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Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Irbesartan and Atorvastatin in Synthetic Mixture

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Abstract: A simple, accurate, rapid and precise reverse phase high performance liquid chromatographic method was developed for simultaneous estimation of Irbesartan and Atorvastatinin synthetic mixture. Inertsil C_{18}, 150 mm x 4.6 mm, 5 µm particle size in gradient mode with mobile phase Acetonitrile: 0.1% Formic acid (40: 60 v/v) and pH adjusted to 3.5 ± 0.1 with orthophosphoric acid was used. The flow rate was 1.0 ml/min and absorbance of individual component was measured at 262 nm. The retention times of Irbesartan and Atorvastatin were found to be 3.993 and 7.733 min, respectively. Linearity for Irbesartan and Atorvastatin was in the range of 400 - 800 and 50- 100µg/ml with correlation coefficient values 0.9995and 0.9994, the percentage recovery obtained was 99.88and 99.70 %, respectively.

Key Words: Irbesartan, Atorvastatin, RP-HPLC, Method validation.

Introduction:

The present study was aimed to develop simple, rapid and precise analytical method for simultaneous estimation of Irbesartan (IRB) and Atorvastatin (ATR).

Irbesartan, an angiotensin II receptor antagonist[(1).] is used mainly for the treatment of hypertension. It is an orally active nonpeptidetetrazole derivative and selectively inhibits angiotensin II receptor type 2. Angiotensin II receptor type1 antagonists have been widely used in treatment of diseases like hypertension, heart failure, myocardial infarction and diabetic nephropathy. IUPAN name of Irbesartan is 2-butyl-3-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1,3-diazaspiro[4.4]non-1-en-4-one.[(2)]

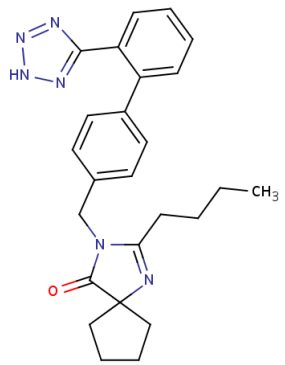
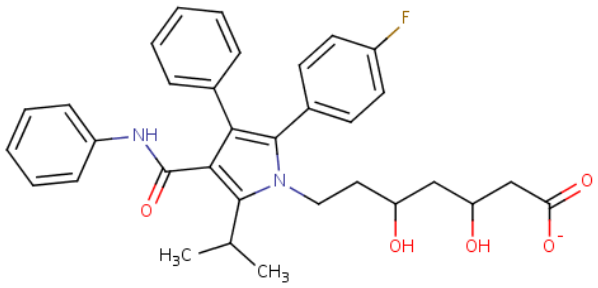


Figure 1 Structure of Irbesartan[(3)]

Irbesartan is white or almost white, crystalline powder. Solubility is given in practically insoluble in water, sparingly soluble in methanol, slightly soluble in methylene chloride.

Atorvastatin is used as lipid-lowering agents used in hyperlipidaemia condition. Atorvastatin selectively and competitively inhibits the hepatic enzyme HMG-CoA reductase.[4]As HMG-CoA reductase is responsible for converting HMG-CoA to mevalonate in the cholesterol biosynthesis pathway, these results in a subsequent



decrease in hepatic cholesterol levels and decreases blood cholesterol level.

Figure 2: Structure of atorvastatin [5]

Atorvastatin is white oral most white, crystalline powder. Solubility is given in practically insoluble in water, soluble in methanol, slightly soluble in methylene chloride.

