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Original Research Article

DEVELOPMENT AND VALIDATION OF A SENSITVE RP-HPLC METHOD FOR ANALYSIS OF MODAFINIL IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

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A simple, rapid, accurate, sensitive and cost-effective reversed phase high performance liquid chromatography (RP-HPLC-PDA) method for the analysis of Modafinil (MOD) in bulk and tablet formulations was developed. A C_{18} reverse phase column (Devlosil) of 250×4.6mm dimensions and 5µm particle size with mobile phase containing Water: Acetonitrile (50:50 v/v) was used at a flow rate of 1 mL/min and the eluents were monitored at 230 nm. The optimised conditions showed a good linear response from 5 to 30µg/mL, with a regression coefficient (R^2) of 0.9994. The limit of detection (LOD) and limit of quantification (LOQ) were 0.2 and 0.7µg/mL, and the percentage recovery and assay were found to be 99.53 and 98.20. Specificity with placebo by 3 D plots showed that the method was specific and free from interfering substances. Therefore, the fully validated method was good enough to carry out routine analysis of MOD in bulk and tablet formulations with high sensitivity, less consumption of organic phase when compared to other published methods.

Key words: Modafinil, Assay, HPLC, Validation, Tablet formulation.

INTRODUCTION:

MOD is chemically 2-[(Diphenyl methyl)-sulfinyl] acetamide (Fig. 1). It is α1-adrenergic agonist and is used for clinical evaluation in hypersomnia and narcolepsy. MOD activates glutamatergic circuits while inhibiting GABAergic neurotransmission. A considered mechanism of action involves brain peptides called Orexin, also known as hypo cretins which causes wake full ness. MOD is not official in any of the pharmacopoeia but is listed in the Martindale - The Complete Drug Reference and Merck Index [1, 2]. Literature survey revealed the estimation of MOD by several analytical techniques. Methods reported earlier include HPLC by UV, PDA and MS detection and most of which were meant for bio analysis [3-10]. Very few HPLC methods have been reported that for the estimation of MOD in the bulk and pharmaceutical formulations[11, 12]. HPLC methods for forced degradation studies were also reported[13]. However, most of the published methods utilized phosphate buffers in the mobile phases and also reported methods were of long chromatographic run, and showed low sensitivity and consumption of more organic solvents for mobilephase[11-13]. Hence, the present investigation was aimed at developing and validation of an LC method for quantification of MOD in bulk and formulations with high sensitivity and short runtime.

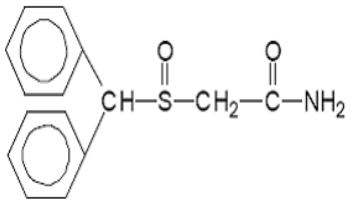

Fig.1.Structure of MOD

EXPERIMENTAL

Chemicals and reagents

MOD was obtained from Sun Pharma, Mumbai as a gift sample. Branded MOD product (Modalert, Sun pharma, India, B # BSJ 2589) containing 200mg of MOD was purchased from a local pharmacy. Acetonitrile and water (HPLC grade) were purchased from E. Merck, Mumbai, India and all the reagents and chemicals used were of HPLC grade.

Equipment

Chromatographic separation was performed on a Shimadzu HPLC system equipped with a LC 10 AVP pump, M20 A PDA detector and LC 10ATVP auto sampler was used with 200µL loop volume. LC solution software was used for analysing the data.

Chromatographic conditions

MOD was separated on a C_{18} analytical column (Devlosil, 250×4.6 mm, 5 µm) under reversed phase condition. The mobile phase, a mixture of acetonitrile and water in 50:50 (v/v) ratio, with 1.0 mL/min flow rate was used. Mobile phases were filtered through membrane filter (Millipore Nylon disc filter of 0.45µm) and sonicated for 5 min in ultrasonic bath before usage. A detection wavelength of 230 nm was used in the analysis.

Preparation of stock and standard solutions

Stock solutions of MOD of strength 1mg/mL were prepared using Acetonitrile. Appropriate volumes of these stock solutions were then further diluted with water as diluent to get the required concentration of standard

solutions at a concentration range of 5-30 µg/mL.

