A validated stability indicating RP-HPLC method for estimation of Armodafinil in pharmaceutical dosage forms and characterization of its base hydrolytic product

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Abstract: The main objective of present study was to develop a RP-HPLC method for estimation of Armodafinil in pharmaceutical dosage forms and characterization of its base hydrolytic product. The method was developed for Armodafinil estimation and base hydrolytic products were characterized. The separation was carried out on C_{18} column by using mobile phase as mixture of water and methanol (45:55%v/v). Eluents were detected at 220nm at 1ml/min. Stress studies were performed with milder conditions followed by stronger conditions so as to get sufficient degradation around 20%. A total of five degradation products were detected and separated from analyte. The linearity of the proposed method was investigated in the range of 20-120µg/ml for Armodafinil. The detection limit and quantification limit was found to be 0.01183µg/ml and 0.035µg/ml respectively. The precision % RSD was found to be less than 2% and the recovery was between 98-102%. Armodafinil was found to be more sensitive to the base hydrolysis and yielded its carboxylic acid as degradant. The developed method was stability indicating assay, suitable to quantify Armodafinil in presence of possible degradants. The drug was sensitive to acid, base & photolytic stress and resistant to thermal & oxidation.

Keywords: Armodafinil, Stability indicating assay, RP-HPLC method, degradation studies.

INTRODUCTION

Chemically, Armodafinil (fig. 5a) is (-) -2-[(R)-(diphenyl methyl) sulfinyl] acetamide. It acts as a stimulant drug and plays a key role in the treatment of narcolepsy. The common cause of Narcolepsy is due to the dysfunctioning of wakefulness promoting and sleep suppressing peptides. Armodafinil shows its mechanism of action by inhibiting the dopamine reuptake, which causes an increased level of extra cellular dopamine occurs (Swanson *et al*., 2003; Wisor *et al*., 2001). It has partial alpha 1B-adrenergic agonist action (Overington *et al*., 2006; Imming *et al*., 2006).

Armodafinil is 'R' isomer of Modafinil. Few stability indicating methods such as HPLC (Vilas *et al.,* 2013), HPTLC have been reported for Modafinil (Gaurang *et al.,* 2013). But those studies were incomplete and not revealed degradation profile. Literature revealed that the methods such as Enantioselective HPLC (Rao *et al.,* 2008), RP-HPLC (Vivek *et al.,* 2011), LC-MS/MS were available for quantification of armodafinil in dosage forms (Mamta *et al.,* 2011; Ramesh *et al.,* 2012). So far, there is no stability indicating assay method on armodafinil has been reported neither to detect possible degradants nor quantification of active ingredients in presence of degradants. In fact, modafinil and armodafinil 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 (Qin *et al.,* 2006), which prompted us to develop new RP-HPLC method (stability indicating) for the armodafinil estimation in pharmaceutical dosage forms along with characterization of major degradation products. The developed method was validated according to the guidelines of ICH Q2 (R2) (ICH guidelines, 1995).

MATERIALS AND METHODS

Materials and reagents

Armodafinil (99.80%), Water, methanol (HPLC grade), and ammonia solution (analytical grade) were purchased from Merck, India. Borosilicate (Class-A) glass wares were used. For isolation and purification, the Flash Chromatography equipped with silica gel as a column material and VWD-UV detection (using software Analogix IF 280 V 5.10) was used.

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Apparatus and chromatographic conditions

HPLC system (Agilent LC 1200 with Ezchrome elite Software) with UV-PDA detector was used and separation was performed using C_{18} (250 × 4.6mm, 5µm). The mobile phase contained a mixture of water: methanol in the ratio of 45:55%v/v. Then it was pumped at flow rate of 1 ml/min. The column condition maintained at ambient temperature and photo diode array detection was set at 220 nm. Samples were introduced into the HPLC column through a Rheodyne injector fitted with 20µl loop.

Forced degradation studies of armodafinil

Forced degradation of Armodafinil drug substance was carried out under neutral (Water), acid (0.1N HCl followed by 1 N HCl), alkaline (0.1 N NaOH), oxidative (0.3% H_2O_2 followed by 3% H_2O_2), thermal (70°C, 48 h) and photolytic (under sunlight) stress conditions.

