Research Article

A Validated Stability-Indicating HPLC assay method for Modafinil HCl in bulk drug

Vilas Chaudhary[1] and Milind Ubale[2*]

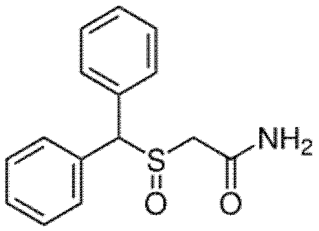
[1]Department of Chemistry, Yogeshwari Mahavidyalaya, Ambejogai, Maharashtra, India.
[2]PG Department of Chemistry, Vasantrao Naik Mahavidyalaya, Aurangabad, Maharashtra, India.

ABSTRACT
An isocratic reversed phase stability-indicating high-performance liquid chromatographic (HPLC) assay method was developed and validated for quantitative determination of Modafinil hydrochloride in bulk drugs. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, using an Thermo Hypersil C18 (250 x 4.6) mm, 5µ column and the mobile phase containing 2.0gm potassium dihydrogen phosphate and 1.0 gm 1-octaneSulfonic acid salt in 1000ml water filter and mixed. Prepare a homogenous mixture of buffer, and Acetonitrile (35:65, v/v). The detection was carried out at wavelength 210 nm. The developed method was validated with respect to linearity, accuracy (recovery), precision, system suitability, selectivity, robustness prove the stability indicating ability of the method.

Keywords: Modafinil hydrochloride, Stability Indicating Assay Methods, Validation.

INTRODUCTION

Modafinil is chemically 2-[(Diphenyl methyl)-sulfinyl] acetamide. It is α1-adrenergic agonist and is used for clinical evaluation in hypersomnia and narcolepsy. It is not official in any of the pharmacopoeia but is listed in the Merck Index and Martindal the complete drug reference.[1]



Chemical Structure of Modafinil

Literature Survey

Literature survey revealed the estimation of Modafinil by several techniques such as simultaneous estimation by HPLC [2] determination of Modafinil in human plasma by solid-phase[3] and liquid-liquid extraction by HPLC[4-5]by RP-HPLCtechniques[6-7] Determination of related substance in Modafinil and determination of Modafinil by a chiral chromatography[8], LC-MS [9,] Electro spray MS[10] and GC-MS[11]. The focus of present study was to develop and validate a rapid, stable and economic RP-HPLC method for the estimation of Modafinil in bulk and its formulation.

EXPERIMENTAL

Material and reagents

Modafinil hydrochloride bulk drug was made available from Ipca Ltd. India (purity 99.8). Sodium dihydrogen phosphate, 1-octaneSulfonic acid was obtained from Qualigens fine chemicals, India Limited. Acetonitrile and methanol were obtained from Rankem laboratories, India. All chemicals and reagent were used as HPLC grades; Milli-Q-Water was used throughout the experiment.

Chromatographic Conditions

A chromatographic system (Systronic) consisting of quaternary solvent delivery pump, a degasser, an auto- injector, column oven and UV detector. The chromatographic column of 250 mm length and internal diameter of 4.6 mm filled with Octadecyl silane Thermo Hypersil C18 stationary phase with particle size 5 micron and pore size 100A□ was used. The instrumental settings were a flow of 1 ml/min; the injection volume was 20 µl.and wavelength 210 nm.

Mobile Phase
The mobile phase containing 2.0 gm potassium dihydrogen phosphate and 1.0 gm 1-octane Sulfonic acid salt in 1000ml are mixed in water and filtered. Homogenous mixture of buffer, and Acetonitrile (35:65, v/v**)** were prepared

Preparation of Standard stock solutions
Standard stock solutions of 500 ppm of Modafinil hydrochloride in mobile phase were prepared in volumetric flasks.

Sample solution
500 ppm of Modafinil hydrochloride in 100ml calibrated flask containing mobile phase.The desired concentration for the drug was obtained by accurate dilution and the analysis was followed up as in the general analytical procedure [12-13]

Selectivity
Selectivity is the ability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include degradants, matrix etc. The selectivity of the developed LC method for Modafinil hydrochloride was carried out in the presence of its degradation products. Stress studies were performed for Modafinil hydrochloride bulk drug to provide an indication of the stability indicating property and selectivity of the proposed method. Intentional degradation was attempted to stress condition exposing it with acid (0.5 N Hydrochloric acid) fig-4, alkali (0.025N NaOH) fig-5, hydrogen peroxide (30%), heat (60 ^{o}C) to evaluate the ability of the proposed method to separate Modafinil hydrochloride from its degraded products. For heat study, study period was 7 days where as for acid, oxidation 48 hr and for base 2 hour. Assay studies were carried out for stress samples against Modafinil hydrochloride reference standard and the mass balance (% assay + % sum of all impurities + % sum of all degraded products) was calculated.

