Reliable and Sensitive Stability Indicating Assay Method of Armodafinil

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ABSTRACT

Objective: A reliable RP-HPLC SIAM (stability indicating assay method) was developed for the approximation of armodafinil in presence of its degradation product in pharmaceutical dosage forms. **Materials and Methods:** Mobile phase composition of water and methanol (10% *v/v* OPA) 55:45 % *v/v* was used for separation by using C$_8$, (250 x 4.6 mm, 5μm) column. Eluents were detected at 225 nm at 1ml/min. Stress studies were performed using acid, base, oxidizing agents, light, and heat to get sufficient degradation about 10-20%. **Results:** A total of six degradation products were detected and were well separated from active drug. The linearity was found in between 10 – 150 mcg/ml. The LOD, LOQ were found as 0.78, 2.37 μg/ml respectively. The % RSD for precision study was less than 2 % the accuracy by the recovery study was 98-102 %. The ARM was found to more sensitive to base hydrolysis followed by acid and photolytic degradation. In acid hydrolysis, four major degradants and rest of them are stress sensitive. **Conclusion:** It is concluded that the developed method

is a specific stability indicating assay, suitable to quantify armodafinil in presence of six degradants. Notably, this method represented degradation product even in thermal and oxidation stress unlike other reported methods.

Key words: Armodafinil, Stability indicating, RP-HPLC method, Degradation studies.

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INTRODUCTION

Armodafinil (ARM) (Figure 1), chemically (-) -2-[(R)-(diphenyl methyl) sulfinyl] acetamide, is used in narcolepsy treatment. It is caused by dysfunction of the orexins, whose neurons are activated by ARM. ARM acts by inhibiting the dopamine reuptake leading to an increased dopamine levels.[1, 2, 3] Because of absence of euphoric or pleasurable effects, ARM has less potential for abuse. ARM has partial alpha 1B-adrenergic agonist effects by stimulating the receptors directly.[4, 5]

ARM is 'R' isomer of modafinil. Few stabilities indicating method such as HPLC.[6] HPTLC.[7] have been reported for modafinil. But those studies were incomplete and did not reveal degradation profile. Literature revealed that methods such as Chiral HPLC.[8] LC-MS/MS.[9, 10] were available for quantification of armodafinil in dosage forms. In fact, modafinil and ARM are structurally similar; there is a necessity to develop new stability indicating assay method for individual isomers. It may due to the disparity in degradations kinetics among isomers or between optically pure isomer and racemic mixture. Few literatures revealed that there is significant difference between enantiomers in both degradation pathway and products.[11, 12] herein we report a new RP-HPLC SIAM for the estimation of ARM. The developed method is validated as per ICH Q2 (R2) Guidelines.[13, 14] There are few methods reported.[16, 17, 18, 19] for ARM in literature, but have revealed no degradation in thermal and oxidation conditions or there was no compliance for degradation studies as per regulatory requirements. In earlier reports there were only 5 degradants reported. However, those methods were not completely validated. Hence, the proposed method could be complete guidance for stability indicating assay of ARM. The carcinogenicity studies for armodafinil was carried out in rat, was considered only marginally adequate as per FDA.

MATERIALS AND METHODS

Materials and Reagents

ARM (99.80%) was a gift sample from Aurobindo Pharma limited, Hyderabad, India. Water, methanol (HPLC grade), and ammonia (analytical grade) were purchased from Merck, India. Borosilicate (Class – A) glass wares were used. All glasswares used were sterilized in hot air oven whenever necessary. All solution was freshly prepared. The ARM API obtained was authenticated by UV and IR spectra.

Apparatus and chromatographic conditions

HPLC system (Waters 2695 (Alliance) Model) with PDA detector was used. SYSTONIC Model: S-926 UV- Visible double beam spectrophotometer was used for measurements of all spectra. Chromatographic separation was performed using C$_8$ (250 × 4.6mm, 5μm). The mobile phase consisted of water: methanol (10% OPA) (55:45 v/v) and was pumped at 1ml/min flow rate. The column condition maintained at ambient temperature and UV detection was set at 225nm. Samples were introduced to HPLC column injector through a Rheodyne fitted with a 20μl loop.

Stress studies of armodafinil

Stress studies of ARM drug substance was carried out under Water (pH 7), acid (0.1N HCl 8h), alkaline (0.01N NaOH 2h), oxidative (3 % H$_2$O$_2$ 4 days), thermal (100 °C, 5 days) and photolytic (under sunlight for 48 h) stress conditions.

Preparation of Stock Solution for stress studies

A 10 mg ARM was weighed and transferred to volumetric flask of 10ml capacity. Add small quantity of methanol for solubility of ARM completely and make up with 0.1N HCL, 3% H$_2$O$_2$ and 0.01N NAOH to get 1000 μg/ml. Thermal stress studies were carried out by heating

samples in hot air oven, at 100°C over a period. Photo degradation was done by exposing the liquid sample (1000μg/ml) under hot sunny light.

