Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 8, 2014

Original Article

STABILITY INDICATING RP HPLC METHOD FOR THE ESTIMATION OF ARMODAFINIL IN TABLET DOSAGE FORM

P. VIVEK SAGAR[1]*, NELOFER BAGUM[1], S. SHOBHA RANI[2]

[1]Department of Pharmaceutical Analysis Care College of pharmacy, Oglapur (v), Atmakur (M), Warangal, Telangana, India, [2]Department of Pharmaceutical Analysis Jawaharlal Nehru Technological University, Kukatpally, Hyderabad, Telangana, India.
Email: viveksagar.p111@gmail.com

Received: 12 Jun 2014 Revised and Accepted: 19 Jul 2014

ABSTRACT

Objective: To develop simple reverse phase HPLC method for the estimation of Armodafinil in tablet dosage form.

Methods: Chromatography was performed by isocratic elution on a Stainless steel Hibar C18 column with dimensions 4.6 x 250 mm, packed with octadecylsilane bonded to porous silica (C18) with particle size 5 micron. Acetonitrile and water in the ratio of 50:50 v/v is used as mobile phase. The flow rate is 1.0 ml/ min and effluent is monitored at 220 nm. Armodafinil was eluted at a retention time of 3.8 minutes.

Results: The standard curve of Armodafinil was linear over a working range of 1–700 µg/ml and gave an average correlation coefficient of 0.999. The limit of quantitation (LOQ) of the drug is 0.1 µg/ ml. Recovery studies were carried out by standard addition method and the recoveries are found satisfactory within the range of 99.3 to 101.5 %. The method is precise with % RSD below

Conclusion: The method is validated in terms of robustness and forced degradation studies were carried out and this method can be applied for routine degradation studies ans quantification in regular laboratories.

Keywords*:* Armodafinil, RP HPLC, Stability indicating assay, Validation.

INTRODUCTION

Armodafinil (Figure 1) (2-[(R)-(diphenylmethyl) sulfinyl] acetamide) is the R-enantiomer of modafinil, which is a racemic mixture of the R- and S-enantiomers. The molecular formula is $C_{15}H_{15}NO_2S$ and the molecular weight is 273.35. Used in treating narcolepsy and shift work sleep disorder (SWSD) and for adjunctive treatment of obstructive sleep apnea/ hypopnea syndrome (OSAHS). The drug is not official in any of the pharmacopoeia. Literature survey revealed that various analytical methods [1-4] were reported for determining the racemic mixture containing both R and S forms of Modafinil. An electrophoretic method [5] and one LC-MS/MS method [6] is reported for determining the R form of Modafinil i. e, Armodafinil. The present study focuses on development of simple, specific, precise, sensitive and economic stability indicating assay method for estimation of Armodafinil in tablet dosage form.

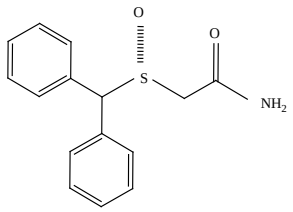


Fig. 1: Structure of Armodafinil

MATERIALS AND METHODS

Reagents and chemicals

Armodafinil tablets were procured form Orchid Pharma limited, Mumbai. Methanol, Acetonitrile, Hydrochloric Acid, Sodium Hydroxide are purchased from MERCK.

Stock solutions and standards

Stock solution of Armodafinil (1 mg/ml) was prepared by dissolving 25 mg of Armodafinil in 25 ml of volumetric flask containing 10 ml of mobile phase. The volume was made up to the mark with mobile phase and the solution was sonicated for about 10 min. Working standard solutions of Armodafinil were prepared by taking suitable aliquots of drug solution from the standard stock solution, 1000µg/ml, and the volume was made up to 10 ml with mobile phase.

Apparatus and chromatographic conditions:

Quantitative HPLC was performed on Waters HPLC system equipped with waters 515 pump and Waters 2489 dual wavelength UV detector. Empower2 software is used for data acquisition. A Stainless steel Hibar column with dimensions 4.6 x 250 mm, packed with Octadecylsilane bonded to porous silica (C18) having particle size 5 micron.

