

## APPLICATION OF VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF ARMODAFINIL IN BULK AND FORMULATION

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Received: 22 May 2017, Revised and Accepted: 22 Jul 2017

### ABSTRACT

**Objective:** The objective of the present study is to develop and validate a simple, rapid, sensitive reverse phase HPLC method for the determination of Armodafinil present in bulk and its pharmaceutical formulations.

**Methods:** The chromatographic separation was achieved by using Hypersil ODS C-18 (150 x 4.6 mm, 5 $\mu$ ) in an isocratic mode with mobile phase methanol: phosphate buffer 3.0 (60:40 %v/v) was used. The flow rate was 1 ml/min and effluent was monitored at 225 nm. The method was validated for validation parameters i.e. linearity, accuracy, precision and robustness according to ICH guidelines.

**Results:** The retention time of Armodafinil was 4.2 min and the linearity range of the method was 500-20000ng/ml with regression ( $r^2$ ) coefficient 0.9998. The method was validated for precision, accuracy, robustness and which were found to be within the acceptable limits according to the ICH guidelines. Also, the method was successfully applied for the estimation of Armodafinil in the marketed formulation of Nuvigil and the recovery was found to be >98%.

**Conclusion:** The developed method possess good selectivity, specificity, there is no interference found in the blank at a retention time of ARM and good correlation between the peak area and concentration of the drugs under prescribed conditions. Hence, the method can be applied for routine analysis of Armodafinil.

**Keywords:** Armodafinil, Method development, Nuvigil, RP-HPLC, Validation

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DOI: <http://dx.doi.org/10.22159/ijcpr.2017v9i5.22162>

### INTRODUCTION

Armodafinil (ARM) is a CNS stimulant used for wakefulness in excessive sleepiness associated with obstructive sleep apnoea. It is chemically (-)-2-[(R)-(diphenyl methyl) sulfinyl] acetamide (C<sub>15</sub>H<sub>15</sub>N<sub>2</sub>O<sub>2</sub>S, MW 273.35) [fig. 1]. ARM is a white to off-white, crystalline powder, very slightly soluble in water, sparingly soluble in acetone and soluble in methanol [1, 2]. ARM is the R-enantiomer of modafinil. Oral administration ARM is readily absorbed. ARM peak concentration (C<sub>max</sub>) achieved in approximately 2 h; this may be delayed by 2–4 h if ARM administered with food. But food has no effect on the overall bioavailability of ARM when compared with the fasting state. ARM is eliminated approximately three times more slowly than the S-isomer of racemic modafinil [3].

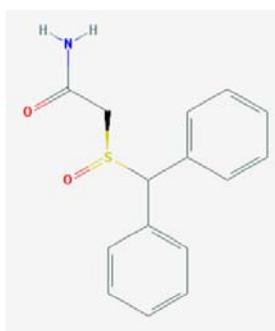


Fig. 1: Chemical structure of armodafinil

There are few methods reported for determination of modafinil in the literature [4-6]. As the ARM is a racemic compound of modafinil,

so, determination of modafinil method may (or may not) be applicable for ARM [4-6].

The literature search revealed that there were no HPLC methods reported. Nageshwararao *et al.* reported enantioselective resolution of synthetic intermediates of ARM along with its related substances [7]. The method was developed by using chiral column and sensitivity was reported at 1540 ng/ml. recently, two methods are reported for ARM in plasma by LC-MS [8, 9]. Hence, in present investigation, we attempted to develop a novel, simple and economic method for determination of ARM in the formulation by employing the high-pressure liquid chromatography method.

### MATERIALS AND METHODS

#### Instrumentation

HPLC-Waters 2695 separation module millennium software, Sonicator-Sharp Analytical, Rotary shaker-Vibramax, Analytical balance-Sartorius, Millipore Direct-Q 3 U. V. pH meter-Systronics.

#### Standards and chemicals

Armodafinil procured from Matrix laboratories Ltd. (Hyderabad, India). Purified water was prepared using a Millipore Direct-Q 3 U. V with pump water purification system. Acetonitrile of HPLC grade were purchased from Merck Ltd. (Mumbai, India), and o-phosphoric acid of A. R. grade was purchased from Asha chemicals pvt. Ltd (Hyderabad, India), Sodium dihydrogen phosphate of A. R. grade was purchased from Asha chemicals pvt. Ltd (Mumbai, India).

