5. RP-HPLC METHOD FOR THE DEVELOPMENT AND VALIDATION OF TELMISARTAN

Telmisartan interferes with the binding of angiotensin II to the angiotensin II AT$_1$-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and adrenal gland.

5.1 Drug Profile:  

Molecular Structure

![Figure 5.1: Structure of Telmisartan (TSN)](image)

**Chemical Name:** 4-((2-n-propyl-4-methyl-6-(1-methylbenzimidazol-2-yl)-benzimidazol - 1 - yl) methyl) biphenyl-2- Carboxylic acid.

**Molecular Formula:** C$_{33}$H$_{30}$N$_4$O$_2$.

**Molecular Weight:** 514.6gms/mol.

**Category:** Antihypertensive agent, Angiotensin II receptor antagonist (ARB)

**Solubility:** It is practically insoluble in water, sparingly soluble in strong acid and soluble in strong base.

**Physical state:** White to half-white amorphous powder.

**Half- life:** 24 hours.

**Absolute bioavailability:** 42%.

**Melting point:** 261 -263ºC

**Mechanism of action:**

Telmisartan interferes with the binding of angiotensin II to the angiotensin II AT$_1$-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and 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.

**Therapeutic uses:** Telmisartan is used in the management of hypertension.
**Dose:** Telmisartan is used in the treatment of essential hypertension. Usually effective dose of Telmisartan is 20, 40 or 80 mg once daily. Some patients may already benefit at a daily dose of 20 mg, in cases where the target blood pressure is not achieved, Telmisartan dose can be increased to a maximum of 80 mg once daily.

**Pharmacodynamics:**

Telmisartan is an orally active nonpeptide angiotensin II antagonist that acts on the AT\(_1\) receptor subtype. It has highest affinity for the AT\(_1\) receptor among commercially available ARB’S and has minimal affinity for the AT\(_2\) receptor. New studies suggest that Telmisartan may also have PPAR\(\gamma\) agonistic properties that could potentially confer beneficial metabolic effects, as PPAR\(\gamma\) is a nuclear receptor that regulates specific gene transcription, and whose target genes are involved in the regulation of glucose and lipid metabolism, as well as anti-inflammatory responses.

This observation is currently being explored in clinical trials. Angiotensin II is formed from angiotensin I in a reaction catalyzed by angiotensin-converting enzyme (ACE, kinase II). Angiotensin II is the principal precursor agent of the renin-angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation, and renal reabsorption of sodium. Telmisartan works by blocking the vasoconstrictor and aldosterone secretory effects of angiotensin II.

**Pharmacokinetics:**

**Absorption:** Rapidly absorb, dose dependent bioavailability 42% (after 40 mg), 58% (after 160mg) and peak plasma concentration 0.5-1 hr.

**Distribution:** Protein binding 99%

**Metabolism:** Under goes conjugation with glucuronic acid to form inactive metabolites.

**Elimination:** <1% excreted via urine.

**Drug interactions:** Telmisartan can increase blood concentrations of digoxin (Lanoxin) and lithium (Eskalith, Lithobid). Therefore blood levels of digoxin and lithium should be monitored and doses adjusted if necessary. Combining telmisartan with potassium-sparing diuretics (for example, spironolactone (Aldactone), triamterene, amiloride), potassium supplements, or salt substitutes containing potassium may lead to hyperkalemia (elevated potassium in the blood). Combining telmisartan or other ARBs with nonsteroidal anti-inflammatory drugs (NSAIDs) in patients who are elderly, fluid-depleted (including those on diuretic therapy), or with
poor kidney function may result in reduced kidney function, including kidney failure. These effects usually are reversible. There have been reports that aspirin and other NSAIDs such as ibuprofen (Advil, Children's Advil/Motrin, Medipren, Motrin, Nuprin, PediaCare Fever, etc.), indomethacin (Indocin, Indocin-SR), and naproxen (Anaprox, Naprelan, Naprosyn, Aleve) may reduce the effects of ARBs.

**Administration:** Telmisartan may be taken with or without food.

**Storage:** Stored at room temperature, 15\&30^\circ c. The tablets should keep in the blister pack packaging until they are used.
5.2 LITERATURE REVIEW:

Seshagiri Rao et al.,⁷⁶ developed a high performance liquid chromatographic method for quantitative estimation of Telmisartan in bulk drug samples and tablet dosage forms. Chromatographic separation of the drug was achieved on a Kromasil C18 column (150 x 4.6 mm; 5μ) using a mixture of phosphate buffer (pH 4.0) and acetonitrile (40:60 v/v) as the mobile phase at a flow rate of 1.0 mL/min. Under optimized conditions, the retention time of the drug was found to be 2.887 min. Good detecting sensitivity for the analyte was observed at 224 nm. The quantitation calibration curve for the drug was linear over the range of 20-60 μg/mL.

Ismail Salama⁷⁷ developed a method based on high performance liquid chromatography (HPLC) for the simultaneous determination of Telmisartan (TELM) and hydrochlorothiazide (HCT) in human plasma using Indapamide as internal standard. The method utilizes proteins precipitation with acetonitrile as only sample preparation prior to RP-HPLC. The analytes were chromatographed on shim-pack cyanopropyl column in isocratic elution with methanol: 10 mM ammonium acetate solution (pH 6.0) (35:65 v/v) as mobile phase at a flow rate of 1 ml/min and the wavelength of detection was 270 nm. The method was validated over the concentration range of 1-10 μg/ml for TELM and 0.31-3.12 μg/ml for HCT in human plasma. Inter- and intra-run precision of TELM and HCT were less than 3.60% and the accuracy was less than 1.868%.

Vijay Kumar et al.,⁷⁸ developed and validated a RP-HPLC method for rapid assay of Telmisartan in serum samples. Isocratic elution at a flow rate of 1.0ml/min was employed on a Equisil, 250 X 4.6mm, 5μ at ambient temperature. The mobile phase consisted of buffer: Acetonitrile (35:65) (V/V). The UV detection wavelength was 282nm and 20μL sample was injected. The retention time for telmisartan was 3.32 min. The percentage RSD for precision and accuracy of the method was found to be less than 2.

Lakshmana Rao et al.,⁷⁹ developed a reverse phase, isocratic HPLC method for the separation and quantification of Telmisartan and Hydrochlorothiazide in pharmaceutical dosage form. The quantification was carried out using ProntoSIL C18-EPS 4.6X150mm, 3μm enhanced polar selectivity column and mobile phase comprised of potassium dihydrogen phosphate buffer pH adjusted to 3.2 ± 0.5 with orthophosphoric acid and acetonitrile in proportion of ratio 55:45 and degassed under ultrasonication. The flow rate was 0.8ml/min and the effluent was monitored at
271nm. The retention time of Telmisartan and hydrochlorothiazide were 5.01±0.5 and 2.94±0.5 respectively. The method was validated in terms of linearity, precision, accuracy, specificity, limit of detection and limit of quantitation. Linearity of Telmisartan and hydrochlorothiazide were in the range of 15.01 to 75.05μg/ml and 5.02 to 25.10μg/ml respectively. The percentage recoveries of both the drugs were 100.8% and 99.5% for Telmisartan and hydrochlorothiazide respectively from the tablet formulation.