Method development and optimization

To develop a suitable HPLC method for the determination of Armodafinil, trials were done with different mobile phases, using water, buffer(0.5 gm potassium dihydrogen phosphate)and acetonitrile in different pH with different compositions of mobile phases (40:60, 50:50, 60:40). The method was optimized finally using combination of Acetonitrile and water in the ratio of 50/50 v/v with a flow rate of 1.0 ml/ min. The drug was eluted at retention time around 3.8 min with symmetric peak shape. Run time was set for 8 minutes. Detection is performed at wavelength 220 nm.

System suitability

For performing system suitability studies, 100% test concentration under degradation conditions was selected. System suitability test was performed by injecting blank solution once and standard solution of 100% test concentration six times in to stabilized HPLC system. The system suitability was established by evaluating the system suitability parameters from the last peak obtained. System suitability parameters include retention factor (k'), repeatability, resolution (R), tailing factor (T) and theoretical plates (N). It was performed by using the concentration of 50µg/ml. The system suitability data was given in the table 1

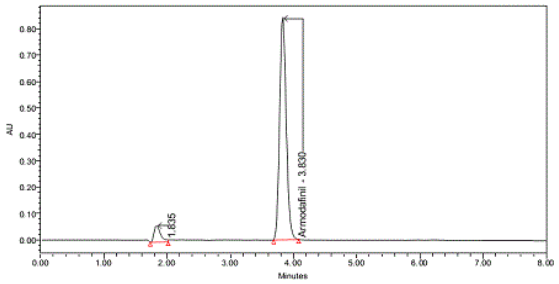
Assay of Armodafinil marketed formulation

Twenty tablets of Armodafinil were weighed and powdered uniformly using mortar and pestle. An accurately weighed sample of

powdered drug containing 25 mg of Armodafinil was dissolved with sufficient quantity of mobile phase in a 25 ml volumetric flask. The volume was made up to the mark finally using the same. The solution is sonicated for 5 minutes. This solution was filtered through 0.45 µm filter paper. The solution obtained was diluted with mobile phase so as to obtain a required concentration. The determinations were carried out in triplicate. The amount of Armodafinil present is calculated by comparing with the standard solution of Armodafinil. The representative chromatograms are shown in figure 2 & 3. the peak areas were mentioned in the table 2.

Table 2: Assay results of Armodafinil

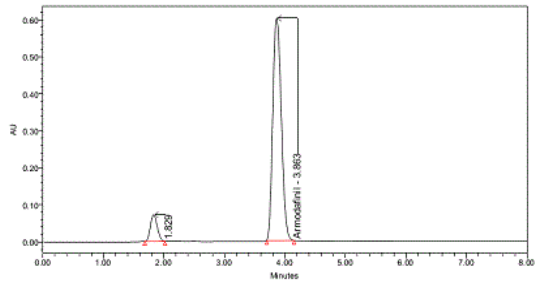
S. No.	Peak areas	
	Standard drug	Tablet formulation
1	6259784	6312897
2	6265017	6318229
3	6262995	6053611
Mean	6262599	6228245
% Assay	99.4%	



Peak results of chromatogram for standard solution (conc.100µg/ml)

Name of the peak	Retention time(min)	Area	Theoretical plates	Tailing factor
Armodafinil	3.830	6259784	6313.568574	1.126

Fig. 2: chromatogram of Armodafinil standard solution (conc. 100µg/ ml)



Peak results of chromatogram for test solution

Name of the peak	Retention time(min)	Area	Theoretical plates	Tailing factor
Armodafinil	3.863	6312897	3519.537733	1.175

Fig. 3: chromatogram of Armodafinil test solution

Validation of the assay method [7-9]

Linearity

Linearity solutions for assay method were prepared from stock solution at concentration levels from 1to 1000µg/ml of analyte concentration. The graph of peak area versus concentration was plotted by least-squares linear regression analysis.

The linear fit of the system was illustrated graphically. The linearity range was found to be 1 - 700 µg/ml. The samples were assayed using the method described above. The standard calibration curve for Armodafinil was constructed using the average peak-area versus the nominal concentrations of the analyte. Linear least-squares regression analysis was performed to assess the linearity.