#### Stock and working solution preparation

**Preparation of standard stock solution:** Accurately weighed and transferred 50 mg of ARM into a 50 ml volumetric flask dissolved and made up of the volume with methanol. Daily working standard solutions of ARM was prepared by suitable dilution of the stock solution with mobile phase.

**Preparation of buffer:** 20 mmol strength of phosphate buffer is prepared by using 620 mg of potassium dihydrogen phosphate in 500 ml HPLC grade water adjust to pH 3.0 by using orthophosphoric acid.

#### Method validation

The validation parameters like linearity, sensitivity, accuracy, precision, recovery and stability, according to the ICH guidelines were carried [10].

Selectivity is studied by comparing the chromatograms obtained from placebo sample with the chromatogram obtained from the tablet. Calibration curves are prepared by assaying standard samples of the drug, ranging from 500-20000 ng/ml. The linearity of the method was determined by plotting the peak area ( $y$ ) of drug versus the nominal concentration ( $x$ ) of the drug. The calibration curves are constructed by least squares linear regression.

Intra-and inter-day accuracy and precision of this method was determined at three different concentration levels in 3 different days. On each day, three replicates were analyzed with independently prepared calibration curves. The accuracy and precision were expressed as percentage accuracy and relative standard deviation (R. SD, %) respectively and calculated by using equations (1) and (2).

$$\text{Accuracy (\%)} = \frac{\text{Mean observed concentration}}{\text{Nominal concentration}} \times 100 \quad \text{Eq. (1)}$$

$$\% \text{RSD} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100 \quad \text{Eq. (2)}$$

The limit of detection (LOD) and limit of quantification (LOQ) are defined as the lowest concentration giving a signal-to-noise ratio (S/N) of at least 3:1 and 10:1 respectively.

The stability of the drug solution was determined for short-term (solution stability) by keeping at room temperature (25 °C) for 24h. Auto sampler stability was determined by storing the samples for 24 h in the auto sampler. Each sample injected three times into HPLC

and concentrations obtained were compared with the nominal values of the QC samples.

#### Analysis of dosage form

20 Tablets (100 mg and 250 mg) each separately, were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to one tablet was transferred into 100 ml volumetric flask and drug was extracted with methanol. This solution was filtered through Whatmann No.1 filter paper and solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined. The sample was injected in five times into HPLC. The amount of drug recovered was calculated from the linearity graph.

### RESULTS AND DISCUSSION

#### Method development

##### Method optimization

The chromatographic method was optimized by changing various parameters, such as pH of the mobile phase, organic modifier and buffer used in the mobile phase and composition of the mobile phase. Water and phosphate buffer in various strengths are tried along with methanol and acetonitrile as organic solvent. A mixture of methanol and phosphate buffer (pH 3.0) was tested as a mobile phase with Hypersil ODS C-18. Increasing the composition of organic phase decrease in retention time, the peak shape of drugs was poor and shoulder peak was observed. The retention time was increased with the reducing the organic phase percentage in the mobile phase. The mobile phase composition of 60:40 v/v methanol: buffer was given good resolution, retention time with a minimal tailing factor in acceptable range. The method was optimized with the mobile phase composition of methanol and phosphate buffer 60:40 (v/v).

Buffer molarity of 10, 20 and 50 mmol was tested. There were no significant changes in the chromatographic response and peak shape with change in buffer molarity. A buffer molarity of 20 mmol was selected for further analysis.