Preparation of stock solution for stress studies

An accurately weighed quantity of 10 mg drug substance was transferred into a 10ml volumetric flask containing methanol, dissolved and finally volume was made up with HCl (0.1-1N), NaOH (0.1N) and H_2O_2 (0.3-3%) respectively, to get a concentration of 1000 µg/ml. Thermal degradation was performed in hot air oven for solid states by means of heating samples over a period at 70°C. Photo degradation was done by exposing the liquid sample (1000µg/ml) to hot sunny light. The detailed procedures were outlined in the following sections.

Hydrolysis

The three types of stock solutions were prepared, namely acidic, basic and neutral stock solutions and which were diluted to get concentration of about 1000µg/ml each. The acidic stock solution was prepared by using hydrochloric acid (1N HCl) and basic stock solution by using sodium hydroxide (0.1N NaOH) and neutral stock solution by using water at room temperature. From the each stock solution, 1ml of sample was withdrawn into 10ml volumetric flask and it was diluted upto 10ml by using mobile phase to get the concentration of 100µg/ml. These samples were neutralized to get the pH 5-6. The sample from acid hydrolysis was neutralized by using 10% Sodium hydroxide and the sample from basic hydrolysis was neutralized by using 10% Hydrochloric acid. After neutralization to pH 5-6, the sample was injected into HPLC column with appropriate control and blanks.

Oxidation

Oxidative stress solution was prepared in 0.3% H_2O_2. From this solution, 1ml was withdrawn and transferred into 10ml volumetric flask. To get the concentration of 100µg/ml, it was diluted upto 10ml by using mobile phase. Then it was injected into column at various intervals against peroxide as a blank.

Thermal degradation

It was performed by using the solid sample. This solid sample was made as a thin layer in the Petri dish and which was exposed to the temperature of about 70°C. After heating, 10 mg of this solid sample was weighed and dissolved in mobile phase at various intervals. This sample solution was diluted to get 100µg/ml and then injected into the system.

Photo degradation

This study was performed by using the liquid sample. From this liquid sample, 1 ml sample was withdrawn and transferred into the 10 ml volumetric flask. It was made dilute up to 10 ml with mobile phase to get the 100 µg/ml and then injected.

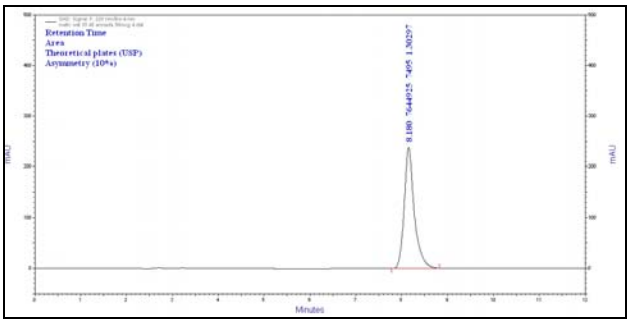


Fig. 1: Optimized chromatogram of armodafinil on C_{18} column

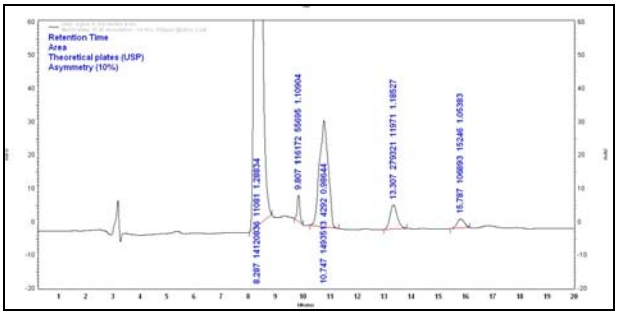


Fig. 2: Acid degradation of Armodafinil (1 N HCl, 24h)

Synthesis, purification and characterization of alkaline degradation product

Base hydrolysis

The 0.003M Armodafinil was prepared and it was dissolved in 10ml methanol. To this solution, 10% Sodium hydroxide was added and it was reflexed for about 2h at 75°C. The acidification technique was used for precipitating the previous resultant mixture by using 1 N HCl, thereby the crude product was obtained. It was recrystallized and purified by using the flash chromatography technique on a silica gel as a column material. For this purification, the mobile phase used was mixture of methanol and ethyl acetate in a gradient mode (from 5% to 50% methanol). The fractions were collected by performing the VWD-UV detection and evaporated by using vacuum dryer.