Recovery and accuracy

The accuracy of the assay method was evaluated at five levels, i. e. 50, 75, 100, 125 and 150% levels (concentrations of 150, 225, 300, 375 and 450µg/ml respectively) in bulk drug sample. The percentages of recoveries were calculated from the slope and *Y*-intercept of the calibration curve obtained. Accuracy/recovery experiments were performed in triplicate. Accuracy was determined by standard addition method. Known amount of different concentrations of pure drug solutions were spiked with solution of pre analysed formulation of concentration 100 µg/ ml.

Precision

The precision was carried out at three levels, intra assay precision of injection, intermediate precision and reproducibility.

Intra assay precision was assessed using 9 determinations covering the range of 50,100 and 150% concentration levels of drug solution.

Intermediate precision (inter day precision) was assessed by inducing typical variations like different days and different columns.

Reproducibility was assessed by different analysts.

Robustness

Robustness of the method was studied under degradation conditions to study the effects of degradants on Armodafinil in changes method conditions. It was carried out by considering deliberate changes in detection wavelength, flow rate, mobile phase ratio. Robustness was carried out by changing detection wavelength by ±3 nm. Robustness was checked by changing the proportion of organic solvent in the mobile phase by ±4%. It was also checked for robustness by change in flow rate by ±0.2 ml/ min.

Forced degradation studies

To study the specificity of the method, pure drug was stressed under different degradation conditions. Degradation studies were carried out by exposing drug for acid hydrolysis, alkali hydrolysis, oxidative degradation, thermal degradation and photolytic degradation. Mobile phase is used as solvent for all degradation studies. All the solutions for degradation studies were prepared by dissolving Armodafinil drug in little amount of mobile phase and the volume was made up to the mark with 0.1N HCl, 0.1N NaOH, 1% H_2O_2. Acid hydrolysis is carried out by exposing the drug to 0.1N HCl. Alkali hydrolysis is carried out by exposing the bulk drug and powdered sample to 0.1N NaOH. Oxidative degradation is carried out by exposing the bulk drug to 1% H_2O_2. Thermal degradation is carried out by exposing the bulk drug in Hot air oven at 50 ^{0}C. Photolytic degradation is carried out by exposing the bulk drug to sun light. The degradation studies were carried at a time interval of 15 minutes. The drug solution was prepared at a concentration of 100µg/ ml.

Acid degradation

10mg of drug was dissolved in a few ml of mobile phase in a 10 ml volumetric flask. The volume was made up to the mark with 0.1N HCl, mixed thoroughly and kept aside. After 15, 30 minutes, solution was mixed and 1 ml of this solution was pipetted into another 10 ml volumetric flask. To this 1 ml solution, 1 ml of 0.1N NaOH was added to neutralize the acid and final volume was made upto the mark with mobile phase and its peak area was observed by injecting into HPLC.

Alkali degradation

10mg of drug was dissolved in a few ml of mobile phase in a 10 ml volumetric flask. The volume was made up to the mark with 0.1N NaOH, mixed thoroughly and kept aside. After 15, 30 minutes, solution was mixed and 1 ml of this solution was pipetted into

another 10 ml volumetric flask. To this 1 ml solution, 1 ml of 0.1N HCl was added to neutralize the alkali and volume was made up to the mark with mobile phase and its peak area was observed by injecting into HPLC.

Photo degradation

Drug powder was exposed to sunlight. After 15, 30 minutes, 10mg of the exposed powder was dissolved in mobile phase in a 10 ml volumetric flask. From this solution, 1 ml was pippeted into another 10 ml volumetric flask and its volume was made upto the mark with mobile phase. The peak area of this solution was observed.

Thermal degradation

Drug powder was exposed to 50^{0}C in a hot air oven. After 15, 30 minutes, 10mg of the exposed powder was dissolved in mobile phase in a 10 ml volumetric flask. From this solution, 1 ml was pippeted into another 10 ml volumetric flask and its volume was made upto the mark with mobile phase. The peak area of this solution was observed.