Siddiqui et al., developed and validated comparative force degradation ultra-performance liquid chromatographic assay method was for Telmisartan and its degradation products. Telmisartan was subjected to acid (0.1M HCl), neutral (water) and alkaline (0.1M NaOH) hydrolytic conditions at 80°C, as well as to oxidative decomposition (H₂O₂) at room temperature. Photolytic studies were carried out by exposing this drug into sunlight (60,000-70,000 lux) for 2 days. Additionally, the solid drug was subjected to 50°C for 60 days in a hot air oven for thermal degradation. The UPLC chromatographic separation was performed on Acquity UPLC BEH C18 column (1.7 μm, 2.1mm×150mm) using isocratic mode (ACN:water, 70:30v/v) at flow rate of 0.2 ml/min and HPLC chromatographic separation was achieved on phenomenex C18 using isocratic mode (ACN:10mM ammonium acetate, Ph 4.5, 85:15v/v) at flow rate of 1.0 ml/min. Telmisartan was found to degrade significantly in acid, base and oxidation, the drug was found to be stable in neutral, thermal and photolytic stress conditions. The ultra performance liquid chromatography (UPLC) and high performance liquid chromatography (HPLC) area %RSD were calculated to be 0.0039 and 0.0015 respectively. The UPLC and HPLC linearity of the proposed method were investigated in the range of 10-50 μg/ml and 30-150 μg/ml. The R² value of UPLC and HPLC were found to be 0.9987 and 0.9989 respectively. Method detection limit (MDL) and Method quantification limit (MQL) were found to be 0.250 μg/ml and 1.20 μg/ml for UPLC and 0.600 μg/ml and 1.900 μg/ml respectively for HPLC. The %R.S.D. values for intra-day and inter-day precision were <1.0%.

Sujana et al., developed and validated a RP High Performance Liquid Chromatographic (HPLC) method of analysis of Telmisartan in pure and pharmaceutical dosage form. The chromatographic conditions comprised of a reverse phase C8 column (4.6 x 150mm, 3.5 μm, Make: X Terra), with a mobile phase composed of Buffer and Methanol (40:60v/v, Adjusted the pH to 3.0 with ortho
Phosphoric acid). Flow rate was 0.5 ml / min. Detection was carried out at 230 nm. The retention time of Telmisartan was 2.6 min. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range 20-100 μg/ml. The values of correlation coefficient, slope and intercept were 0.9998, 2.326 and 6.708 respectively.

Osman et al., developed and validated a reversed-phase High performance liquid chromatographic (RP-HPLC) method for the determination of Telmisartan in bulk and pharmaceutical dosage form. Chromatographic separation of Telmisartan was achieved on a reverse phase C18 column using a mobile phase consisting of acetonitrile: phosphate potassium buffer (pH= 3): methanol in the ratio of 40:20:40 v/v. The mobile phase was pumped at a flow rate of 1 ml/min and the eluents were monitored at 295 nm.

Amit et al., An approach of forced degradation study was successfully applied for the development of a stability indicating assay method for simultaneous determination of Telmisartan and Indapamide in a formulation in the presence of its degradation products. The method showed adequate separation of Telmisartan and Indapamide from their associated main impurities and degradation products. Separation was achieved on an Amazon C18, 5 micron, 150 x 4.6 mm the mobile phase (Buffer: Acetonitrile: Methanol) (45+25+30) KH2PO4 & Triethaylamine pH 3.0 with ortho phosphoric acid buffer flow rate of 1 ml/min and UV detection at 285 nm. Comprehensive stress testing of Telmisartan and Indapamide Rt = 4.7 min, 10.7 min was according to the International Conference on Harmonization (ICH) guideline Q1A (R2). The method was validated in terms of linearity, precision, accuracy, Specificity, robustness, and solution stability. The linearity of the proposed method was investigated in the range of 6-22.5 microg/ml (R²= 0.999) for Telmisartan and 11.2-42 microg/ml (R² = 0.9997) for Indapamide.

Charde et al., developed and validated a reverse phase high performance liquid chromatographic (RP-HPLC) method for determination of Telmisartan in tablet dosage form was. Chromatographic separation was achieved on a 250 × 4.6 mm, 5μ, Waters symmetry column in gradient mode, with mobile phase consisting of a mixture of solution (10 mM potassium dihydrogen phosphate, pH 3.5 ± 0.01): acetonitrile (64:40) was used. The quantitation performed at flow rate of 1.0 ml/min at 230 nm and run time was 12 min. The analytical method was validated as per ICH guideline for linearity, accuracy, precision, specificity, limit of detection, limit of
quantification, robustness and stability and method can be extended to the analysis of Telmisartan in tablet formulations. The relative standard deviation values for precision was less than 2%, and % recovery was greater than 98% for Telmisartan.

Rajeswari A. et al.,\textsuperscript{144} developed a reverse phase HPLC method for the simultaneous estimation of Amlodipine besylate and Telmisartan. A Phenominex-luna C18 column (250x4.6 mm i.d 5\(\mu\)) was used with a mobile phase containing a mixture of acetonitrile and phosphate buffer in the ratio of 56:44%v/v. pH was adjusted with orthophosphoric acid to 4. The flow rate was 1ml/min and the eluents were monitored at the detector wavelength of 236nm. The retention times of Amlodipine besylate and Telmisartan were found to be 4.32 and 5.32 minutes respectively.

Lakshmi Surekha et al.,\textsuperscript{145} developed and validated a RP–HPLC method for the determination of Telmisartan in Pharmaceutical dosage form. Separation was achieved under optimized chromatographic condition on a Zorbax-SB-18;(ODS) column (150 X 4.6 mm i.d., particle size 3.5\(\mu\)). The mobile phase consisted of Pentane sulphonic acid sodium salt mono hydrate, add 1ml of Perchloric acid and adjust the pH-2.7±0.05 with Triethyl amine: Methanol in the ratio 40: 60 v/v. An isocratic elution at a flow rate of 1.2 ml/ min at ambient temperature. The detection was carried out at 230 nm using waters UV Visible detector. The calibration curve was linear in the concentration range of 4–20mg/ ml (R\(^2\)═0.9999). The limit of detection and the limit of quantification were found to be 0.2515 mg/ml and 0.6623 mg/ml respectively. The amount of Telmisartan present in the formulation was found to be 99.95. The method was validated statistically using SD and %RSD and the values are found to be within the limits and recovery studies were performed and the percentage recoveries was found to be 99.55± 0.7211 %.

