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DEVELOPMENT AND VALIDATION OF RP-LC METHOD FOR ARMODAFINIL IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid Chromatography assay method has been developed for estimation of Armodafinil in tablet formulations. The separation was achieved by using column Delvosil ODS –UG-5 C18 (250×4.6 mm, 5µ), in mobile phase consisted of acetonitrile and pH 2.5 phosphate buffer, adjusted to pH 2.5 with the help of dilute orthophosphoric acid in the ratio of (60:40, v/v). The flow rate was 1.2 mL/min-1 and the separated Armodafinil was detected using UV detector at the wavelength of 220 nm. Column temperature 35°C and sample temperature ambient and injection volume 10µl. The retention time of Armodafinil, was noted to be 4.45 min respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

KEYWORDS: Liquid Chromatography; Armodafinil, Validation.

1.0 INTRODUCTION

Armodafinil is a wakefulness-promoting agent for oral administration. Armodafinil is the R-enantiomer of Modafinil which is a mixture of the R and S enantiomers. Chemically, it is 2-[(R)-(diphenyl methyl) sulfinyl] acetamide with molecular formula C15H15NO2S. Armodafinil is used for the treatment of narcolepsy and shift work sleep disorder, and as an adjunctive treatment for obstructive sleep apnea.[1] Armodafinil is mostly metabolized by Hydrolytic deamidation, S-oxidation and aromatic ring hydroxylation, subsequent glucuronide conjugation of the hydroxylated products.

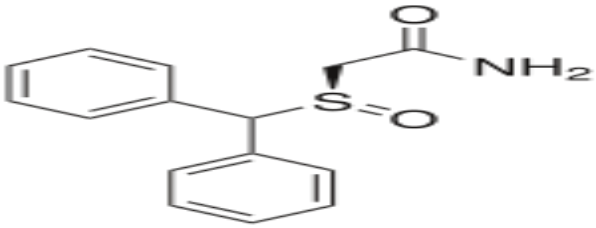

Fig.1.1 The structure of Armodafinil

Literature survey reveals that few analytical methods have been reported for the estimation of Armodafinil such as UV.[2] HPLC.[3] Chiral chromatography.[4], LCMS/MS.[5], Capillary electrophoresis.[6] but Modafinil revealed several methods based on different technique, such as; HPLC with UV detection.[7], LCMS.[8], GC-MS.[9], HPLC with UV detection assay for its quantification in plasma and serum.[10-14] and Chiral Chromatography.[15]

The aim of present work is to develop a simple, specific, sensitive, accurate and stability indicating HPLC analytical procedure for the analysis of Armodafinil and validated as per ICH guidelines.[16]

2.0 EXPERIMENTAL

2.1. Chemicals and Reagents

Analytical-grade Potassium dihydrogen phosphate, ortho phosphoric acid, were from Merck Chemicals Mumbai, India. Acetonitrile, Methanol and Water, both HPLC-grades, were from Merck Chemicals. Mumbai, India. Millex syringe filters (0.45 µm) were from Millex-HN, Millipore Mumbai, and India.

2.2. Instrumentation

Waters 2489 U.V-Visible detector/2695 Separation Module, equipped with Empower 2 software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) Centrifuge Eppendorf 5810 were use in the present assay.

2.3 Preparation phos phate Buffer pH 2.5: About 6.85 g of Potassium dihydrogen phosphate dissolved in 1000.0 mL of Milli-Q water and adjust the pH to 2.50±0.05 with ortho phosphoric acid. The solution was filtered through 0.45µ filter paper and degassed.

2.4 Mobile phase preparation

600 volumes of buffer and 400 volumes of filtered and degassed acetonitrile were mixed and sonicated.

2.5 Diluent preparation

650mL of water, 350mL of Acetonitrile, and 10mL of acetic acid were mixed and sonicated to degas.

