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Development and Validation of RP-HPLC method for determination of Modafinil in bulk and dosage form

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ABSTRACT

A reverse phase high performance liquid chromatographic method was developed for the determination of Modafinil in bulk and dosage form. The separation was effective on a Hypersil ODS C_{18} column (250 mm x 4.6 mm; 5µ) using a mobile phase mixture of Buffer:Acetonitrile in a ratio of 55:45 (v/v) at a flow rate of 1.0ml/min. The detection was made at 220nm. The retention time of modafinil was found to be 4.80±0.06 min. Calibration curve was linear over the concentration range of 20-120µg/ml of modafinil. The proposed method was validated as per the ICH guidelines. The method was accurate, precise, specific and rapid and thus found to be suitable for the quantitative analysis of modafinil in the bulk and dosage form.

***Key Words*:** Method development, validation, Modafinil, Tablets, Hypersil C_{18} Column, RP-HPLC.

INTRODUCTION

Modafinil (The Merk Index, 2001) belongs to the class narcoleptics. It is chemically 2- [(diphenyl methyl)-Sulfinyl]acetamide. A literature survey reveals a few methods for quantification of modafinil and its acid metabolite in human plasma (Moachon and Matinier, 1994); determination of modafinil and its two metabolites in human plasma using solid-phase extraction (Burnat *et al.*, 1998); quantitative analysis of modafinil in plasma and urine (Schwertner *et al.*, 2005); method to separate and quantitate the enantiomers (d- and l-) of modafinil in human serum (Donovan *et al.*, 2003); determination of enantiomers of modafinil and its two major metabolites using a bi-dimensional HPLC system by coupling chiral column (Cass and Galatti, 2007); method for separation and determination of related substance, *viz*, sulphide, sulphoxide, sulphones, acid and ester derivatives of modafinil (Nageswara *et al.*, 2007); determination of optical isomers of modafinil and modafinil acid (YU and Chovan, 1999) etc. The present investigation by the authors describes a rapid, accurate, precise and specific RP-HPLC method for the determination of modafinil from bulk sample and pharmaceutical dosage form. It is not official in any of the pharmacopoeia. The detector responses were linear in the concentration range of 20-120µg/ml of drug. The method was validated as per ICH guidelines.

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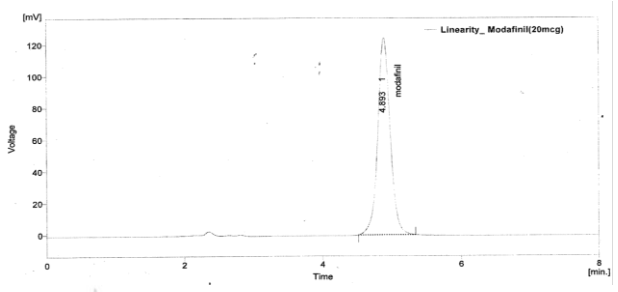
EXPERIMENTAL

Chromatographic Conditions

Shimadzu with high pressure liquid chromatographic instrument provided with a Hypersil ODS C_{18} column (250 mm x 4.6 mm; 5µ) and LC 20 AD Pump and Prominence SPD 20A UV-deuterium detector was employed in the study. A 20µL Hamilton injection syringe was used for sample injection. Data acquisition was performed by using Spinchrome software, Shimadzu Class VP version 6.12 SPS data system. HPLC grade water, methanol, acetonitrile were purchased from E. Merck Co., Mumbai, India, and Potassium dihydrogen ortho phosphate, dipo-



Result Table (Uncal - Linearity_ Modafinil(20mcg))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	4.893	1548.888	124.350	100.000
	Total	1548.888	124.350	100.000

Column Performance Table (From 50% - Linearity_ Modafinil(20mcg))

	Reten. Time	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Resolution [-]
1	4.893	0.187	1.036	3807	-

Figure 1: Chromatogram of Modafinil 20 µg/ml.

tassium hydrogen ortho phosphate AR grade were purchased from S D Fine Chem Limited, Mumbai, India.

Drug samples

The reference sample and branded formulation of modafinil was supplied by M/s Orchid Pharmaceuticals, Chennai, India.

Mobile phase

Accurately 1.36g of potassium dihydrogen phosphate was weighed out and dissolved in 550ml of water. 0.3g of dihydrogen potassium phosphate was weighed out and dissolved in 450ml of water. Both solutions were mixed to prepare buffer solution. The solution was filtered through 0.45µ membrane filter and was degassed. A freshly prepared binary mixtures of buffer:acetonitrile in a ratio of (55:45) V/V was used as the mobile phase. Methanol was used as diluent for preparing the working solution of the drug. The mobile phase was filtered through 0.05µ membrane filter and sonicated by using Power Sonicator, model no:405, Hwashin Technology, Korea before use. The flow rate of the mobile phase was maintained at 1ml/min. The column temperature was maintained at 25°C and the detection of the drug was carried out at 220nm.

Preparation of stock and working standard solution of modafinil

About 100mg of modafinil was weighed accurately and transfer in to 100ml volumetric flask the solution was sonicated and filter through Whatman filter paper, resulting solution was diluted with the mobile phase to get a working standard solution of 100 µg/ml of modafinil.