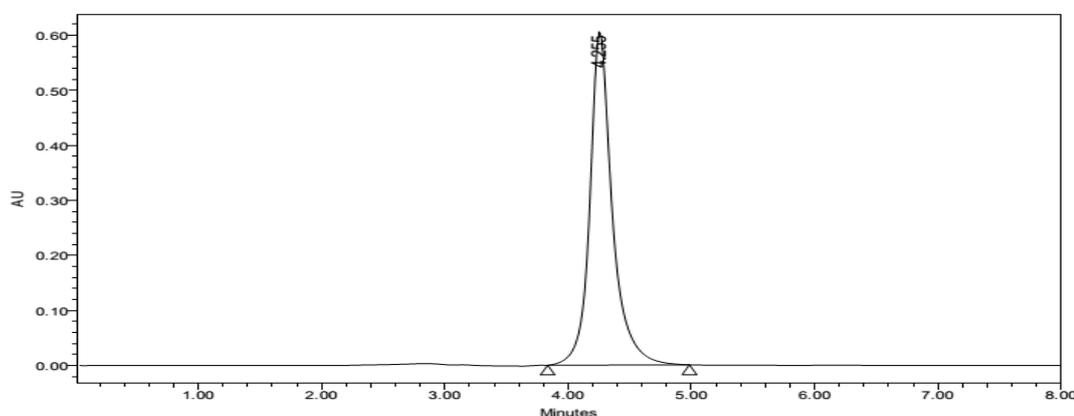


Fig. 2: Standard chromatogram of armodafinil

After several trials, the method was optimized as a mixture of 20 mmol potassium dihydrogen phosphate buffer (pH 3.0) and methanol (40:60 v/v), at a flow rate of 1 ml/min, at 225 nm for run time 8 min. These chromatographic conditions achieved a satisfactory resolution, retention time and tailing for ARM. The [fig. 2] shows that standard chromatogram of ARM.

#### System suitability

To check the system suitability, working stock standard of individual drugs were injected HPLC to determine the individual retention times of drugs. Then working standard solution was injected five times and we considered relative standard deviation (RSD) for five consecutive injections  $\leq 2$ , the resolution between two adjacent

peaks  $\geq 2$  and tailing factor  $< 2$  acceptable values [11]. Resolution (R), relative standard deviation from five replicate injections of working standard mixture solution, tailing factor (T) and retention time drug was presented in [table 1]. System suitability test confirmed that the chromatographic system was adequate for the analysis planned to be done.

#### Method validation

##### Selectivity

The selectivity of the present method is established by checking the blank sample and observed the chromatogram. There is no interference found at retention time ARM in the blanks concludes the selectivity of the method.

Table 1: System suitability parameters of ARM

Parameters (n=5)	Results	Required limits
Retention time in minutes (R <sub>t</sub> )	4.22±0.01, 0.36	RSD≤2
Theoretical plates (N)	5756±64, 1.12	N>2000
Tailing Factor (T)	1.17±0.01, 1.48	T≤2

### Linearity

The linearity of this method is evaluated by linear regression analysis, which is calculated by the least square method and the drug is linear in the concentration range of 500-20000 ng/ml. Calibration standards are prepared by spiking required volume of working standard (100µg/ml)

solution into different 10 ml volumetric flasks and volume made up with methanol to yield concentrations of 500, 1000, 2000, 5000, 10000, and 20000ng/ml of ARM. The resultant peak area of the drug was measured. The calibration curve is plotted between peak areas of the drug against the concentration of the drug. The [fig. 3] shows the linearity graph regression coefficient (r<sup>2</sup>) including the slope and y-intercept.

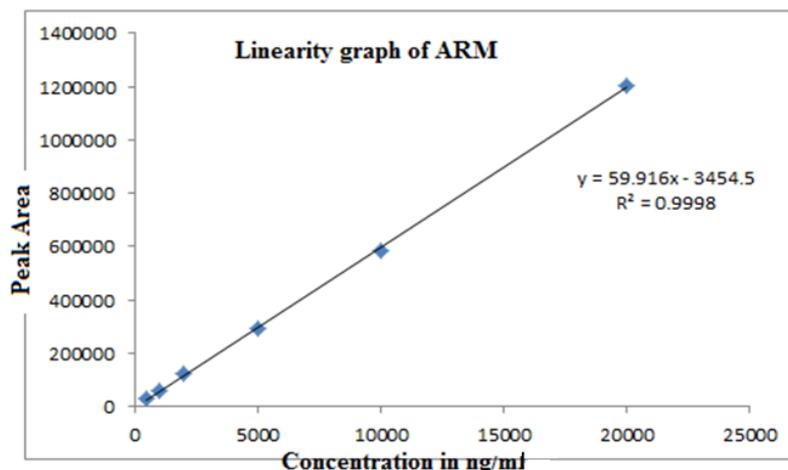


Fig. 3: Linearity graph of armodafinil

### Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) are defined as the lowest concentration giving a signal-to-noise ratio of at least 3-fold and 10-fold, respectively. The LOD and LOQ of this method were verified based on the standard deviation of response and slope and found to 144.74ng/ml and 438.62ng/ml respectively.

### Intra-day and Inter-day precision and accuracy

The intra-and inter-day precision and accuracy of this method is determined by analyzing replicates of QC samples at three concentrations on 3 different days. The coefficients of variation for the intra-and inter-day precision were <2%. The intra-and inter-day accuracies are 95.80-104.56%. The low levels of coefficients of variation (0.18%-1.85%) [table 2], indicate the method is accurate and precise.