2.6 Standard preparation

Accurately weighed and transferred about 25.0 mg of Armodafinil working standard into 100mL volumetric flask and about 5mL of Acetonitrile is added and sonicated to dissolve for 2 min. Dilute to the volume with diluent and mix well. Further dilute 5.0mL of the above solution into 25mL volumetric flask with diluent and mixed well (50µg/mL)

2.7 Sample preparation

Weighed accurately 5 tablets and directly transfer into a 500mL volumetric flask then added about 300mL of diluent. The solution was sonicated for 20 minutes with intermediate shaking. Maintained the sonicated bath temperature below 25°C throughout the sonication and centrifuge the solution at about 4000 rpm for 10 min. Pipette out 2mL of above solution into 100mL volumetric flask and dilute to volume with diluent and mixed well. The sample solution was filtered through 0.45µ nylon filter and inject into HPLC system.

2.8 Chromatographic conditions

Chromatographic analysis was performed on Delvosil ODS UG-5 C_{18}. 250x4.6 mm, 5µ (Make: Thermo) column. The mobile phase consisted of pH 2.5 phosphate buffer and Acetonitrile in the ratio of 60:40%v/v. The flow rate was 1.2 mL/min, column oven temperature 35°C, the injection volume was 10 µL, and detection was performed at 220 nm using a photodiode array detector (PDA).

3.0 RESULTS AND DISCUSSION

Method development

Spectroscopic analysis of compound Armodafinil showed that maximum UV absorbance (λmax) at 220 nm respectively. To develop a suitable and robust LC method for the determination of Armodafinil, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Agilent Zorbax AQ C_{18} with the following different mobile phase compositions like that Buffer and acetonitrile in the ratio of 40:50 v/v 50:50 v/v & 55:45. It was observed that when Armodafinil was injected, Peak Tailing, not satisfactory.

For next trial Delvosil ODS UG-5 C_{18}. 250x4.6 mm, 5µ column used and the mobile phase composition were changed slightly. The mobile phase composition was buffer and acetonitrile in the ratio of 60:40 v/v. respectively as eluent at flow rate 1.2 mL/min. UV detection was performed at 220nm. The retention time of Armodafinil is 4.44 minutes and the peak shape was good.

The chromatogram of Armodafinil standard using the proposed method is shown in **(Fig: 1.2)** system suitability results of the method are presented in **Table-1.1.**

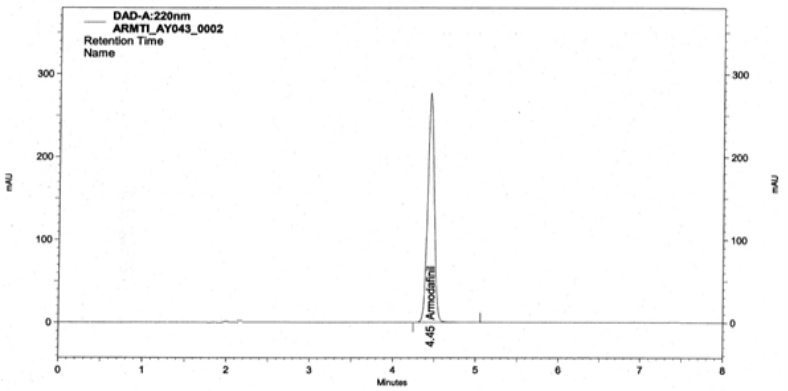

Figure 1.2: Chromatogram showing the peak of Armodafinil

4.0 Method validation

The developed RP-LC method extensively validated for assay of Armodafinil using the following parameters.

4.1 Specificity

Preparation of blank solution

Mixed 650mL water and 350mL acetonitrile and 10mL of acetic acid and sonicate to degas.

Preparation of Placebo solution

Placebo solution was prepared in duplicate by weighing the equivalent amount of excipients present in the finished drug product and analysed as per proposed method. Interference due to placebo was evaluated for each of the placebo preparations.

Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic

conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution **(Fig: 1.3)** showed no peak at the retention time of Armodafinil peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Armodafinil in Armodafinil tablets. Similarly chromatogram of placebo solution **(Fig: 1.4)** showed no peaks at the retention time of Armodafinil peak. This indicates that the placebo used in sample preparation do not interfere in estimation of Armodafinil in Armodafinil tablets.

