

Research Article



Development and Validation of RP-HPLC Method for the Estimation and Separation of Valsartan, Losartan and Irbesartan in Bulk and Pharmaceutical Formulation

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ABSTRACT

A simple, rapid, and precise reversed-phase High performance liquid chromatographic (RP-HPLC) method has been developed and validated for the determination and separation of Valsartan, Losartan and Irbesartan in bulk and pharmaceutical dosage form. Chromatographic separation of these drugs was achieved on a reverse phase C18 column using a mobile phase consisting of acetonitrile: phosphate potassium buffer (pH= 3). The mobile phase was pumped at a flow rate of 1.5 mL/min and the eluents were monitored at 254 nm. The method was successfully validated in accordance to ICH guidelines acceptance criteria for (linearity, accuracy, precision, selectivity, limit of detection, limit of quantification) all validation parameters were within the acceptance range. The proposed method was found to be suitable and accurate for quantitative determination and separation of mixture of three drugs in bulk samples and pharmaceutical preparation and it can be used for the quality control of formulation products.

Keywords: Estimation, Irbesartan, Losartan, RP-HPLC, Validation, Valsartan.

INTRODUCTION

There are new highly selective, non-peptide angiotensin II type 1(AT1)-receptor antagonist that lower blood pressure through blockade of the rennin angiotensin – aldosterone system (RAAS). They are widely used in treatment of Hypertension.¹⁻³

These drugs are called angiotensin receptor blockers (ARBs). They can selectively block the angiotensin II type 1 (AT1) receptors, causing inhibition of the action of angiotensin II on vascular smooth muscle, ultimately leading to a reduction in arterial blood pressure. It is useful in treatment of mild to moderate hypertension and well tolerated with lower incidence of cough than ACE inhibitors.¹⁻³ The drug substances studied in this research are: Valsartan, Losartan and Irbesartan, they are methyl biphenyl derivatives used as angiotensin II antagonists.⁴

Losartan potassium is (2-Butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl)biphenyl]-4-yl]methyl]-1H-imidazole-5-methanol.⁴

Irbesartan is 2-Butyl-3-[[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1,3-diazaspiro[4.4] non-1-en-4-one.⁴

Valsartan is n-[p-o-1h-tetrazole-5-yl phenyl]benzyl-n-valeryl-L-valine.⁴

These three drugs have similar chemical structures figure 1.

MATERIALS AND METHODS

Reference standards of Irbesartan, Losartan and valsartan were obtained from sigma Aldrich Chemical Co. (Hamburg, Germany).

-The pharmaceutical tablets sample was purchased from the Community Pharmacy.

Chemicals and solvents

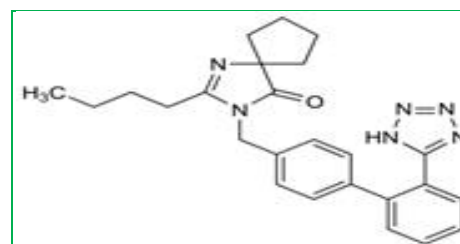
Potassium dihydrogen phosphate and ortho phosphoric acid were obtained from BDH Laboratory Supplies (Poole) UK. HPLC Solvents (Acetonitrile, Methanol, water) were purchased from the Merck, Germany

pH solutions: Hana company, Hungary.

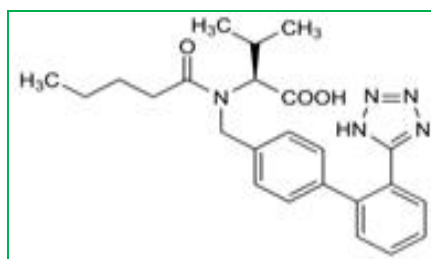
Instrument and Equipments

JASCO high pressure liquid chromatography system provided with two pump (PU-980 Intelligent HP) and UV /VIS detector (UV 254 nm), and manual injector (20 µl loop).