Linearity and construction of calibration curve

The quantitative determination of the drug was accomplished by a standard method. The column was equilibrated with the mobile phase for at least 30 min prior to the injection of the drug solution. Linearity of the peak area response was determined by taking measurement at six concentration prints (6 replicates at each point) working dilution of modafinil in the range of 20-120µg/ml were prepared by taking suitable aliquots of working standard solution in different 10ml volumetric flasks and diluting up to the mark with the mobile phase. Twenty micro lot quantity of the dilution was injected each time into the column at a flow rate of 1.0ml/min. Each dilution was injected 6 times in to the column. The drug in the elutes was monitored at 220 nm and the corresponding chromatograms were obtained. From these chromatograms the mean peak areas were calculated and a plot of concentration over the peak area was constructed. The regression of the plot was completed by least squares regression method. A linear relationship in the range was found to the 20-120 µg/ml of the drug between the concentration of modafinil and respective peak area. This regression equation was later used to estimate the amount of modafinil in pharmaceutical dosage form. A representative chromatogram for the separation of modafinil is given in figure 1.

Preparation of sample solution

Twenty tablets of modafinil were weighed and powdered uniformly in a mortar. An accurately weighed portion from this powder equivalent to 100mg of modafinil was transferred into 100ml volumetric flask. The contents of the flask were sonicated for about 15 min for complete solubility of the drug and the volume was made up to 100ml with mobile phase. Then the mixture was filtered through a 0.45µ membrane filter. From the above solution 1ml aliquot was taken into a separate 10ml volumetric flask and diluted up to the volume with

Table 1: Calibration data of the proposed method.

Concentration of Modafinil (µg/ml)	Mean peak area (n=6)	
20	1548.88	Concentration range
40	3122.309	(µg/ml) = 20-120
60	4346.061	Correlation coefficient
80	5727.505	(r^2) = 0.9991
100	7031.031	Slope (m) = 66.153
120	8188.787	Intercept (b) = 363.386

the mobile phase and mixed well. The above solution (20µL) was then injected six times into the column. The mean peak area of the drug content in the formulation was calculated by the regression equation of the calibration plot.

RESULTS AND DISCUSSION

The present study was aimed at developing a sensitive, precise and accurate HPLC method for the analysis of modafinil in bulk drug and in pharmaceutical dosage form and forced degradation. In order to achieve optimum separation of the component peaks, mixtures of acetonitrile with phosphate buffer in different combinations were tested as mobile phase on a C_{18} stationary phase. A binary mixture of buffer:acetonitrile in a proportion of 55:45 v/v was selected as the chromatographic peaks were well defined and resolved with no tailing. The retention time obtained for modafinil was 4.80±0.06 min. Each of the samples was injected six times and the sample retention times were observed in all cases. The peak areas of modafinil were reproducible as indicated by low coefficient of variation. A good linear relationship (r^2 = 0.9991) was observed between the concentration of modafinil and the respective peak areas. The regression curve was constructed by linear regression fitting and its mathematical expression was Y = 66.153X + 363.386 (Where Y gives peak area and X is the concentration of the drug). The regression characteristics are given in table 1. When modafinil solutions were analyzed by the proposed method for finding out intra and inter-day variation, low co-efficient of variation was observed. The absence of additional peaks indicated non-interference of common excipients used in the tablets.

Table 2: Accuracy data (Triplicate values at 80,100,120 percent levels).

*Amount taken (µg)	*Amount found (µg)	*Percent recovery	*Mean percentage recovery
90	90.033	100.03	100.03
110	109.48	99.527	99.527
130	129.085	99.296	99.296

*Each value is a mean of three readings

High recovery values obtained from the different dosage form by the proposed method indicates the method is accurate. The drug content in tablets was quantified using the proposed analytical method are given in table 2.

The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicated the robustness of the method. The robustness study results are presented in table 3. The lowest value of LOD and LOQ as obtained by the proposed method indicates the sensitivity of the method. The standard solution of the drug was stable up to 24 hrs as the difference in percent assay during the above period is within limit. System suitability parameters were studied with six replicates standard solution of the drug and the calculated parameters are within the acceptance criteria. The tailing factor, the number theoretical plates are in the acceptable limits. The system suitability results are shown in table 4.

The system precision was established by six repli-

Table 3: Robustness Study.

Variations	Chromatographic parameters		
	Tailing Factor	Theoretical Plates	Retention Time
Flow rate at 0.9ml/min	1.035	3411	5.388
pH of Mobile phase at 4.5	1.035	3411	5.630
Wave length at - 220nm	1.055	3645	4.873

Table 4: System Suitability Parameters.

Parameters	Value
Theoretical Plates (h)	3420
Tailing factor (T)	1.017
LOD (µg/ml)	2.356
LOQ (µg/ml)	7.140

Table 5: Forced Degradation.

Drug Name	Condition	Time	RT (min)	Area	% Degradation	% of Active drug Present after Degradation
Modafinil	Control Sample	00	4.986	7983.69	0.2%	99.8%
	Acid Degradation	24	4.980	7897.38	7.85%	92.15%
	Alkaline Degradation	24	4.980	6835.49	7.38%	92.62%
	Thermal Degradation	24	4.960	7181.54	4.55%	95.45%

cate injections of the standard solution containing analytes of interest. The value of relative standard deviation (0.666) was found to be within the limit, indicating the injection repeatability of the method. The method precision was established by carrying out the analysis six times using the proposed method. The relative standard deviation (0.662) was found to be within the limit, indicating the injection repeatability of the method.

The specificity of the HPLC method was determined by the complete separation of modafinil. When it was subjected to forced degradation as per ICH guidelines which was carried out with 0.1N HCL, 0.1N NaOH and Heat degradation at 105°C. The method does not permit detection of degradation product for modafinil. The results of specificity data for degradation study are given in table 5.

Hence it can be concluded that the proposed HPLC method is sensitive and reproducible for the analysis of modafinil in pharmaceutical dosage form with short analysis time of 8 min.

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