Results

System suitability

The system suitability of Armodafinil was ascertained good under degradation conditions and didn't show any significant change with %RSD less than 2. The degradants peak and Armodafinil peak has resolution greater than 2 in all the conditions. This shows no interference of degradant peak on Armodafinil peak. The tailing factor for Armodafinil was always less than 2.0 with plate count more than 2000.

Linearity

Linear calibration plot for assay method was obtained over the calibration ranges tested, i. e. 1- 700 µg/ml and the correlation coefficient obtained was greater than 0.999. The results show that an excellent correlation existed between the average peak area and concentration of the analyte(Table 3).

Table 1: System suitability data for Armodafinil

Retention time (Rt)	3.810
Peak area	4982685
USP plate count(N)	4677.0355
USP tailing factor(T)	1.186
% RSD of(n= 6)	1.3

Results of method validation experiments

Table 3: Linearity of Armodafinil

Conc.(µg/ml)	Peak Area	Conc.(µg/ml)	Peak Area
1	91760	300	12505210
5	307010	400	16251659
50	2989697	500	20323095
100	4837989	600	24402243
200	8843917	700	28711674

Recovery and accuracy

The percentage recovery of Armodafinil in bulk drug samples was ranged from99.3-101.5 which indicates that the method isaccurate (Table 4).

Precision

From the results shown in precision Tables5, 6, 7 & 8, it was found that pooled and % RSD was less than 2%; which indicates that the proposed method has good reproducibility.

Table 4: Accuracy data for Armodafinil

Spiked Levels	Standard		Test peak area(conc. 100µg/ml)	Spiked		% Recovery	Mean % Recovery
	Conc. (µg/ml)	Peak Area		Conc. (µg/ml)	Peak Area		
50%	150	9829413	6358316	250	16263079	101.3	
		9698922	6371291		16153903	101.4	101.5
		9788409	6318229		16152568	101.9	
75%	225	14928123	6372897	325	21324411	100.5	
		14259859	6214178		20650587	101.6	100.8
		14333226	6343288		20660537	100.3	
100%	300	19488942	6325669	400	25776514	100	
		19661406	6213901		26130545	101.5	100.3
		19508556	6357126		25743117	99.6	
125%	375	22164423	6153611	475	28395215	100.6	
		23975972	6229245		30391629	101	100.4
		23917241	6342612		30170864	99.8	
150%	450	26011432	6139224	550	31665322	98.3	
		27629712	6373759		33829352	99.5	99.3
		28262352	6335217		34620376	100.2	

Table 5: Intra assay precision data for Armodafinil

% Level	Peak Area	Amount Obtained(µg/ml)	% Obtained
	9530370	152.5	101.7
50	9448723	151.3	100.9
	9551190	153	102
	18424051	296.1	98.7
100	18647226	299.7	99.9
	18817822	302.4	100.8
	27536746	442.9	98.4
150	27629712	444.4	98.7
	28262352	454.6	101
Pooled RSD			1.3

Table 6: Intermediate precision data of Armodafinil for day 1 and day 2

S. No.	Day 1		Day 2	
	Retention Time	**Peak Area**	**Retention Time**	**Peak Area**
1	3.810	4982685	3.832	4827119
2	3.822	5043391	3.869	4975624
3	3.827	4956253	3.817	4853891
4	3.832	4959394	3.859	4942266
5	3.863	4857086	3.846	4931394
6	3.878	4967299	3.813	4920865
	% RSD	1.2	**% RSD**	1.1

Table 7: Intermediate precision data of Armodafinil for Column 1 and Column 2

S. No.	Column 1		Column 2	
	Retention Time	**Peak Area**	**Retention Time**	**Peak Area**
1	3.845	22081819	3.915	22250028
2	3.848	22129248	3.917	22317532
3	3.869	21926638	3.919	22317228
4	3.872	21908561	3.912	22198769
5	3.874	21882375	3.921	22102983
6	3.878	22117781	3.917	22043224
	% RSD	0.5	**% RSD**	0.5

