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Protein Binding Study of Expired and Extant Losartan Formulations by UV Spectroscopy.

[*1]Sajna John, [2]Abdul Hafeedh, [3]Vahab K M , [4]Hyma M H , [5]Nifla Shan E P, [6]Adarsh B S, [7]Gopika V C

*[*1]Sajna John, Assistant Professor, Department of Pharmaceutical Analysis, ELIMS College of Pharmacy, Thrissur, Kerala*
[2]Abdul Hafeedh, Department of Pharmaceutical Analysis, ELIMS College of Pharmacy, Thrissur, Kerala
[3]Vahab K M, Department of Pharmaceutical Analysis, ELIMS College of Pharmacy, Thrissur, Kerala
[4]Hyma M H, Department of Pharmaceutical Analysis, ELIMS College of Pharmacy, Thrissur, Kerala
[5]Nifla Shan E P, Department of Pharmaceutical Analysis, ELIMS College of Pharmacy, Thrissur, Kerala
[6]Adarsh B S, Department of Pharmaceutical Analysis, ELIMS College of Pharmacy, Thrissur, Kerala
[7]Gopika V C, Associate Professor Department of Pharmaceutical Analysis, ELIMS College of Pharmacy, Thrissur, Kerala
Corresponding Author: Sajna John

Abstract
Untreated, high blood pressure increases the risk of heart attack, stroke and other serious health problems. Losartan is drug of choice in hypertension. Protein binding study of drugs are important as it effects the pharmacokinetic properties. Protein binding study of standard drug Losartan and the extant and expired formulations were studied using UV SPECTROSCOPY using carbonate buffer of pH 7.4 as solvent at a lamda max of 264nm and 227nm respectively.
Keywords: Losartan, bovine serum albumin, uv spectroscopy, protein binding

I. INTRODUCTION

Blood pressure is the force of circulating blood against the walls of blood vessels, mainly caused by the heart pumping blood through the body. Hypertension, commonly known as high blood pressure, is a prevalent health condition affecting millions of people worldwide. Often referred to as the silent killer, hypertension can lead to serious complications if left untreated.

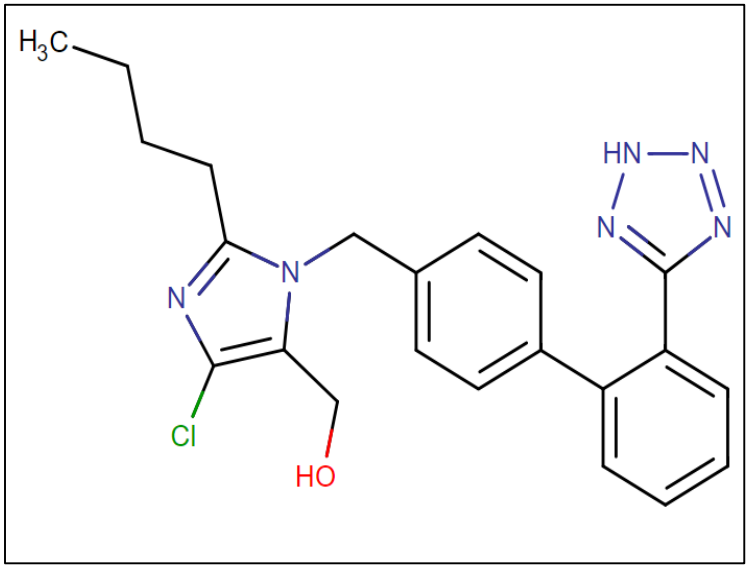
Hypertension is a medical term used to describe consistently elevated blood pressure levels. Blood pressure is the force exerted by blood against the walls of arteries as the heart pumps it around the body. When this pressure remains high over an extended period, it can cause damage to the arteries and lead to severe health issues such as heart disease, stroke, and kidney problems. In order to avoid complications, it is important to start treatment as soon as possible, achieve the goals in the shortest time possible and ensure treatment adherence.

