CHAPTER - II

2.1 LITERATURE REVIEW OF MODAFINIL

Moachon G, etal 19, 1994: A sensitive and selective high performance liquid chromatographic (HPLC) method for the simultaneous quantification of Modafinil and its acid metabolite in human plasma has been developed. The method is based on liquid-liquid extraction followed by isocratic reversed – phase HPLC with ultraviolet absorbance detection at 236 nm. The eluent used was acetonitrile: water: acetic acid (150:420:12 v/v/v). The run time was 45 min. The method provided a detection limit of 0.04 mg/L for Modafinil and acid metabolite, a quantization limit of 0.13 mg/L for Modafinil and 0.14 mg/L for acid metabolite. A good linear relationship was obtained in the concentration range studied (0.1-20 mg/L) for both compounds and the method was sufficiently accurate and precise to support clinical pharmacokinetic studies. To our knowledge this is the first described method for determination of Modafinil and its acid metabolite in plasma.

Burnat P, etal 20, 1998: A simple procedure for the simultaneous determination of Modafinil, its acid and sulfoxide metabolites in plasma is described. The assay involved an extraction of the drug, metabolites and internal standard from plasma with a solid-phase extraction using C₁₈ cartridges. These compounds were eluted by methanol. The extract was evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was redissolved in 250 μl of mobile-phase and a 30 μl aliquot was injected via an automatic sampler into the liquid chromatography and eluted with the mobile-phase (26%, v/v acetonitrile in 0.05 M orthophosphoric acid buffer adjusted to pH 2.6) at a flow-rate of 1.1 mL/min on a C8 Symmetry cartridge column (5 μm, 150 mm x 3.9 mm, Waters) at 25°C. The eluate was detected at 225 nm. Intra-day coefficients of variation ranged from 1.0 to 2.9% and inter-day coefficients from 0.9 to 6.1%. The limits of detection and quantization of the assay were 0.01 μg/ml and 0.10 μg/ml respectively.
Gorman SH, 1999: Modafinil, DL-2-[(diphenylmethyl) sulfinyl] acetamide (Provigil), which is chiral at its sulfur atom, is a novel wake-promoting agent currently being developed as the race mate in the United States by Cephalon, Inc. In order to characterize the pharmacokinetic properties of each enantiomer, a stereo specific high-performance liquid chromatography (HPLC) method has been developed for simultaneous determination of D- and L-Modafinil in human plasma. The analytes are extracted from plasma into a mixture of hexane-methylene chloride-triethylamine (55:45:2, v/v/v) and then resolved on an EM Separations ChiraDex beta-cyclodextrin column at 12°C using an isocratic mobile phase of 0.020 M, pH 3.0 phosphate buffer-acetonitrile (84:14, v/v). D- and L-Modafinil, and the internal standard, 3, 3-diphenylpropylamine, are monitored by UV detection at 225 nm. The two major circulating metabolites, Modafinil acid and Modafinil sulphone, have been shown not to interfere with the assay. Using 0.200 ml of plasma for extraction, the quantifiable range of the assay is 0.100 to 15.0 µg/ml for each enantiomer. The utility of the assay for the characterization of D- and L-Modafinil pharmacokinetics in humans after single and multiple oral doses of racemic Modafinil have been demonstrated.

Schwertner, et al, 2005: Modafinil (provigil) is a new wake-promoting drug that is being used for the management of excessive sleepiness in patients with narcolepsy. It has pharmacological properties similar to that of amphetamine, but without some of the side effects associated with amphetamine-like stimulants. Since Modafinil has the potential to be abused, accurate drug screening methods are needed for its analysis. In this study we developed a high performance liquid chromatography procedure (HPLC) for the quantitative analysis of Modafinil in plasma and urine (phenylthio) acetic acid was used as an internal standard for the analysis of both plasma and urine. Modafinil was extended from urine and plasma with ethyl acetate and ethyl acetate-acetic acid (100:1v/v), respectively and analysed on a C18 reverse phase column with methanol: water: acetic acid (500:500:1v/v) as the mobile phase. Recoveries from urine and plasma were 80.0 and 98.9%, respectively and the limit of quantization was 0.1@ mg/l at 233nm. Forty-eight 2-h post-dose urine samples from sham controls and from individuals taking 200 or 400mg of Modafinil...
were analyzed without knowledge of drug administration. All 16-placebo urine samples and all 32 2-h post-dose urine samples were correctly classified. The analytical procedure is accurate and reproducible and can be used for therapeutic drug monitoring, drug abuse screening and Pharmaco kinetic studies.

