

RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF VALSARTAN IN BULK, PHARMACEUTICAL FORMULATION AND HUMAN SERUM

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Abstract

Valsartan is one of angiotensin II antagonists which are major development in hypertension management in over decade, because of its excellent lower side effect and specificity in its action As well as effectiveness, the research purposed to develop HPLC reversed phase for separation and determination of Valsartan in raw materials and tablets, chromatographic separation was achieved on column (C18) using isocratic elution (at flow rate 1.5ml/min) by using 10mM potassium dihydrogen phosphate buffer (pH= 3) and acetonitrile (60:40)v/v as the mobile phase and ultraviolet detector set at 254nm, this method had been validated and accepted, then this method had applied on human serums after using solid liquid extraction for determination of Valsartan in group of patients using it for treatment.

Keywords: high performance liquid chromatography, assay, Valsartan, antagonists.

Introduction

Valsartan is angiotensin receptor blocker, it 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 (Finkel. Richard et al., 2009; Laurence L. Brunton et al., 2006; Rang et al., 2001; British National Formulary (BNF), 2009). Valsartan is one of methyl biphenyl derivatives used as angiotensin II antagonist (Antony C Moffat et al., 2004; David A. William's Thomas and Lemke, 2002). **Valsartan** is

n-[p-o-1h- tetrazole-5-yl phenyl)benzyl-n-valeryl-L-valine (John H. Block et al., 2004).

International references mention various analytical methods for the determination of valsartan alone or with other medicines in pharmaceutical preparations and human serum by HPLC (Macek et al., 2006; Tian et al., 2008). The aim of this study is to develop sensitive, accurate and precise reverse phase HPLC method for the estimation and separation Valsartan in raw material, tablets, and then it will be applied on human serums after using solid liquid extraction for determination of Valsartan in group of patients using it for treatment.

Materials and Methods

Reference standard of valsartan was obtained from sigma Aldrich Chemical Co (Hamburg, Germany). The pharmaceutical tablets sample were purchased from the Community Pharmacy.

Human serum samples were obtained from a hospital laboratory

Chemicals and solvents

Potassium dihydrogen phosphate and ortho phosphoric acid were obtained from BDH Laboratory Supplies (Poole)UK.

HPLC Solvents (Acetonitrile, water, methanol) were purchased from the Merck, Germany pH solutions: were purchased from Hana company, Hungary.

Sensitive balance Sartorius TE64 model (sensitivity of 0.0001 g) Volumetric flasks, Pipette and glass of different sizes.

Centrifuge type HERMLE Z230A - cartridge 18: RP-Adsorbex from Darmstadt. F.R. Merck Company for serum sample.

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. C18 column (250X4.6) mm Eurospher, 5µm from - Knauer Germany.

Chromatographic conditions

Mobile phase: acetonitrile: 10M 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

Preparation of solution

Preparation of stock solution of Valsartan (0.1 mg / ml)

100 mg standard of Valsartan was weighted and placed in a 1000 ml volumetric flask, then it was dissolved by an amount of acetonitrile and water until it completely Dissolved and Completed the volume with acetonitrile, solution of concentration 0.1 mg / ml was obtained.

Preparation of Standards (50 µg / ml)

50 ml from the stock solutions 0.1 mg / ml of Valsartan was 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 (50 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 valsartan (Food and Drug Administration; 1997; USP Pharmacopoeia, 30/2007, 32 /2009 Editions; European Pharmacopoeia 6.0 Edition 2007; Food and Drug Administration.1997; Brith pharmacopoeia 2009).

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 (Food and Drug Administration; 1997; USP Pharmacopoeia, 30/2007, 32 /2009 Editions; European Pharmacopoeia 6.0 Edition 2007; Food and Drug Administration.1997; Brith pharmacopoeia 2009).

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 (50 µg/ml) (Food and Drug Administration; 1997; USP Pharmacopoeia, 30/2007, 32 /2009 Editions; European Pharmacopoeia 6.0 Edition 2007; Food and Drug Administration.1997; Brith pharmacopoeia 2009).

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 (Food and Drug Administration; 1997; USP Pharmacopoeia, 30/2007, 32 /2009 Editions; European Pharmacopoeia 6.0 Edition 2007; Food and Drug Administration.1997; Brith pharmacopeia 2009).