Hypertension frequently coexists with hyperlipidaemia and both are considered to be major risk factors for developing cardiac disease ultimately resulting in adverse cardiac events. This clustering of risk factors is potentially due to a common mechanism. Further, patient compliance with the management of hypertension is generally better than patient compliance with hyperlipidaemia. It would therefore be advantageous for patients to have a single therapy which treats both of these conditions with help of fixed dose combination of Irbesartan and atorvastatin.[6, 7]

The review of literature regarding quantitative analysis of Irbesartan and atorvastatin revealed that no attempt was made to develop analytical methods for Irbesartan and atorvastatin. Some spectrometric methods and chromatographic methods have been reported for the estimation of the individual drugs.The focus of the present study was to develop and validate a rapid, stable, specific, and economic high performance liquid chromatographic method for the estimation of Irbesartan and atorvastatin in Synthetic mixture.[8, 9]

Materials and Methods

Chemicals and Reagents

Irbesartanraw material was received as gift sample from CTX Life science, Surat. Atorvastatinraw material was received as gift sample from J.B. Chemicals, Ankleshwar. Methanol HPLC Grade (Finar),HPLC Grade Water, Acetonitrile HPLC Grade (Finar), Potassium dihydrogen Phosphate (Merck),Ortho Phosphoric Acid AR Grade (Merck) was used for development purpose.

Instruments and Chromatographic conditions

Chromatographic analysis was carried out on a prominence liquid chromatograph (UFLC Shimadzu Corporation, Tokyo, Japan) with LC-2010AHT series binary pump systems, Auto injection system, temperature controller (system controller and a UV detector (LC-2010). CLASS-VP (version 2.31) software was used to acquire and process the data.Semi micro analytical balance (Sartorius CD2250, Germany) was used for weighing purpose. Unisphere C_{18} column (Agela Tech.) (5 µm, 250mm × 4.6 mm i.d) was used for method development purpose.HPLC water was obtained using arium®611VF (Sartorius).Magnetic stirrer (Remi) was used for mixing purpose.PH tutor (313927, Eutech Instruments) was used for pH measurement. Sonication of solutions was done using Ultrasonic cleaner (D 120/1H, Trans-O-Sonic).Nylon membrane filters (0.22 µm, 47 mm D) were used for filtration purpose.

All volumetric glassware used was calibrated.

Preparation of Reagents and Standards

Mobile phase

The mobile phase consisted of mixture of Acetonitrile: 0.1% formic acidin ratio of (40: 60, v/v) and adjust pH 3.5 with Ortho phosphoric acid. The mode for was Gradient. The mobile phase was filtered through a 0.22 µm nylon membrane filter and degassed prior to use.

Preparation of Standard Stock Solutions

Preparation Standard solution of Irbesartan

A standard stock solution of IRB (1000 µg/ml) was prepared separately by dissolving 10mg of drug in 10ml mobile phase - Acetonitrile: 0.1% Formic acid (40:60, v/v).

Preparation working standard solution of Irbesartan

400µg/ml of IRB stock solution was prepared by diluting 4 ml stock solution to 10 ml with mobile phase - Acetonitrile: 0.1% Formic acid (40:60, v/v)

Preparation Standard solution of Atorvastatin

A standard stock solution of ATR (1000µg/ml) was prepared separately by dissolving 10mg of drug in 10ml mobile phase - Acetonitrile: 0.1% Formic acid (40:60, v/v).1 ml of stock solution diluted up to 10 ml of mobile phase give 1000µg/ml solution of Atorvastatin as a stock solution.

Preparation Standard solution of Irbesartan and Atorvastatin in combination

4 ml from working standard stock solutions of IRB (1000µg/ml) and 0.5 ml from working standard stock solutions of ATR (100µg/ml) were taken in a common volumetric flask diluted up to 10ml with mobile phase - Acetonitrile: 0.1% Formic acid (40:60, v/v) to make final concentration IRB (400µg/ml) and ATR (50µg/ml).

Preparation of Test (formulation) Solution

Composition of synthetic mixture

- Irbesartan : 160 mg
- Atorvastatin : 20 mg
- Microcyrstalline cellulose: 65.6 mg
- Hydroxymethayl cellulose : 12mg
- Magnesium stearate : 7 mg
- Starch : 23mg
- Magnessium carbonate : 75.5 mg

Polysorbate80: 1.2 mg all the expients were mixed in 10ml volumetric flask and sonicate for 15min. make up the volume with Distilled Water. The solution was filtered through Whatman filter paper No. 42.