R.NageswaraRao, et al\textsuperscript{23}, 2007: A reversed-phase high-performance liquid chromatographic (RP-HPLC) method for determination and evaluation of purity of Modafinil in bulk drugs using Kromasil C\textsubscript{18} column with Acetonitrile: 0.02 M ammonium acetate as a mobile phase in gradient elution mode at 30\degree C and detection at 225 nm using photodiode array detector has been developed. The effects of pH, temperature and the percent of organic modifier on resolution were studied. Related substances, viz, sulphide, sulphoxide, sulphones of the Modafinil, acid and ester derivatives, were separated and quantified. The method was found to be simple, rapid, selective and capable of detecting all process related impurities at trace levels in the finished products of Modafinil with detection limits of 0.6-2.4 \times 10^{-8} g. the method was validated with respect to accuracy, precision, linearity, ruggedness, and limits of detection and quantification. It was found to be suitable not only for monitoring the reactions during the process development but also quality assurance of Modafinil.

Quezia B. Cass, et al\textsuperscript{24}, 2007: Coupled-column using restricted access media as the first dimension in order to exclude macromolecules and retain micro molecules has been successfully used for a number of biological fluids. This paper describes the first method developed and validated for the analysis in a single run of the enantiomers of Modafinil and its two major metabolites. The method was developed using a bidimensional HPLC system by coupling a restricted access medium (RAM) bovine serum albumin (BSA) column (1.0 cm \times 0.46 cm i.d.) to an amylase tris[(S)-1-phenylethylcarbamate] chiral column. The method was fully validated and showed good linearity, precision, accuracy, sensitivity and selectivity, allowing it to be used for pharmacokinetic studies. The quality of the performance of both columns was maintained with over 280 plasma injections of 100\mu l.
2.2 DRUG PROFILE OF MODAFINIL

a. Chemical structure:

![Chemical structure of Modafinil](image)

b. Nomenclature : 2-(diphenylmethane) sulfinylacetamide.

c. Molecular Formula : C₁₅H₁₅NO₂S.

d. Molecular weight : 273.35g.mol⁻¹.

e. Therapeutic category : Central nervous system stimulant used in the treatment of narcolepsy.

f. Solubility : It is a white to off-white, crystalline powder that is practically insoluble in water and cyclohexane. It is sparingly to slightly soluble in methanol and acetone.

g. Adverse effects : Back pain, headache, nausea, nervousness, stuffy nose, dizziness, diarrhea, anxiety, anxious, stomach upset.

h. Storage Conditions : Store at room temperature between 20°C and 25°C.

i. Formulations Available: modafil, modalert, madapro, modatec.
2.3 EXPERIMENTAL DETAILS OF MODAFINIL

2.3.1 INSTRUMENTS AND COLUMNS

- Shimadzu high pressure liquid chromatograph provided with a LC 20 AD Pump and Prominence SPD 20A UV-deuterium lamp detector.
- Data acquisition was performed by using Spin chrome software, Shimadzu Class VP version 6.12 SPS data system.
- Power Sonicator, model no: 405, Hwashin Technology, Korea.
- The column used in the development for determination is Hypersil ODS C₁₈ (250 mm x 4.6 mm; 5 µ).

2.3.2 CHEMICALS USED

- HPLC grade water, methanol and acetonitrile were purchased from E.Merck Co., Mumbai, India, and Potassium dihydrogen ortho phosphate and dihydrogen potassium phosphate AR grade were purchased from SD Fine Chem. Limited, Mumbai, India.