Validation

The method was validated according to International Conference on Harmonization[14] guidelines for:

Linearity

The linearity of MOD responses in the concentration ranges of 5 to 30µg/mL was determined by preparing and injecting solutions at an injection volume of 20µL and the data was given in Table 2.

Precision

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of the same standard concentration 20µg/mL. The data was given in Table 3.

Accuracy

Accuracy (Recovery) of the method was tested by spiking 80, 100 and 120% of MOD working standard. The accuracy of the analytical method was established in triplicate across its range according to the assay procedure and the data was given in Table 4.

Assay

20 tablets were weighed individually and powdered in a mortar. A blend of powder equivalent to 25mg of MOD was transferred to a 25 mL standard flask and 10 mL of Acetonitrile was added and sonicated to extract and dissolve the MOD. The volume was made up with Acetonitrile, filtered with a 0.45µ syringe filter and 1mL of the filtrate was diluted to obtain a final concentration of 20µg/mL with diluent. 20 µL of the solution was injected into the HPLC system.

Robustness

Method robustness was determined by analyzing same sample at normal operating conditions and also by changing some operating analytical conditions such as, mobile phase composition, flow rate. The data was given in Tables 6 and 7.

LOD and LOQ

The LOD and LOQ values determined by the formulae LOD = 3.3 σ/m and LOQ = 10 σ/m (Where, σ is the standard deviation of the responses and m is mean of the slopes of the calibration curves)

Specificity

This was done by spiking pure substances (drug substance or drug product) with appropriate levels of impurities and/or excipients and demonstrating that the assay result is unaffected by the presence of these materials (by comparison with the assay result obtained on unspiked samples)

RESULTS AND DISCUSSION

The development of the RP-HPLC method for the determination of drugs has received considerable attention in recent years because of its importance in routine quality control analysis. In the present investigation, different analytical columns with various stationary phases and mobile phase combinations were tested to develop a highly sensitive LC method, for the analysis of MOD in bulk and formulations. Initial trials were carried out using C_{18} Phenomenex

column (250 x4.6 mm) with methanol: water (50:50 v/v) at a flow rate of 1mL/min as mobile phase and the peak was eluted at 4.1 min with good shape and symmetry etc however, the peak response was too low. Same mobile phase was tried using Devlosil RP Aqueous column (250 x4.6 mm) but resulted in bad peak shape. In another trial mobile phase of Methanol: 0.1% v/v formic acid in water (50:50) at a flow rate of 1 mL/min was studied using same column but peak tailing was observed.

Finally a mobile phase consisting of acetonitrile: water (50:50v/v) resulted in good peak shape and response when compared to the previous trials. With these conditions the retention time of MOD was 4.1 min at a flow-rate of 1 mL /min and the injection volume was 20 µL. All the trials were performed at 230 nm. Fig 2 shows a typical chromatogram obtained under these conditions along with UV spectrum and purity index.

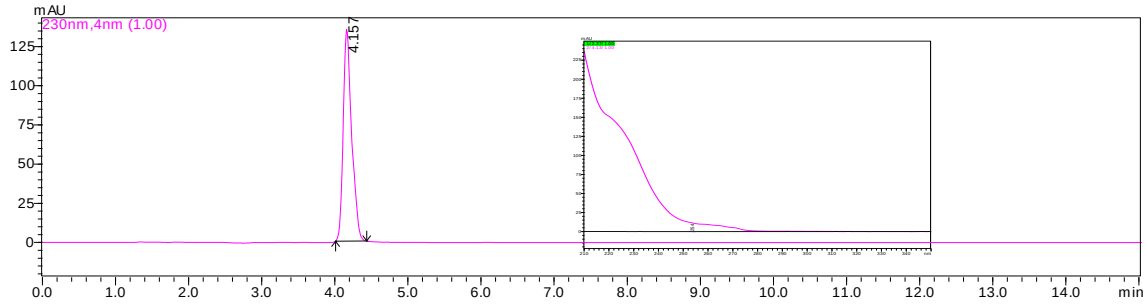

Fig.2. MOD chromatogram (20µg/mL) with UV spectrum and peak purity index*
(*Peak Purity Index:1.0000 ; Single Point Threshold :0.9999921 ; Min.peak purity index: 78).

System suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (*tR*), number of theoretical plates (*N*) and tailing factor (*T*) were evaluated for 20 µg/mL MOD concentration at different injection volumes. The results given in Table 1 were within acceptable limits. A typical overlay chromatogram of MOD is presented in Fig 3.