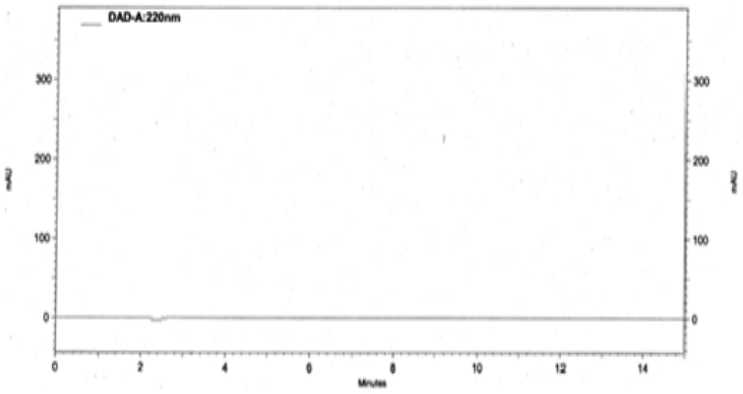

Fig: 1.3 Chromatogram showing the no interference of diluent for Armodafinil

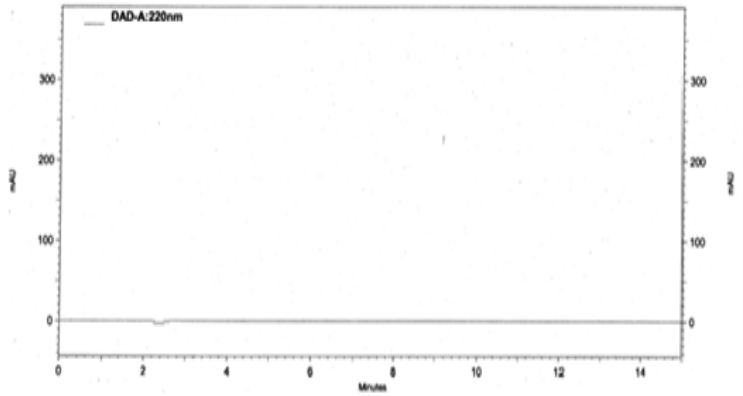

Fig: 1.4 Chromatogram showing the no interference of placebo for Armodafinil

Table 1.1: System suitability parameters for Armodafinil by proposed method

Name of the Compound	Retention Time	Theoretical plates	Tailing factor
Armodafinil	4.45	15193	1.02

4.2 System precision

The standard solution was prepared as per the test method, injected into the HPLC system for six times and evaluated the % RSD for the area responses. The data were shown in **Table: 1.2.**

Table: 1.2 System precision data for Armodafinil

No. of injections	Peak area response
1	1515654
2	1515420
3	1514722
4	1514045
5	1514069
6	1514550
Average	1514743
SD	673.4
% RSD	0.04

4.3 Method precision

The precision of test method was evaluated by doing assay for six samples of Armodafinil tablet as per test method. The content in mg and % label claim for Armodafinil for each of the test preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The data were shown in **Table: 1.3.**

Table: 1.3 Method precision data for Armodafinil

No. of injections	Armodafinil
	Percentage assay
Preparation 1	98.9
Preparation 2	99.9
Preparation 3	100.7
Preparation 4	100.7
Preparation 5	101.7
Preparation 6	103.3
Average	**100.9**
SD	**1.5**
%RSD	**1.5**

4.4 Linearity of detector response

The linearity of an analytical method is its ability to obtain test results which has a definite mathematical relation to the concentration of analyte. The linearity of response for Armodafinil was determined in the range of 20 to 150% (10.2, 20.4, 30.6, 51.1 and 76.6µg/ml for Armodafinil). The calibration curve of analytical method was assessed by plotting concentration versus peak area and represented graphically. The correlation coefficient [r2] was found to be 0.999. Therefore the HPLC method was found to be linear standard curve were calculated and given in **Figure: 1.5** to demonstrate the linearity of the proposed method. From the data obtained which given in **Table: 1.4** the method was found to be linear within the proposed range.

