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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE DETERMINATION OF MODAFINIL IN BULK AND TABLET DOSAGE FORMS

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ABSTRACT

The validated reverse phase liquid chromatographic method developed and validated for the quantification of Modafinil in bulk drug and in Pharmaceutical dosage form. Separation was achieved under optimized chromatographic condition on a PhenomenaxLunaC_{18} (ODS) column (150 X 4.6 mm i.d., particle size 5µ). The mobile phase consisting of phosphate buffer pH 3.0: Acetonitrile (60:40, v/v). An isocratic elution was achieved at a flow rate of 1 ml/ min at ambient temperature. The detection was carried out at 225nm using Shimadzu UV-Visible detector SpD-10AVP. The retention time of Modafinil was found to be 3.2 min. The calibration curve was linear in the concentration range of 5-30µg/ ml (r^2= 0.9999). The limit of detection and the limit of quantification were found to be 0.580531 µg/ml and 1.75918µg/ml respectively. The amount of Modafinil present in the formulation was found to be 99.79 ± 0.8075. The method was validated statistically using the SD, %RSD and SE and the values were found to be within the limits. The recovery studies were performed and the percentage recoveries were found to be 101.10±1.635%. So, the proposed method was found to be simple, specific, linear, and rugged. Hence it can be applied for routine analysis of Modafinil in the Pharmaceutical formulation.

KEYWORDS: Modafinil**,** RP-HPLC Method, Validation, System suitability tests.

INTRODUCTION

Modafinil acts as a eugeroic for the treatment of narcolepsy (sleepiness), sleep disorder due to different shift work and more day-time sleepiness which was associated with OPA (obstructive sleep apnea).[1-4] It was administered by the oral route. It acts by inhibiting selectively and weakly the dopamine reuptake process and indirectly promotes the releasing of histamines and orexin neurological peptides from the tuberomammillary nucleus and lateral hypothalamus, respectively and leads to contribution to heightened-arousal.

Chemically Modafinil designated as 2-[(diphenylmethyl) sulfinul] acetamide **Fig. 1** having a molecular mass of 273.35 g/mol.[5,6]

Modafinil activates the cytochrome-P450 enzymes CYP-1A2, CYP-3A4, and CYP-2B6, as well as inhibition of CYP-2C9 and CYP-2C19 *in-vitro*. Modafinil also produces P-glycoprotein (Pgp) material which affects drug transportation by this glycoprotein (as digoxin). The bioavailability of modafinil is greater than 80% of the administered dose. An *in-vitro* study of the medicament indicates that 60% of the drug is bound only to plasma proteins in the clinical concentration level. The percentage sometimes changes with change in the concentration. C_{max} occurs nearly at 2-3 h after drug administration. In the presence of food, the drug will show slow absorption, but it will not affect total AUC. The half-life of the drug was approximately between 10–12 h range, which was differed by CYP-genotypes, functioning of liver and kidney. The drug is metabolized in the liver and resulting inactive metabolite is excreted in the urine.[7-9]

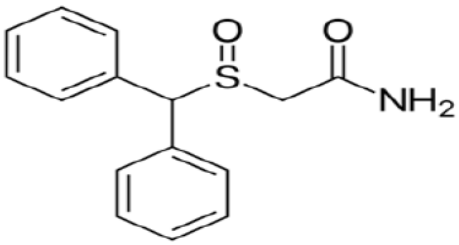

Fig 1: Chemical structure of Modafinil.

2.0. MATERIALS AND METHODS

2.1. Chemicals

Acetonitrile used was of HPLC grade from E. Merck, India. HPLC grade water was obtained using millipore water purification system. Working standard of Modafinil with potency of 99.67% was obtained from Dr. Reddy's Laboratories, Hyderabad. Other chemicals were

analytical grade of above 99% purity. All volumetric ware was pre-calibrated by the manufacturer (Borosil) and was of grade A. HPLC grade water was obtained using millipore water purification system. Commercial tablets containing Modafinil (Tekturna-150mg) were procured from the local chemist shop.