Purification

The product was purified by flash chromatography using

silica gel as a column material and the mobile phase used was mixture of methanol and ethyl acetate (from 5% to 50 % methanol) in gradient mode. VWD-UV detection was carried out for collection of fractions. At the end of the elution, fractions were examined based on UV characteristics and TLC based identity of compound fractions were combined and evaporated in vacuum dryer. The spectral methods were used for the isolation and characterization of the product. This product was compared with standard Armodafinil solution to determine the degradation product in stress conditions.

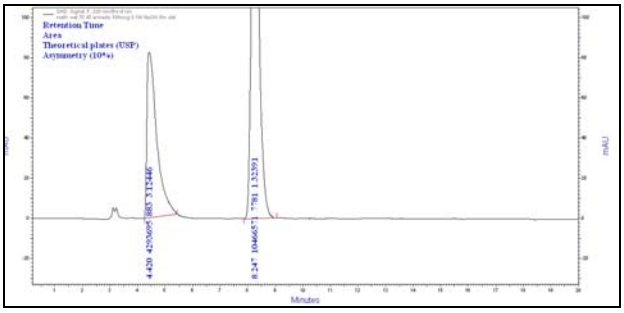


Fig. 3: Base degradation of Armodafinil (0.1N NaOH, 3h)

Base degradant (D1)

2-[(diphenyl methyl) sulfinyl] acetic acid; Molecular formula: $C_{15}H_{14}O_3S$; Molecular weight: 274.75g/mole; M.P: 145-147^oC; λmax: 218 nm; IR (KBr, cm^{-1}): 3430 (OH str), 3061 (Ar-H str), 2872 (CH_2-str), 1710 (C=O str, COOH), 1596, 1576 (C=C str), 1274 (C-O str), 1002 (OH bending), 693 (Ar-H, out of plane deformation). ^{1}H-NMR ($CDCL_3$, δ ppm): 4.02 (s, 1H, CH), 7.12-7.74 (m, cpx, 10H, Ar-H), 11.9 (s, 1H, OH exchangeable). Mass (ESI) 275 (M+H).

RESULTS

The best conditions for proposed RP-HPLC method were selected for the quantification of armodafinil in pharmaceutical dosage forms and characterization of possible degradation products. The developed method was validated according to ICH guidelines (table 2).

DISCUSSION

Method development and optimization of chromatographic conditions

In this present study, the reverse phase mode was used for separation of Armodafinil by using the varying concentrations of % aqueous phase ranged from 10% to 45%v/v. The drug was retained and the peak shape was good. It was noted that the retention time of Armodafinil was affected by % aqueous in mobile phase. To get the desired retention time for stability assay, the 45% aqueous in mobile phase was optimized to achieve retention about 8.1min. In order to achieve efficient elution of armodafinil, various trials were done by varying pH with appropriate buffers, but there was no significant enhancement in theoretical plates when compared to the run in absence of buffers.

Hence the chromatographic conditions were optimized on C_{18} (Agilent, 250 x 4.6mm, 5µ) column with UV- DAD detection by using the mobile phase of water and methanol in the ratio of 45:55% v/v. The injection volume was about 20µl and it was detected at 220 nm. The optimized chromatogram was shown in fig. 1. It was observed that the method has showed all degradant's peaks from Armodafinil in various conditions. It was revealed that the specificity of the method. The results were indicated that the 5 (D1-D5) major degradation products were observed with retention times of 4.4 min (D1), 9.8 min (D2), 10.4min (D3), 13.3min (D4) and 15.7 min (D5) respectively. The resolution was found to be more than 2. The percent degradation and the assay of the active substance after degradation studies was calculated based on control and zero hour injection as shown in table 1.

