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RP-HPLC Method Development and Validation for the Estimation of Armodafinil and Valsartan

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ABSTRACT

The current manuscript deals with development and validation of RP-HPLC method for estimation of Armodafinil and Valsartan. The proposed method is simple, selective, reproducible, sensitive and accurate with good precision. Some of the methods were proved to be superior to most of the reported methods. All these proposed methods for estimation of selected drugs such as Armodafinil and Valsartan were successfully applied either in bulk or pharmaceutical formulations. The precision of the method was estimated by analyzing sample solutions. Six multiple samples (from a homogeneous lot) were analysed and content of Valsartan is determined as mg/ tab of the tablet. The RSD of the content was found to be well within the limits (i.e. %RSD<2%). The linearity was investigated in the range of 1 to 200 mcg/ml using six different concentrations. The areas obtained at 250 nm for Valsartan were fitted to a straight line by the method of least squares. Linear regression analysis for Valsartan was calculated and was found to be 0.9999. Accuracy was investigated in the concentration range of 80-120% of the standard concentration for Valsartan. The percentage recovery values obtained lie within the standard limit of 98 % to 102%. The proposed method can be used as alternative method to the reported ones for the routine determination of selected drugs under the study in bulk and pharmaceutical dosage forms.

INTRODUCTION

Chromatography is a separation process that is achieved by distributing the components of a mixture between two phases, a stationary phase, and a mobile phase. Those components held preferentially in the stationary phase are retained longer in the system than those that are distributed selectively in the mobile phase. Consequently, solutes are eluted from the system as local concentrations in the mobile phase in the order of their increasing distribution coefficients with respect to the stationary phase; ipso facto, a separation is achieved. The technique was originally developed by Russian botanist M.S.Tswett in 1903. The definite breakthrough for liquid chromatography of low molecular weight compounds was the introduction of chemically modified small diameter particles (3 to 10 μm) e.g., octadecyl groups bound to silica in the late 1960's.

High-performance liquid chromatography (HPLC) is a form of column chromatography used frequently in biochemistry and analytical chemistry. It is also sometimes referred to as High-Pressure Liquid Chromatography. The name "HPLC" originally referred to the fact that high pressure is required to generate the flow required for liquid chromatography in packed columns. In the beginning, instrument components only had the capability of generating pressures of 500psi (35 bars). The early 1970's saw a tremendous leap in technology. These new "HPLC" instruments could develop up to 6,000psi (400 bar) of pressure, and included improved detectors and columns. HPLC really began to take hold in the mid to late 1970's. With continued advances in performance, the name was changed to High Performance Liquid Chromatography (HPLC).

MATERIALS AND METHODS

LIST OF INSTRUMENTS USED

Table 1: List of Instruments

Sr.No	Instruments/Equipments/Apparatus
1. 1.	HPLC-Waters Separation Module 2695 with Waters 2696 PDA
2. 2.	UV-Visible Spectrophotometer (Agilent 8543).
3. 3.	Electronic Balance (Mettler toledo)
4. 4.	Ultra Sonicator (Enertech)
5. 6.	Symmetry -C18 (250 X 4.6mm, 5 µm) column
6. 7.	Zorbax -C18 (150 X 4.6mm, 5 µ) column
7. 8.	x-Terra-RP18, 4.6×150mm, 5µm
8. 9.	pH Analyzer (744 Metrohm)

LIST OF CHEMICALS, REAGENTS AND STANDARDS USED:

Table 2: List of Chemicals, Reagents and Standards

Sr.No	Chemicals / Reagents / Standards	Grade	Specification
1.	Sodium dihydrogen phosphate	AR	99.0%
2.	Methanol	HPLC	99.8%
3.	Acetonitrile	HPLC	99.8%
4.	Water	NA	NA
5.	Armodafinil working standard	AR	99.0%
6.	Armodafinil sample	NA	99.92 (w/v)
7.	Valsartan working standard	NA	99.96 (w/w)
8.	Valsartan sample	NA	99.93 (w/v)
13.	Hydrogen peroxide	NA	NA
14.	Sodium Hydroxide	NA	NA
15.	Hydrochloric Acid	NA	NA
16.	Orthophosphoric Acid	NA	NA

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Table 3: Optimized Chromatographic Conditions

Parameters	Method
Stationary phase (column)	Symmetry C18 (250 x 4.6 x 5µ)
Mobile Phase	Buffer (0.02M NaH2PO4 PH- 2.5): ACN (58: 42)
Flow rate (ml/min)	1.0
Run time (minutes)	20
Column temperature (°C)	Ambient
Volume of injection loop (µl)	10
Detection wavelength (nm)	250
Drugs RT (min)	9.349

ANALYSIS:

Preparation of Mobile Phase:

Preparation of 0.02 M buffer:

Accurately weighed 3.01gm of Sodium dihydrogen orthophosphate and dissolved in one liter of millipore water and pH adjusted to 5.8.Adjust the prepared Buffer at pH 5.8.