Lakshmana Rao et al.,\textsuperscript{146} developed aRP-HPLC method for the simultaneous determination of Telmisartan and Hydrochlorothiazide in pharmaceutical dosage forms. Telmisartan has absorption maxima at 296 nm and hydrochlorothiazide has absorption maxima at 280 nm. For the simultaneous estimation of Telmisartan and hydrochlorothiazide the detection wavelength was taken as 271 nm. Linearity for detector response was observed in the concentration range of 50 to 150 % of test concentration. Correlation coefficient (R\(^2\)) for calibration curve was found to be 1.0. Retention times were found to be 5.79 min and 2.85 min for Telmisartan and hydrochlorothiazide respectively. Percent recovery was found to be within the range of 98.0 % to 102.0%. The percent RSD for the analyzed tablet and recovery studied
was less than 2. The results of recovery studies were found to be linear in the range 50 % to 150 % of test concentration.

**Shrivastava et al.,** developed and validated a isocratic reversed-phase high-performance liquid chromatographic method (RP-HPLC) for the simultaneous estimation of Amlodipine and Telmisartan in combined dosage form. The chromatographic separation was achieved by using mobile phase acetonitrile and 0.05M sodium dihydrogen phosphate buffer (60:40) adjusted to pH 6.0, a C-18 column, perfectsil target ODS3 (150 mm x 4.6 mm i.d., 5 µm). The mobile phase was pumped at a flow rate of 0.8 ml/min and the eluents were monitored at 254 nm. Retention times were 4.0 min and 8.2 min for Amlodipine and Telmisartan respectively. The method was validated in terms of accuracy, precision, linearity, range and specificity, limit of detection and limit of quantitation. Linearity for Amlodipine besylate and Telmisartan was established in the range of 5-30 and 10-60 µg/ml, respectively. The recoveries for the two compounds were above 96%.

**Narasimha Rao et al.,** developed and validated a reverse phase high-performance liquid chromatography method for analysis of Ramipril and Telmisartan in pure and pharmaceutical dosage form. The method was developed on symmetric C18 (4.6 x 150mm, 5µm, Make: Zorbax), with a mobile phase of phosphate buffer (pH 3.0): Acetonitrile (55:45) %v/v. The effluent was monitored by Waters HPLC model containing Alliance 2695 with 2487 detector, variable wavelength prominence UV/ VIS detector SPD-20A (VP series). Calibration curve was linear over the concentration range of 1.25-6.25µg/ml for Ramipril and 10-50µg/ml for Telmisartan. Recovery of Ramipril and Telmisartan was found to be in the range of 99.1 - 100.2%. The limit of detection (LOD) and quantification (LOQ) were 2.97 and 9.84 for Ramipril and 3.0 and 9.95 for Telmisartan, respectively.

**Yogesh et al.,** developed a RP-HPLC method for the simultaneous estimation of Ramipril and Telmisartan in tablet dosage forms, using UV-detector. The developed method was validated as per ICH guidelines and specificity, linearity & range, accuracy, precision and robustness was performed. Specificity was determined by comparing the results obtained by running the placebo solution with that of standard and method was found to be specific due to no interference between placebo peaks and drugs peaks. Linearity range was found to be 4 to 16µg/ml and 32 to 128µg/ml of Ramipril and Telmisartan respectively. The method was found to be linear in the range of 4 to 16µg/ml and 32 to 128µg/ml for Ramipril and Telmisartan respectively.
In the linearity study, regression equation and coefficient of correlation for Ramipril and Telmisartan were found to be \( y = 924480x - 151831 \), \( R^2 = 0.9997 \) and \( y = 2901878.3558x + 3803877 \), \( R^2 = 0.9996 \) respectively.

Jaydeep et al., developed and validated a reversed phase high performance liquid chromatographic (HPLC) method for the determination of Telmisartan in small volumes of rat plasma. Biological sample preparation involving simple extraction with organic solvent, followed by dilution with mobile phase was adopted to eliminate any chromatographic solvent effects. The method was proven to be linear over a plasma concentration range of 10 to 1000 ng/mL with a mean correlation coefficient of 0.9942. The limit of detection and the limit of quantification of the newly developed method were determined to be 1 ng/ml and 10 ng/ml, respectively. The method was successfully applied to assess pharmacokinetic parameters of Telmisartan in Wister rats following a single oral dose (1.8 mg/kg, b.w.).

Vijayamirtharaj R et al., developed a RP-HPLC method for the determination of Telmisartan and Atorvastatin calcium in bulk and in formulation using UV detector. Selected mobile phase was a combination of Acetonitrile: Buffer (0.01M Potassium dihydrogen phosphate) 65:35 pH 4.00 (adjusted with Orthophosphoric acid) and the wavelength selected was 250 nm. The flow rate was kept at 2.0 ml/min, and the injection volume was 10 μL. The separation was performed at ambient temperature. Retention time of Telmisartan and Atorvastatin calcium was found to be 3.72 and 6.14 minutes respectively. Linearity of the method was found to be 319-480 μg/ml for Telmisartan and 86-130 μg/ml for Atorvastatin calcium. The correlation coefficient of Telmisartan was found to be 0.9998 and the correlation coefficient of Atorvastatin calcium was found to be 0.9999. Accuracy of the method was determined through recovery studies by adding known quantities of standard drug to the pre analyzed test solution and was found to be 98.92-100.02 for Telmisartan and 99.93-100.96 for Atorvastatin calcium respectively. The system suitability parameters such as theoretical plates and tailing factor were found to be 6347, 1.652 and 9720, 1.394 respectively for Telmisartan and Atorvastatin calcium.

Sahoo et al., developed a high performance liquid chromatography method for the simultaneous quantitative determination of Telmisartan and Chlorthalidone from their combination drug product. It involves a Xterra 150 mm x 4.6 mm, 5 μm, C-18 column. The separation is achieved on a simple isocratic method. The mobile phase contains a mixture of potassium dihydrogen phosphate buffer pH 2.5 (0.025M):
acetonitrile in the ratio 60:40, \(v/v\). The flow rate is 1.0 mL min\(^{-1}\) and the column is maintained at normal temperature. The detector wavelength is 235 nm. The retention times of Chlorthalidone and Telmisartan are 2.5 minutes and 4.4 minutes respectively. The total runtime for the separation of the two active compounds is 6.0 minutes.

A. Kottai Muthu et al.,\(^{153}\) developed a method for the simultaneous determination of Amlodipine and Telmisartan in bulk drug and pharmaceutical dosage by RP-HPLC method. Separation was performed on a 5\(\mu\)m Nucleodur\(^\circledR\) C18 column (250 \(\times\) 4.6mm ID) with acetonitrile: phosphate buffer at pH 4.5 (60:40v/v) in isocratic elution in less than 9 min with a flow rate of 1.3 ml min\(^{-1}\). Good sensitivity for all analytes was observed with UV detection at 238 nm. The method allowed quantification over the 1-11\(\mu\)g/ml range for Amlodipine and 8-80\(\mu\)g/ml range for Telmisartan. The method has been applied, without any interference from excipients or endogenous substances, for the simultaneous estimation of these two compounds in bulk drug and in tablets.