- The reference sample of Modafinil supplied by M/s Orchid chemicals and Pharmaceuticals Ltd, Chennai, India, and branded formulation purchased from local market.

2.3.3 SELECTION OF CHROMATOGRAPHIC METHOD

Selection of chromatographic method in general is done taking into consideration of several parameters like the nature of the drugs, molecular weight and solubility. Since the drugs selected are polar in nature, RP-HPLC was selected for initial chromatographic condition because of its simplicity and suitability.

2.3.4 SELECTION OF WAVE LENGTH ($\lambda_{max}$)

An ideal wavelength is one that uses good response for the drugs to be detected Modafinil in diluents the spectra was scanned on UV- visible spectrophotometer in the range of 200 nm to 400 nm against diluents as blank. The maximum absorbance of Modafinil was found to be 220 nm.
2.3.5 PREPARATION OF MOBILE PHASE

Weighed accurately 1.36gms of potassium dihydrogen phosphate and dissolved in 550 mL of water and 0.3 gms of dihydrogen potassium phosphate was weighed and dissolves in 450 mL of water. Mix both solutions adjust pH to 4.5. The buffer solution was filtered through 0.45µ membrane filter and degassed. A freshly prepared binary mixture of buffer: acetonitrile in a ratio of (55:45) V/V was used as the mobile phase and also methanol which 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 25°C was maintained the detection of the drug was carried out at 220 nm.

2.3.6 PREPARATION OF STOCK AND WORKING STANDARD SOLUTION

Weighed accurately about 100 mg of Modafinil and transfer in to 100 mL volumetric flask the solution was sonicated and filter through what’s man filter paper, resulting solution was diluted with the mobile phase.

Standard preparation: Transfer 10 mL of standard stock solution into 100 mL volumetric flask and dilute to volume with diluent.

2.3.7 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 100 mg of Modafinil was transferred into 100 mL 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 100 mL 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 10 mL volumetric flask and diluted up to the volume with the mobile phase and mixed well.
2.3.8 CHROMATOGRAPHIC CONDITIONS

Carry out the method for HPLC, using the following conditions.

Column : HYPERSIL ODS C<sub>18</sub>, 250mm X 4.6 mm, 5µ,
Flow rate : 1.0 mL/min
Wavelength : 220 nm
Column temperature : 25°C
Injection volume : 20 µL
Run time : 10 Minutes
Diluent : Mobile phase
Elution : Isocratic
Needle wash : Water: Acetonitrile 90:10 (v/v)

2.4 VALIDATED RP-HPLC METHOD FOR MODAFINIL

2.4.1 ACCURACY

For accuracy determination, three different concentrations were prepared separately i.e.80%, 100%, and 120% of analyte and the chromatograms were recorded for the same. The results obtained for recovery were found to be within the limits. Hence the proposed method was found to be accurate and precise.

Acceptance criteria: The % recovery of Modafinil at each spike level should be in between 98.0% - 102.0%.
Table no: 2.4.01 Accuracy Data for Modafinil

<table>
<thead>
<tr>
<th>S No</th>
<th>Accuracy 80% (µV² Sec)</th>
<th>Accuracy 100% (µV² Sec)</th>
<th>Accuracy 120% (µV² Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection-1</td>
<td>6722.809</td>
<td>8260.229</td>
<td>9643.708</td>
</tr>
<tr>
<td>Injection-2</td>
<td>6803.336</td>
<td>8179.889</td>
<td>9695.007</td>
</tr>
<tr>
<td>Injection-3</td>
<td>6703.336</td>
<td>8158.953</td>
<td>9665.502</td>
</tr>
<tr>
<td>Average</td>
<td>6743.16</td>
<td>8199.69</td>
<td>9668.072</td>
</tr>
</tbody>
</table>

