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Protein Binding Study of Expired and Extant Telmisartan Formulations by UV Spectroscopy

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A B S T R A C T

Anti-hypertensive drug Telmisartan is a prominent drug in the treatment and management of hypertension. Serum albumin is major carrier protein and its binding with drugs is important to examine the change in pharmacokinetic properties which directly influence the bio availability, sustained drug release and toxicity of drugs. Interaction of Telmisartan with BSA has been studied systematically using UV spectroscopy by fixing the carbonate buffer of pH 7.4 as solvent and at a suitable wavelength 232nm. To gain an insight into the interaction with BSA, the spectra of the Losartan and Telmisartan and BSA were examined in concentration in linearity range. Stability studies and intraday studies were done. The changes in peak intensity, absorbance and peak area were observed for both standard drug and for formulations. The detectable differences were compared with standard. This method can be used successfully for the interaction study with BSA.

Keywords: Bovine serum albumin, Telmisartan, UV visible Spectroscopy, interaction study.

1. Analytical methods

Analytical methods are intended to establish the identity, purity, physical characteristics and potency of drugs. These methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. 1The methods used for the qualitative and quantitative determination of the concentration of a compound using various techniques are titrations, spectroscopies, chromatography, and gravimetric analysis

There are two main types of Analytical methods.

- Qualitative (identification)
- Quantitative (estimation)

1.1 UV-Visible spectrophotometer

UV-Vis spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. Beer-Lambert's law8

Beer-Lambert's law is the fundamental law that governs the quantitative spectrophotometric analysis. The Beer-Lambert Law can be used to calculate the concentration of a sample and is frequently used in absorption and transmission tests on samples. Light travels through a cuvette containing a sample to evaluate absorption. used in modern laboratories for evaluating pharmaceuticals, organic chemistry, and quantification-based studies. The combination of Beer's law and Lambert's law is known as Beer-Lambert's law. According to this law, the thickness of the absorbing media and the solution's concentration is related to how much light is absorbed and how intense it is. Mathematically, Beer-Lambert's law is expressed as,

$$A=\varepsilon bc$$

Where,

A= Absorbance or optical density

ε = Absorptivity or extinction coefficient

b= Path length of radiation through sample

c= Concentration of solute in solution

The molar absorptivity at a specified wavelength of substance in solution is the absorbance at that wavelength of a 1mol-1cm-1 solution in a 1cm cell. Another form of the Beer-Lambert's proportionality constant is the specific absorbance, which is the absorbance of a specified path length. The most common form in pharmaceutical analysis is the A1%1cm, which is the absorbance of a 1g/100mL (1%w/v) solution in a 1cm cell. The Beer-Lambert's equation therefore takes the form:

A=A1%1cmbc

Where c is in g/100mL and b is in cm. The units of A1% are dlg-1cm-1.

1.2 Hypertension

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.

Combination therapy for hypertension1

When hypertensive patients do not achieve adequate control of their blood pressure, the options to try and achieve required treatment goals are to increase the dose of monotherapy (which increases the risk of side effects) or to use drug combinations with minimum side effects. 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. The use of combination therapy provides greater protection to a target organ than increasing the dose of monotherapy.

1.3 Protein binding

As a protein bound drug is neither metabolized nor excreted hence it is pharmacologically inactive due to pharmacokinetic and pharmacodynamics inertness. Protein binding is the term used to describe the process by which drug and protein complexes form. Binding of drugs to proteins is generally reversible and irreversible. Reversible generally involves weak chemical bonds such as Hydrogen bonds, hydrophobic bonds, Ionic bonds, Vander waals forces. Irreversible drug binding results from covalent binding and is frequently the cause of the drug's carcinogenicity or tissue toxicity.

1.4 Interaction of Bovine serum albumin

Serum albumin (SA) is a multifunctional protein with extraordinary ligand binding capacity, making it a transporter molecule for a diverse range of metabolites, drugs, nutrients, metals and other molecules. Mechanistic interaction insights studies of the drug help in determining the factors that influence the protein conformational changes, protein folding, ligand binding activity and structural elucidation. Over the last decades, interaction studies of drugs with serum albumin proteins have attracted great interest to reach a step closer to preclinical trials.