Finally the solution had concentration 16000µg/ml for IRB and 2000µg/ml for ATR. from that pipette out 1ml in 10 ml volumetric flask and volume was made up to mark with mobile phase - Acetonitrile: 0.1% Formic acid (40:60, v/v) to make final concentration IRB (10µg/ml) and ATR (250µg/ml).

Chromatogram of the Test solution containing 560µg/ml of IRB and 70µg/ml of ATR was recorded and peak areas were noted for estimation of IRB and ATR.

Validation of the Developed Method[(10)]

The developed method of analysis was validated as per the ICH guidelines for the parameters like system suitability, specificity, linearity, precision, accuracy and system suitability, limit of detection (LOD) and limit of quantitation (LOQ).

Selection Of Wavelength

Selection of wavelength was carried out using UV spectrophotometer. Both the drugs were detected at 262nm. (Figure 3)

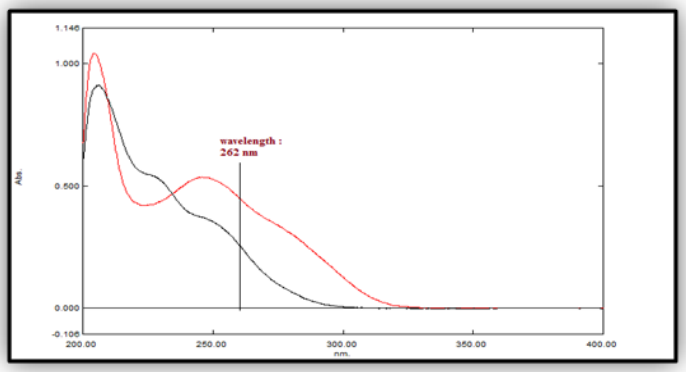


Figure 3: Spectra taken in UV Spectrophotometer in Mobile Phase: Acetonitrile: 0.1% Formic acid (40:60, v/v)

Mobile Phase Selection

Various mobile phases with different ratio of different solvents and pH were used are shown in Table 1. The mixture of acetonitrile and 0.1% formic acid in ratio of (40: 60, v/v) provided optimum polarity for proper migration, separation and resolution of Irbesartan and Atorvastatin peaks. Under these conditions, the eluted peaks were well defined, resolved and free from tailing.

Due to the non-polar nature of the stationary phase more polar component Atorvastatin will be eluted first because of its more affinity towards the polar mobile phase and less polar component Irbesartan will be eluted later due to its more affinity towards non-polar stationary phase.

Table 1 Optimization of Mobile Phase

Trial	Mobile Phase	Ratio V/V	Remark
1.	ACN : 0.05 M KH_2PO_4	70 : 30	ATR peak is not observed
2.	ACN : 0.1% Formic acid	60 : 40	No separation observed and broadening of IRB and ATR peak
3.	ACN : 0.1% Formic acid	50 : 50	Separation of peak observed but tailing in IRB peak
4.	ACN : 0.1% Formic acid (pH-4.5 with o-phosphoric acid)	40 : 60	Separation of peak observed but broadening of IRB and ATR peak
5.	ACN : 0.1% Formic acid (pH-3.5 with o-phosphoric acid)	40 : 60	Peak sharpness was good, More theoretical plates, Less tailing and good resolution