### Robustness

Robustness of the method was done by changing slight variation in the parameters like mobile phase composition, flow rate and wavelength. Present method didn't show any significant change when the critical parameters were modified. The tailing factor of drug was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the

specificity of the method, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

### Ruggedness

Ruggedness is studied along with precision and accuracy of batches where the effect of the column, and analyst change are observed. The observed value for column variation and results obtained for precision and accuracy are within the acceptance criteria (i.e. there are no changes in the retention time, recovery and precision of the drug) according to ICH [10].

### Stabilities

The stability of drug was studied at different conditions for quality control (QC) of samples. The samples were analyzed and compared with freshly analyzed QC samples, no difference were found in accuracy and precision concludes that there was no degradation of drugs during the analysis.

### Application of method for assay of dosage form

Amount of the drug in the formulation has been determined and the percentage recovery was found to be >98.21%. The data were shown in [table 3]. The [fig. 3], shows the chromatogram of ARM from formulations.

Table 4: Intra and inter-day accuracy and precision of ARM

Concentration (ng/ml)	Intra-day (n=6)		Inter-day (n=9)	
	mean±SD	RSD (%)	mean±SD	RSD (%)
1500	104.62±0.55	0.53	104.56±1.20	1.15
8000	98.12±1.34	1.36	95.80±1.77	1.85
15000	100.16±0.40	0.40	99.32±0.18	0.18

Values expressed mean±SD

Table 3: Recovery study from formulation (n=5) of ARM

Brand name	Labeled amount(mg)	Calculated amount (mg) $\pm$ SD	Assay (%)
Nuvigil	100	98.21 $\pm$ 0.76	98.21
	250	252.54 $\pm$ 1.07	100.98

Values are expressed in mean $\pm$ SD, n=5

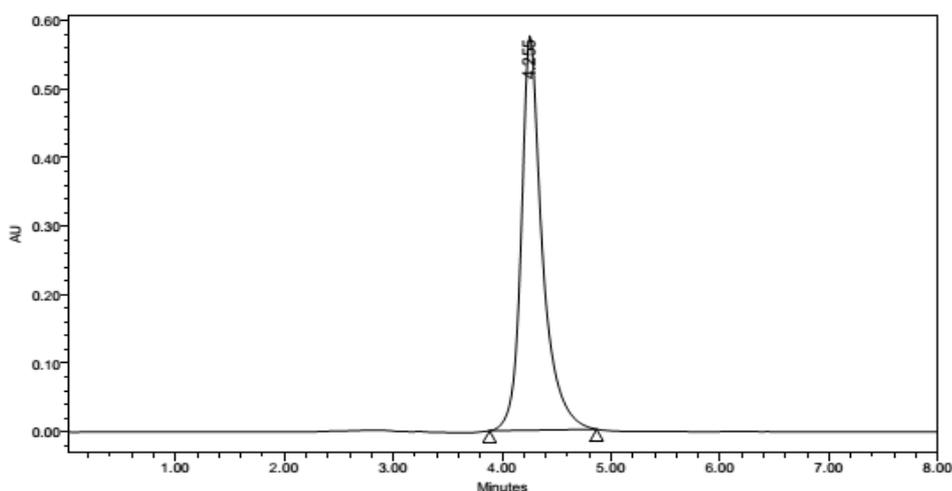


Fig. 4: Chromatogram of armodafinil from formulation

## CONCLUSION

The developed method possess good selectivity, specificity, there is no interference found in the blank at a retention time of ARM and good correlation between the peak area and concentration of the drugs under prescribed conditions and also the accuracy of the was found to be >95.80%. The observation of % RSD less than 2 for both intra-and inter-day measurements also indicates a high degree of precision. In the present method, a Hypersil ODS, C-18 column has been used at flow rate of 1 ml/min and pH 3.0. A linearity range of 500-20000 ng/ml ARM; this linearity range covers all the strengths of ARM. The method was successfully applied for analysis of ARM from its formulation and also it can be helpful for further investigative studies ARM.

## ACKNOWLEDGEMENT

Author is thankful to Adept Pharma and Bioscience Excellence Private Limited for providing all types of facilities for the research.

## CONFLICT OF INTERESTS

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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## How to cite this article

- Devi Ramesh, Mohammad Habibuddin. Application of the validated RP-HPLC method for the determination of armodafinil in bulk and formulation. *Int J Curr Pharm Res* 2017;9(5):158-161.