2. Drug Profile

TELMISARTAN

Telmisartan interferes with the binding of angiotensin II to the angiotensin II AT1-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland. As angiotensin II is a vasoconstrictor, which also stimulates the synthesis and release of aldosterone, blockage of its effects results in decreases in systemic vascular resistance. Telmisartan does not inhibit the angiotensin converting enzyme, other hormone receptors, or ion channels.Studies also suggest that telmisartan is a partial agonist of PPARγ, which is an established target for antidiabetic drugs. This suggests that telmisartan can improve carbohydrate and lipid metabolism, as well as control insulin resistance without causing the side effects that are associated with full PPARγ activators.

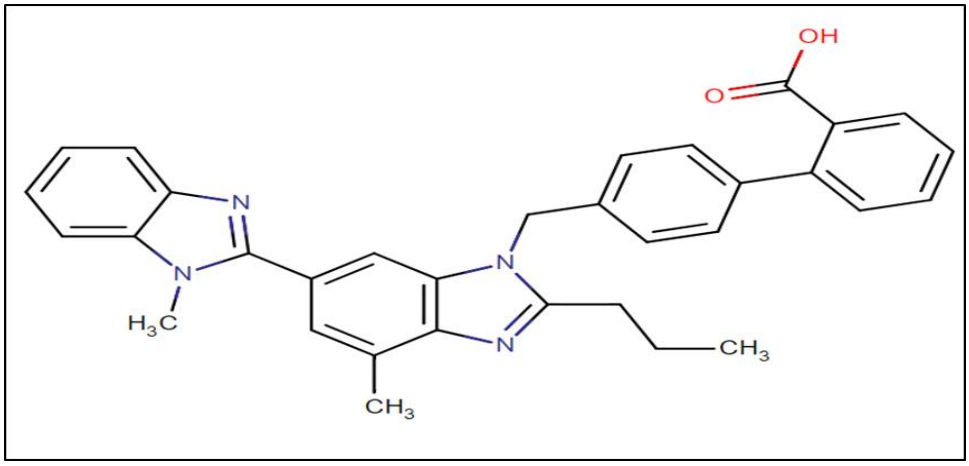

Figure 1: Molecular structure of Telmisartan

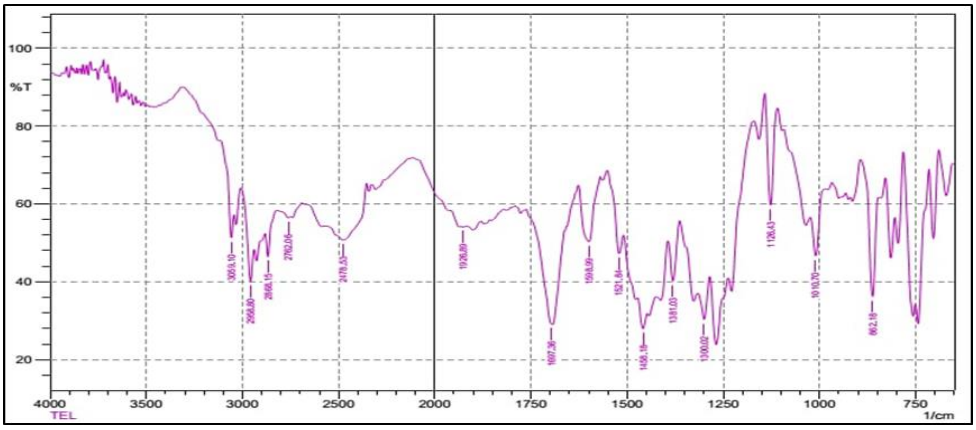

Figure 2: IR Spectrum of Telmisartan

2. Aim and Objectives

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

The objective of the present work is,

- To study the spectral characteristics of Losartan, Telmisartan and BSA by UV visible spectroscopy.
- To perform the interaction study of Losartan, Telmisartan and BSA in standard drugs and in expired and extant formulations.
- To compare the interaction of standard drug with BSA and also with the expired and extant marketed formulation of drug with BSA under normal conditions.