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 and filtered then injected in HPLC(Food and Drug Administration; 1997; USP Pharmacopoeia, 30/2007, 32 /2009 Editions; European Pharmacopoeia 6.0 Edition 2007; Food and Drug Administration.1997; Brith pharmacopeia 2009).

Preparation of Standard Solutions Series for Determination the Recovery from the Serum

Series of standard solutions were prepared in the following concentrations (1, 2, 4, 8, 16 µg/ml) of Valsartan by diluting its stock solutions 0.1mg/ml.

Preparation of Series of Serum Standard Solutions

The above-mentioned series of standard solutions were prepared with duplicated concentrations. 1 ml of series solutions was added to 1 ml drug-free serum to obtain a new series of serum standard solutions with the same concentrations of the previous series, Centrifuge for half an hour.

Extraction

Extraction was done by liquid/solid extraction method using C18 cartridge in the following way:

The cartridge was preconditioned with 3 ml of methanol and withdrawn then with 3ml of water and withdrawn.

Serum sample was applied on the solid phase. Compounds were eluted with 10 ml mixture of acetonitrile and methanol (3:2)

The solutions were run through 0.45 µm HPLC filters then injected directly to HPLC.

Results of validation test

Tab (1):system suitability of Valsartan , Tab(2) shows method validation results of Valsartan Figure (1) shows chromatogram Of Valsartan , Figure(2):shows Liner Regression Equation of Valsartan

Samples test result

Pharmaceutical Preparations

Results of tablets samples of Valsartan

Table(3) shows the results of analysis of the tablet samples of Valsartan.

The percentage of active substance in each sample was calculated from the peaks areas of samples and standard solutions.

Serum samples

Standard serum solutions series were first injected using the mentioned Chromatographic conditions, Linearity was good in the range of (1 – 16) µg/ml.

Peak areas of the standard serum series were compared with peak areas of standard series solutions and the recoveries were calculated (table4). Figure(3) shows Chromatogram of a Serum Sample of valsartan (5µg /ml).

Discussion and results

In the proposed method, the retention time of Valsartan was found to be 15.82 min.

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.

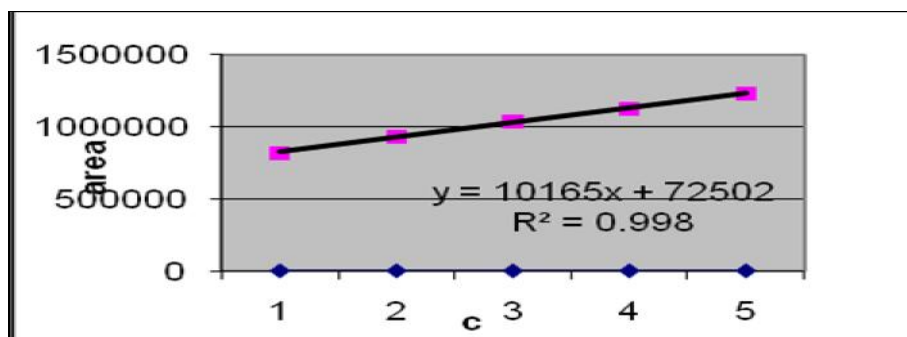
The linearity results showed good with Regression factor is ($R^2 = 0.9988$).

The Limit of detection is 0.11µg/ml , Limit of Quantification is 0.37 µg/ml that indicated the

Tab (1) system suitability of Valsartan

Standard NO	Capsity factor	plates	Retention time	Area	Asymmetry		
1	24.21	7575.38	15.767	1037247	0.995		
2	24.54	7574.8	15.770	1031145	0.998		
3	24.31	7576.2	15.825	1021458	0.987	Average	1030898
4	24.45	7574.7	15.797	1015897	0.988	SD	12970.03
5	24.11	7576.4	15.763	1048741	0.997	RSD	1.26