Gandhi et al.,\(^{154}\) developed and validated a reverse-phase high-performance liquid chromatographic method for the simultaneous quantification of Telmisartan and Cilnidipine as the bulk drug and in tablet dosage forms. Separation was carried out on Jasco HPLC system equipped with HiQ sil C18 HS column (250 \(\times\) 4.6 mm i.d.) and PDA detector using Methanol: 40 mM Potassium dihydrogen ortho phosphate buffer (pH 3) (90:10, v/v)) as the mobile phase, and detection was carried out at 245 nm. Results were linear in the range of 1-10\(\mu\)g/ml for Cilnidipine and 5-30\(\mu\)g/ml for Telmisartan.

Basaveswara Rao et al.,\(^{155}\) developed and validated a reverse phase high performance liquid chromatographic method for the estimation of Telmisartan in tablet dosage form. The expected separation and peak shapes were obtained on chromosil C18 (250 mm x 4.6 mm, 5 \(\mu\)m) column. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like methanol, water and acetonitrile with or without different buffers in different combinations were tested as mobile phases on a chromosil C18 column. A mixture of methanol : 0.1% orthophosphoric acid : acetonitrile in the ratio of 80:05:15 v/v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was better defined and resolved and was almost free from tailing. The flow rate was 1.5 ml/min and effluents were monitored at 256 nm. The retention time for Telmisartan was 2.7 min. Recovery of Telmisartan from tablet formulation was found to be 99.41\%.
Amit Suryakant Tapkir et al.\textsuperscript{156} developed a method for the simultaneous determination of Telmisartan and Clorthalidone from pharmaceutical formulation by reversed-phase high performance liquid chromatography. The separation was carried out on C18 column using mobile phase consisting of a mixture of acetonitrile: methanol and pH adjusted to 3.4 with orthophosphoric acid in the ratio (80:20 v/v). The flow rate was maintained at 1ml/min. The UV detection was carried out at a wavelength of 225 nm. The retention time for Telmisartan and Clorthalidone was found to be 3.1 min and 4.6 min respectively. Linear response obtained for Telmisartan was in the concentration range 10-60 μg/ml ($R^2 = 0.999$) and Clorthalidone in the range 10-50μg/ml ($R^2 = 0.999$). The relative standard deviation in the tablets was found less than 2% for six replicates.

Joshi Pryanka et al.\textsuperscript{157} developed a reverse phase High Performance Liquid Chromatographic method for simultaneous estimation of Metoprolol and Telmisartan in tablet dosage form on RP C-18 Column (Hypersil Gold, 25cm x 4.6mm, 5μm) using Acetonitrile and buffer (0.05M KH2PO4, pH 3.0±0.02, 35:65 v/v) as mobile phase at a flow rate of 1.0 ml/min and the detection wavelength was 225nm. The retention time for Metoprolol and Telmisartan was found to be 3.71 and 10.02 min. respectively. Detection response for both Metoprolol and Telmisartan were found to be linear in concentration range of 29.88-69.72mcg/ml and 24.12-56.27mcg/ml respectively in the linearity study, regression equation and coefficient of correlation for Metoprolol and Telmisartan were found to be ($y = 33409x + 5518$, $R^2 =0.9999$) and ($y = 111545x – 5850.4$, $R^2 = 0.9999$).

S. Rajitha et al.\textsuperscript{158} developed a reverse phase high performance liquid chromatographic method for simultaneous estimation of Telmisartan and Amlodipine Besylate in tablet dosage form. Symmetry C18 4.6 x 250mm, 5μm particle size was used. The method was carried out in gradient program using mobile phase, 0.02M Potassium dihydrogen orthophosphate: acetonitrile (30:70 v/v) adjusted to pH-5 using dilute ortho phosphoric acid. Flow rate was adjusted to 1.0ml/min and effluents were monitored at 245nm. The retention time obtained for Amlodipine Besylate and Telmisartan was 2.325 and 3.523 min respectively. The calibration curves were linear in the concentration range of 32-96μg/ml for Telmisartan and 4-12μg/ml for Amlodipine.

Agey et al.\textsuperscript{159} described two methods for simultaneous estimation of Telmisartan and Amlodipine in binary mixture by using UV Spectrophotometry and RP-HPLC.
The first method was based on UV-spectrophotometric determination of two drugs which involves absorbance measurement at 297.0 nm (λmax of Telmisartan) and 362.0 nm (λmax of Amlodipine) in methanol: water (70:30); linearity was obtained in the range of 8-40 μg/ml and 1-5 μg/ml for Telmisartan and Amlodipine respectively. The second method was based on RP-HPLC and separation of the two drugs was achieved on phenomenex C18 column with acetonitrile and phosphate buffer, pH 2.9 (gradient program). The method was linear with entire range of Telmisartan (16-48 μg/ml) and Amlodipine (2-6 μg/ml) with coefficient of correlation was above 0.995 for both methods.

Vekariya et al., developed a reverse phase HPLC method for the estimation of Telmisartan in tablet dosage form. Luna 5 μ C18, 250 × 4.6 mm, particle size 5 μm, with mobile phase consisting of 5 mM Phosphate buffer: Acetonitrile (60:40, v/v), pH 7.4 was used. The flow rate was 1 ml/min and the effluents were monitored at 295 nm. The retention time was 7.02 min. The detector response was linear in the concentration of 2-14 μg/ml. The respective linear regression equation being Y=61480X-10188. The limit of detection and limit of quantification was 0.06 and 0.18 mcg/ml respectively. The percentage assay of Telmisartan was 100.28 ± 0.93 %.

**5.3 MATERIALS & METHODS**

**Instrumentation:**
Chromatographic separation was performed using Phenomenex C-18compact LC system integrated with a variable wavelength programmable UV detector and a Rheodyne injector equipped with 25μl fixed loop. The separation was performed on a reverse phase C18 column [Phenomenex C-18, 250×4.6mm, 5μm]. Quantitative HPLC was performed on a gradient High Pressure Liquid Chromatography (Shimadzu HPLC class VP series) with two LC-10 AT, VP pumps, variable wavelength programmable UV/Visible detector SPD-10A, VP, CTO-10ASVP column oven (Shimadzu), SCL-10A, VP system controller (Shimadzu) and on column [Phenomenex C18 (250 mm x 4.6 mm) I.D.; particle size 5 mm]. The HPLC system was equipped with the software “class VP series version 5.03 (Shimadzu).