Table 8: Reproducibility data for Armodafinil

S. No.	Analyst 1		Analyst 2	
	Retention time	**Peak area**	**Retention time**	**Peak area**
1	3.901	25458196	3.900	25224810
2	3.903	25554856	3.903	26154098
3	3.903	25324321	3.904	25894457
4	3.904	25592739	3.906	26085712
5	3.920	25472533	3.906	26071259
6	3.921	25414751	3.907	25938737
	% RSD	0.4	**% RSD**	1.3

Table 9: Robustness data for change in wavelength

S. No	λ_{max} -220 nm		λ_{max} - 217 nm		λ_{max} - 223 nm	
	Retention Time	**Peak Area**	**Retention Time**	**Peak Area**	**Retention Time**	**Peak Area**
1	3.806	3775757	3.875	3832275	3.798	3603294
2	3.817	3628570	3.894	3883298	3.807	3461902
3	3.820	3711842	3.867	3773320	3.812	3543550
4	3.837	3772145	3.909	3812237	3.829	3598687
5	3.842	3707342	3.929	3895566	3.834	3538528
6	3.846	3750440	3.962	3911544	3.838	3578669
% RSD		1.5	-	1.4	-	1.5

Table 10: Robustness data for change in mobile phase composition

S. No.	Normal MP		ACN: -4%; Water: +4%		ACN: +4%; Water: -4%	
	Retention Time	**Peak Area**	**Retention Time**	**Peak Area**	**Retention Time**	**Peak Area**
1	3.932	3881751	4.103	3805520	3.852	3893374
2	3.919	3913373	4.114	3873667	3.892	3885448
3	3.874	3795148	4.117	3849376	3.879	3845856
4	3.937	3889966	4.124	3864902	3.895	3818582
5	3.930	3890511	4.134	3829262	3.869	3868084
6	3.953	3923926	4.142	3900418	3.881	3901546
% RSD		1.2	-	0.9	-	0.8

Robustness

The percent recovery of Armodafinil was good under most conditions and didn't show any significant change when the critical parameters were modified under degradation conditions. The degradants peak and Armodafinil peak has resolution greater than 2 in all the conditions. This shows no interference of degradant peak on Armodafinil peak.

The tailing factor for Armodafinil was always less than 2.0 with plate count more than 2000 and with %RSD less than 2. The component was well eluted under all the changes carried out. Considering the modifications in the system suitability parameters, as well as carrying the experiment at room temperature may conclude that the method conditions were robust (Tables 9,10,11).

Table 11: Robustness data for change in flowrate

S. No.	Flow rate-1 ml/min		Flowrate-0.8 ml/min		Flowrate-1.2 ml/min	
	Retention Time	Peak Area	Retention Time	Peak Area	Retention Time	Peak Area
1	3.816	3966950	4.813	5039838	3.225	3313690
2	3.806	3915545	4.824	5015856	3.237	3302692
3	3.821	3985012	4.857	5089164	3.231	3354139
4	3.822	3969882	4.823	4916595	3.257	3386897
5	3.832	3966345	4.842	5053443	3.262	3388427
6	3.863	3993207	4.885	5031944	3.282	3374513
% RSD		0.7	-	1.2	-	1.1
USP Plate count		3219.21	-	3528.07	-	3753.59
USP Resolution		8.613	-	10.495	-	7.997
USP Tailing		1.108	-	1.112	-	1.129

Table 12: Stability data for Armodafinil

Days	Retention time	Peak area	% Stability
1	3.856	7184293	100
	3.979	7169924	99.7
2	3.981	7140816	99.3
	3.904	7114240	99
3	3.869	7096528	98.7
	3.884	7053627	98.1
4	3.882	7025064	97.7

Stability of drug solution:

The stability was checked by diluting the stock solution to 100µg/ ml. It was checked for 4 days at interval of 12 hours under normal laboratory conditions (25 ±1 ^{0}C). The drug was stable up to 36 hours and has no significant change in analyte composition and peak areas (Table 12).

Forced degradation studies:

Specificity was studied by exposing the sample solutions to stress conditions i. e. 0.1N HCl, 0.1N NaOH, photolytic degradation and thermal degradation.