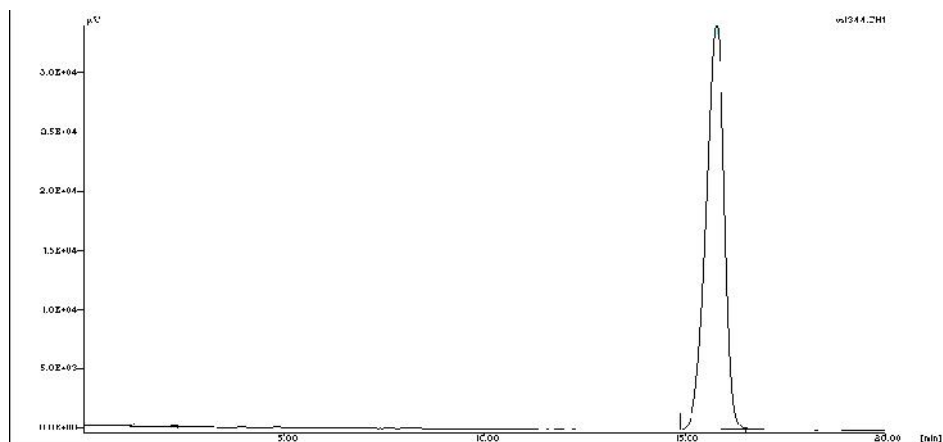
Figure(1): Liner Regression Equation

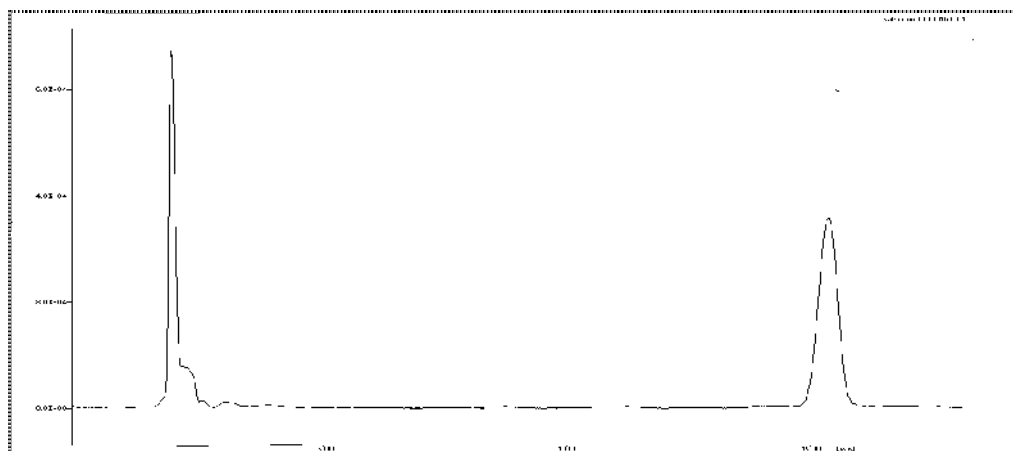


Tab(2): the results validation of Valsartan

Name	Limit of Quantification	Limit of detection	Robustness			selectivity	Precision	
			Flow rate				Intrmediate Precision	Repeatability
Valsartan	0.37 µg/ml	0.11 µg/ml	1.4	1.5	1.6	100.29	100.65 RSD=1.55	100.77 RSD=1.35
			RSD=0.99	RSD=1.02	RSD=1.01			
			100.18	100.15	100.36			

Figure(2) :chromatogram of Valsartan



Figure(3) : Chromatogram of a Serum Sample of Valsartan (5µg /ml)**Tab(3):** Results of tablet Samples of tablets

Factory	Tablet sample	Average of Standard Peak Area*	Average of sample Peak Area**	Percentage of the Active Substance %
A	Valsartan 80	1041126	1030898	100.99
B	Valsartan 80	1025854	1034552	99.15
C	Valsartan 160	1022456	1031567	99.12

* Average of five injections of the standard; ** Average of three injections of the sample

Tab(4): Recovery Results of the Serum Samples

Number of samples	Concentration µg/ml	Recovery
1	1	90.45
2	2	89.91
3	4	95.55
4	8	90.4
5	16	89.9

method is sensitive so it was applied on serum sample .

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 recovery percentages of spiked serum samples of Valsartan were between 89 and 95.

The results were found to be accurate, reproducible and free from interference and better than the earlier reported methods.

Conclusion

A new, valid, sensitive, accurate and rapid analytical method has been developed in this study for the assay and separation of Valsartan using column C18. 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 Valsartan in raw materials as well as in tablets .It could be also used for the determination of their concentration in human serum in the range (1-16) µg/ml.

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