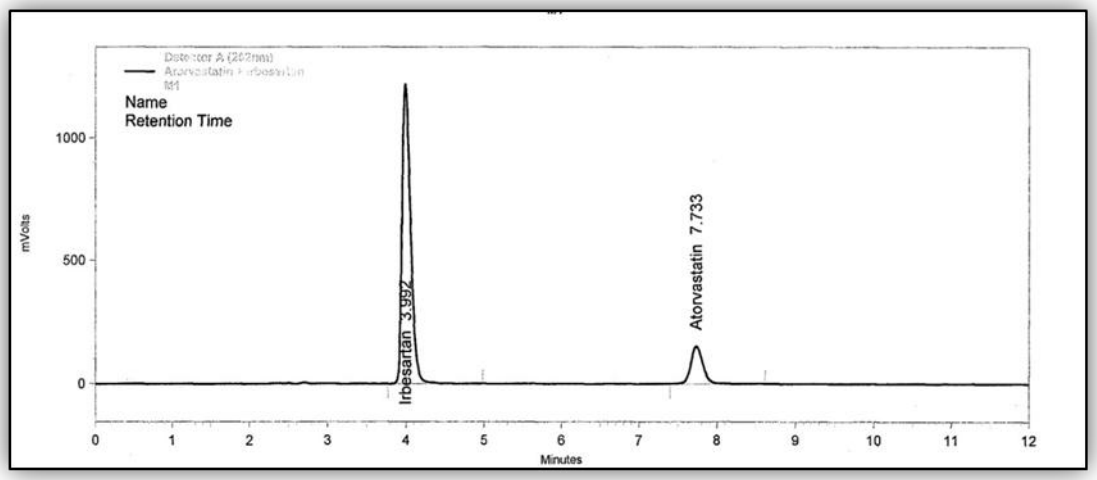


Figure 4: Chromatogram of IRB and ATR in the ratio of 400: 50

1. Linearity andRange

Linearity in the concentration range was400-800µg/mland 50-100µg/ml for IRB and ATR, respectively. (Table2)

Correlation coefficient(r^2) for calibration curve of IRB and ATR was found to be 0.9995 and 0.9994,respectively

The regression line equation for IRB and ATR are as following,

y = 26907x – 100000 for IRB _____ (1)

y = 35715x - 323106for ATR _____ (2)

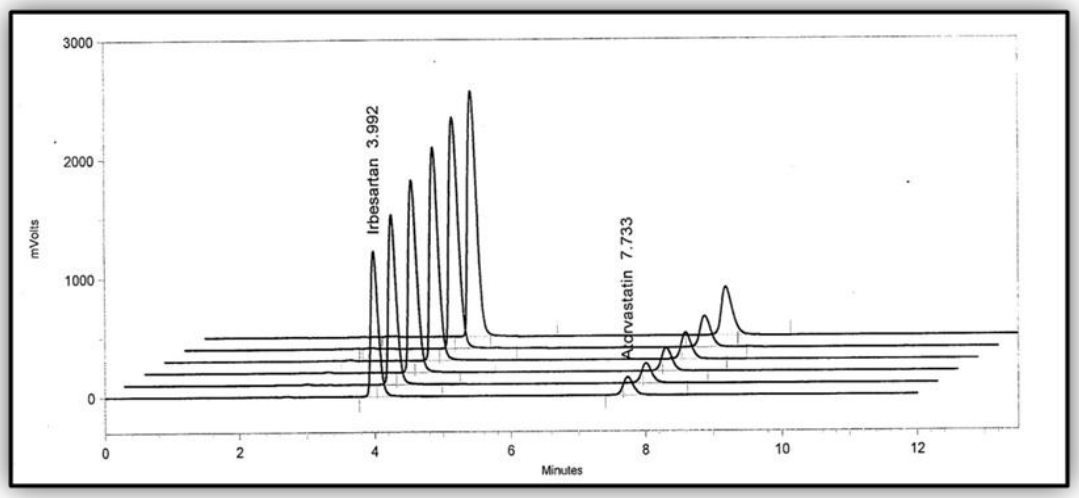


Figure 5: Overlain chromatogram for five concentration of IRB(400-800µg/ml) and ATR (50-100µg/ml)

Table 2 Calibration data for IRB and ATR*(n=6)

Sr	Concentration (µg/ml)		Peak Area* ± SD IRB	Peak Area* ± SD ATR
	IRB	ATR		
1	400	50	9242236±982	1457491±781
2	480	60	11525689±949	1815554±756
3	560	70	13638920±977	2201458±753
4	640	80	15963852±654	2523585±697
5	720	90	17958623±818	2875682±897
6	800	100	19985685±759	3258262±774