RESULTS AND DISCUSSION
Optimization of chromatographic conditions
The main target for the development of chromatographic method was to get the reliable method for the quantification of Modafinil hydrochloride from bulk drug and which will be also applicable for the degradable products. Initially, we took the effort for the development of HPLC method quantification of standard Modafinil hydrochloride from bulk. For this purpose, we have used Water nova pack C18(150X4.6)mm,5µ, Kromasil C18(150X4.6)mm,5µ, Inertsil ODS 3V C18(250X4.6)mm,5µ and Kromasil C18(250X4.6)mm,5µ,Star ODS-II C18 (250X4.6)mm,5µ and Grace Alpha C18 (250mm x 4.6)mm,5u Out of these used HPLC column, Thermo Hypersil C18 (250 x 4.6)mm,5u found to comparatively better and gave the graph with better gaussian shape at retention time 4.86 min. To improve the shape and width of the graph, for the above columns different solvents and buffer taken for trials such as 0.1M KH_2PO_4 and Acetonitrile (60:40,v/v) in these trials peak shape is not good,another trials 0.01M Ammonium acetate P^H-5.9 and acetonitrile(20:80,v/v) peak shape not found well, trials Acetonitrile and water (80:20, v/v) column temperature 35 ^{o}C peak shape not found good, trials K_2HPO_4,Methanol and water (10:70:20,v/v/v)column temperature 35 ^{o}C, trials 1.0gm KH_2PO_4 and 0.45gm 1-Hexa sulphonic acid sodium salt make P^H-3.5 Ortho phosphoric acid and methanol(25:75, v/v) peak shape obtained but retention is not good, finally try for the mobile phase containing 2.0gm potassium dihydrogen phosphate and 1.0 gm 1-octaneSulfonic acid salt in 1000ml waterfilter and mixed. Prepare a homogenous mixture of buffer, and acetonitrile (35:65, v/v).

Table 1: Summary of Forced degradation result

Stress condition	Time	Assay of active Substance %	Remarks
Acid Hydrolysis (0.5 N HCl)	48 Hrs	99.03	No Degradation
Base Hydrolysis (0.025 N NaOH)	2 Hrs	77.14	Degradation
Oxidation (30% H_2O_2)	48 Hrs	99.10	No Degradation
Thermal (80°C)	7 days	99.00	No Degradation
Photolytic degradation	1.2Lux million Hrs	97.45	negligible degradation

Method Validation

System suitability

For system suitability studies, five replicate injections of acid, base and oxidative degraded solutions were used and the RSD of peak area ratio, resolutions, tailing factor and number of theoretical plates of the peak were calculated. The system suitability results are shown in Table 2.

Table 2: System suitability reports

Compound (n=3)	Retention Time	% RSD	USP tailing	Theoretical plates
Modafinil HCl	4.86	0.87	1.00	6871

Precision

The precision of the method was studied by determining the concentrations of the drug Modafinil hydrochloride in the tablet for six times .[14] The results of the precision study (Table 3) indicate the reliability of the method (RSD %< 2)

Table 3: Results of the Linearity study and Precision

Ingredient	Precision (% RSD)	Linearity (µg/ml)	Slopes* (n= 3)	Coefficients of correlations
Modafinil HCl	0.84	80-120	2417.23	0.99950

*Standard deviation shown in parentheses

Accuracy (Recovery test)

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 80%, 100% and 120%. The recovery samples were prepared as aforementioned procedure. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for Modafinil hydrochloride ranged from 100.13% to 101.17% (Table 4). The average recoveries of three levels nine determinations for Modafinil hydrochloride were 100.24- 100.66%.

Table 4: Results of the Recovery Tests for the Modafinil HCl

Level of Addition (%)	Amount added (n = 3) (ppm)	% Recovery*	% Average recovery^
80	50	100.12	100.24
100	100	100.11	100.41
120	150	101.17	100.27

* RSD shown in parenthesis.