Mobile phase preparation/Diluent:

A mixture of Buffer (58%) and 42% of Acetonitrile was prepared after filtration.

Preparation of Standard Solution:

Accurately about 5 mg of the Valsartan working standard was weighed and transferred into 50 ml clean, dry standard volumetric flask. To this about 20 ml of diluent was added and then it was kept in an ultrasonic bath to dissolve. The volume is made up to the mark with the diluent and mixed well. This yielded a standard stock solution with concentration 100 ppm of Valsartan. This working standard solution was analyzed using the HPLC conditions mentioned above.

Buffer Preparation: Accurately weigh and transfer about 2.72gmof potassium dihydrogen orthophosphate in 1000 ml of purified water and mix. Adjust the solution pH to 4.0 with dilute orthophosphoric acid. Filter the solution through 0.45µ membrane filter.

Mobile phase preparation: Mixture of buffer and Acetonitrile in the ratio of 650: 350 was prepared after filter and degassed.

Diluent preparation: use mobile phase as diluent.

STANDARD PREPARATION: (for Armodafinil tablets 50mg and 250 mg)

Accurately weighed and transferred about 25mg of Armodafinil working standard into a 100 ml volumetric flask to it 60ml of methanol was added and mixture was sonicated to dissolve. Solution was cooled to room temperature and diluted with methanol. 5 ml of above solution was transferred to 25 ml of volumetric flask and diluted with diluent.

SAMPLE PREPARATION: (for Armodafinil tablets 50mg)

Accurately weighed and powered 20 tablets and transferred, equivalent to 250mg of armodafinil into 250ml volumetric flask. 160 ml of methanol was added and sonicated for 45 minutes with occasional shaking. The mixture was cooled to room temperature and diluted with methanol, filtered through 0.45µm membrane filter.5ml of above solution transferred to 100 ml volumetric flask and diluted with diluent.

Weigh and finely powder not fewer than 20 tablets. Transfer accurately weighed portion of the powder, equivalent to 250mg of Armodafinil into a 250 ml volumetric flask. Add about 160ml of methanol and sonicate for 45 minutes with occasional shakings. Cool the solution to room temperature and dilute to volume with methanol. Filter the solution through 0.45µm membrane filter. Transfer 5ml the above filter solution into a 100 ml volumetric flask. Dilute to volume with diluent.

SAMPLE PREAPARTION: (for Armodafinil tablets 250mg)

Accurately weighed and powered 20 tablets and transferred, equivalent to 1250 mg of armodafinil into 250ml volumetric flask. 160 ml of methanol was added and sonicated for 45

minutes with occasional shaking. The mixture was cooled to room temperature and diluted with methanol, filtered through 0.45µm membrane filter. 2 ml of above solution transferred to 100 ml volumetric flask and diluted with diluent.

Weigh and finely powder not fewer than 20 tablets. Transfer accurately weighed portion of the powder, equivalent to 1250mg of Armodafinil into a 250 ml volumetric flask. Add about 160ml of methanol and sonicate for 45 minutes with occasional shakings. Cool the solution to room temperature and dilute to volume with methanol. Filter the solution through 0.45µm membrane filter. Transfer 2ml the above filtered solution into a 200 ml volumetric flask. Dilute to volume with diluent.

STANDARD PREPARATION: (for Armodafinil tablets 150mg)

Accurately weighed and transferred about 24mg of Armodafinil working standard into a 100 ml volumetric flask and added 60ml of methanol and sonicated to dissolve. Cooled the solution to room temperature and diluted to volume with methanol. Transferred 5 ml of the above solution into a 25 ml volumetric flask and diluted to volume with diluent.

SAMPLE PREPARATION: (for Armodafinil tablets 150mg)

Accurately weighed and powered 20 tablets and transferred, equivalent to 750mgof armodafinil into 250ml volumetric flask. 160 ml of methanol was added and sonicated for 45 minutes with occasional shaking. The mixture was cooled to room temperature and diluted with methanol, filtered through 0.45µm membrane filter.4mlof above solution transferred to 100 ml volumetric flask and diluted with diluent.

