysis of at least 3 times each at 3 concentration levels (low-, mid-, and high-levels).
5. Carry over -It was evaluated by analysing a blank sample following the highest concentration calibration standard.
6. Dilution Integrity- It was evaluated by taking 1 mL spiked plasma of conc. 15  $\mu\text{g/mL}$  and it was tested upon subsequent dilution.
7. Stability Study-The stability of the Modafinil solutions and plasma samples was also evaluated during method validation. Modafinil stability was evaluated using two concentration levels i.e. at LQC, HQC. 3 Types of stability studies were performed i.e. Freeze and Thaw stability, Short term stability and Long term stability.
  - Short term stability - A stock solution was kept at room temperature for 4 hours and checked for its stability.
  - Long term stability-A stock solution was kept in deep freezer for 30 days and checked for its stability.
  - Freeze thaw stability-The stability of low and high quality concentration samples was determined after three freeze thaw cycles. The percent degradation was determined by comparing area.

## **Results and Discussion**

The method was validated in terms of limit of quantification, Recovery, Selectivity, Precision, accuracy and stability

### **1. Selectivity**

It was evaluated using blank plasma samples. The absence of interference at Modafinil retention time was confirmed as shown in Table 1.

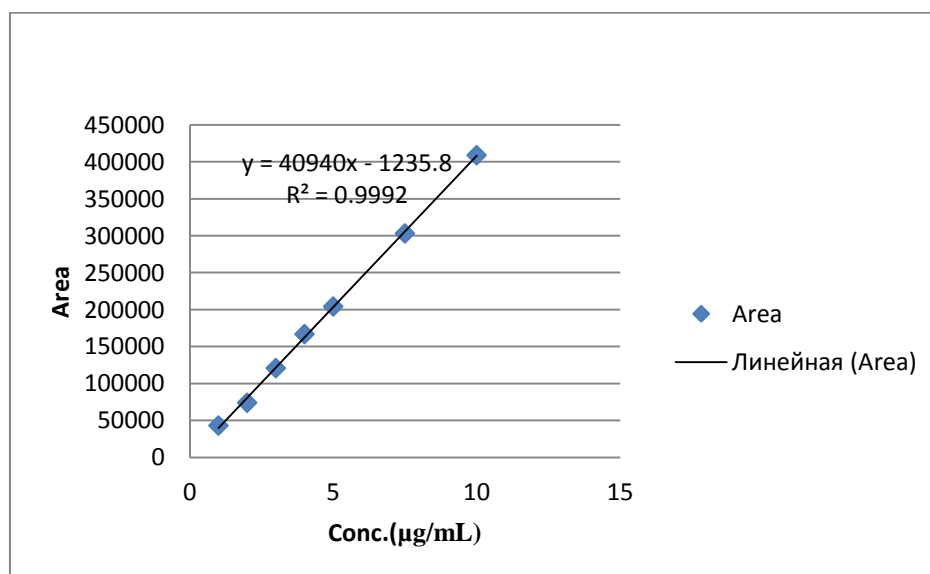
**Table 1: Results for selectivity.**

Replicate No.	Nominal concentration (LLOQ) (1µg/mL)		
	Area of Modafinil	Calculated Concentration	
		µg/mL	% Recovery
1	42123.93	1.0477	104.77
2	43242.07	1.026	102.6
3	42017.25	1.0611	106.11
4	47015.07	0.9532	95.32
5	44986.5	1.0583	105.83
6	44087.00	0.9735	97.35
Mean		1.0199	101.99
SD		0.042	4.2
% CV		4.11	

**2. Linearity** –Linearity was validated over a range of 1-10µg/ml. Best fit line equation was  $y = 40940x - 1235.8$ , the correlation coefficient for Plasma spiked with Modafinil ( $r^2$ ) 0.9992. Representative calibration curve is shown in Figure.5, as shown in Table 2.

**Table-2: Linearity Studies of Modafinil.**

Sr. No.	Conc.(µg/mL)	Area	S.D. (For n=5)	% Recovery
1	1	43876.95	2578.907	102.92
2	2	80575.15	6632.464	94.79
3	3	122266.8	1898.057	97.89
4	4	165435.3	1347.344	99.02
5	5	205370.00	4825.997	98.122
6	7.5	322047.1	11252.84	102.21
7	10	417438.9	14313.84	99.21
$r^2$		0.9992		



**Fig.5: Calibration curve of Modafinil.**

### 3. Accuracy

Accuracy was measured by using minimum 5 determination per 3 concentration i.e. at LQC, MQC, HQC level ranged from 86.93%-108.31%, which is within acceptance limit 85%-115% while at LLOQ level ranged from 98.25%-116.17% which is within acceptance limit 80%-120%, as shown in Table 3.

**Table 3: Results of Accuracy for Modafinil.**

Replicates	Calculated conc.			
	At LLOQ (1 µg/mL)	At LQC (3 µg/mL)	At MQC (5 µg/mL)	At HQC (7.5 µg/mL)
1	1.167	2.76	5.00	7.571
2	1.08	3.04	4.96	7.68
3	0.98	2.74	4.5	7.90
4	1.07	3.16	4.34	7.75
5	1.02	2.75	4.409	8.123
Mean	1.06	2.89	4.64	7.80
SD	0.06	0.175	0.28	0.1916
%CV	5.66	6.02	6.03	2.45
% Mean Accuracy	106.57%	96.33	92.80	104.00

### 4. Precision

#### A) Inter day Precision

The Inter day Precision was evaluated in five replicates for four different concentration of Modafinil on three consecutive days (fresh samples were prepared every day.) The % CV of calculated concentration for all Quality control samples of LLOQ, LQC, MQC, HQC concentration level ranged from 1.37-6.03 as shown in Table 4.