^ Average recovery = the average of three levels, nine determinations

Calibration and linearity

Linearity test solutions for the method were prepared from Modafinil hydrochloride stock solutions at six concentrations levels from tested from 80% to 120% of the targeted level of the assay concentration Modafinil hydrochloride. Standard solutions containing 80-120 µg/ml of Modafinil hydrochloride in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area verses the concentration data was treated by least-squares linear regression analysis, the calibration graphs were found to be linear in the mentioned concentrations the slopes and correlation coefficients are shown in Table –3.

Robustness

To determine the robustness of the developed method experimental condition were purposely altered and the resolution between Modafinil hydrochloride and acid degraded product were evaluated. The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution, it was changed by 0.2 unit from 0.8 to 1.2ml/min while the other mobile phase component were held as stated in chromatographic conditions. The effect of percent organic strength on resolution was studied by varying acetonitrile from –10 to +10 % while other mobile phase components were

held constant as stated in chromatographic condition. The effect of column temperature on resolution was studied at 25 and 35°C instead of 30°C while the other mobile phase components were held constant stated in chromatographic condition. The results are shown in table-5

Table 5: Results of robustness study

Sr. No.	Parameters	Variations	Resolutions between Modafinil HCl and base degraded product
1	Temperature	25 °C 35 °C	8.21 7.68
2	Flow rate	0.8 ml/min 1.2 ml/min	8.02 8.94
3	Mobile phase	40.5 ml 49.5 ml	3.7 3.3

LOD and LOQ (Sensitivity)

A series of solutions in the range 0.2–1.0% of the assay concentration (40 µg mL−1) were prepared by dilution of the standard solutions. Each solution (20 µL) was injected five times, the areas were measured for the drug peak, and the standard deviation for the five injections was calculated for each concentration. And the values were used for calculation of the LOD and LOQ. The results are shown in table-6

Table 6: Results of the LOD and LOQ

Name	%LOD	%LOQ
Modafinil HCl	0.17	0.26

Stability of analytical solution

The stability of the standard solutions and the sample solutions was tested at intervals of 24, 48 and 72 h. The stability of solutions was determined by comparing results of the assay of the freshly prepared standard solutions. The RSD for the assay results determined up to 72 h for Modafinil hydrochloride was 0.35 %. The assay values were within ± 2 % after 72 h. The results indicate that the solutions were stable for 72 h at ambient temperature.

CONCLUSION

The method developed for quantitative determination of Modafinil hydrochloride is rapid, precise, accurate and selective. The method was completely validated showing satisfactory data for all method-validated parameters tested. The developed method is stability indicating and can be used for assessing the stability of Modafinil hydrochloride as bulk drugs. The developed method can be conveniently used for the assay determination of Modafinil hydrochloride in bulk drugs and pharmaceutical dosage form.

ACKNOWLEGDEMENT

The authors are grateful to University Grant Commission (UGC) New Delhi for financial support and thankful to Ipca Ltd. India for gift samples of Modafinil hydrochloride