Losartan reversibly and competitively prevents angiotensin II binding to the AT1 receptor in tissues like vascular smooth muscle and the adrenal gland. Losartan and its active metabolite bind the AT1 receptor with 1000 times more affinity than they bind to the AT2 receptor. The phenomenon of complex formation of drug with protein is called as protein binding of drug. As a protein bound drug is neither metabolized nor excreted hence it is pharmacologically inactive due to its pharmacokinetic and pharmacodynamics inertness. The unbound drug alone is supposed to exhibit the pharmacological activity and/or the side effect by diffusing from the blood to the extravascular active sites. Bovine serum albumin is commonly used as a model protein for human serum albumin due to its strong structural similarity, low procurement cost and ease in availability. The resemblance between BSA and HSA is 86% with respect to amino acid sequences and 75.6% in terms of identity, Because of high structural similarity with HSA and similarity in ligand binding patterns with HAS have led to the use of BSA as a model protein for studying the interactions between drugs and plasma proteins

II. Aim and Objectives

The aim of the project is to perform a study on interaction of Losartan with bovine serum and evaluation of expired and extant marketed formulations employing the UV-Visible spectrophotometric method.

III. Drug Profile



Structure of Losartan

IV. RESEARCH METHODOLOGY

4.1 Standard drugs

Losartan and Bovine serum albumin were obtained as gift sample from Micro labs limited.

4.2 Test products

Losartan tablet: RePACE 25 (Formullation-1), RePACE 25(Expiry-1) (expired on 2019)

4.3 Chemicals and solvents used

Distilled water, Sodium bicarbonate, Sodium carbonate, Sodium hydroxide, Hydrochloric acid

4.4 Instruments used

Analytical balance - (Wensar ISO 9001:2000 Certified), UV-Visible spectrophotometer – (Shimadzu 1900), pH-meter (Eutech), Sonicator – HE (HPLC Engineers)

4.5 Selection of wavelength

An ideal wavelength is the one that gives maximum absorbance and good response for the drugs detected at lower concentration also. UV absorption spectra of Losartan, Telmisartan and BSA were measured using a UV-1800 spectrophotometer (Shimadzu) with a 1 cm path length cuvette and 1 nm slit width shows major and minor peaks. Losartan and BSA shows significant peak at 264nm and 227nm respectively.

4.6 Preparation of carbonate buffer

Dissolve 8.4gm of Sodium bicarbonate and 10.6 gm of Sodium carbonate in sufficient water to produce 500ml. 0.1N Hcl and 0.IN NaOH solutions were used for adjusting the pH of carbonate buffer to 7.4. Freshly prepared buffer solutions were used for the entire studies.

4.6.1 Preparation of 0.1M sodium hydroxide

4.0gm of sodium hydroxide pellets was weighed and dissolved in a small amount of distilled water then made up the volume to 1000ml.

4.6.2 Preparation of 0.1M Hydrochloric acid

8.33ml of concentrated hydrochloric acid was measured and diluted with distilled water to 1000ml.

4.6.3 Preparation of standard drug (Losartan) solution

Weighed accurately 25mg of the Losartan RS, and transferred it into 25ml standard flask, dissolved completely. From the above solution take 1ml and made upto 10ml in the freshly prepared carbonate buffer solution of 7.4pH to get a concentration of 100 µg/ml.

The solution is sonicated for 10 min and filtered using whatmann filter paper. An aliquot solution was then diluted the carbonate buffer to get final concentration of 3µg/ml, 6µg/ml, 9µg/ml, 12µg/ml and 15µg/ml.

4.6.4 Preparation of standard BSA solution

Weighed accurately 100 mg of the BSA, and transferred it into a 100ml standard flask, dissolved completely and made up to 100ml in the freshly prepared carbonate buffer solution of 7.4pH to get a concentration of 1000g/ml. The solution is sonicated for 10 min and filtered using whatmann filter paper. An aliquot solution was then diluted with carbonate buffer to get a final concentration of 20ug/ml, 40ug/ml, 60ug/ml, 80ug/ml and 100ug/ml.

4.7 Calibration plot of Losartan in carbonate buffer

Accurately pipetted out 0.3ml, 0.6ml, 0.9ml, 1.2ml and 1.5ml of standard solution into 5 labelled standard flasks of 10ml and volume was made up to the mark with carbonate buffer. The absorbance of each solution was measured with carbonate buffer as blank and this data reveals that Beer-Lambert's law was obeyed from 3-15 µg/ml.