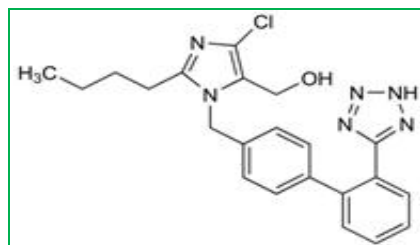
- An Ultrasonic device T310 Germany
- pH meter model Orion 410 A
- Magnetic stirrer model Labinco L33
- Filters 0.45 µm from Whatman Inc.
- HPLC filters 0.45 µm from Sartorius stedium biotech company.
- C8 column (250X4.6) mm Eurospher, 5µm from - Knauer Germany.



Irbesartan



Valsartan



Losartan

Figure 1: Chemical Structures of Losartan, Irbesartan, Valsartan.

Some spectroscopic, High performance liquid chromatographic (HPLC) methods using the most commonly available columns reported earlier for the determination of each Losartan, Valsartan, Irbesartan alone or with diuretics or with other drugs⁴⁻⁸, But the HPLC methods for separation and determination of mixture Of these three medicines were few and using gradient elution.⁹ The aim of this study is to develop a rapid, sensitive, accurate and precise reverse phase HPLC method for the estimation and separation mixture of Valsartan, Losartan, Irbesartan in raw material and tablets using isocratic elution.

Accuracy solutions

Tablet excipients (Sorbitol, Kollidon CL, Talc, Aerosil 200, Calcium arachinate) I was added to the standard solutions of Valsartan, Losartan, Irbesartan.

Nine samples were divided into three groups containing respectively 80%, 100% and 120% of standard solution Concentration.¹⁰⁻¹³

Repeatability solutions

Tablet samples were analyzed. Nine samples were prepared and divided into three groups containing respectively 80%, 100% and 120% of standard solution concentration of Valsartan, Losartan and Irbesartan.¹⁰⁻¹³

Preparation of solution

Preparation of stock solution of Valsartan, Losartan, Irbesartan (1 mg / ml)

100 mg standard of Losartan, Valsartan, Irbeartan were weighted and placed in a 100 ml volumetric flask, then they were dissolved by an amount of acetonitrile and water until they completely Dissolved and Completed the volume with acetonitrile, solutions of concentration 1 mg / ml were obtained.

Preparation of Standards (0.1 mg / ml)

10 ml from the stock solutions 1 mg / ml of Valsartan, Losartan, Irbesartan were taken by calibrated pipette and placed in a 100 ml volumetric flask and completed volume of the solution with acetonitrile to get the concentration of 0.1 mg / ml (100 µg / ml)

Preparation of validity test Solutions

Standard linearity solutions

Five sequential concentrations were Prepared from the stock solution containing respectively 80%, 90%, 100%, 110% and 120% of the standard solution concentration of Losartan, valsartan, Irbesartan.¹⁰⁻¹³

Chromatographic conditions

Mobile phase: acetonitrile: phosphate potassium buffer (pH=3): in the ratio of 40:60 v/v. Flow rate: 1.5 ml / min

UV-detector: 254 nm, Column: C18, Eurospher (250X4.6) mm, 5µm Column, Temperature: 25°C, injection volume: 20 µl.

Intermediate precision solutions

Samples were prepared in the same way mentioned in the repeatability solutions. Assay was carried out after two weeks in the same experimental conditions.¹⁰⁻¹³

Selectivity solution

A drug-free sample (excipients only) was prepared in mixture of acetonitrile and water; three samples containing excipient and active ingredient were prepared in 100% standard solution concentration (100 µg/ml).¹⁰⁻¹³

Robustness solutions

Three tablet samples containing 100% of standard solution concentration were analyzed. The sample was injected at different flow rates 1.4, 1.5, and 1.6 ml/min.¹⁰⁻¹³

Preparation of the standard solution mixture of Valsartan, Losartan, Irbesartan

10 ml of each of the stock solutions of Valsartan, Losartan, Irbesartan were Taken and Placed all in a100 ml volumetric flask, and completed to the volume of the solution with acetonitrile.