Table.1. For System suitability data(n=3)

Injection volume (µL)	Retention time (min)	Tailing factor	Theoretical plates (N)	Capacity factor
10	4.129	1.316	4894.348	**2.121**
20	4.157	1.389	5172.962	**2.104**
30	4.163	1.392	4804.456	**2.125**
40	4.176	1.410	4881.593	**2.125**
50	4.189	1.429	4894.526	**2.127**
Mean	4.189	1.3872	4889.577	**2.1204**
% RSD < 2	0.54	0.37	1.72	**0.44**
Limits		**< 2**	**> 2000**	**1-5**

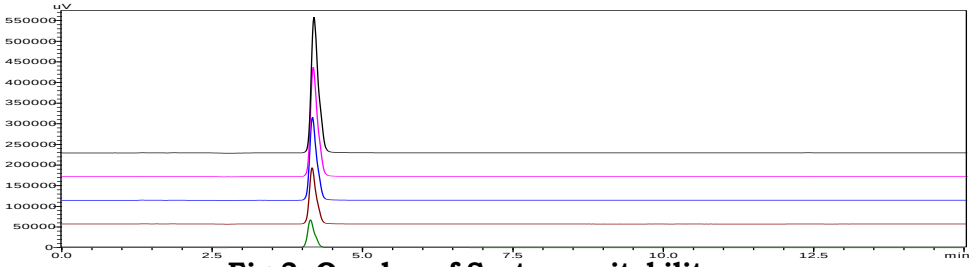

Fig.3. Overlay of System suitability

Linearity:

A linear relationship was evaluated across the range (5-30 µg/mL) of the analytical procedure in triplicate. The range of concentrations was selected based on 80-120 % of the test concentration (for assay). Peak area and concentrations were subjected to least square regression analysis to calculate regression equation. The regression coefficient (R^2) was found to be 0.9994 with correlation coefficient (R) of 0.9997 and shows good linearity. The data of the calibration curve was given in Table 2.

Table.2.Linearity data for MOD (n=3)

SI:NO	Concentration (µg/mL)	Area ± SD
1.	5	262692 ± 2266.635
2.	10	516956 ± 2180.652
3.	15	727099 ± 2952.503
4.	20	986089 ± 5484.857
5.	25	1237960 ± 21317.13
6.	30	1474338 ± 22963.85
y = 48459x + 19496 R^2 = 0.9994		

Precision:

Precision studies were carried out in terms of repeatability. Six determinations of 100 % concentration at 20µg/mL level was evaluated and the data given in Table 3 and shown Figure 4. The % RSD was found to be less than 2 and fulfilled the ICH guidelines criteria.

Table.3.Precision data of MOD for 20µg/mL

Injection	Area	Retention Time
Injection 1	983127	4.155
Injection 2	982236	4.148
Injection 3	993549	4.150
Injection 4	986575	4.145
Injection 5	993566	4.150
Injection 6	984537	4.146
Standard deviation	5089.661	0.003578
%RSD	0.5155	0.08263

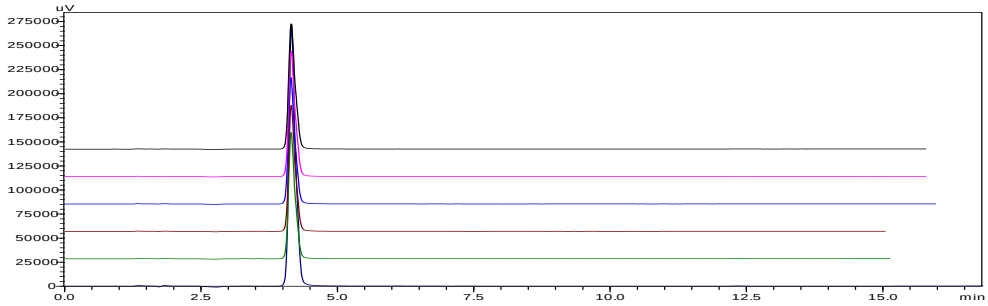

Fig.4. Overlay of Precision

Accuracy

Accuracy of the method was examined by performing recovery studies by standard addition method and the analyte peak is evaluated by 3D plot of the chromatogram in order to confirm the existence of one component at 4.1 min elution time of MOD as the impurities are not available (Fig 5). The recovery of the added standard to the drug product sample was calculated and it was found to be 98.57 - 99.53%, which indicates a good accuracy of the method to that of the labelled claim. The obtained recovery results were given in Table 4.