**Table 4: Results of Precision(Interday) for Modafinil.**

Conc. Level	% CV		
	Day 1	Day 2	Day 3
At LLOQ	5.66	3.51	3.8
At LQC	6.02	1.37	3.04
At MQC	6.03	2.35	2.845
At HQC	2.45	1.8	5.132

**B) Intraday precision**

Repeatability of the method was evaluated in five replicates on the same day for four different concentration of Modafinil (1, 3, 5, 7.5 µg/mL). The %CV of calculated concentrations for all quality control samples at LQC, MQC, HQC concentration levels ranged from , which is within acceptance limit 15%, and at LLOQ levels ranged from 2.1-4.83 as shown in Table 5.

**Table 5: Results of Precision(Intraday) for Modafinil.**

Conc. Level	At LLOQ	At LQC	At MQC	At HQC
% CV	4.83	2.1	2.22	3.9

**5. Recovery**

Recovery was evaluated by replicate analysis of at least 3 times each at 3 concentration levels (low-, mid-, and high-levels). Overall % recovery was found to be 94.092%, as shown in Table 6.

**Table 6: Results of Recovery for Modafinil.**

Conc. Level	Area		% Recovery
	Standard	Spiked Plasma	
LLOQ	47271.78	42199.343	89.269
LQC	146205.3	141376.5	96.697
MQC	249130.4	238545.37	95.127
HQC	317978.2	306245.75	96.31
Mean			94.092

**6. Carry-over**

Carry-over evaluated by analysing a blank sample following the highest concentration calibration standard. % Carry over is 4.468, which is within limit as shown in Table 7.

**Table 7: Results of Carry-over for Modafinil.**

Replicates	Area		% Carry Over
	At HQC	At Blank	
1	305965.5	12899.962	4.468
2	323461.9	13921	
3	277948.468	13725.918	

Mean	302458.6227	13515.6267	
SD	18745.51972	442.56	
%RSD	6.198	3.274	

### 7. Dilution integrity

Dilution integrity were evaluated by taking 1 mL spike plasma of conc. 15 µg/mL and it was diluted by adding 2 mL plasma in it. % Recovery of diluted samples was within the range of 95%-102.57% as shown in Table 8.

**Table 8: Results of Dilution Integrity for Modafinil.**

Conc. (µg/mL)	Calculated conc.	% Recovery
5	4.75	95.00
	4.95	99.18
	5.128	102.57
	4.91	98.22
	4.76	95.2
	4.8996	98.034

### 8. Stability

Drug Stability in biological fluid is a function of storage conditions, chemical properties of drug, the matrix and the container system. Stability procedure should evaluate the stability of analyte during sample collection and handling after long term(frozen at intended storage temp. )and short term (room temp.) storage conditions, as shown in Table 9.

#### A. Freeze and thaw stability-

Freeze and thaw stability of spiked quality control samples was determined after 3 Freeze and thaw cycles stored at -5 °C ±0 °C. Compared them to the freshly spiked quality control sample to assess stability.

The mean % stability for HQC(7.5 µg/mL) and LQC(3 µg/mL) was found to be 98.59% and 96.42% respectively, which is within acceptance limit of 85-115%.

#### B. Long Term stability-

Long Term stability of LQC and HQC was determined for period of 1 month stored at 4 °C, comparing them to the freshly weighed stock solution assessed for stability. The % mean stability for HQC(7.5 µg/mL), LQC(3 µg/mL) was found to be 98.09%, 96.71% respectively, which is within the acceptance limit 85-115%.



**C. Short term stability-**

Short term temp.stability of spiked quality control samples was determined for a period of 4 hrs. stored at room temperature. Comparing them against the freshly spiked quality control samples assessed stability.

The % mean stability for HQC and LQC are found to be 97.41%, 99.19% respectively, which is within acceptance limit of 85-115%.

**Table 9: Results of Stability for Modafinil.**

Stability	% Stability	
	At LQC	At HQC
Freeze and Thaw Stability	96.42	98.59
Long Term Stability	96.71	98.09
Short Term Stability	99.19	97.41

**Discussion**

There are a few papers available in literature for HPLC determination of Modafinil in human plasma. In work of Long Shan et al, Modafinil eluted at retention time 14.81 min, but in current work it is 6.79±0.1 min which requires less time. We propose protein precipitation method for plasma sample treatment; which is simpler and economical as compared to solid phase extraction or liquid-liquid extraction mentioned in work by McKinney AR et al and Gorman S.H. et al respectively. Thus the developed method is rapid and simple.

**Conclusion**

A new, simple and rapid method for the quantification of Modafinil in human plasma using HPLC with UV detection has been developed. The method reported here uses a simple and effective extraction technique with good and reproducible recovery. It is suitable for application to a pharmacokinetic, bioequivalence studies for the estimation of Modafinil from plasma.

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