Preparation of samples (Tablets)

Three tablet samples solutions were prepared in mixture of acetonitrile and water with concentration of 100% of standard solutions of Valsartan, Losartan and Irbesartan and filtered then injected in HPLC.

Results of validation test

Table 2 show method validation results of Irbesartan, Losartan and Valsartan.

Figures 1, 2, 3 show chromatograms Of each Irbeartan, Losartan and Valsartan.

Results of separation of mixture of three drugs

Table 1: The results of separation of mixture of Valsartan, Losartan, Irbesartan.

Samples test results

Results of tablets samples of losartan, Valsartan, Irbesartan

Table shows the results of analysis of the tablet samples of losartan, Valsartan, Irbesartan. The percentage of active substance in each sample was calculated from the peaks area of samples and standard solutions.

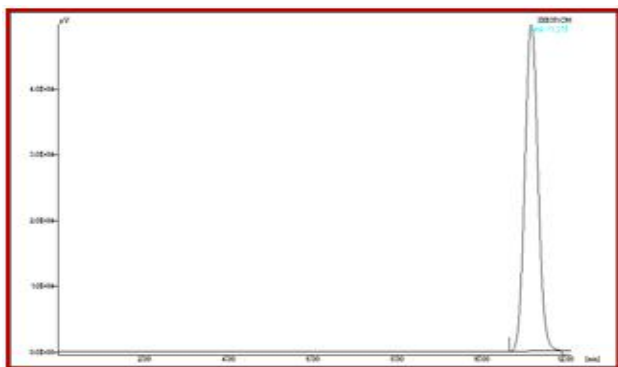


Figure 1: Chromatogram Of Irbesartan

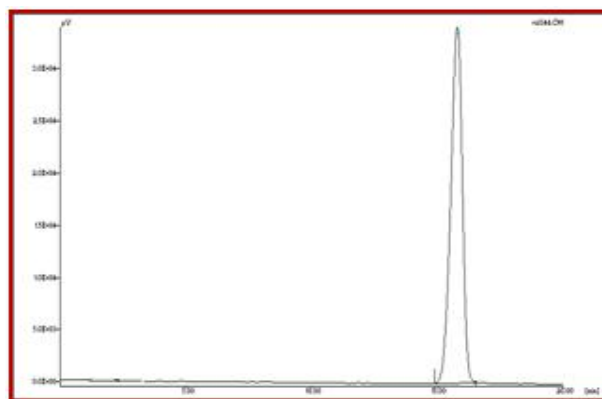


Figure 2: chromatogram Of Valsartan

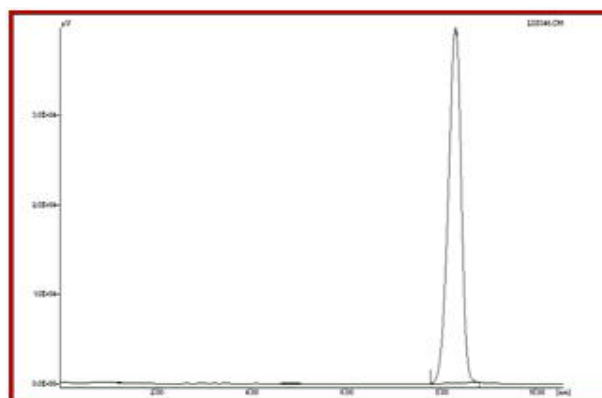


Figure 3: Chromatogram of Losartan

Table 1: The results of separation of mixture of Valsartan, Losartan, Irbesartan

Name	Theoretical Plates	Capacity factor	Tailing factor	Resolution	Retention time
Losartan	6829.774	17.83	0.99	-	8.35
Valsartan	7980.511	28.167	1.09	3.071	11.22
Irbesartan	8872.08	24.33	0.98	7.876	15.82