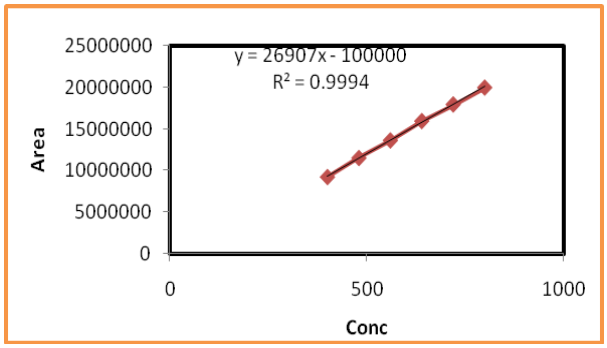


Figure 6: Calibration curve for IRB

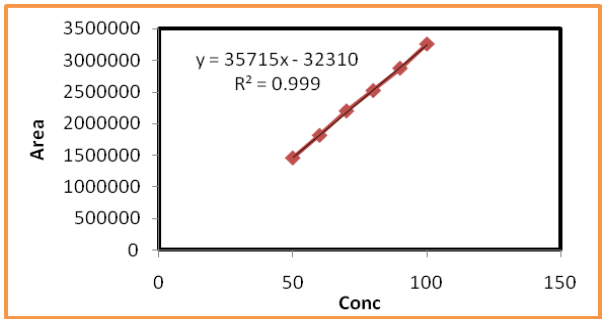


Figure 7: Calibration curve for ATR

2. Precision

I. Intraday precision

The data for intraday precision for combined standard solution of IRB and ATR is presented in Table3.

The% R.S.D was found to be0.010-0.017 % for IRB and 0.020-0.058 % for ATR.

These % RSD value was found to be less than±1.0 indicated that the method is precise.

Table 3 Intraday precision data for estimation of IRB and ATR*(n=3)

Conc. (µg/ml)		Peak area* ± SD IRB	% RSD	Peak area* ± SD ATR	% RSD
IRB	ATR				
400	50	9243885±1573	0.017	1456946±850	0.058
560	70	13639476±1583	0.011	2202412±813	0.037
800	100	19987538±1998	0.010	3257610±670	0.020

II. Interday precision

The data for inter day precision for combined standard solution of IRB and ATR is presented in Table 4.

The % R.S.D was found to be0.010-0.021 % for IRB and 0.023-0.054 % for ATR.

These % RSD value was found to be less than±1.0 indicated that the method is precise.

Table 4 Interday precision data for estimation of IRB and ATR*(n=3)

Conc. (µg/ml)		Peak area* ± SD IRB	% RSD	Peak area* ± SD ATR	% RSD
IRB	ATR				
400	50	9244374±1616	0.017	1458349±793	0.054
560	70	13637965±1708	0.021	2207496±1003	0.045
800	100	19986106±1919	0.010	3258843±768	0.023

3. Accuracy

Accuracy of the method was determined by recovery study from synthetic mixture at three levels (80%, 100%, and 120%) of standard addition.

The% recovery values are tabulated in Table 5 and 6.

Percentage recovery for IRB and ATR by this method was found in the range of99.98 to 100.26% and99.81 to 100.04%, respectively.

The value of % RSD within the limit indicated that the method is accurate and percentage recovery shows that there is no interference from the excipients.

Table 5 Recovery data of IRB*(n=3)

Conc.of IRB from formulation (µg/ml)	Amount of Std.IRB added (µg/ml)	Total amount of IRB (µg/ml)	Total amount of IRB found (µg/ml) Mean*± SD	% Recovery (n=3)	% RSD IRB
320	256	576	575.6±0.102	99.9974	0.011
320	320	640	641.1±0.213	100.2648	0.015
320	384	704	703.8±0.099	99.98366	0.009

Table 6 Recovery data of ATR*(n=3)

Conc. Of ATR from formulation (µg/ml)	Amount of Std.ATR added (µg/ml)	Total amount of ATR (µg/ml)	Total amount ofATR found (µg/ml) Mean*± SD	% Recovery (n=3)	% RSD ATR
40	32	72	72.01±0.098	100.0495	0.04
40	40	80	79.8±0.113	99.8125	0.023
40	48	88	87.9±0.105	99.8536	0.035

4. Limit of detection and quantitation

The LOD for IRB and ATR was conformed to be 0.033µg/mland 0.101µg/ml, respectively.