*Amount added (µg/mL)*

- 90
- 110
- 130

*Amount Recovered (µg/mL)*

- 90.033
- 109.48
- 129.00

% *Recovered*

- 100.04
- 99.53
- 99.30

Over all mean of three levels % recovery

- 99.62

*Each value is a mean of three readings*

Figure no: 2.4.1.1 Chromatogram of Modafinil accuracy - 80%
Figure no: 2.4.1.2 Chromatogram of Modafinil accuracy - 100%

Figure no: 2.4.1.3 Chromatogram of Modafinil accuracy - 120%
2.4.2 PRECISION

The precision of an analytical procedure express the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed in terms of variance, standard deviation, coefficient of variation of a series of measurement.

SYSTEM PRECISION

System precision was determined by injecting six homogenous preparation solutions into HPLC System Concentration 100 µg/mL. The mean, standard deviation and % RSD for peak areas of Modafinil from standard solutions were calculated. The % RSD Modafinil was found to be below 1. Hence the method is said to be Precise.

Acceptance criteria: The % relative standard deviation (RSD) of Modafinil peak areas should not be more than 2
<table>
<thead>
<tr>
<th>S No</th>
<th>Name</th>
<th>RT (min)</th>
<th>Area(μV² Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Injection-1</td>
<td>4.880</td>
<td>7053.235</td>
</tr>
<tr>
<td>2</td>
<td>Injection-2</td>
<td>4.887</td>
<td>7060.875</td>
</tr>
<tr>
<td>3</td>
<td>Injection-3</td>
<td>4.890</td>
<td>7060.556</td>
</tr>
<tr>
<td>4</td>
<td>Injection-4</td>
<td>4.887</td>
<td>7163.569</td>
</tr>
<tr>
<td>5</td>
<td>Injection-5</td>
<td>4.880</td>
<td>7111.111</td>
</tr>
<tr>
<td>6</td>
<td>Injection-6</td>
<td>4.887</td>
<td>7060.875</td>
</tr>
<tr>
<td></td>
<td><strong>Average</strong></td>
<td>4.880</td>
<td>7089.869</td>
</tr>
</tbody>
</table>

Standard Deviation: 0.00455  47.23576

% RSD: 0.093  0.666

Figure no: 2.4.2.5 Chromatogram of Modafinil system precision
2.4.3 METHOD PRECISION

Method precision was determined by injecting six sample solutions of Single batch were analysed as per test method Concentration 100µg/mL. The mean, standard
deviation and % RSD for peak areas of Modafinil from sample solutions were
calculated. The % RSD Modafinil was found to be below 1. Hence the method is said
to be Precise for the estimation of Modafinil.

Acceptance criteria: The % relative standard deviation (RSD) of Modafinil peak
areas should not be more than 2.

Table no: 2.4.03 Method precision data for Modafinil

<table>
<thead>
<tr>
<th>S No</th>
<th>Name</th>
<th>RT (min)</th>
<th>Area(µV² Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Solution-1</td>
<td>4.883</td>
<td>7114.654</td>
</tr>
<tr>
<td>2</td>
<td>Solution-2</td>
<td>4.883</td>
<td>7121.672</td>
</tr>
<tr>
<td>3</td>
<td>Solution-3</td>
<td>4.873</td>
<td>7125.057</td>
</tr>
<tr>
<td>4</td>
<td>Solution-4</td>
<td>4.890</td>
<td>7055.016</td>
</tr>
<tr>
<td>5</td>
<td>Solution-5</td>
<td>4.887</td>
<td>7015.902</td>
</tr>
<tr>
<td>6</td>
<td>Solution-6</td>
<td>4.883</td>
<td>7121.672</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>4.8832</td>
<td>7086.46</td>
</tr>
</tbody>
</table>

Standard Deviation

<table>
<thead>
<tr>
<th>% RSD</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.132</td>
<td>0.688</td>
</tr>
</tbody>
</table>

Figure no: 2.4.3.6 Chromatogram of Modafinil Method precision
2.4.4 LINEARITY

Linearity for Modafinil was determined in the range of 20μg/mL - 120 μg/mL. A graph was plotted with concentration on X-axis and peak area on Y-axis and correlation coefficient was determined. The method was linear from the concentration of 20μg/mL- 120μg/mL for the estimation of Modafinil.