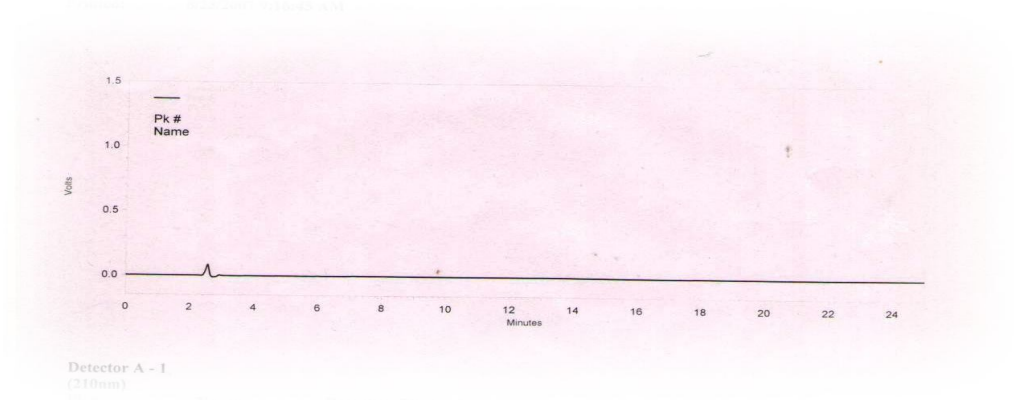


Fig. 1: A Typical Chromatogram of Modafinil Blank

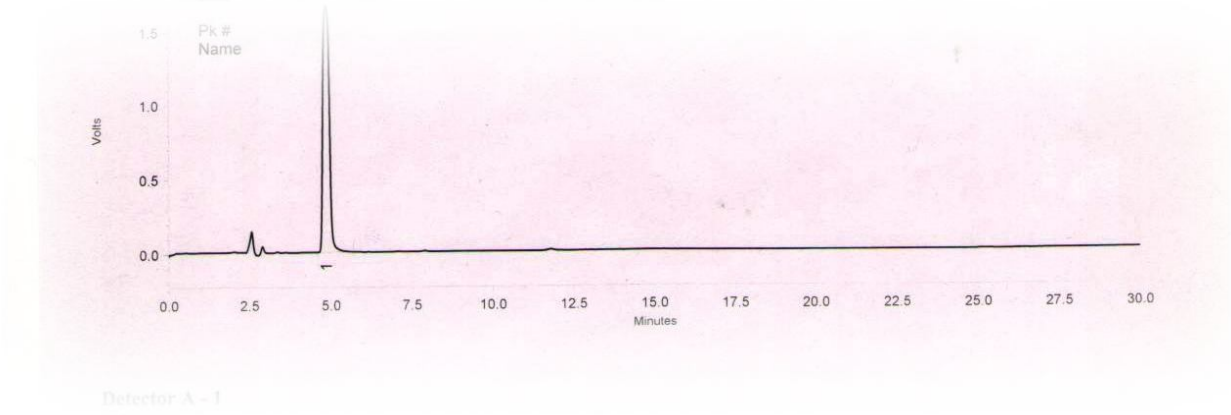


Fig. 2: A Typical Chromatogram of Modafinil Sample Preparation

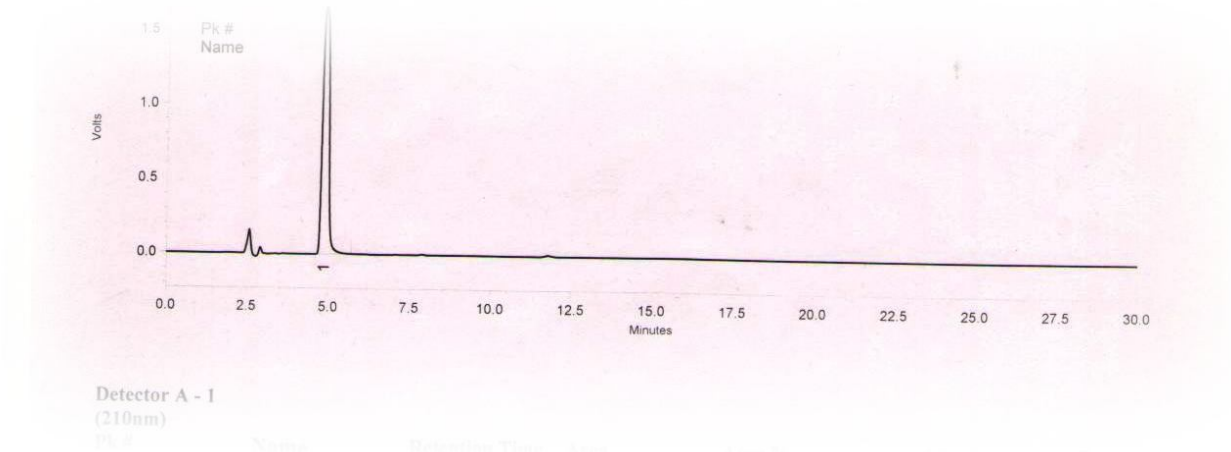


Fig. 3: A Typical Chromatogram of Modafinil Standard Preparation

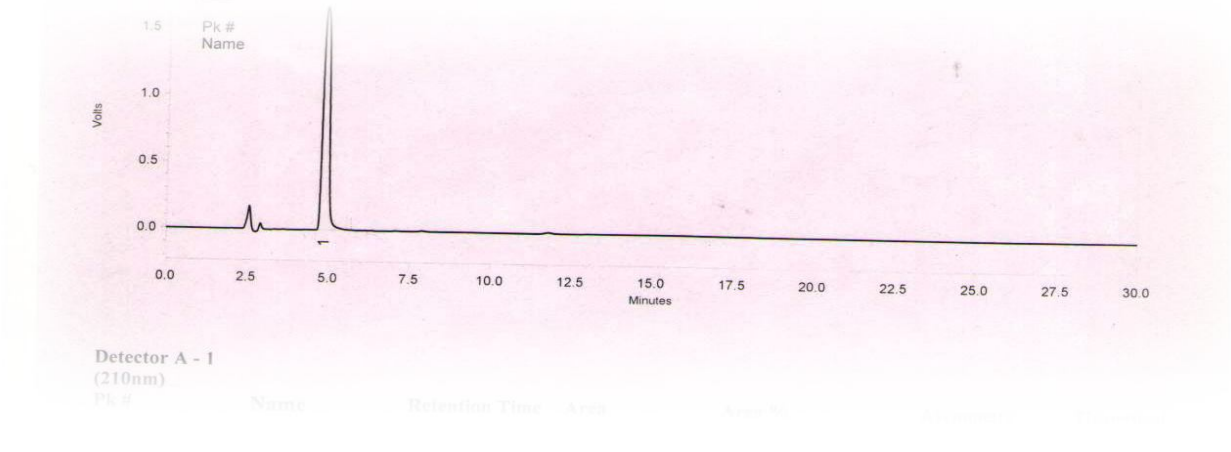


Fig. 4: A Typical Chromatogram of Modafinil Acid Degradation

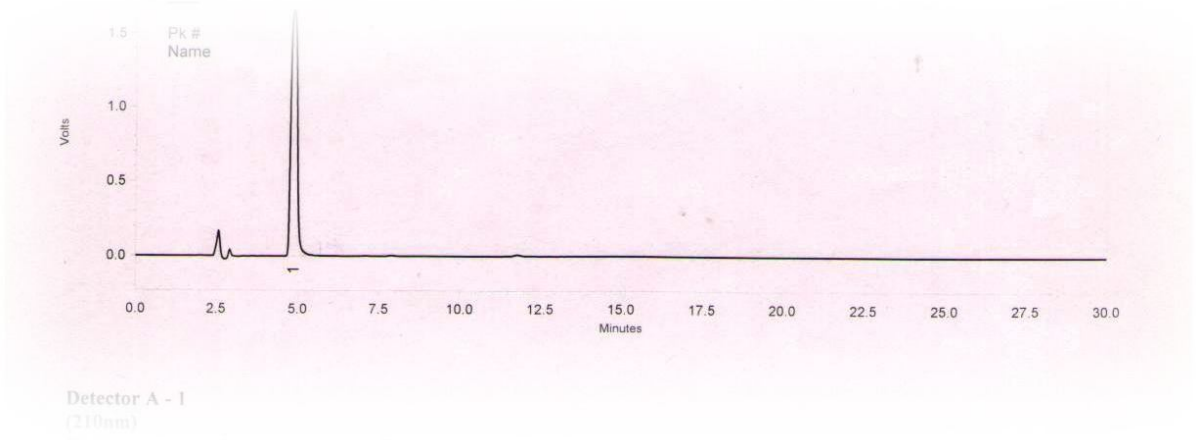


Fig. 5: A Typical Chromatogram of Modafinil Alkali Degradation

REFERENCES

1. Royal Pharmaceutical society of Great Britain, Martindale, Thirty-first Edition,1 Lambeth High Street London SE17 Jn England, The extra pharmacopoeia (1996) 435-36.
2. 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.
3. 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.
4. Gorman SH. Determination of modafinil, Modafinil acid and modafinil sulfone in human plasma utilizing liquid–liquid extraction and high-performance liquid chromatography. J Chromatogr B Anal Tech Biomed Life Sci. 2002; 767(2):269-276.
5. 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.
6. 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.
7. 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.
8. 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.
9. 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 ofChromatography B. 2008; 873(1): 119-123.
10. McKinney AR, Suann CJ and Stenhouse AM. A Method for the Detection of Modafinil and its Major Metabolite, ModafinilAcid, in Equine Urine by Solid-Phase Extraction and Positive Ion Electrospray Ionisation LiquidChromatography/Mass Spectrometry. RCM. 2005; 19(10): 1217-1220.

11. Tseng YL, Uralets V, Lin CT and Kuo FH. Detection and Quantification of Modafinil in Human Urine by Gas Chromatography–Mass Spectrometry (GC–MS). Journal of Pharmaceutical and Biomedical Analysis. 2005; 39(5):1042-1045.
12. U.S. Pharmacopoeial Convension Inc., 28th Review Rockville, MD, United States Pharmacopoeia (2005) 1196-1198.
13. ICH Q2B: Validation of Analytical Procedures: Methodology May 1997.
14. Validation of Compendial Methods, United States PharmacopeiaConvention: Rockville. United States Pharmacopeia (2002).