**Table 5.1: Chromatographic conditions for TSN**
<table>
<thead>
<tr>
<th><strong>Parameters</strong></th>
<th><strong>Method</strong></th>
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<tr>
<td>Stationary phase (column)</td>
<td>C&lt;sub&gt;18&lt;/sub&gt; column (Phenomenex C&lt;sub&gt;18&lt;/sub&gt;, 250×4.6mm, 5µm)</td>
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<tr>
<td>Mobile phase</td>
<td>Methanol: Buffer [80:20] [pH 5.8], v/v</td>
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<tr>
<td>IS</td>
<td>3.2 minutes</td>
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**PREPARATION OF THE TELMISARTAN STANDARDS**

**Standard Solution Preparation:**

Accurately weigh and transfer 25mg of Telmisartan working standard into 25 ml volumetric flask and add about 25ml of methanol and sonicate to dissolve completely and make volume up to the mark with the same solvent (stock solution).

Further pipette 1ml into 10ml volumetric flask and make up the volume to 10ml with diluent; it is further diluted with diluent to obtain 1ug/ml, 3ug/ml, 5ug/ml, 7ug/ml, 9ug/ml and 10ug/ml respectively.

**Internal Standard Solution Preparation:**

Accurately weigh and transfer 25mg of Eprosartan Mesylate working standard into 25 ml volumetric flask and add about 25ml of methanol and sonicate to dissolve completely and make volume up to the mark with the same solvent (stock solution).

Further pipette 1ml into 10ml volumetric flask and makeup the volume to 10ml with diluent; it is further diluted with diluent to obtain 1ug/ml, 3ug/ml, 5ug/ml, 7ug/ml, 9ug/ml and 10ug/ml respectively.

**Sample Solution Preparation:**

Equal volumes of same concentrations of the Active Pharmaceutical Ingredient (API) and IS were taken and vortexed to get the final mixtures which were analysed by using HPLC.

**METHOD DEVELOPMENT:**
**Purpose:**
The purpose of method development is to optimize the chromatographic conditions by conducting various trials that result in a sensitive, precise, accurate and reliable method that enables the estimation of Telmisartan in pharmaceutical formulations.

**Procedure:**
In developing a HPLC method, a systematic study of effects of various parameters was undertaken by varying one parameter at a time and controlling all other parameters. The following studies are to be conducted for this purpose.

**A) Selection of Mode of separation:**
The first consideration when developing HPLC method is to determine the solubility of sample components. As Telmisartan and Eprosartan Mesylate were soluble in polar solvents, RP-HPLC mode was chosen.

**B) Selection of Mobile phase:**
In order to get symmetric peaks and optimum resolution between the components, a number of experiments were carried out by varying different parameters like nature and ratios of components of mobile phase by changing one at a time and keeping all other parameters constant. Standard solutions containing Telmisartan were injected into the column and run using different mobile phases. Different mobile phases of different proportions of the organic phase and buffers of different pH were tried at different flow rates for the better elution and separation of the drugs. Each mobile phase was filtered through 0.45µ membrane filter and sonicated for 15 min before the trials. Standard solutions were injected into the column after obtaining a steady baseline to get well resolved and stable peaks for both the drugs. After performing trials with different mobile phases, both the drugs were found to separate well with stable retention times when run with a mobile phase of combination methanol and phosphate buffer of pH 5.8 in a ratio of 80:20, v/v at a flow rate of 0.8ml/min. so, this mixture was selected as the mobile phase for the chromatographic method development because of the sharp symmetrical peaks and reproducible retention times obtained.

**C) Selection of Stationary phase:**
Selection of appropriate stationary phase helps to improve the efficiency of the method. In the present study, in order to get better peak resolution with less tailing factor and more theoretical plates, C\textsubscript{18} column (Phenomenex C-18, 250*4.6mm, 5µm) was selected.

**Preparation of Optimized Mobile Phase:**
Preparation of Potassium Dihydrogen Phosphate Buffer:
Weigh 0.68 grams of potassium dihydrogen phosphate into a 500 ml beaker, dissolve and dilute to 500 ml with HPLC grade water. Adjust the pH to 5.8 with 10% v/v ortho phosphoric acid.

Preparation of Mobile Phase:
Filter the above prepared buffer with 0.2 µ filter under vacuum. Mix the filtered buffer 200 ml (20%) and methanol 800 ml (80%) and degas in ultrasonic water bath for 10 minutes.

Diluent Preparation:
Mobile phase as a diluent

Recommended procedure
After systematic and detailed study of the various parameters involved, as described under results and discussion in this chapter, the following procedure was recommended for the determination of Telmisartan in bulk samples and pharmaceutical formulations using Eprosartan mesylate as an internal standard.

Method:
Five sets of the drug solutions and IS’s were prepared in mobile phase containing Telmisartan and Eprosartan mesylate at a concentration range of 1-11 µg/ml. The contents of the mobile phase were filtered before use through 0.45 µm membrane filter, degassed by sonication. Prior to injection of the drug, the mobile phase was pumped for about 30 min to saturate the column thereby to get the base line corrected. Then 20 µl of each of the drug solutions were injected for 5 times. Quantity determinations were made by comparisons of the peak area from a standard injection. The amount of Telmisartan present in the sample was calculated through the standard calibration curve.

5.4 ANALYTICAL METHOD DEVELOPMENT
To develop a suitable (specific) and robust LC method for the determination of Telmisartan, different mobile phases were employed to achieve the best separation and resolution. The method development started with symmetry C18 (50 × 2.1 mm, 3.5 µm) with following mobile phase compositions like 80:20, 70:30 & 60:40 methanol and buffer (pH 7) were carried out. Methanol and buffer in ratio 60:40 was good compared to other. 60 ml of methanol and 40 ml buffer of different pH’s were
then tried, out of those mobile phase composition 60:40 at pH of 5.8 gave good peak shapes with less retention times. The retention time of Telmisartan and IS were 4.8 and 3.18 mins. The chromatograms of Telmisartan and ISblank, placebo and standard were shown in the Figures 5.2, 5.3 & 5.4 respectively.

Figure 5.2: A typical Chromatogram of Telmisartan Blank

Figure 5.3: A typical Chromatogram of Telmisartan Placebo

Figure 5.4: A typical Chromatogram of Telmisartan Standard

5.5 METHOD VALIDATION:

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The developed LC method is extensively validated for Telmisartan using the following parameters

5.5.1 Specificity:

**Purpose:**
Specificity of a method was determined by testing standard substances against potential interferences. Triplicate injections of 100% test concentration (5µg/ml Telmisartan + 50µg/ml Eprosartan Mesylate) were given to the system for checking the interferences of excipients if any.

**Procedure:**
Common excipients that are usually present in the formulation such as lactose, microcrystalline cellulose and magnesium stearate have been added to the sample solution and injected into the HPLC system by following the test method conditions.

5.5.2 System Suitability:

**Purpose:**
System suitability tests were carried out on each day of validation to evaluate the components of the analytical system in order to show that the performance of the system meet the standards required by the method.