Acceptance criteria: The correlation coefficient (CC) value should not be less than 0.9999

Table no: 2.4.04 Linearity data of the Modafinil

<table>
<thead>
<tr>
<th>Concentration of Modafinil (μg/mL)</th>
<th>peak area (μV² Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1548.88</td>
</tr>
<tr>
<td>40</td>
<td>3122.309</td>
</tr>
<tr>
<td>60</td>
<td>4346.061</td>
</tr>
<tr>
<td>80</td>
<td>5727.505</td>
</tr>
<tr>
<td>100</td>
<td>7031.031</td>
</tr>
<tr>
<td>120</td>
<td>8188.787</td>
</tr>
</tbody>
</table>

Concentration range (μg/mL) = 20 – 120
Correlation coefficient (r²) = 0.9991
Slope (m) = 66.153
Intercept (b) = 363.386

Figure no: 2.4.4.7 Calibration curve for Modafinil
Figure no: 2.4.4.8 Chromatogram of Modafinil Linearity - 20µg/mL

Figure no: 2.4.4.9 Chromatogram of Modafinil Linearity - 40µg/mL
Figure no: 2.4.4.10 Chromatogram of Modafinil Linearity - 60µg/mL

Figure no: 2.4.4.11 Chromatogram of Modafinil Linearity - 80µg/mL
Figure no: 2.4.4.12 Chromatogram of Modafinil Linearity - 100µg/mL

Figure no: 2.4.4.13 Chromatogram of Modafinil Linearity - 120µg/mL
2.4.5 RUGGEDNESS (INTERMEDIATE PRECISION)

The Ruggedness of the method has been verified by analysing the six samples of the same batch for method precision as per test method by different analyst using different instrument, different days. The analyst’s prepared six samples of the same batch by two different analysts. Calculated % RSD for two different analysts in six samples for ruggedness results with the method precision.

Acceptance criteria: The %RSD for two different analysts in six samples should not be more than 2.

Table no: 2.4.05 Ruggedness data for Modafinil

<table>
<thead>
<tr>
<th>S No</th>
<th>Name</th>
<th>Analyst-1</th>
<th>Analyst-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RT (min)</td>
<td>Area (μV² Sec)</td>
</tr>
<tr>
<td>1</td>
<td>Ruggedness-1</td>
<td>4.953</td>
<td>8232</td>
</tr>
<tr>
<td>2</td>
<td>Ruggedness-2</td>
<td>4.956</td>
<td>8236</td>
</tr>
<tr>
<td>3</td>
<td>Ruggedness-3</td>
<td>4.952</td>
<td>8230</td>
</tr>
<tr>
<td>4</td>
<td>Ruggedness-4</td>
<td>4.953</td>
<td>8238</td>
</tr>
<tr>
<td>5</td>
<td>Ruggedness-5</td>
<td>4.952</td>
<td>8234</td>
</tr>
<tr>
<td>6</td>
<td>Ruggedness-6</td>
<td>4.958</td>
<td>8242</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>4.954</td>
<td>8235</td>
</tr>
<tr>
<td></td>
<td>Standard Deviation</td>
<td>0.0024</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>% RSD</td>
<td>0.0494</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Figure no: 2.4.5.14 Chromatogram of Modafinil Ruggedness Analyst -1

Figure no: 2.4.5.15 Chromatogram of Modafinil Ruggedness Analyst -2
2.4.6 ROBUSTNESS

To evaluate the robustness, the following small deliberate variations are made in the method and analysed the sample in triplicate.

1. Flow rate (±10%)
2. Wave length (±2 nm)
3. pH of Mobile phase

The system suitability was evaluated in each condition and compared the results with method precision results. The method is robust for change in flow rate, wave length and buffer variation.