4.8 Calibration plot of BSA in carbonate buffer

Accurately pipetted out 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1ml of standard solution into 5 labeled standard flasks of 10ml and volume was made up to the mark with carbonate buffer. The absorbance of each solution was measured with carbonate buffer as blank and this data reveals that Beer-Lambert's law was obeyed from 20-100µg/ml.

4.9 Interaction study of both Extant and Expired formulation with BSA

Losartan/Re-PACE-25/ Extant F-1 and Expired E-1

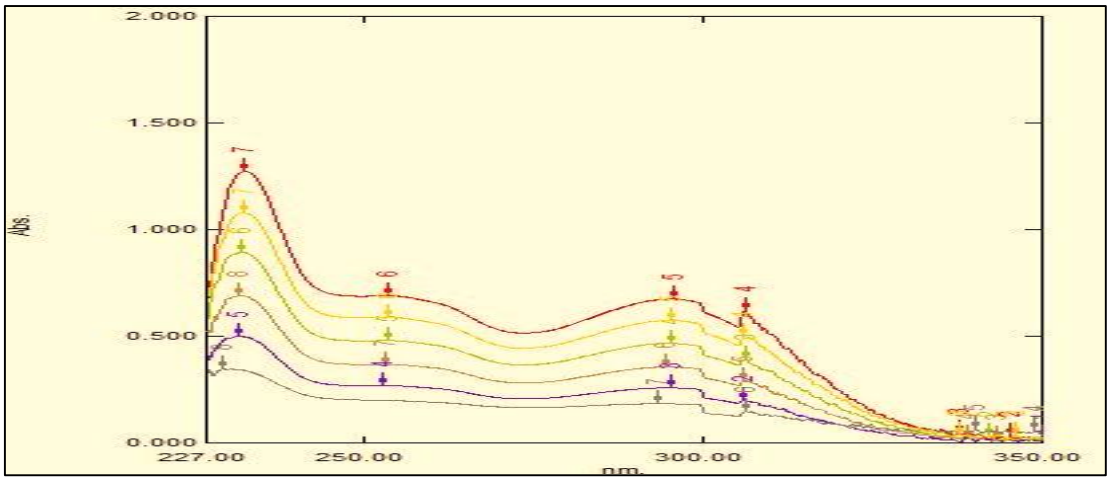
A quantity of powder equivalent to 25 mg of F-1 and E-1 were taken and transferred to 25 ml standard flask and made up to the volume with carbonate buffer of pH 7.4 which is then sonicated for 10mins. Filter the solution by using whatmann filter paper. Pipette out 1ml of this solution to a 10ml standard flask and made upto the volume with carbonate buffer of pH 7.4. From the above solution again pipette out 1.5ml and make up to 10ml to get a final concentration of 12µg/ml for both F-1 and E-1 respectively and allows binding with BSA of concentration 80µg/ml.

Changes in spectral characters of F-1 and E-1 due to interaction with BSA were examined by scanning the solutions. Changes in peak intensity, peak areas and shift in wave length were studied.

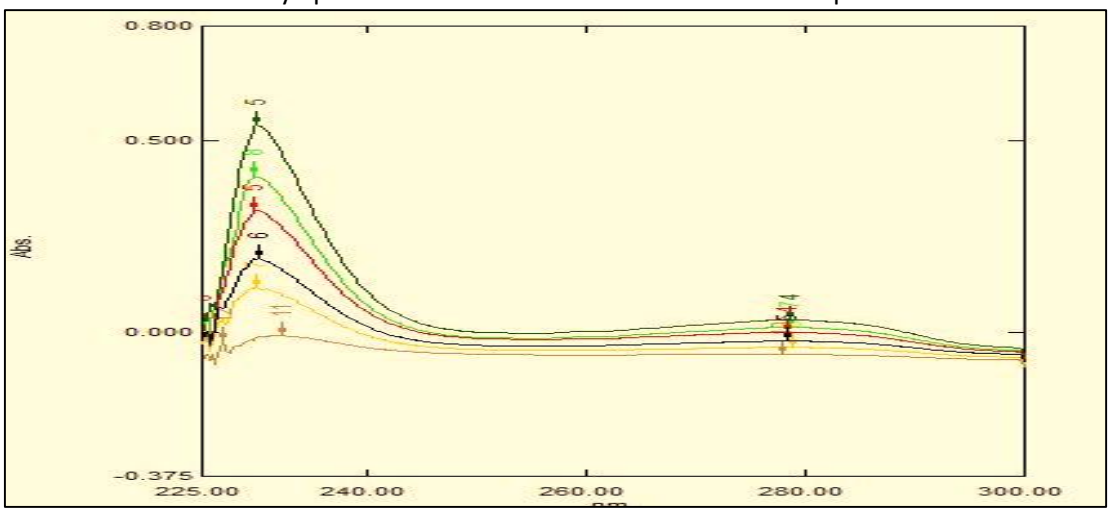
Comparison of interactions of standard drug with that of Extant and Expired formulation with BSA.