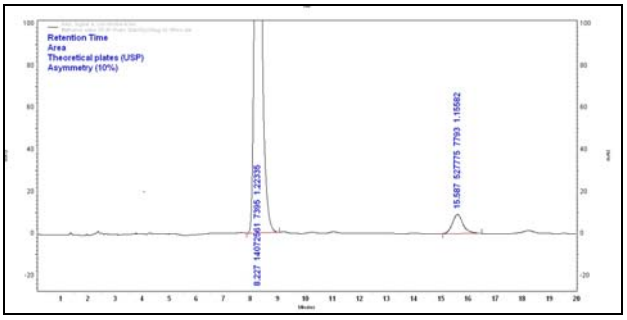
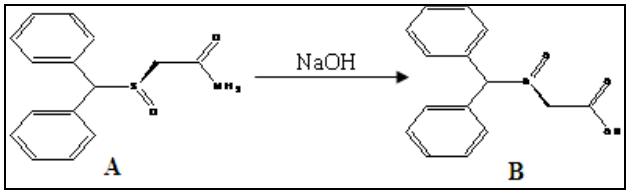


Fig. 4: Photolytic degradation of armodafinil in water (20 h)

Validation parameters

These parameters like linearity, accuracy, precision, specificity, robustness, limit of detection and limit of quantification were performed according the guidelines of ICH (Q2). The validation parameters were reported in table 2.



a) (-)-2-[(R)-(diphenyl methyl sulfynil)] acetamide; b) 2- [(benz hydryl sulfinyl)] acetic acid (degradant - D1)

Fig. 5: (a & b): Structure of Armodafinil and its base hydrolytic degradant

Specificity and selectivity

For confirming the specificity and selectivity of the developed method, the degradation studies were performed on the Armodafinil. All degradants adequately separated from armodafinil, thus the specificity of the method was proven.

Linearity and range

The linearity studies for Armodafinil were performed at different concentrations ranged from 20µg/ml to

120µg/ml (20, 40, 60, 80, 100 and 120µg/ml). The correlation coefficient was found to be 0.9990 with the regression coefficient of y=147975.3629x + 284476.9333 (table 3).

Accuracy

The standard addition method was used for performing the accuracy by recovery studies and was conducted at 80, 100 and 120% levels as detailed in table 4. Each concentration was analyzed in replicate. The recovery

Table 1: Stress degradation studies for Armodafinil

Stress Conditions	No. of Degradants	Degradation products at t_R (% area)					% Degradation	% Assay
1N HCl (24 h)	4	--	0.71	9.24	1.72	0.66	12.52	87.37
1N NaOH (3 h)	1	27.34	--	--	--	--	33.28	65.41
3% H_2O_2 (96 h)	--	--	--	--	--	--	--	98.24
Thermal (70^0C, 48 h)	--	--	--	--	--	--	--	99.43
Photolytic (Sun light (20h)	1	--	--	--	--	1.23	11.79	87.01

Table 2: Validation Parameters

Parameter	Armodafinil
Retention time (t_R)	8.1±0.1min
LOD (µg/ml)	0.012±0.01µg/ml
LOQ (µg/ml)	0.035±0.01 µg/ml
Linearity	20-120µg/ml, r^2=0.9991
Accuracy	101.06 %
Intraday precision	1.15-1.49 (%RSD)
Inter day precision	0.12-0.94 (%RSD)
Robustness	Organic phase (±2%)
System Suitability	Theoretical plate = 7496 ±146 System precision % RSD = < 0.5 Tailing factor = 1.15±0.1

Table 3: Linearity for Armodafinil

S. No.	Concentration (µg/ml)	Peak area Mean ± SD (n=3)	% RSD
1	20	3208400±8590	0.27
2	40	6170101±24345	0.41
3	60	9087801±26305	0.29
4	80	12259831±33290	0.27
5	100	15344439±24863	0.16
6	120	17785942±36075	0.20

% RSD = relative standard deviation; SD = standard deviation

Table 4: Recovery studies (n = 6)

Amount (µg/ml)	Recovery Level	Amount added (µg/ml)	Amount recovered Mean ± SD ((µg/ml)	% Recovery
50	80%	90	39.58±0.33	98.96
	100%	100	50.53±0.39	101.06
	120%	110	59.51±0.35	99.09

Table 5: Intra and Inter-day Precision

Drug	Amount (µg/ml)	Intraday (n=3)		Inter day (n=3)	
		Amount found Mean ±SD	% RSD	Amount found Mean ± SD	% RSD
Armodafinil	20	3206948±3706	0.16	3191205±44527	1.39
	50	7606613±51556	0.68	7566592±113196	1.49
	120	17700001±167495	0.95	17628247±202950	1.15

results were found to be in between 98.96 and 101.06%.