Weigh and finely powder not fewer than 20 tablets. Transfer accurately weighed portion of the powder, equivalent to 750mg of Armodafinil into a 250 ml volumetric flask. Add about 160ml of methanol and sonicate for 45 minutes with occasional shakings. Cool the solution to room temperature and dilute to volume with methanol. Filter the solution through 0.45µm membrane filter. Transfer 4ml the above filtered solution into a 250 ml volumetric flask. Dilute to volume with diluent.

RESULTS

SYSTEM SUITABILITY

Diluent Chromatogram

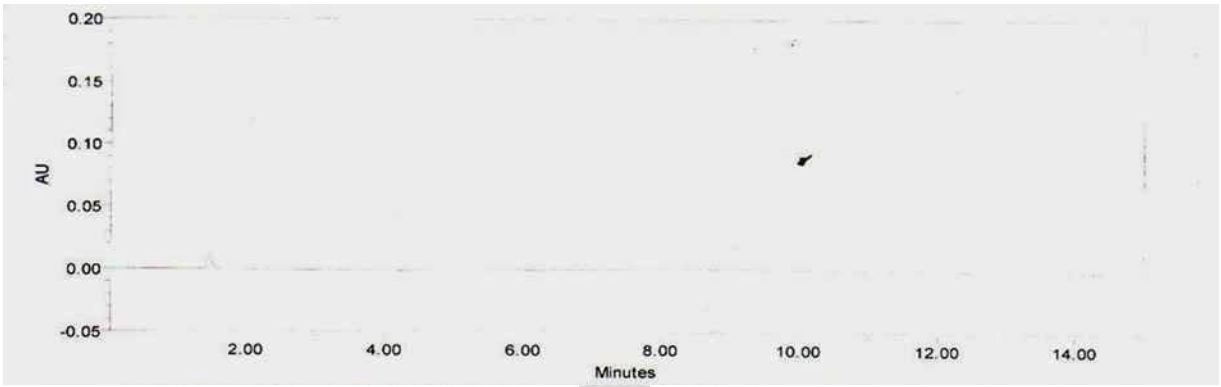
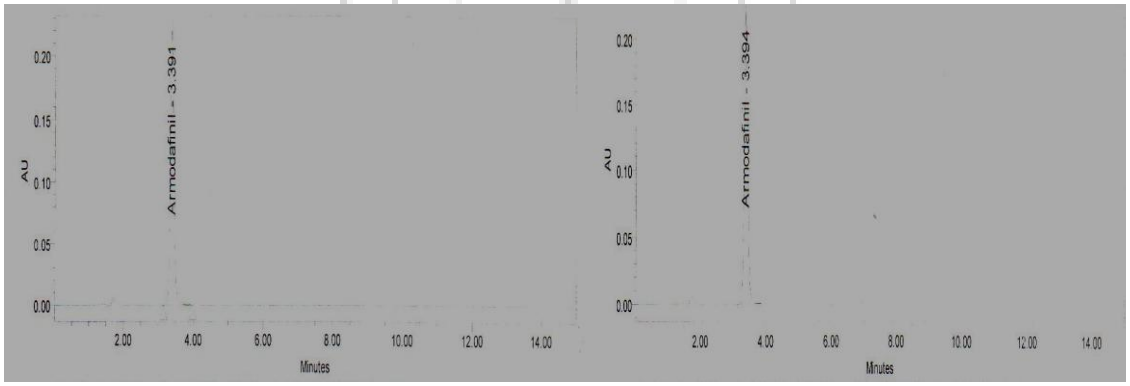
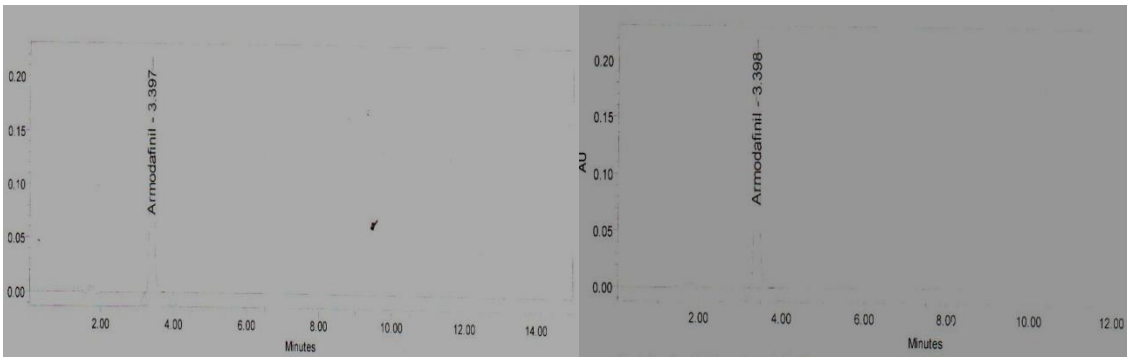
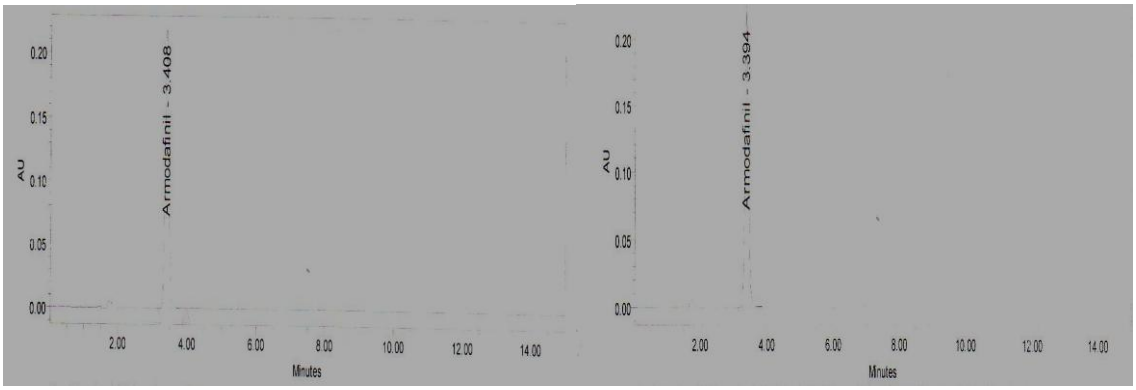