Hydrolysis stress studies

The 1000 μg/ml stock solutions were prepared in Basic (0.01N NaOH), Acidic (0.1N HCl) and water (pH 7) at room temperature. Samples of 1ml were taken from stock solutions at specific intervals of time and made to 100μg/ml with mobile phase. The pH of acidic sample was adjusted to 3 Ammonia (NH₃) and pH of basic sample was adjusted to pH 3.0 with glacial acid. After adjusting the pH, the sample was injected onto HPLC column with appropriate control and blank solutions.

Oxidation

The sample for oxidation stress studies (1000 μg/ml) were prepared in 3 % H_2O_2. Sample of 1ml was withdrawn from stock solution and made to 100μg/ml with mobile phase and injected onto HPLC column at different time intervals against blank.

Thermal stress studies

Thermal degradation studies were performed by exposing the solid sample to100°C. At varied intervals of time 10 mg of the samples were taken and made to 100μg/ml and injected.

Photo degradation

1000 μg/ml ARM in water was exposed to direct sunlight for 48h. 1 ml of samples were taken at varied time and made to 100 mcg/ml and injected onto the system with appropriate blanks.

RESULTS

The optimized condition was done by using C_8 column, pH 3 mobile phase, water and methanol (10%OPA) within the ratio of 55:45 % *v/v* at 225 wavelengths. In Figure 2 the optimized chromatogram was shown. Totally there were 6 degradants observed during stress studies with RTˢ of 4.4 min (D1), 6.6 min (D2), 9.8min (D3), 10.4min (D4), 13.3 min (D5) and 15.7 min (D6) respectively. In acid stress studies four degradants were formed (D3, D4, D5, D6), base stress studies - one degradant (D1), photolytic studies - one degradant(D6), Oxidative stress studies - one degradant(D2), Thermal stress studies - one degradant(D4). Out of 6 degradants D1, D2, D4 are specific to base, oxidation, thermal respectively. D6 is common degradant in both acid and photolytic stress studies. All peaks have resolution more than 2. In Table 5 the %degradation was shown.

Validation Parameters

As per ICH (Q2) guidelines method was validated regarding to specificity, accuracy, linearity, precision, robustness, LOD, LOQ and shown in Table 4.

Specificity and Selectivity

Specificity and selectivity was there for all degradants which were obtained during stress studies. All degradants were separated properly from ARM, so that the method is specific.

Linearity and range

ARM linearity was studied in the range of 10-150 mcg/ml at six different concentrations such as 10, 20, 40, 60, 80, 100, 120, 150μg/ml. R² value was found to be 0.9994 as shown in Table 1.

Accuracy

Recovery studies using spike method was performed for Accuracy by using lactose solution to prove specificity in presence of excipients. Study was conducted at 80, 100 and 120 % levels as detailed in the Table 2. Recovery results were in between 99.37 to 100.3%.

Table 1: Linearity Range Data for Armodafinil.

Concentration (μg/ml)	Peak area Mean ± SD (n=3)	% RSD
10	1534696 ± 12813	0.83
20	3086506 ± 3655	0.11
40	6191672 ± 8087	0.13
60	9130919 ± 10685	0.11
80	12250248 ± 39298	0.32
100	15345283 ± 137657	0.89
120	18678534±109555	0.58
150	22926079±55633	0.24

% RSD = relative standard deviation; SD = standard deviation

Table 2: Recovery Studies.

Amount (μg/ml)	Recovery Level	Amount spiked (μg/ml)	Amount recovered (μg/ml)	% Recovery (n = 3)
	80 %	80	79.5 ± 0.57	99.37
100	100 %	100	100.3 ± 0.36	100.30
	120 %	120	120.1 ± 0.39	100.08

Table 3: Intra and Inter-Day Precision.

Drug	Amount (μg /ml)	Intraday (n=3)		Interday (n=3)	
		Amount found Mean ±SD	% RSD	Amount found Mean ± SD	% RSD
	20	3082880 ± 1156	0.17	3077213 ±9739	0.31
ARM	50	7678476±65636	0.85	7718476±99992	1.29
	100	15358694±18317	0.11	15392027±75867	0.49

Table 4: Validation Parameters.

Parameter	Armodafinil
Retention time	8.2±0.1min
LOD(μg/ml)	0.78 ±0.01μg/ml
LOQ(μg/ml)	2.37 ±0.01 μg/ml
Linearity	10-120 μg/ml, r²=0.9994
Accuracy	100.3%
Intraday precision	0.11-0.85(%RSD)
Inter day precision	0.31-1.29(%RSD)
Robust ness	Organic phase (±2%) %RSD 1.56 Flow rate (± 0.1ml) %RSD 1.47
System Suitability	7496 ±146 < 0.5 1.15±0.1

Precision

At triplicate concentrations of 20, 50, 100μg/ml intra-day and inter-day precision results were obtained. The % RSD value for both precision was less than 2% and shown in Table 3.