Table 2: Method validation results of Irbesartan, Valsartan and Losartan

Name	Limit of Quantification	Limit of detection	Robustness			selectivity	Precision		Accuracy	linearity	RT
			Flow rate				Intermediate Precision	Repeatability			
Irbesartan	0.34 µg\ ml	0.1 µg\ml	1.4	1.5	1.6	100.29	100.65 RSD=1.55	100.77 RSD=1.35	100.65	0.998	11.23
			0.99	0.98	1.01						
			100.91	101.7	100.23						
Losartan	0.4 µg\ ml	0.12 µg\m	0.98	0.997	0.99	100.15	100.03 RSD=1.01	100.55 RSD=1.52	78.100	0.998	8.31
			100.69	100.06	100.01						
Valsartan	0.37 µg\ ml	0.11 µg\ml	0.98	1.01	0.98	100.3	101.5 RSD=1.78	100.97 RSD=1.62	100.51	0.99	15.76
			100.18	100.15	100.36						

RESULTS AND DISCUSSION

In the proposed method, the retention time of Losartan was found to be 8.35min. The retention time of Irbesartan was found to be 11.33 min. The retention time of Valsartan was found to 15.82 be min.

The resolution was above of 1.5 that indicates to good separation. The number of theoretical plates calculated was above of 2000 which indicates efficient performance of the column.

The high percentage of recovery indicates that the proposed method is highly accurate. The precision results showed good reproducibility with percent relative



standard deviation (RSD %) are below 2.0. This indicated that the method is highly precised.

The results of assay indicate that the amount of each drug in the tablets is within the requirements of 90–110% of the label claim. No interfering peaks were found in the chromatogram of the formulation within the run time

indicating that excipients used in tablet formulations did not interfere with the estimation of the drugs by the proposed HPLC method. The results were found to be accurate, reproducible and free from interference and better than the earlier reported methods.

Table 3: Results of tablet Samples of Losartan, Irbesartan and Valsartan

Factory	Tablet	Average of standard peak area	Average of sample peak area	Percentage of active substance
A1	Losartan 25mg	725550	721916	100.5
B1	losartan 50 mg	723190	737116	98.1
D1	losartan 50 mg	721916	727421	99.2
A2	Valsartan 80 mg	1033629	1038218	99.58
B2	Valsartan 160 mg	1074924	1031988	104.1
D2	Valsartan 80 mg	1019414	1035519	98.4
A3	Irbesartan 150 mg	1047327	1031988	100.2
B3	Irbesartan 150 mg	1034367	1035519	99.3
D3	Irbesartan 150 mg	1048789	1038182	101.02

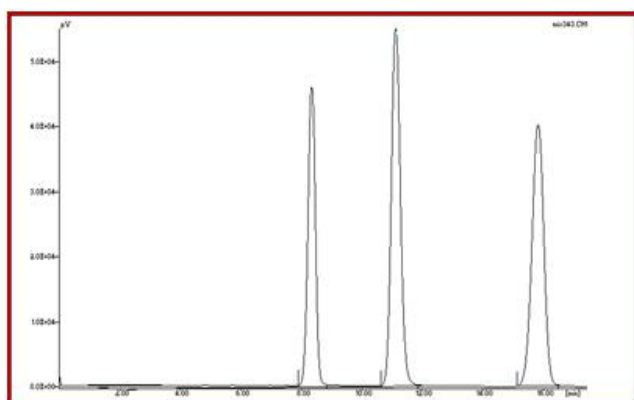


Figure 4: Chromatogram of mixture of three medicines

CONCLUSION

A new, valid, sensitive, accurate and rapid analytical method has been developed in this study for the assay and separation mixture of Valsartan, Losartan, Irbesartan using column C8 mobile phase: acetonitrile: phosphate buffer (40:60) v / v, UV detector at 254nm, and flow rate 1.5 ml / min, This analytical method seem to be a good one for the determination of three compounds in raw materials.

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