Table.4.Accuracy data of 20µg/ML

Level of recovery (%)	Amount present (µg/mL)	Amount added (µg/mL)	% Recovery (Mean ± SD)	% RSD
80	20	18	98.57 ± 0.104	0.10
100	20	20	99.33 ± 0.557	0.51
120	20	24	99.35 ± 0.361709	0.34

Assay

The amount present in the each tablet was calculated by comparing the area of standard MOD and tablet sample. The content of drug in the formulation was found to be within the limits.

Table.5.Assay Results (n=3)

Formulation	Labelled Amount (mg)	Amount found (mg) (Mean ± SD)	% Assay	% RSD
MOD Tablets (Modalert® 200mg)	200	196.4 ± 0.152753	98.2	0.07780

Robustness

As part of the robustness, deliberate changes in the flow rate, mobile phase composition, was made to evaluate the impact on the method. Retention times were significantly changed with flow rate and mobile phase compositions (Tables 6 and 7).

Table.6.Robustness data relating to flow rate change

S.NO	Flow rate (mL/min)	Retention time (min)	Plate Count	Tailing factor
1	0.8	3.9min	4933.4	1.5
2	1.0	4.1min	4882.9	1.3
3	1.2	4.2min	4910.0	1.5

Table.7.Robustness data relating to mobile phase composition change

S.NO	Mobile phase	Retention time (min)	Plate Count	Tailing factor
1	2 % less	3.8min	4976.4	1.5
2	actual	4.1min	4882.9	1.3
3	2 % more	4.4min	4848.9	1.5

Sensitivity

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve. LOD was found to be 0.2µg/mL and LOQ was found to be 0.7µg/mL indicating high sensitivity of the method.

Specificity

The specificity of the method was established by spiking diluent solution of

commonly used excipients in the form of tablet and showed no peaks within the retention time of MOD i.e. 4.1 min and also over the range of 10 min as shown Figure 6.

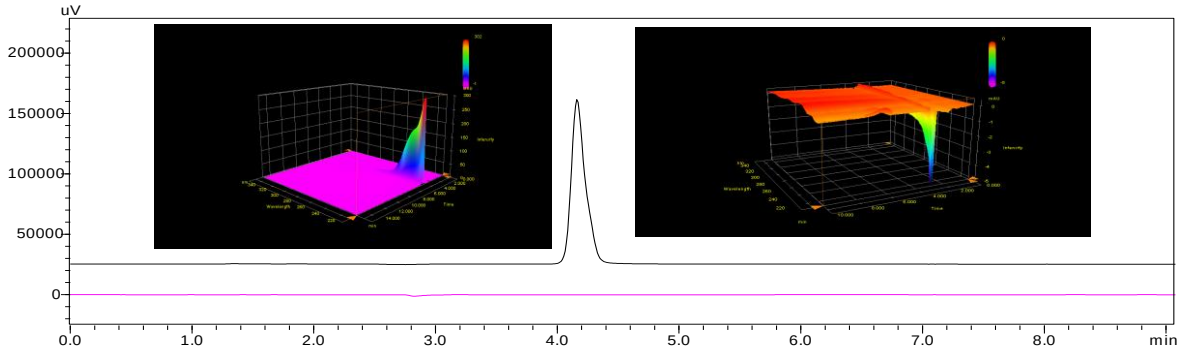

Fig.6.Overlay of standard and placebo chromatogram along with 3D view

CONCLUSIONS

Finally, it can be concluded that the proposed RP-HPLC-PDA method was validated fully as per the International Conference on Harmonisation (ICH) Guidelines, and found to be applicable for routine quality control analysis for the estimation of MOD. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The method provides selective quantification of MOD without interference from blank and placebo. The proposed method is sensitive, reproducible, reliable, rapid and specific and also has the unique advantage of LC conditions being compatible with MS detection. Therefore, this method can be employed in quality control to estimate the amount of MOD in bulk and dosage forms.

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