Robustness test

By changing certain properties such as flow rate, mobile phase robustness test was performed for developed method. The method was robust for flow rate parameter tested.

Table 5: Stress Degradation Studies for Armodafinil.

Stress		t_R (min) of Degradation products						% degradation	% Assay
Condition	No. of	(% area)							
	Degradants	4.4	6.61	9.8	10.4	13.3	15.7		
		D1	D2	D3	D4	D5	D6		
0.1N HCL (8 h)	4	--	--	0.75	9.7	1.82	0.69	14.4	85.5
0.01N NaOH(2h)	1	27.9	--	--	--		--	31.7	68.2
3% H$_2$O$_2$ (4 days)	1	--	3.43	--	--	--	--	18.4	81.5
Thermal (100°C, 5 days)	1	--	--	--	10.2	--	--	10.5	89.4
Photolytic(48h)	1	--	--	--	--	--	1.23	8.29	91.7

Limit of detection and limit of quantification

LOQ and LOD were found to be 2.37, 0.78 μg/ml, respectively.

DISCUSSION

By using reverse phase mode with water and methanol as a mobile phase the drug was subjected to separation by varying % aqueous phase from 10 % to 55 %. The ARM was separated properly on chromatogram with good peak shape. 55% of aqueous phase was taken for optimization with RT of 8.2 min. To achieve efficient elution of ARM, various trials were done by varying pH with appropriate buffers, but there was significant enhancement in theoretical plates and Tailing factor (more than 7000, 1.30) when 10% orthophosphoric acid (OPA) used in mobile phase. In comparison to earlier methods there was one additional impurity revealed in peroxide degradation.

Stress Degradation

Acidic Degradation

When treated with 0.1N HCl at room temp for 8h, 14.4% degradation was observed with formation of four degradants at retention time (in min) of D3 at 9.80, D4 at 10.47, D5 at 13.30 and D6 at 15.78 with respective area percentage of 0.71 %, 9.70%, 1.8%2 and 0.69 %. The assay of ARM was about 85.5 % as shown in Figure 3.

Basic degradation

When ARM was exposed to 0.01N NaOH, the drastic degradation 31.7 % was takes place within 2 h. The percentage area of D1 was 27.9 % at retention time of 4.48 min. The assay of ARM was about 68.2 % shown in Figure 4.

Neutral Degradation

No degradation was observed when exposed neutral condition for 7 days at room temperature.
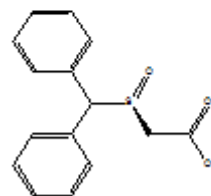
Photolytic degradation

When ARM was exposed to sun light for a period of 48 hours, 8.29 % degradation was observed with formation of one degradant. The retention time (t$_R$) of D6 was found to be 15.5 min. The assay of ARM was about 91.7 % as shown in Figure 5.

Oxidative degradation

ARM showed degradation of 18.4% in 3 % H$_2$O$_2$ for 4 days. The percentage area of D2 was 3.43 % at retention time of 6.61 min. The assay of ARM was about 81.5 % shown in Figure 6.
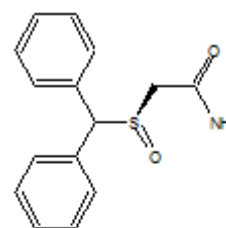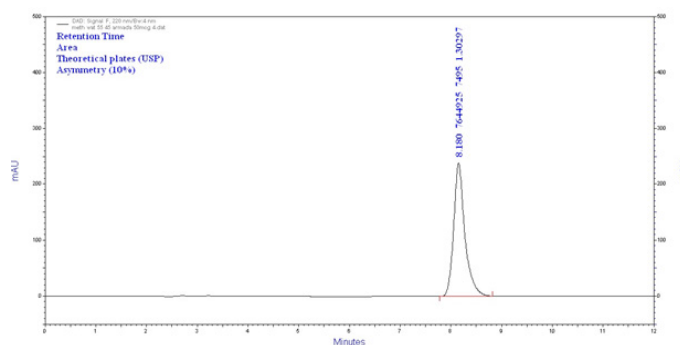

Figure 1: Structure of armodafinil and its base hydrolytic degradant; a) (-)-2-[(R)-(diphenylmethylsulfynil)] acetamide b) 2- [(benzhydrylsulfinyl)] acetic acid (degradant - D1)

Figure 2: Optimized Chromatogram of Armodafinil.