2.1 Materials and methods

Telmisartan and Bovine serum albumin were obtained as gift sample from Micro labs limited and Biocon limited respectively. Telmisartan tablet: Telma 40 (Formulation-2), (Telma 40 (Expiry-2)(expired on 2021)

3. Analytical methodologies

3.1 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 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. Telmisartan and BSA shows significant peak a 232nm and 227nm respectively.

3.2 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.

3.3 Preparation of standard drug (Telmisartan) solution

Weighed accurately 100mg of the Telmisartan RS, and transferred it into a 100ml standard flask, dissolve completely. From the above solution take 1ml and made up to 100ml in the freshly prepared carbonate buffer solution of 7.4pH to get a concentration of 10µg/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 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml and 12 µg/ml

3.4 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.

3.5 Calibration plot of Telmisartan in carbonate buffer

Accurately pipetted out 0.4ml, 0.6ml, 0.8ml, 1ml and 1.2ml 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 4 -12 µg/ml.

Statistical evaluation of calibration plot Telmisartan

The calibration curve was plotted with absorbance on the Y-axis and concentration on the X-axis. A linear plot was obtained within this concentration range.

3.6 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.

Statistical evaluation of calibration plot BSA

The calibration curve was plotted with absorbance on the Y-axis and concentration on the X-axis. A linear plot was obtained within this concentration range.

3.7 Intraday studies

Changes in absorbance, peak intensity, peak areas and shift in wave length were studied at zero minutes and repeated in 30minutes, 60minutes time intervals for Telmisartan.

3.8 Interaction study of both Extant and Expired formulation with BSA

Telmisartan/Telma-40/ Extant F-2 and Expired E-2

A quantity of powder equivalent to 100mg of Telmisartan F-2 and E-2 was taken and transferred to 100 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 100ml standard flask and made upto the volume with carbonate buffer of pH 7.4. From the above solution again pipette out 1.2 ml and make up to 10ml to get a final concentration of 10µg/ml for both F-2 and E-2 respectively and allows binding with BSA of concentration 80µg/ml. respectively and allows binding with BSA of concentration 80µg/ml.

Changes in spectral characters of F-2 and E-2 due to interaction with BSA were examined by scanning the solutions. Changes in peak intensity, peak areas and shift in wave length were studied.

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

The comparison of the interactions of standard drug Telmisartan with BSA with and that of extant and expired were studied. The absorbance, peak area and shifts in peaks obtained by the interaction of standard Telmisartan is compared with that produced by extant and expired