2.2. Instrumentation

The validated method utilized a Shimadzu HPLC system containing SPD-10 ATVP pump and SPD-10AVP UV-Visible detector with an isocratic elution technique at a flow rate of 1ml/min on a Phenomenax LunaC_{18} column (250 X 4.6 mm i.d., 5µ) at ambient temperature. A rheodyne injector with 10 µl loop was used for injecting the sample. Shimadzu balance[4] was used for weighing purpose in this method.

2.3. Chromatographic conditions

The analysis was carried out with UV detection at 293 nm (fig.2) using a 20 µl Injection volume. Assay was performed using a C18 reversed-phase column eluted with phosphate buffer pH 3.0: Acetonitrile (60:40, v/v) at a flow rate of 1.0 ml/ min. Chromatography was carried out at ambient temperature. The solvents were mixed, filtered through a membrane filter of 0.45 micron pore and degassed in ultrasonic bath prior to use.

2.4. Standard solution preparation[5-11]

Standard stock solutions were prepared by dissolving 10 mg of Modafinil working standard in 8.0 ml of mobile phase and diluting to 10.0 ml with the same to obtain concentration of 1000 µg/ml. It was filtered through a.22µmembrane filter. The stock solution was protected from light using aluminum foil and stored for 1 week at 40C and was found to be stable during this period.

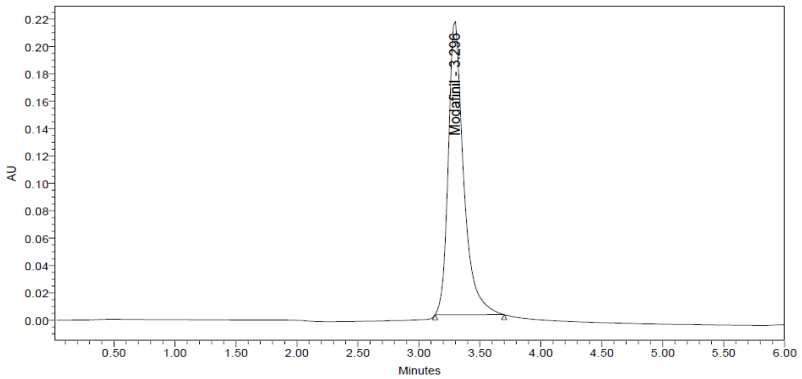

Fig.2 Chromatogram of standard solution.

2.5. Procedure for analysis of tablet formulation[12]

20 Tablets of the product under study were weighed, crushed and mixed in a mortar. A portion of powder equivalent to the weight of 100.00mg was accurately weighed and transferred to a dry 100 ml A-grade volumetric flask and 100 ml mobile phase was added. The volumetric flask was sonicated for 20 min to effect complete dissolution of Modafinil and made up to the volume with mobile phase. Suitable aliquots of solution were filtered through a 0.45 µm nylon filter. This was further diluted with mobile phase to yield concentration of Modafinil in the range of linearity (15ppm).Each of standard and test preparation was injected into the chromatograph and the responses recorded (Fig.3.)

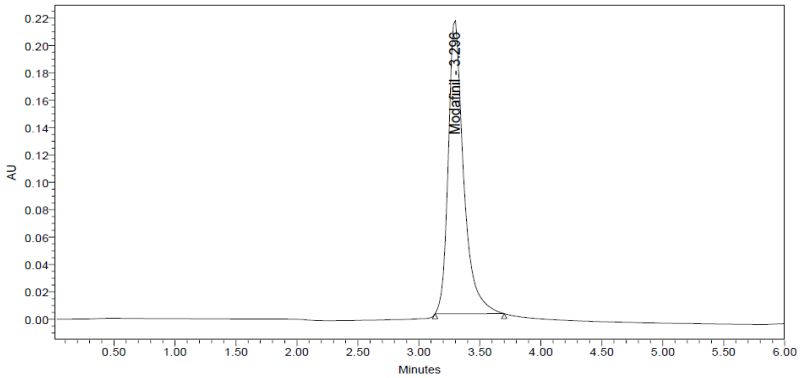

Fig.3.Chromatogram of Modafinil sample solution.