Table: 1.4 Linearity studies for Armodafinil by proposed method

Level no.	Armodafinil		
	Linearity concentration	Concentration (µg / ml)	Average area response
1	20%	10.21	283612
2	40%	20.43	566584
3	60%	30.65	848712
4	100%	51.08	1423316
5	150%	76.62	2129948
Correlation coefficient:			**1.000**
Slope (m):			**27834**
Intercept (y):			**-1624**

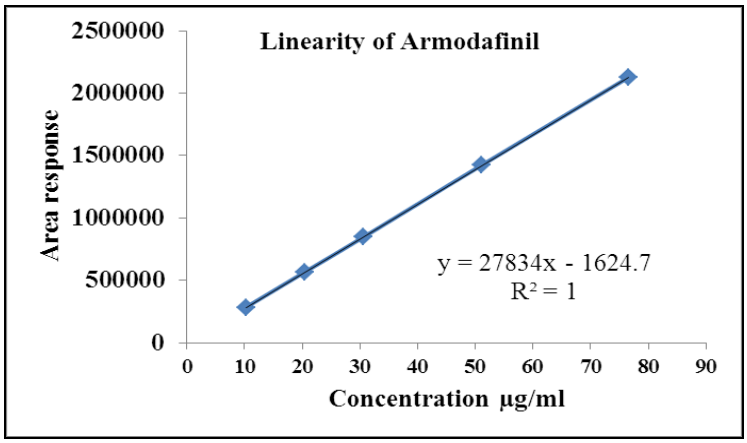

Figure: 1.5 Calibration curve for Armodafinil

4.5 Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of Armodafinil, analyzed as per the proposed method. The mean percentage recovery for 50%, 100%, 150% level was found to be 100.2, 100.6and 101.4. %RSD was found to be 0.1, 0.1 and 0.3 respectively. They are within the acceptance limits. Therefore, the HPLC method for the determination of assay of Armodafinil in formulation was found to be accurate. The data obtained which given in **Table: 1.5** the method was found to be accurate.

Table: 1.5 Recovery studies for Armodafinil by proposed method

Levels	Response 1	Response 2	Mean response	Amount added	Amount recovered	%Recovery	Mean % recovery	%RSD
50%	702279	703954	703116	622.80	624.56	**100.3**	**100.2**	**0.1**
	702901	702408	702654	622.90	624.15	**100.2**		
	701833	702480	702156	622.90	623.71	**100.1**		
100%	1411539	1411765	1411652	1245.99	1253.94	**100.6**	**100.6**	**0.1**

	1409871	1411013	1410442	1246.19	1252.87	**100.5**		
	1410929	1409955	1410442	1245.99	1252.87	**100.6**		
150%	2144697	2147578	2146137	1875.40	1906.37	**101.7**	**101.4**	**0.3**
	2139819	2139144	2139481	1875.60	1900.46	**101.3**		
	2136185	2133925	2135055	1875.30	1896.53	**101.1**		

4.6 Limit of Detection and Limit of Quantification

For the present developed HPLC method Limit of Detection was found to be 0.043µg/mL and Limit of Quantification was found to be 0.13µg/mL for Armodafinil. LOD and LOQ were determined based on signal to noise ratio.

5.0 CONCLUSION

An RP-HPLC method for estimation of Armodafinil was developed and validated as per ICH guidelines.

A simple, accurate and reproducible reverse phase HPLC method was developed for the estimation of Armodafinil in bulk drugs and formulations. The optimized method consists of mobile phase pH 2.5 phosphate buffer and Acetonitrile in the ratio of 60:40%v/v with Delvosil ODS-UG C_{18} (150 × 4.6mm, 5µ) column. The retention time of Armodafinil was found to be 4.44min. The developed method was validated as per ICH Q2A (R1) guideline. The proposed HPLC method was linear over the range of 10.2-76.6 µg/ml, the correlation coefficient was found to be 1.000. Relative standard deviation for method precision was found to be 1.5. Limit of Detection was found to be 0.043µg/ml and Limit of Quantification was found to be 0.13µg/ml respectively.

We have developed a fast, simple and reliable analytical method for determination of Armodafinil in pharmaceutical preparation using RP-LC. As there is no interference of blank and placebo at the retention time of Armodafinil. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and Linearity. It allows reliably the analysis of Armodafinil in its different pharmaceutical dosage forms.

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