Figure 1: Chromatogram of Diluent







S. No.	Sample Name	Vial	Inj	Name	RT	Area	USP Tailing	USP Plate Count
1	Armodafinil_Assay_Standard(50-250 mg)	50	1	Armodafinil	3.391	1603465	1.16	5224
2	Armodafinil_Assay_Standard(50-250 mg)	50	2	Armodafinil	3.394	1601522	1.15	5107
3	Armodafinil_Assay_Standard(50-250 mg)	50	3	Armodafinil	3.397	1599865	1.15	5111
4	Armodafinil_Assay_Standard(50-250 mg)	50	4	Armodafinil	3.398	1605703	1.15	5121
5	Armodafinil_Assay_Standard(50-250 mg)	50	5	Armodafinil	3.408	1600061	1.16	5163
6	Armodafinil_Assay_Standard(50-250 mg)	50	6	Armodafinil	3.394	1601102	1.15	5096
Mean					3.397	1601953	1.15	5136.8
Std.Dev.					0.006	2244.94		
%RSD					0.2	0.14		

Armodafinil Assay Calculations

Armodafinil Formulations (mg)	Test Area	Test Wt.	Avg. Wt.	Label Amount(mg)	% of result	Statistical analysis
250	1672308	3251.9	625.9	250	100.6	AVG. =100.65 SD =0.070711 %RSD=0.07025
	1672937	3429.7	625.9	250	100.7	
150	1570716	1891.5	376.6	150	100.4	AVG. =99.6 SD =1.1313 %RSD=1.1359
	1546276	1892.4	376.6	150	98.8	
50	1574525	618.5	125.1	50	99.5	AVG. =99.2 SD =0.4242 %RSD=0.4276
	1564664	618.7	125.1	50	98.9	

VALSARTAN

SYSTEM SUITABILITY

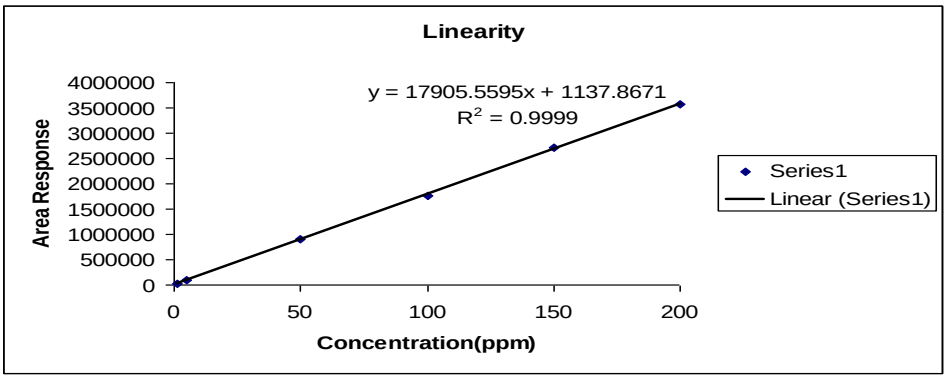
Concentration	Injection	Area	Retention time
100 ppm	Injection-1	1757046	9.335
	Injection-2	1756314	9.342
	Injection-3	1756437	9.41
	Injection-4	1760266	9.398
	Injection-5	1765267	9.387
	Injection-6	1773636	9.385
STATISTICAL ANALYSIS	Avg	1761494	9.376167
	Std	6860.933	0.03059
	%Rsd	0.389495	0.326256
	TAILING FACTOR	0.9	
	PLATE COUNT	$7.3 * 10^3$	
Acceptance Criteria: RSD should be not more than 2.0 %			