Thermal degradation

ARM was exposed to heat in oven at 100°C for 5 days, it was showed 10.5% degradation with formation of one degradant at retention time of 10.69 min. The assay of ARM was 89.4% shown in Figure 7.
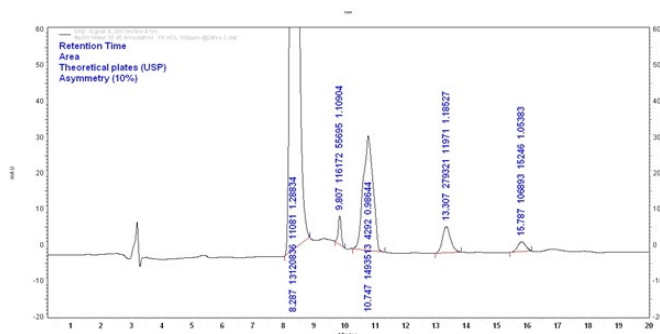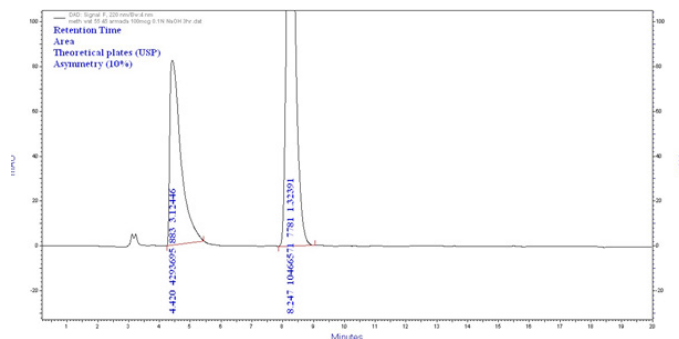
Figure 3: Acid degradation(0.1N HCl, 8h).
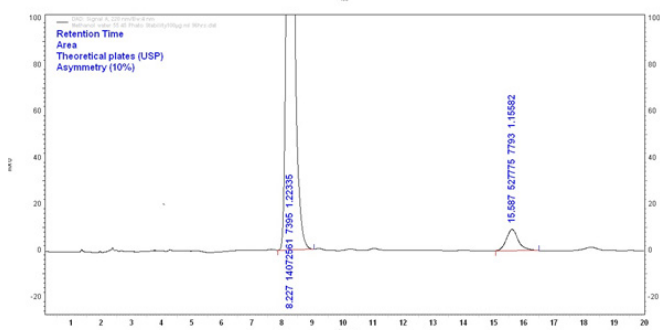

Figure 4: Base degradation(0.01N NaOH, 2h).

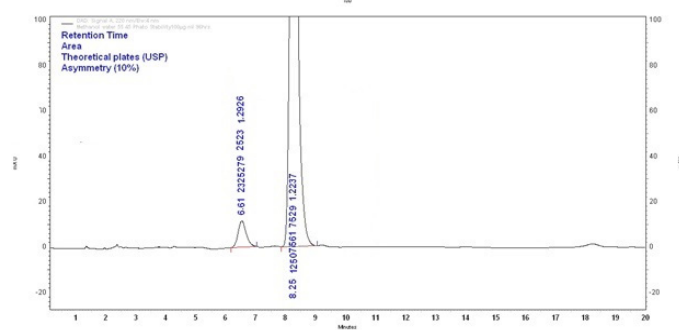**Figure 5:** Photolytic degradation (48h).

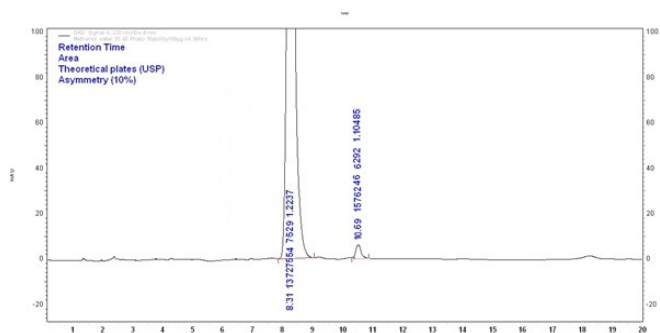**Figure 6:** Oxidative degradation (3% H2O2 4 days).

Figure 7: Thermal degradation (1000C, 5 days).

A total of six degradants were identified in all stress degradation, the drug was more sensitive for basic stress then followed by acid stress, photolytic stress. The ARM was resistant in Neutral conditions.

CONCLUSION

A new RP-HPLC SIAM method was developed for the estimation of armodafinil in dosage form and validated according to ICH guidelines. This method revealed formation of six possible degradation products after conducting stress studies. The ARM was found to more sensitive to base hydrolysis followed by acid and photolytic degradation. This method is most suitable for stability study of drug after marketing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATION USED

SIAM: Stability Indicating Assay Method; **ARM:** Armodafinil.

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