3.0. METHOD VALIDATION[13-17]

3.1. Linearity

A series of standard curves were prepared over a concentration range of 5 -30µg/ml by diluting the standard stock solution of Modafinil (1mg/ml) in mobile phase. The data from peak area versus drug concentration plots were treated by linear least square regression analysis and r^2 was found 0.9999(Fig.4). The standard curves were evaluated for intra-day and inter-day reproducibility. Each experiment was repeated in triplicate.

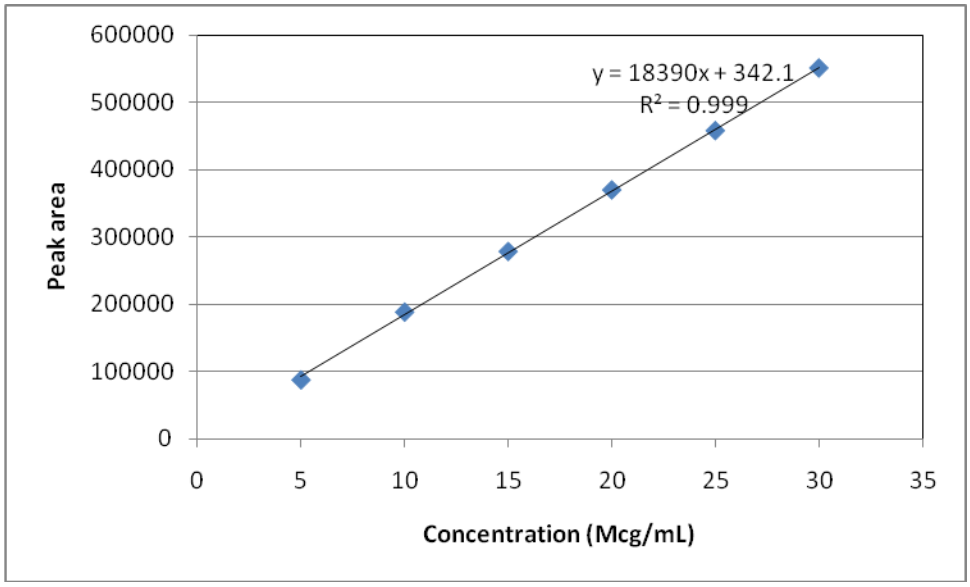

Fig.4.Calibaration curve of Modafinil.

3.2. Precision

Precision was measured in accordance with ICH recommendations. The precision study was carried out by injecting sample preparation of 15µg/ml concentration six times.

Table 1: Precision studies.

No.of Injection	% Assay*
1	99.48
2	99.23
3	100.35
4	99.68
5	99.85
6	99.11
Mean	99.79 ± 0.8075
SD	0.8075
%RSD	8077

3.3. Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples Modafinil (15 µg/ml) were spiked with known amount of standard so as to get three different levels (66.33, 88.33% and 100%) and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. Recovery (%), RSD (%) was calculated for each concentration.

Table.2. Recovery studies of Modafinil.

Amount Present (µg/ml)	Amount added* (µg/ml)	Amount found* (µg/ml)	% recovery*	Average ± S.D	% RSD
19.96	2.003	21.97	101.35	101.10± 1.635	1.654
19.96	6.009	26.06	101.84		
19.96	10.015	29.97	100.13		

(*n=6)

3.4. Limit of detection and limit of Quantitation

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ) values, the blank sample was injected six times and the peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level (S/N = 3:1) while ten times the noise level gave the LOQ (S/N=10:1).

3.5. Ruggedness

The ruggedness of the method was demonstrated by analysis of the samples as for precision study by a second analyst. The RSD of the two sets of data indicates the ruggedness of the method. Further, the t-test was performed on the data and the difference was found to be not significant.

Table.3. Ruggedness Analysis.

Analyst 1 Sample	% Assay	Analyst 2 Sample	% Assay
1	100.04	1	99.85
2	100.22	2	98.91
3	99.84	3	100.45
4	100.35	4	100.03
5	100.67	5	99.47
6	100.41	6	99.49
*Mean	100.25	Mean	99.75
SD	0.283	SD	0.193
RSD	0.271	RSD	0.159

3.6. Robustness

The robustness of the method was determined to assess the effect of small but deliberate changes of the chromatographic conditions on the determination of Modafinil. The different variations are in flow rates by ± 0.1 mL/min, in wavelength by ± 2 nm and in temperature by ± 5 °C. The concentration of the solution analyzed was 15µg/mL.