METHOD PRECISION

Concentration	Sample no	Injection 1	Injection 2	Average
100 ppm	Sample-1	1755394	1756443	1755918
	Sample-2	1772277	1772237	1772257
	Sample-3	1728206	1771459	1749832
	Sample-4	1786190	1786276	1786233
	Sample-5	1719461	1721105	1720283
	Sample-6	1811502	1817307	1814404
STATISTICAL ANALYSIS			Average	1766487.83
			Std	32382.5221
			%Rsd	1.83315852
Acceptance Criteria: RSD should be not more than 2.0 %				

LINEARITY

Sample	Concentration	Inj 1	Inj 2	Avg
1	1	20813	20918	20755
2	5	93149	96272	94287
3	50	904888	896324	897863
4	100	1768520	1764689	1767713
5	150	2701998	2709753	2709660
6	200	3572932	3577105	3576763



ACCURACY

Sample ID	Concentration (µg/ml)		%Recovery of	Statistical Analysis	
	Pure drug	Formulation	Pure drug		
S_1 : 80 %	160	200	99.98	Mean	99.88
S_2 : 80 %	160	200	99.82	SD	0.085
S_3 : 80 %	160	200	99.85	% RSD	0.085
S_4 : 100 %	200	200	99.76	Mean	99.34
S_5 : 100 %	200	200	99.51	SD	0.5316
S_6 : 100 %	200	200	98.74	% RSD	0.5351
S_7 : 120 %	240	200	99.73	Mean	99.73
S_8 : 120 %	240	200	99.94	SD	0.215
S_9 : 120 %	240	200	99.51	% RSD	0.2156
Acceptance Criteria: RSD should be not more than 2.0 %					

DISCUSSION

The %RSD value, plate count and tailing factor results were found to be well within limits as per the ICH guidelines. The precision of the method was estimated by analysing sample solutions. Six multiple samples (from a homogeneous lot) were analysed and content of Valsartan is determined as mg/ tab of the tablet. The RSD of the content was found to be well within the limits (i.e. %RSD<2%). The linearity was investigated in the range of 1 to 200 µgm/ml using six different concentrations. The areas obtained at 250 nm for Valsartan were fitted to a straight line by the method of least squares. Linear regression analysis for Valsartan was calculated and was found to be 0.9999. Accuracy was investigated in the concentration range of 80-120% of the standard concentration for Valsartan. The percentage recovery values obtained lie within the standard limit of 98 % to 102%.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Valsartan from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their

respective label claims and they suggested non – interference of formulation excipients in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Valsartan in dosage forms and can also be used for dissolution or similar studies.

The proposed method is simple, selective, reproducible, sensitive and accurate with good precision. Some of the methods were proved to be superior to most of the reported methods. All these proposed methods for estimation of selected drugs such as Armodafinil and Valsartan were successfully applied either in bulk or pharmaceutical formulations.

The proposed methods can be used as alternative method to the reported ones for the routine determination of selected drugs under the study in bulk and pharmaceutical dosage forms.

ACKNOWLEDGEMENTS

I humbly present this work to the eternal almighty. Indeed my final work is done with the help of primitive persons at heart. So it is my bounded duty to promulgate them individually.

I wish to extend my sincere thanks **Sri. L. Venkateshwarlu**, Principal, Vikas College of Pharmacy, Jangaon for his valuable ideas which made me to finish this work.

ABBREVIATIONS

HPLC	High performance Liquid Chromatography
NMT	Not More Than
%	Percent
AUC	Area Under Curve
LC	Liquid Chromatography
PDA	Photodiode Array
ICH	International Conference on Harmonization
GR	General reagent
C18	Octadecyl
UV	Ultraviolet
ml	Milliliter
Min	Minute

MeOH	Methanol
µl	Micro-Liter
µ	Micron
µg	Microgram
N	Normality
M	Molar
ppm	Parts per million
nm	Nanometer
RSD	Relative Standard Deviation
Fig	Figure
cv	Coefficient of variation
HIV	Human Immunodeficiency Virus

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