4. Results and Discussion

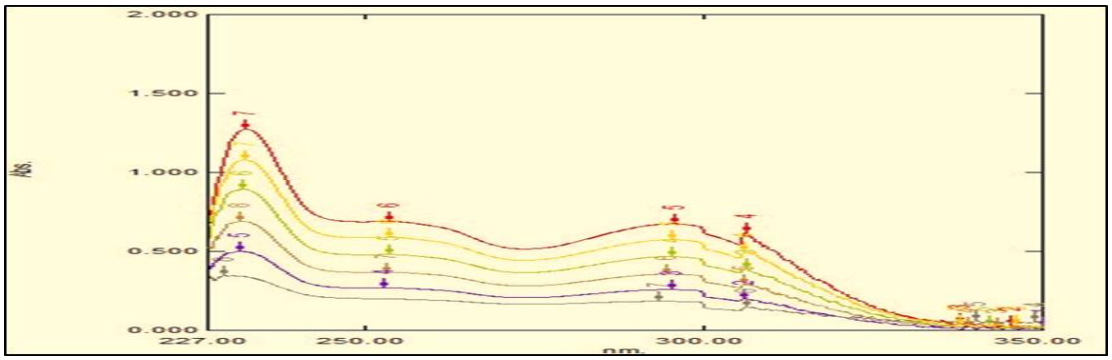

Figure 3: Overlay Spectrum of Telmisartan in Carbonate Buffer of pH 7.4

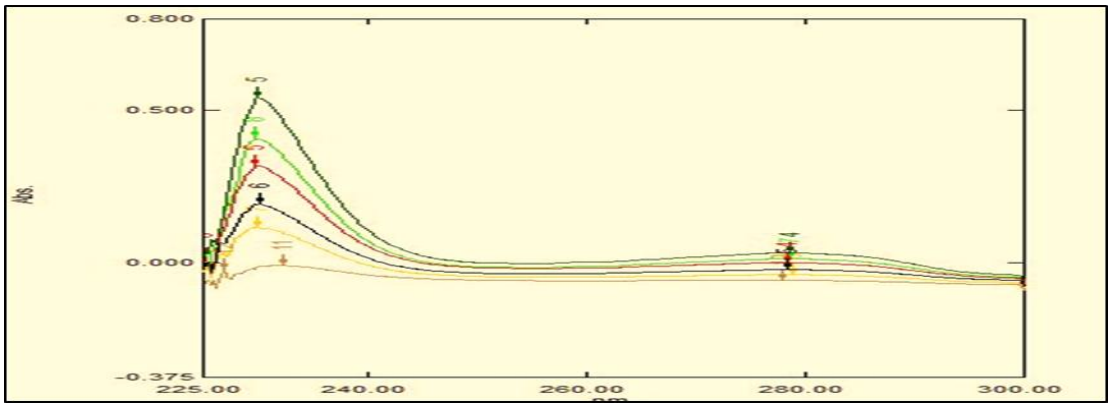

Figure 4: Overlay Spectrum of BSA in Carbonate Buffer of pH 7.4

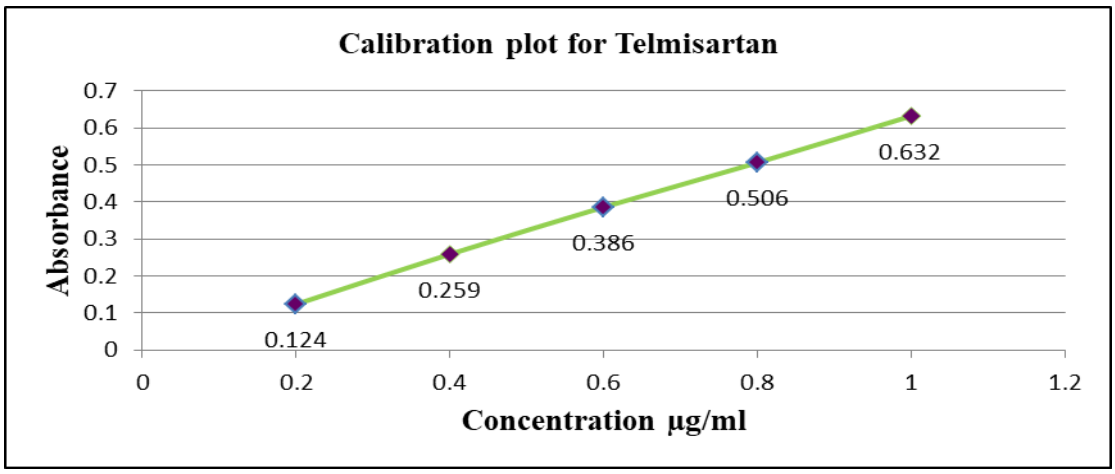

Figure 5: UV Calibration plot of Telmisartan

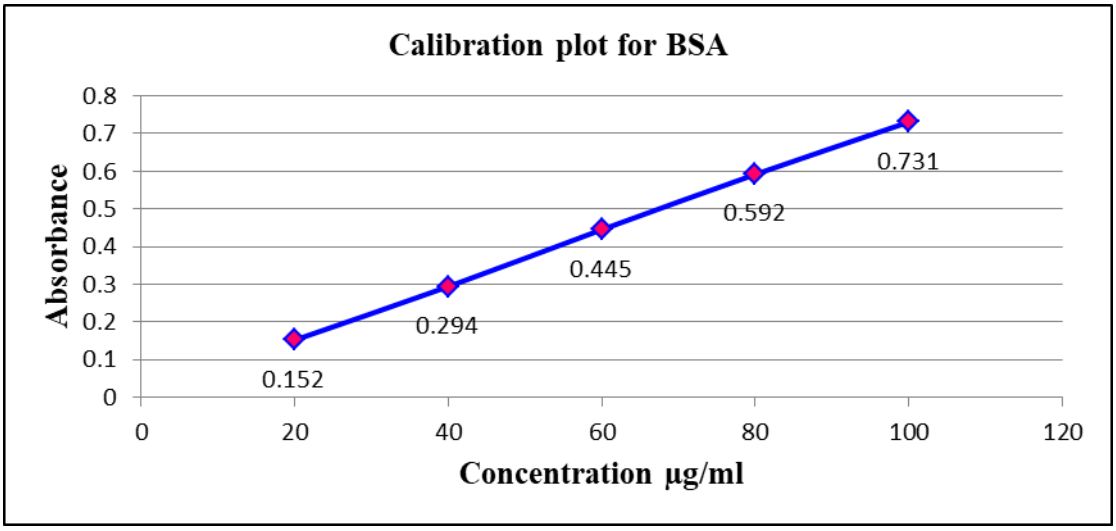

Figure 6: UV Calibration plot of BSA

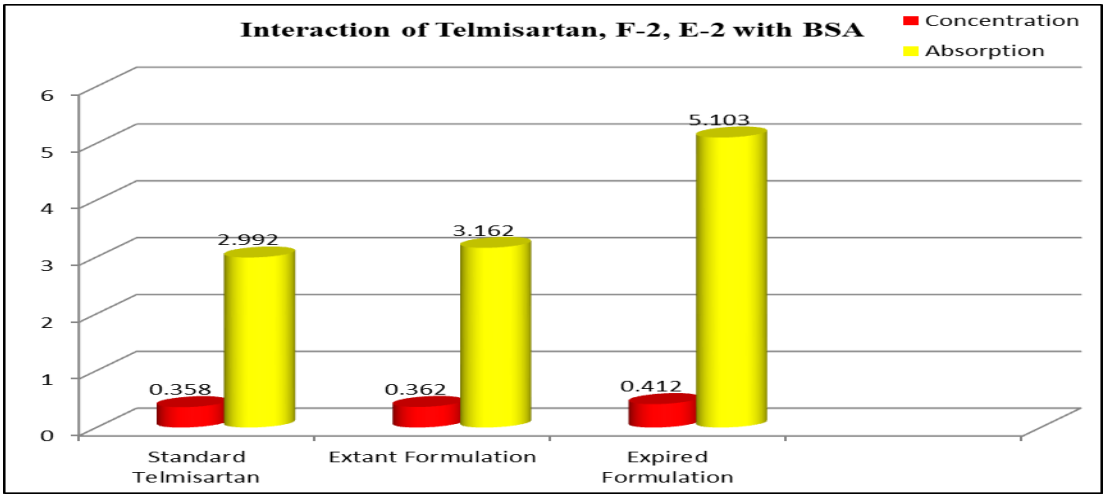

Figure 7: Comparison of Absorbance and Peak area of standard Telmisartan, F-2, E-2 with BSA

Table 1: Calibration data of Telmisartan and BSA

Telmisartan λ **max 232**		**Bovine Serum Albumin** λ **max 227**	
Conc. µg/ml	**Absorbance**	**Conc.** µg/ml	**Absorbance**
4	0.124	20	0.152
6	0.259	40	0.294
8	0.386	60	0.445
10	0.506	80	0.592
12	0.632	100	0.731

Table 2: Intraday interaction study of standard Losartan and Telmisartan with BSA

Concentration µg/ml	**Absorbance 0 min**	**Absorbance 30 min**	**Absorbance 1 hr**
Telmisartan 10 µg/ml	0.632	0.592	0.532

Table 3: Interaction study of standard drug Telmisartan, F-2 and E-2 with BSA

Drug	**Absorbance**	**Peak Area**	**Shift in peak**
Standard Telmisartan	0.358	2992	232
F-2	0.362	3162	232
E-2	0.412	4103	232

5. SUMMARY AND CONCLUSION

Telmisartan 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. Protein binding of standard drug along with the extant and expired formulation was considered in the present study by employing UV Visible spectroscopy.

- Interaction of drug with BSA was studied by fixing carbonate buffer of pH 7.4 as solvent.
- Calibration curve of Telmisartan and BSA was plotted in the obtained λmax and the curves found to be linear in the selected concentration range 4-12µg/ml and 20-100µg/ml for Losartan, Telmisartan and BSA respectively.
- To elucidate the interaction of Telmisartan with BSA, the spectra were examined in a concentration in linearity range (12 and 10 µg/ml)
- Stability and the intraday studies were carried out.
- 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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