3.7. System suitability tests

The chromatographic systems used for analyses must pass the system suitability limits before sample analysis can commence. The capacity factor (K), injection repeatability, tailing factor (T), theoretical plate number (N) and reference solution (Rs) for the principal peak were the parameters tested on a 15 µg/mL sample of Modafinil to assist the accuracy and precision of the developed HPLC system.

4.0. RESULTS

4.1. Linearity

Peak area versus drug concentration was plotted to construct a standard curve for Modafinil and linearity was shown in concentration range of 5µg/ml to 30µg/ml. The polynomial regression for the calibration plots showed good linear relationship with coefficient of correlation, $r^2 = 0.9999$.

4.2. Precision

System precision is the measure of the method variability that can be expected for a given analyst performing the analysis. Precision of the method was determined with the product. The precision study was carried out by injecting sample preparation of 15µg/ml concentration and assayed in six replicate determinations for each of the six weighing amounts. The results for precision are shown in Table 1, indicating that acceptable precision was achieved for Modafinil as revealed by relative standard deviation data (RSD<2.0% in all of the levels)

4.3. Accuracy

The % recovery was calculated for triplicate samples and for all levels and mean recovery was calculated. The mean recovery was well within the acceptance limit hence the method was accurate, as depicted in Table 2.

4.4. Limit of detection and limit of Quantitation

The LOD was calculated to be 0.5805µg/ ml and the LOQ was calculated to be 1.7591 of the placebo mixtures with the peak of Modafinil was observed.

4.5. Ruggedness

The % assay and RSD for samples prepared by second analyst was calculated and found within limit. Then RSD of analyst 1 and analyst 2 was calculated and found within limit. This proved that the method is rugged, as depicted in Table 3.

4.6. Robustness

The results of the analysis (% RSD ranged from 0.059 to 1.361 %) of the samples under the conditions of the above variations indicated the nature of robustness of the method.

4.7. System suitability tests

The results of the system suitability tests assure the adequacy of the proposed HPLC method for routine analysis of Modafinil. The capacity factor (k) was found to be 1.905, indicating that the Modafinil peak is well resolved with respect to the void volume. The RSD of six consecutive injections performed under the precision test (Table 4) was found to be 0.43% and thus shows good injection repeatability. The tailing factor (T) for Modafinil peak was found to be 0.7, reflecting good peak symmetry. The theoretical plate number (N) was found to be 8536, thus demonstrating good column efficiency.

Table 4: System Suitability Parameter.

S.No.	Parameter	Suitable Values
1.	Retention Time	3.2
2.	Tailing factor	0.7
3.	Asymmetrical factor	1.23
4.	Theoretical plates	8536
5.	Capacity factor	1.905
6.	HETP	0.03256

4.8. Specificity

The chromatograms obtained showed separation of the analyte from the excipients was complete, i.e. there was no interference from the excipients under the chromatographic conditions used for the analysis. No interference.

RESULTS AND DISCUSSION

A simple, selective, rapid and precise RP-HPLC method for the estimation of Modafinil in bulk material and in pharmaceutical formulation has been developed and validated. The linearity range was determined by external standard calibration method in the concentration range of 5-30µg/ml. The correlation co-efficient was found to be 0.9999 indicated that the concentrations of Modafinil had good linearity. The LOD and LOQ were found to be 0.580531 µg/ml and 1.75918 µg/ml, respectively. The system suitability parameters like capacity factor, asymmetric factor, tailing factor, HETP and number of theoretical plates were calculated and it was observed that all the values are within the limits. The percentage of Modafinil present in formulation was found to be 99.79 ± 0.8075. Further the precision of

the method was confirmed by the repeatable analysis of formulation. The % RSD was found to be 0.8077. It indicated that the method has good precision. The percentage recovery of Modafinil present in formulation was found to be 101.10±1.635and the percentage RSD value was found to be 0.8700. The low percentage RSD value indicated that there is no interference due to excipients used in formulation. Hence, the accuracy of the method was confirmed.