The comparison of the interactions of standard drug Losartan with BSA and that of extant and expired were studied.

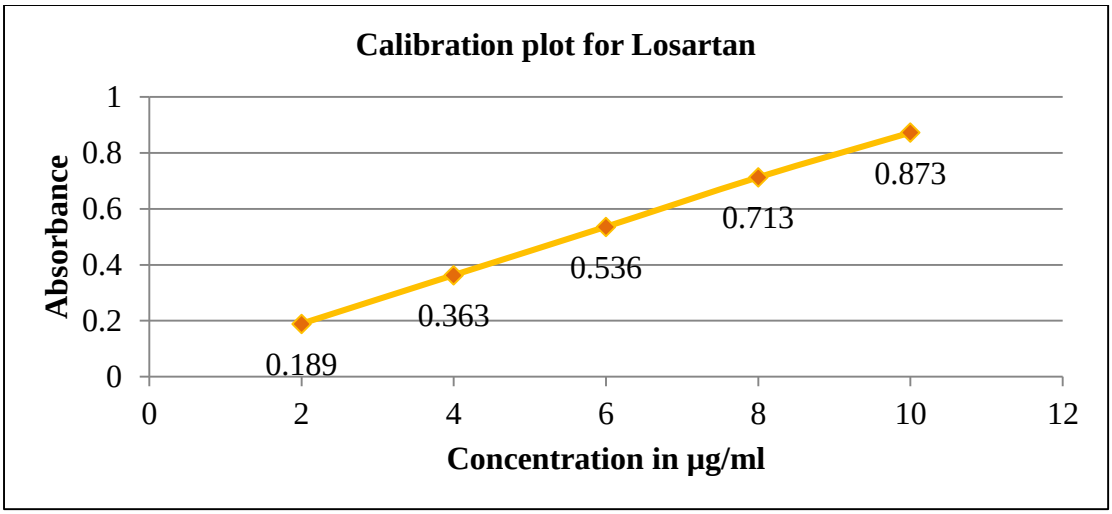
V. RESULTS AND DISCUSSION



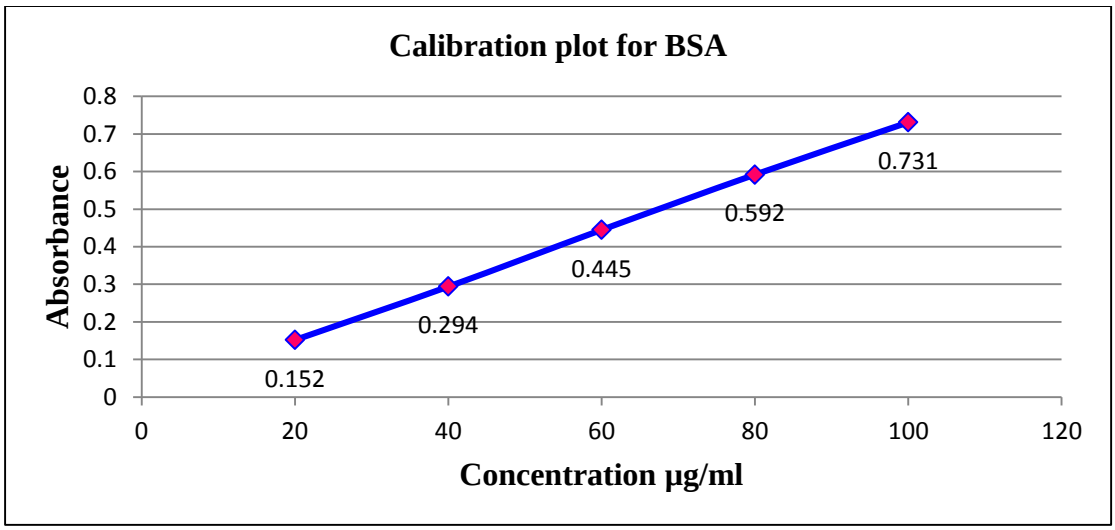
Overlay Spectrum of Losartan in Carbonate Buffer of pH 7.4