Table no: 2.4.06 Robustness data for Modafinil

<table>
<thead>
<tr>
<th>Variations</th>
<th>Chromatographic parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tailing Factor</td>
</tr>
<tr>
<td>1. Flow rate at 0.9 mL/min</td>
<td>1.035</td>
</tr>
<tr>
<td>2. Flow rate at 1.1 mL/min</td>
<td>1.012</td>
</tr>
<tr>
<td>1. pH of Mobile phase at 4.3</td>
<td>1.092</td>
</tr>
<tr>
<td>2. pH of Mobile phase at 4.7</td>
<td>1.006</td>
</tr>
<tr>
<td>1. Wave length at 218 nm</td>
<td>1.012</td>
</tr>
<tr>
<td>2. Wave length at 222 nm</td>
<td>1.055</td>
</tr>
</tbody>
</table>

2.4.7 SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components that might be expected to be present, such as impurities, degradation products, and matrix components.

Procedure: Separately injected mobile phase, standard preparation, sample solution into the HPLC system to examine peak is not affected by mobile phase.

Specificity by degradation studies: To confirm that during stability study or throughout the shelf life, any degradation product if found will not interfere with
the main peak of Modafinil. Also the forced degradation study will help to identify the type of degradation (whether alkali hydrolysis, acid hydrolysis and dry heat) for each of the degradants.

Forced Degradation study was carried out by treating the sample under the following conditions. Twenty tablets of Modafinil were weighed and powdered uniformly in a mortar. An accurately weighed portion from this powder equivalent to 100 mg of Modafinil was transferred into 100 mL 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 100 mL with mobile phase. Then the mixture was filtered through a 0.45µ membrane filter.

Acid degradation: 10 mL of the above stock solution was transferred into 100 mL volumetric flask, and added 50 mL of diluent with intermediate shaking for 15 min. To this flask 5 mL of 0.1N HCl was added and sonicated for 30 minutes neutralized with 5mL of 0.1N NaOH and diluted to volume with diluent and was analysed as per the test method.

Alkali degradation: 10 mL of the above stock solution was transferred into 100 mL volumetric flask, and added 50 mL of diluent with intermediate shaking for 15 min. To this flask 5 mL of 0.1N NaOH was added and sonicated for 30 minutes neutralized with 5 mL of 0.1N HCL and diluted to volume with diluent and was analysed as per the test method.

Thermal degradation: The Drug substance was taken in Petri dish and exposed to a temperature of 105°C for 24 hrs. Then the sample was taken and diluted with the diluent for further analysis. Treated sample was analysed as per the test method.

Acceptance criteria: Chromatogram of degradants should not show any peak at the retention time of analyte peak. There is no interference due to degradants at the retention time of analysis.

Observation: No peaks are observed at the time of retention time of Modafinil.

Conclusion: The method was found to be specific.
Table no: 2.4.07 Forced Degradation data for Modafinil

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Condition</th>
<th>Time (hrs)</th>
<th>RT (min)</th>
<th>Area (μV² Sec)</th>
<th>% Degradation</th>
<th>% of Active drug Present after Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MODAFINIL</td>
<td>Acid Degradation</td>
<td>24</td>
<td>4.980</td>
<td>7897.38</td>
<td>7.85</td>
<td>92.15</td>
</tr>
<tr>
<td>MODAFINIL</td>
<td>Alkaline Degradation</td>
<td>24</td>
<td>4.980</td>
<td>6835.49</td>
<td>7.38</td>
<td>92.62</td>
</tr>
<tr>
<td>MODAFINIL</td>
<td>Thermal Degradation</td>
<td>24</td>
<td>4.960</td>
<td>7181.54</td>
<td>4.55</td>
<td>95.45</td>
</tr>
</tbody>
</table>

Figure no: 2.4.7.16 Chromatogram of Modafinil Forced Degradation specificity - Acid
Figure no: 2.4.7.17 Chromatogram of Modafinil Forced
Degradation specificity - Alkaline