Percentage degradation level of 10% was considered for degradation studies. Degradant peaks at 1.73 minutes was observed by acid hydrolysis, a degradant peak at 1.75 minutes was observed by alkali degradation, a degradant peak at 1.48 minutes was observed by photolytic degradation and a degradant peak at 1.68 minutes was observed by thermal degradation. From the results obtained (shown in tables 13, 14, 15, 16), it was found that the drug is stable for 30 minutes in acidic and basic medium. The drug has thermal and photolytic stability for one hour. By the chromatograms obtained during the degradation studies it was found that there is no interference of the degradant peak with the analyte peak. Hence the method is stability indicating.

Table 13: Degradation data for Armodafinil in acidic condition

S. No.	Time of Exposure	Peak Area of Armodafinil	% of Stable Armodafinil	% Degraded
1	0 hour	6783903	100	0
2	30 min	5445913	80.2	19.8
3	1 hour	5316242	78.3	21.7

Table 14: Degradation data for Armodafinil in Alkaline condition

S. No.	Time of Exposure	Peak Area of Armodafinil	% of Stable Armodafinil	% Degraded
1	0 hour	6783903	100	0
2	30 min	5036633	74.2	25.8
3	1 hour	4220732	62.2	37.8

Table 15: Photo degradation data for Armodafinil

S. No.	Time of Exposure	Peak Area of Armodafinil	% of Stable Armodafinil	% Degraded
1	0 hour	6783903	100	0
2	30 min	6032936	88.9	11.1
3	1 hour	5851660	86.2	13.8

CONCLUSION

The proposed method was found to be simple, rapid, sensitive, precise, robust and accurate for determination of Armodafinil in formulation. The proposed method was very simple as the preparation of mobile phase is simpler. The method is very sensitive as the LOQ concentration was very low. The method was found linear over wide range of concentration.

The method can be used for routine analysis of Armodafinil in the presence of degradants.

ACKNOWLEDGEMENTS

The authors are thankful to the management and authorities of Department of pharmaceutical Analysis, Care college of pharmacy for providing the necessities required for carrying out the present

research work and also MSN laboratories, Hyderabad for providing the gift sample of Armodafinil.

REFERENCES

1. Quezia B Cass, Cristiane K Kohn, Silvana A Calafatti, Hassan Y Aboul-Enein. An enantioselective assay for (±)-modafinil. J Pharm and Biomed Ana 2001;26(1):123-30.
2. Jennifer L, Robert J, John S, DeVane C. Donovan, Malcolm, Markowitz, Lindsay. Chiral Analysis of d-and l-Modafinil in Human Serum:Application to Human Pharmacokinetic Studies. J Ther Drug Monit 2003;25(2):197-202.
3. 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 Pharm Biomed Anal 2008;46(5):937-44.
4. Khaldun M, L A, Rohana B. Muhammad IdirisSaleh. Enantios elective determination of modafinil in pharmaceutical formulations by capillary electrophoresis and computational calculation of their inclusion complexes. J Mic Chim Acta 2009;166(3-4):311-7.
5. Suyun W. Wei Xiaojuan Zhou, YibingJi and Bingren Xiang. Enantiomeric Separation and Determination of the Enantiomeric Impurity of Armodafinil by Capillary Electrophoresis with Sulfobutyl Ethercyclodextrin as Chiral Selector. J Molecules 2012;17(1):303-14.
6. New LC, S M Ramesh, Devi; Ramakrishna, Singirikonda; Habibuddin, Mohammad. Development and Validation of MS/for the Determination of armodafinil in Human Plasma. J Current Pharm Ana 2012;8(3):295-305.
7. H. IC. Validation of analytical procedure: Methodology Q2B, Tripartite Guidelines. J Pharm Biomed Anal 1996.
8. ICH harmonized tripartite guideline. Impurities in New Drug products Q3B R2 current step 4 versions dated 2 June 2006.
9. H. IC. Guideline, Validation of analytical procedures:Text and Methodology Q2 (R1);November. J Pharm Biomed Anal 2005.