Precision

The precision studies (intraday and inter day) were obtained from linearity at 3 different concentrations i.e. 20, 50 and 120µg/ml. The % RSD value for both precision was less than 2% and the results were shown in table 5.

Robustness

The robustness was determined for present developed method by analyzing the samples under altered method parameters such as change in flow rate, mobile phase composition and wavelength. The method was robust for mobile phase parameter and not complied with flow rate.

Detection limit and quantification limit

Both of these limits were determined by based on signal to noise ratio (S/N ratio). The S/N ratio for LOD was taken as 3:1 and for LOQ, it was taken as 10:1. The values of LOD and LOQ were found to be 0.012, 0.035 µg/ml, respectively.

Forced degradation

Acid induced degradation

In this degradation study, the initial degradation was performed in 0.1N HCl for more than 3 days at room temperature and it was noted that the drug showed less than 2% of drug degradation even after 3 days at room temperature. The degradation studies with 1N HCl for 24 h at room temperature, it was observed that the 12.52% degradation for a total of four degradants D2 (9.80min), D3 (10.47min), D4 (13.30min) and D5 (15.87 min) with respective area percentage of 0.71%, 9.24%, 1.72% and 0.66%. The assay of armodafinil was about 87.37% as shown in fig. 2.

Base induced degradation

The degradation studies with 0.1N NaOH, it was observed that the 33.28% in 3h with the formation of 1 degradation product. The percentage area of D1 was 27.34% at retention time of 4.48min. The assay of armodafinil was about 65.44% shown in fig. 3.

Neutral degradation

No degradation was observed when exposed to neutral condition for 96h at room temperature.

Oxidative degradation

The degradation studies with 0.3% H_2O_2 for 120 h and 3 % H2O2 for 96h, it was observed that the drug showed negligible degradation. Even after there was no degradants observed.

Thermal degradation

These degradation studies were performed by exposing the drug to the dry heat in oven at 70^{o}C for 48h and it was observed that the drug showed negligible degradation. The assay of Armodafinil was found to be 99.43%.

Photolytic degradation

It was performed by exposing the drug to the sun light for 20 h and it was noted that the 11.79% degradation with the formation of one degradant. The retention time (t_R) of D5 was found to be 15.72min. The assay of Armodafinil was found to be about 87.01% as shown in fig. 4. The mass balance was fall in between 97-99%.

A total of five degradants were identified in all stress degradation, the drug was more sensitive for basic stress then followed by acid stress, photolytic stress. The % degradation was relatively low in oxidation, hydrolysis and thermal, indicating that the drug was almost stable. Armodafinil was found to be more resistance towards oxidative and thermal degradation. The acid and base hydrolysis products are not similar, and significantly differ in their structure.

Identification of degradants

In the present forced degradation study, the major degradant (D1) was synthesized and purified by Flash Chromatography. Structure of degradant was elucidated as 2-[(benz hydryl sulfinyl)] acetic acid (fig. 5b). It was supported by the evaluation of ammonia during reflux and spectral characteristics. Spike analysis of synthesized D1 with Armodafinil standard confirmed that the retention time (t_R) of elucidated degradant at 4.4 min. For looking, the D1 has yellowish silky crystals like structure. The solubility studies indicated that the D1 was soluble in methanol and sparingly soluble in water gave effervescence with sodium carbonate, which indicated that the presence of -COOH group in the structure. It might be due to the amide hydrolysis of Armodafinil under alkaline condition.

CONCLUSION

From the results, it could be concluded that this method revealed five degradation products and among them, base hydrolytic product was characterized. The armodafinil was found to be more sensitive to the base hydrolysis followed by acid and photolytic degradation.

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