Figure no: 2.4.7.18 Chromatogram of Modafinil Forced
Degradation specificity - Thermal
2.4.8 ANALYSIS OF MARKETED FORMULATIONS

The fixed chromatographic conditions were applied for the estimation of MODAFINIL (Modfil-100 mg) formulation by RP-HPLC method. Twenty tablets of Modafinil were weighed and powdered uniformly in a mortar. An accurately weighed portion from this powder equivalent to 100 mg of Modafinil was transferred into 100 mL 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 100 mL with mobile phase. Then the mixture was filtered through a 0.45μ membrane. From the above solution 10 mL aliquot was taken into a separate 100 mL volumetric flask and diluted up to the volume with the mobile phase and mixed well. Initially inject 20μL of blank solution, placebo solution and sample solution. Disregard the peaks due to blank and placebo if any.

RECORDING OF CHROMATOGRAMS

The standard solutions stabilize the system until stable baseline is obtained. Initially inject the blank solution and placebo. The standard chromatograms were recorded by injected standard solutions and the peak areas of standard chromatograms were noted. A calibration graph was plotted using peak area Vs concentration. Then the sample solution was injected and the amount of Modafinil present in the formulation was calculated from the calibration curve. The amount of Modafinil present in per tablet was found to be 99.80 ± 0.065 mg. Total label claim for MODFIL formulation was 100 mg.

Table no: 2.4.08 Analysis of marketed formulation (Assay) data for Modafinil

<table>
<thead>
<tr>
<th>Drug</th>
<th>Quantity claim (mg/tablet)</th>
<th>*Quantity found (mg/tablet) ± SD</th>
<th>* % Assay found ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modafinil</td>
<td>100</td>
<td>99.8 ± 0.065</td>
<td>99.8 ± 0.065</td>
</tr>
</tbody>
</table>

*Each value is a mean of three readings
Figure no: 2.4.8.19 Chromatogram of Modafinil Assay - Sample

Figure no: 2.4.8.20 Chromatogram of Modafinil Assay - Standard
2.4.9 LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

The LOD and LOQ of the developed method were determined by analyzing progressively low concentration of the standard solutions using the developed methods. The LOD is the concentration of the analyte that gives a measurable response (Signal to noise ratio 3.3). The LOD of Modafinil was found to be 2.356 µg/mL. LOQ is the lowest concentration of the analyze which gives repose that can be accurately quantified (Signal to noise ratio 10). The LOQ of the Modafinil was found to be 7.140 µg/mL.

2.5 RESULTS AND DISCUSSION OF MODAFINIL

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 C18 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 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 system precision was established by six replicate injections of the standard solutions containing analytes of interest. The value of relative standard deviation was found to be 0.666 within the limit, indicating the injection repeatability of the method. The method precision was established by carrying out the analyte six times using the proposed method. The relative standard deviation was found to be 0.688 within the limit, indicating the injection repeatability of the method. The lowest value of LOD and LOQ as obtained by the proposed method indicates the sensitivity of the method.
For accuracy determination, three different concentrations were prepared separately i.e. 80%, 100%, and 120% of analyte and the chromatograms were recorded for the same. The results obtained for recovery were found to be within the limits. Hence the proposed method was found to be accurate and precise. Six samples of the same batch were prepared by two different analysts. Calculated % RSD for two different analysts in six samples for ruggedness (Intermediate precision) results with the method precision. The system suitability was evaluated in each condition and compared the results with method precision results. The method is robust for change in flow rate, wave length and buffer variation of mobile phase πH.

To confirm that during stability study or throughout the shelf life, any degradation product if found will not interfere with the main peak of Modafinil. Also the forced degradation study will help to identify the type of degradation (whether alkali hydrolysis, acid hydrolysis and thermal) for each of the degradants. No peaks are observed at the time of retention time of Modafinil. The method was found to be specific. The formulation was calculated from the calibration curve. The amount of Modafinil present in per tablet was found to be 99.80 ± 0.065 mg. Total label claim for MODFIL formulation was 100 mg.