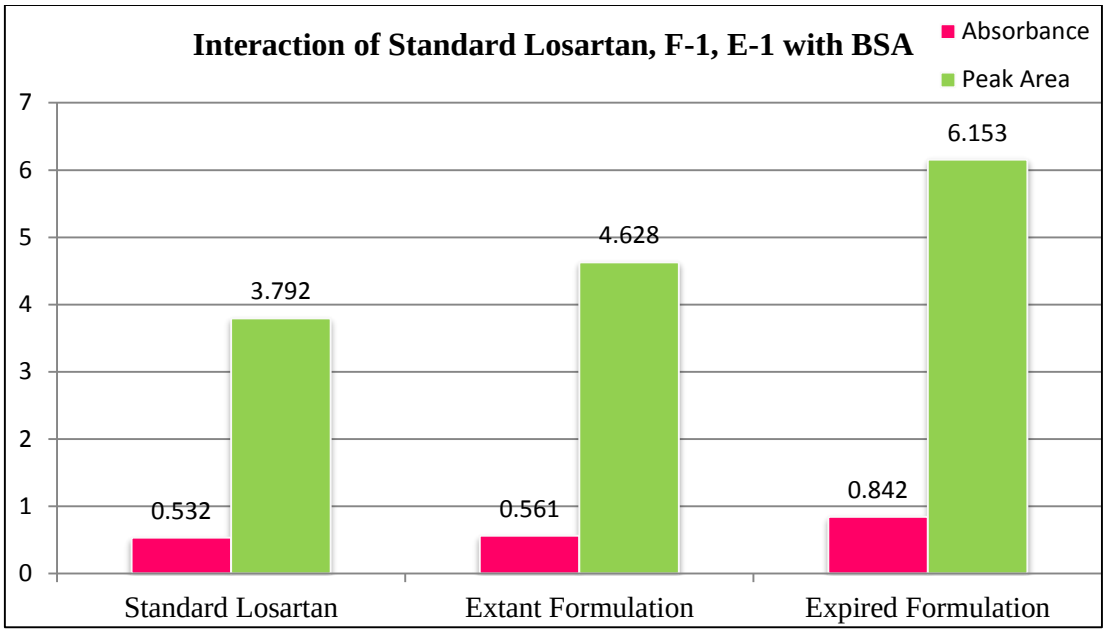
Overlay spectrum of BSA in carbonate buffer of pH 7.4



UV Calibration plot of Losartan



UV Calibration plot of BSA



Comparison of absorbance and peak area of standard Losartan with F-1, E-1 with BSA

Interaction study of standard drug Losartan, F-1, E-1 with BSA

Drug	Absorbance	Peak Area	Shift in peak
Standard Losartan	0.532	5792	264
F-1	0.561	5928	264
E-1	0.842	8153	264

Calibration data of Losartan and BSA

Losartan λ max 264		Bovine Serum Albumin λ max 227	
Conc. µg/ml	Absorbance	Conc. µg/ml	Absorbance
3	0.189	20	0.152
6	0.363	40	0.294
9	0.536	60	0.445
12	0.713	80	0.592
15	0.873	100	0.731

VI. CONCLUSION

Losartan is a drug which is an extensively used drug so it is crucial to enlighten pharmacokinetic properties as it is recommended drug in multi regiment therapy in the management of hypertension. The absorbance, peak area and shifts in peaks were observed for standard as well as for the extant and expired formulation. Samples undergo greater changes when compared with that of standard.

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