The HPLC system was equilibrated using the initial mobile phase composition, followed by six replicate injections of the 100% concentration containing 5 and 50µg/ml. The chromatogram was represented in the Figure 5.5 and result was reported in the Table 5.2.

### Table 5.2: System suitability data of TSN

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No.of theoretical plates (N)</td>
<td>DRUG 23400</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IS 16235</td>
</tr>
<tr>
<td>2</td>
<td>Tailing factor (T)</td>
<td>DRUG 1.194</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IS 1.349</td>
</tr>
<tr>
<td>3</td>
<td>Retention time (min)</td>
<td>DRUG 4.8±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IS 3.18±0.2</td>
</tr>
<tr>
<td>4</td>
<td>%RSD</td>
<td>DRUG 0.975</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IS 0.824</td>
</tr>
</tbody>
</table>
5.5.3 Linearity:

**Purpose:**
The purpose of this study is to verify that the detector response is directly proportional to the concentration.

**Procedure:**
Measure the peak areas of the respective concentration levels at 296nm. Plot a graph of peak area ratio versus concentration (on X-axis concentration and on Y-axis peak area) and calculate the correlation coefficient. Calibration curve was represented in the Figure 5.6. The result was reported in the Table 5.3. Chromatograms representing the linearity were shown in the Figures 5.7-5.9.

**Table 5.3: Calibration data of TSN**

<table>
<thead>
<tr>
<th>Conc.(µg/ml)</th>
<th>Peak Area (PA) Drug(D)</th>
<th>I.S</th>
<th>PA(D)/I.S</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32550</td>
<td>122810</td>
<td>0.2650</td>
</tr>
<tr>
<td>3</td>
<td>96555</td>
<td>118376</td>
<td>0.8156</td>
</tr>
<tr>
<td>5</td>
<td>157070</td>
<td>126502</td>
<td>1.2416</td>
</tr>
<tr>
<td>7</td>
<td>210253</td>
<td>119668</td>
<td>1.7569</td>
</tr>
<tr>
<td>9</td>
<td>263288</td>
<td>118355</td>
<td>2.2245</td>
</tr>
<tr>
<td>11</td>
<td>326493</td>
<td>116543</td>
<td>2.8014</td>
</tr>
</tbody>
</table>

Slope 0.248

Intercept 0.024

Correlation coefficient 0.998
Figure 5.6: Standard calibration curve of Telmisartan

Figure 5.7: Chromatogram showing linearity of Telmisartan Inj-1

Figure 5.8: Chromatogram showing linearity of Telmisartan Inj-2

\[ y = 0.248x + 0.024 \]

\[ R^2 = 0.998 \]
5.5.4 Precision:

Purpose:
The purpose of this study is to establish that the developed RP-HPLC method is precise for the analysis of Telmisartan in Pharmaceutical formulations. The precision of the method was verified by performing repeatability and intermediate precision studies.

Repeatability:
The precision of the method was assessed by the six replicate injections of the 100% test concentration (5μg/ml of Telmisartan and 50μg/ml of IS) analyzed at 296nm on the same day and % relative standard deviation (%RSD) was calculated. The results were reported in the Table 5.4. Chromatogram represent the precision was shown in the Figure 5.10.

Table 5.4: Repeatability data of TSN

<table>
<thead>
<tr>
<th>Conc.[μg/ml]</th>
<th>Peak area(API)</th>
<th>Peak area(IS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection 1</td>
<td>124504</td>
<td>32547</td>
</tr>
<tr>
<td>Injection 2</td>
<td>121631</td>
<td>32485</td>
</tr>
<tr>
<td>Injection 3</td>
<td>123342</td>
<td>32258</td>
</tr>
<tr>
<td>Injection 4</td>
<td>121717</td>
<td>32473</td>
</tr>
<tr>
<td>Injection 5</td>
<td>122860</td>
<td>32989</td>
</tr>
<tr>
<td>Injection 6</td>
<td>124704</td>
<td>32576</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>122810.8</strong></td>
<td><strong>32550.4</strong></td>
</tr>
<tr>
<td><strong>Std dev</strong></td>
<td><strong>1197.899</strong></td>
<td><strong>268.3967</strong></td>
</tr>
<tr>
<td><strong>% RSD</strong></td>
<td><strong>0.975402</strong></td>
<td><strong>0.824557</strong></td>
</tr>
</tbody>
</table>
Intermediate Precision:
The precision of the method was assessed by the six replicate injections of the 100% test concentration (5μg/ml of Telmisartan and 50μg/ml of IS) and analyzed at same time on two different days at their selected analytical wave length of 296nm. The variation of the results on different days was analyzed and %RSD was calculated.

Table 5.5: Intermediate precision data of TSN

<table>
<thead>
<tr>
<th>Conc. [μg/ml]</th>
<th>Day 1 (5μg/ml and 50μg/ml)</th>
<th>Day 2 (5μg/ml and 50μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak area (API)</td>
<td>Peak area (IS)</td>
</tr>
<tr>
<td>Injection 1</td>
<td>124504</td>
<td>32547</td>
</tr>
<tr>
<td>Injection 2</td>
<td>121631</td>
<td>32485</td>
</tr>
<tr>
<td>Injection 3</td>
<td>123342</td>
<td>32258</td>
</tr>
<tr>
<td>Injection 4</td>
<td>121717</td>
<td>32473</td>
</tr>
<tr>
<td>Injection 5</td>
<td>122860</td>
<td>32989</td>
</tr>
<tr>
<td>Injection 6</td>
<td>127540</td>
<td>32754</td>
</tr>
<tr>
<td>Average</td>
<td>122810.8</td>
<td>32550.4</td>
</tr>
<tr>
<td>Std dev</td>
<td>1197.899</td>
<td>268.3967</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.975402</td>
<td>0.824557</td>
</tr>
</tbody>
</table>

Figure 5.10: Chromatogram showing Repeatability of TSN

Figure 5.11: Chromatogram showing Intermediate precision of TSN
5.5.5 Accuracy:

**Purpose:**
The purpose of this study is to express the extent of closeness of the results obtained by the proposed method to that of true value. Recovery studies were performed by standard addition method for verifying the accuracy of the proposed method. A known quantity of pure drug was added to pre-analysed sample and contents were reanalysed by the proposed method and the percent recovery was reported.

**Procedure:**

**Preparation of 80% recovery sample:**
2 ml and 4 ml of standard stock solution ‘B’ of Drug & IS (4 µg/ml & 40 µg/ml) was added to 2.5 & 5 ml mixture (5 µg/ml & 50 µg/ml) of sample stock solution in a 50 ml volumetric flask and volume was made up to the mark with mobile phase to get 80% recovery sample (9 µg/ml & 90 µg/ml).