The LOQ for IRB and ATR was conformed to be 0.028 µg/ml and 0.086µg/ml, respectively. The obtained LOD

and LOQ results are presented in Table 7.

Table 7 LOD and LOQ data of IRB and ATR

Parameter	IRB	ATR
LOD (µg/ml)	0.033	0.028
LOQ (µg/ml)	0.101	0.086

5. Robustness

The obtained Ruggedness and Robustness results are presented in table8

The% R.S.D was found to be 0.0072-0.021 for IRB and0.011-0.041% for ATR.

These % RSD value was found to be less than±1.0 indicated that the method is precise. No significant changes in the Peak area were observed, proving that the developed method is rugged and robust.

Table 8 Robustness and Ruggedness data of IRB and ATR*(n=3)

No.	Factor	Level	Peak area* ± SD	%RSD	R_{t*} ± SD	%RSD
IRB (560µg/ml)						
1.	Change in the pH of mobile phase	3.3	13637566 ± 983	0.0072	7.725 ± 0.003	0.41
		3.7	13648654 ± 1156	0.0084	7.765 ± 0.003	0.56
2.	Change in the Flow Rate (ml/min)	0.9	13635674 ± 1667	0.012	7.321 ± 0.003	0.45
		1.2	13632690 ± 1502	0.011	8.215 ± 0.005	0.23
3.	Change in mobile phase ratio	42:58	13635379 ± 956	0.021	7.756 ± 0.008	0.25
		38:62	13636673 ± 1801	0.013	7.732 ± 0.004	0.32
ATR (70µg/ml)						
1.	Change in the pH of mobile phase	3.3	2206801 ± 254	0.011	3.698 ± 0.004	0.45
		3.7	2198692 ± 908	0.041	3.856 ± 0.003	0.11
2.	Change in the Flow Rate (ml/min)	0.9	2205965 ± 652	0.029	3.562 ± 0.003	0.22
		1.2	2205990 ± 472	0.021	4.325 ± 0.005	0.15
3.	Change in mobile phase ratio	42:58	2205845 ± 663	0.029	3.823 ± 0.008	0.14
		38:62	2206037 ± 442	0.020	3.995 ± 0.004	0.23

5 Application of the Proposed Method for Analysis of IRB and ATRIN Synthetic Mixture

Chromatogram of the Test solution containing 560µg/ml of IRB and 70µg/ml of ATR was recorded and peak areas were noted for estimation of IRB and ATR, respectively.

The concentration of IRB and ATR in synthetic mixture was determined against the standard IRB and ATR.

The results from the analysis of synthetic mixture containing Irbesartan (560µg/ml)and Atorvastatin (70µg/ml) in combination are presented in table in 9.

The percent assay shows that there is no interference from excipients and the proposed method can successfully applied to analysis of commercial formulation containing IRB and ATR. The %assay values are tabulated in Table 9.

Table 9 Analysis data of commercial formulation*(n=3)

Sr. No.	Formulation (synthetic mixture)		Peak area IRB	%Assay* IRB±SD	Peak area ATR	%Assay* ATR±SD
	IRB	ATR				

1	560	70	13635695	99.88 ± 0.201	2206523	99.70 ± 0.086
2			13635482		2205689	
3			13637585		2205231	

Table 10 Summary of validation parameters

Parameters	**RP-HPLC Method**	
	Irbesartan	**Atorvastatin**
Concentration	400-800	50-100
Regression equation	y = 26907x – 100000	y = 35715x - 323106
Correlation	0.9995	0.9994
Accuracy(%Recovery)	100.07	99.99
Intra-dayPrecision	0.010-0.017	0.020–0.058
Inter-dayprecision	0.010-0.021	0.023-0.054
LOD(µg/ml)	0.033	0.023
LOQ(µg/ml)	0.101	0.086
Ruggedness and Robustness	0.0072-0.021	0.011-0.041
%Assay	99.88	99.70

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Conclusion:

A new, reverse phase high-performance liquid chromatography method has been developed for estimation of Irbesartan and Atorvastatin in synthetic mixture. The method was validated by employment of ICH (18) guidelines. The validation data is indicative of good precision and accuracy, and prove the reliability of the method.

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