CONCLUSION

The developed RP-HPLC method was validated and the system suitability studies were performed and all parameters combined with the simplicity and ease of operation ensures that the validated method can successfully used for routine analysis of Modafinil in bulk and tablet dosage formulation.

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REFERENCES

1. Available from: http//www.pubmed.pharmacol.com/ Modafinil.
2. Available from: http//www.Rx list .com/ Modafinil.
3. Ford J. L., Raja-Siahboomi A. R., In Encyclopedia of Pharmaceutical Technology, Marcel Dekker, New York, 2002; 717-728.
4. Anonymous., Shimadzu LC-10 ATVP High performance liquid chromatography Instruction Manual, Shimadzu Corporation., Kyoto, Japan, 2001; 11-2.
5. Budavari S. The Merk index, 13th Eds., Merk & Co., inc., Whitehouse station, Eds.,in: NJ, 2001; 1112.
6. Royal Pharmaceutical society of Great Britain, Martindale, Thirty-first Edition,1 Lambeth High Street London SE17 Jn ngland, The extra pharmacopoeia, 1996; 435-36.
7. Moachon G, Matinier D. Simultaneous determination of modafinil and its acid metabolite by high-performance liquid chromatography in human plasma. J Chromatogr B Biomed Appl, 1994; 654(1): 91-96.
8. Burnat P, Robles F. High-performance liquid chromatographic determination of modafinil and its two metabolites in human plasma using solid-phase extraction. J Chromatogr B Biomed Sci Appl, 1998; 706(2): 295-304.

9. Gorman SH. Determination of modafinil, Modafinil acid and modafinil sulfone in human plasma utilizing liquid– liquidextraction and high-performance liquid chromatography. J Chromatogr B Anal Tech Biomed Life Sci, 2002; 767(2): 269-276.
10. Gorman SH. Determination of the D-and L-Enantiomer of Modafinil in human plasma utilizing liquid-liquid extraction and high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl, 1999; 730(1): 1-7.
11. Schwertner HA, Kong SB. Determination of modafinil in plasma and urine by reversed phase high-performance liquidchromatography. J Pharm Biomed Anal, 2005; 37(3): 475-479.
12. Nageswara Rao R, Dhananjay D, Shinde and MVN Kumar T. Development and validation of a reversed phase liquid chromatographic method for separation and determination of related-substances of modafinil in bulk drugs. Talanta, 2007; 73(3): 407-414.
13. Cass QB, Ferreira Galatti T. A method for determination of the plasma levels of Modafinil enantiomers, (+/-)-modafinic acid and modafinil sulphone by direct human plasma injection and bidimensional achiral-chiral chromatography. J PharmBiomed Anal, 2008; 46(5): 937-944.
14. Rao RN, Shinde DD, Talluri MVNK and Agawane SB. A Highly Sensitive and Specific Liquid Chromatography/Tandem Mass Spectrometric (LC–MS/MS) Method for Investigating the Pharmacokinetics of Adrafinil in Rats. Journal of Chromatography B. 2008; 873(1): 119-123.
15. McKinney AR, Suann CJ and Stenhouse AM. A Method for Rowe R. C., Sheskey P. J., Owen S. C., Handbook of Pharmaceutical Excipients, 5th edition., Pharmaceutical Press: Grayslake, 2006.
16. Mendham J., Denny R.C., Barnes J.D., Thomas M.J.K., Vogel's, Text book of Quantitative Chemical Analysis, 6th edition, Pearson Education Pvt. Ltd., New Delhi, 2002, 261-263,268,277,653,654.
17. USP 32, The United States Pharmacopeia/The National Formulary, United States Pharmacopeial Convention: Rockville, 32nd edition, 2009.
18. FDA, Guidance for Industry: Dissolution testing of immediate release solid oral dosage forms, U.S. Food and Drug Administration: Rockville, 1997.
19. Code Q2A, Text on Validation of Analytical Procedures. ICH Tripartite Guidelines, Geneva, Switzerland, 1994; 1-5.

20. Code Q2B, Validation of Analytical Procedures, Methodology, ICH Tripartite Guidelines, Geneva, Switzerland, 1996; 1- 8.