**Preparation of 100% recovery sample**
2.5 ml and 5 ml of standard stock solution ‘B’ of Drug & IS (5 µg/ml & 50 µg/ml) was added to 2.5 & 5 ml mixture (5 µg/ml & 50 µg/ml) of sample stock solution in a 50 ml volumetric flask and volume was made up to the mark with mobile phase to get 100% recovery sample (10 µg/ml & 100 µg/ml).

**Preparation of 120% recovery sample**
3 ml and 6 ml of standard stock solution ‘B’ of Drug & IS (6 µg/ml & 60 µg/ml) was added to 2.5 & 5 ml mixture (5 µg/ml & 50 µg/ml) of sample stock solution in a 50 ml volumetric flask and volume was made up to the mark with mobile phase to get 120% recovery sample (11 µg/ml & 110 µg/ml).

The solutions were filtered through 0.45 µ membrane filter and then they were subjected to analysis by RP-HPLC method under the described chromatographic conditions. Recovery studies were carried out in triplicate at each level. The results obtained were compared with expected results and were statistically validated. The result was reported in the Table 5.6. Chromatograms represent the % recovery were shown in the Figures 5.12-5.14.

Measure the peak areas of the standard concentration (1 µg/ml, 5 µg/ml, and 10 µg/ml) at 296 nm. Calculate the % RSD and the % recovery of the concentrations.
Table 5.6: Accuracy data of TSN

<table>
<thead>
<tr>
<th>Recovery level (%)</th>
<th>DRUG</th>
<th>IS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount added (µg/ml)</td>
<td>Amount found (µg/ml)</td>
</tr>
<tr>
<td></td>
<td>Standard</td>
<td>Test</td>
</tr>
<tr>
<td>80%</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>100%</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>120%</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Mean recovery</td>
<td>99.18-100% w/w</td>
<td>99.94-100.08% w/w</td>
</tr>
</tbody>
</table>

Figure 5.12: Chromatogram showing Accuracy - 80%

Figure 5.13: Chromatogram showing Accuracy - 100%
5.5.6 Robustness:

**Purpose:**
The purpose of this study is to determine the capability of the proposed method to remain unaffected by small deliberate variations in method parameters. Robustness of the method was verified by altering the chromatographic conditions like detection wavelength and flow rate.

**Procedure:**
Small changes in the operational conditions were allowed and the extent to which the method was robust was estimated. Deviations of ±2nm in the detection wavelength and ±0.2 ml/min in the flow rate were tried individually, and percentage Relative standard deviation was calculated statistically. The results were reported in the Tables 5.7-5.8. Chromatograms represent the robustness (variation in parameters) were shown in the Figures 5.15-5.18.

Solutions of 100% test concentration with the specified changes in the operational conditions were injected to the instrument in triplicate and the % RSD of the chromatographic peak area was separately for each variable.

**Table 5.7: Robustness data of change in flow rate**

<table>
<thead>
<tr>
<th>Flow rate</th>
<th>Peak area</th>
<th>Mean</th>
<th>SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug</td>
<td>IS</td>
<td>Drug</td>
<td>IS</td>
</tr>
<tr>
<td>0.6ml/min</td>
<td>32649</td>
<td>3</td>
<td>11654</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>32548</td>
<td>7</td>
<td>32548</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>32757</td>
<td>3</td>
<td>11436</td>
<td>11436</td>
</tr>
</tbody>
</table>
Table 5.8: Robustness data of change in wavelength

<table>
<thead>
<tr>
<th>Wave length (nm)</th>
<th>Peak area</th>
<th>Mean</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug IS</td>
<td>Drug IS</td>
<td>Drug IS</td>
<td></td>
</tr>
<tr>
<td>294</td>
<td>326493 116565</td>
<td>326517.66</td>
<td>1043.21</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>325487 115471</td>
<td>115471</td>
<td>1088.39</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>327573 114367</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>298</td>
<td>97471 271455</td>
<td>97456.66</td>
<td>99.27</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>97351 270537</td>
<td>271524</td>
<td>1023.76</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>97548 272581</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.17: Chromatogram showing variation in wavelength – 294nm

Figure 5.18: Chromatogram showing variation in wavelength – 298nm

5.5.7 Limit of Detection (LOD):

The main purpose of this study is to evaluate the sensitivity of the proposed method. Detection limit was determined based on the standard deviation of the response and slope. The detection limit may be expressed as $3.3\sigma/s$, where $\sigma$ is the standard deviation (SD) of the response, $s$ is the slope of the calibration curve.

5.5.8 Limit of Quantitation (LOQ):

A specific calibration curve should be studied using samples, containing an analyte in the range of quantitation limits. The residual SD of a regression line or the SD of y-intercepts or regression lines may be used as SD. LOQ may be expressed as $10\sigma/s$. Results of LOD and LOQ were reported in Table 5.9.
5.5.9 Assay of Marketed Formulation of Telmisartan

**Purpose:**
The purpose is to estimate the amount of Telmisartan present in pharmaceutical formulations

**Procedure**
25µl of 50µg/ml sample solution of Telmisartan was injected into the chromatographic system, the chromatogram was recorded and peak area was measured. The assay values were calculated using the regression equation.

The result was reported in the Table 5.10. Chromatogram representing the assay was shown in the Figure 5.19.

### Table 5.10: Assay Data of Telmisartan Marketed Formulation

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Label claim</th>
<th>Amount recovered</th>
<th>% Amount found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telmisartan 20</td>
<td>20mg</td>
<td>19.84</td>
<td>99.2</td>
</tr>
</tbody>
</table>

**Figure 5.19: Chromatogram showing Assay of TSN**

5.6 RESULTS AND DISCUSSION:

A RP-HPLC method has been developed and validated by the author for estimation of Telmisartan using Eprosartan Mesylate as internal standard. The author has developed this method based on the use of Phenomenex C_{18} column and mobile phase.
composition of methanol and phosphate buffer, pH 5.8 in ratio 80:20 v/v. The method was validated over a linear concentration range of 1–11µg/ml and with a correlation coefficient of \( R^2 \) - 0.998 for Telmisartan. The %RSD of the peak response of six replicate injections of standard concentration was found to be below 2, indicating that the proposed method is precise. The percentage recoveries of API from dosage forms ranged from 99.5 to 101.4% w/w for Telmisartan and IS, indicating that the proposed method to be accurate. The LOD & LOQ values were found to 0.5µg/ml and 0.8µg/ml for Telmisartan indicating the sensitivity of the method. Thus the results of analysis of pharmaceutical formulations reveal that the proposed method is suitable for their analysis with virtually no interference of the usual additives presented in pharmaceutical formulations. The proposed method (RP-HPLC) is simple, sensitive and reliable and can be used for the routine determination of Telmisartan in bulk samples and pharmaceutical formulations depending upon the need of the specific and arising situation.

5.7 CONCLUSION
A simple, specific, accurate and precise reverse phase high performance liquid chromatography method has been developed which can be used for quantitative estimation of Telmisartan for routine analysis of individual and combination of drugs. Method was validated as per ICH Q2 (R1) so it can be used by pharmaceutical industries.
6 RP-HPLC METHOD FOR THE DEVELOPMENT AND VALIDATION OF VALSARTAN

Valsartan is used to treat high blood pressure (hypertension) and heart failure. It is also used to improve the chance of living longer after a heart attack lowering high blood pressure helps to prevent strokes, heart attacks and kidney problems.

6.1 Drug Profile: \(^{130-32}\)

Molecular structure

![Figure 6.1: Structure of Valsartan (VST)](image)

**Chemical Name:** N-(1-oxopentyl)-N-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]–4 yl]methyl] –L-valine.

**Molecular Formula:** C\(_{24}\)H\(_{29}\)N\(_{5}\)O\(_{3}\)

**Molecular Weight:** 435.52 gm/mol.

**Category:** Antihypertensive agent, Angiotensin II receptors antagonist (ARB).

**Solubility:** Soluble in methyl acetate, n-butyl acetate, acetonitrile, N,N- dimethyl formamide, dichloromethane, chloroform, alcohol and poorly soluble in water.

**Physical state:** White to off-white crystalline powder.

**Half-life:** 6 hours.

**Absolute bioavailability:** About 25% (range 10%- 35%).

**Melting point:** 116-117°C

**Mechanism of Action:**

Angiotensin II is formed from angiotensin I in a reaction catalyzed by angiotensin-converting enzyme (ACE, kinase II). Angiotensin II is the principal precursor agent of the renin-angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation, and renal reabsorption of sodium. Valsartan blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1
receptor in many tissues, such as vascular smooth muscle and the adrenal gland. Its
action is therefore independent of the pathways for angiotensin II synthesis.

Blockade of the angiotensin II receptor inhibits the negative regulatory
feedback of angiotensin II on renin secretion, but the resulting increased plasma renin
activity and angiotensin II circulating levels do not overcome the effect of Valsartan
on blood pressure.

**Therapeutic uses:**
Valsartan is used to treat high blood pressure (hypertension) and heart failure. It is
also used to improve the chance of living longer after a heart attack lowering high
blood pressure helps to prevent strokes, heart attacks, and kidney problems. This drug
may also be used to protect the kidneys from damage due to diabetes.

**Dose:**

**Table 6.1: Dosage of Valsartan**

<table>
<thead>
<tr>
<th>Indication</th>
<th>Starting dose</th>
<th>Dose range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult hypertension</td>
<td>80 or 160 mg</td>
<td>80 - 300 mg once daily</td>
</tr>
<tr>
<td>Pediatric hypertension (6-16 yrs)</td>
<td>1.3 mg/kg once daily (upto 40 mg)</td>
<td>1.3 - 2.7 mg/kg once daily (upto 40-160 mg)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>40 mg twice daily</td>
<td>40-160 mg twice daily</td>
</tr>
<tr>
<td>Post-myocardial infarction</td>
<td>20 mg twice daily</td>
<td>20-160 mg twice daily</td>
</tr>
</tbody>
</table>

**Pharmacodynamics:**
Valsartan belongs to a class of antihypertensive agents called angiotensin II receptor
blockers (ARBs). Valsartan is a specific and selective type-1 angiotensin II receptor
(AT1) antagonist which blocks the blood pressure increasing effects angiotensin II via
the renin-angiotensin-aldosterone system (RAAS). RAAS is a homeostatic
mechanism for regulating hemodynamics, water and electrolyte balance. During
sympathetic stimulation or when renal blood pressure or blood flow is reduced, renin
is released from granular cells of the juxtaglomerular apparatus in the kidneys. Renin
cleaves circulating angiotensinogen to angiotensin I, which is cleaved by angiotensin
converting enzyme (ACE) to angiotensin II. Angiotensin II increases blood pressure
by increasing total peripheral resistance, increasing sodium and water reabsorption in
the kidneys via aldosterone secretion and altering cardiovascular structure.
**Pharmacokinetics:**

**Absorption:**

Peak Plasma Concentration was reached in 2 to 4 hours after dosing. Valsartan shows biexponential decay kinetics following intravenous administration, with an average elimination half-life of about 6 hours. Absolute bioavailability is about 25% (range 10%- 35%). Protein binding was found to be 94 - 97% bound to serum proteins, primarily serum albumin. Food decreases the exposure (as measured by AUC) to Valsartan by about 40% and peak plasma concentration (\(\lambda_{\text{max}}\)) by about 50%. AUC and \(\lambda_{\text{max}}\) values of Valsartan increase approximately linearly with increasing dose over the clinical dosing range. Valsartan does not accumulate appreciably in plasma following repeated administration.

**Distribution:**

The steady state volume of distribution of Valsartan after intravenous administration is small (17 L), indicating that Valsartan does not distribute into tissues extensively. Valsartan is highly bound to serum proteins (95%), mainly serum albumin.

**Metabolism:**

Valsartan, when administered as an oral solution, is primarily recovered in feces (about 83% of dose) and urine (about 13% of dose). The recovery is mainly as unchanged drug, only about 20% of dose recovered as metabolites. The primary metabolite, accounting for about 9% of dose, is valeryl 4-hydroxy Valsartan. The enzyme(s) responsible for Valsartan metabolism have not been identified but do not seem to be CYP 450 isoenzymes.

**Elimination:**

Following intravenous administration, plasma clearance of Valsartan is about 2 L/h and its renal clearance is 0.62 L/h (about 30% of total clearance).

**Over dosage:**

The most likely manifestations of over dosage hypotension and tachycardia; bradycardia could occur from parasympathetic (vagal) stimulation. If symptomatic hypotension should occur, supportive treatment should be instituted.

**Adverse Reactions:**

Adverse effects have been reported most frequently with Valsartan include nausea, diarrhoea, headache, dizziness, dose-related orthostatic hypotension, rash, angioedema, hyperkalemia, myalgia, respiratory tract disorders, back pain, GI
disturbances, fatigue, increase in BUN and serum creatinine, abdominal pain, dry
cough, LFT elevations.

**Potentially Fatal:**
Blood dyscrasias (e.g. neutropenia).

**Drug interactions:**
Increased risk of renal impairment and hyperkalemia with NSAIDs and
cyclosporine. Increased risk of hypotension with general anaesthetics, clozapine,
dopamine agonists and other antihypertensives. Increased risk of lithium toxicity.

**Contraindications:**
Hypersensitivity, severe hepatic impairment, cirrhosis or biliary obstruction; primary
hyperaldosteronism. Pregnancy (2\textsuperscript{nd} and 3\textsuperscript{rd} trimesters) and lactation.

**Administration:**
May be taken with or without food.

**Storage:**
Should be stored at room temperature, 15-30°C (59-86°F).