<table>
<thead>
<tr>
<th>Title</th>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Review of work done on cilnidipine and telmisartan</td>
<td>33</td>
</tr>
<tr>
<td>2.2</td>
<td>Review of work done on azelnidipine and olmesartan</td>
<td>40</td>
</tr>
<tr>
<td>2.3</td>
<td>Review of work done on ambrisentan and tadalafil</td>
<td>50</td>
</tr>
<tr>
<td>2.4</td>
<td>References</td>
<td>55</td>
</tr>
</tbody>
</table>
2.1 Review of work done on cilnidipine and telmisartan

2.1.1 Review of work done on cilnidipine

Tan Shuzhen et al.,\(^1\) prepared a novel fluorescent probe for the determination of cilnidipine via an emulsion polymerization technique, using Triton-X 100 as an emulsifier, EDMA as a cross-linker, butyl methacrylate as a monomer, and potassium persulfate as an initiator. 1-Naphthaleneboronic acid was encapsulated; the resultant particles were used as a fluorescence probe for cilnidipine assay based on fluorescence quenching. The sensitivity of 1-naphthaleneboronic acid encapsulated probe to cilnidipine was largely improved in comparison with that of free 1-naphthaleneboronic acid. The probe showed a linear response toward cilnidipine over the concentration range of 2.0×10\(^{-7}\) to 1.1×10\(^{-5}\) mol/l with high sensitivity, fast response time, and good selectivity.

Zhang A Xianhua et al.,\(^2\) established HPLC–MS/MS for the determination of cilnidipine, in human plasma. The reversed-phase chromatographic system was interfaced with a turbo ion spray source. Nimodipine was employed as the IS. Sample extracts following protein precipitation were injected into the HPLC–MS/MS system. The analyte and IS were eluted isocratically on a C\(_{18}\) column, with a mobile phase consisting of methanol and ammonium acetate (96:4, v/v). Quantification was performed using multiple reaction monitoring of the transitions \(m/z\) 491.2→122.1 and \(m/z\) 417.1→122.1 for cilnidipine and for the IS, respectively. The analysis time for each run was 3.0 min. The calibration curve fitted well over the concentration range of 0.1–10 ng/mL. The recoveries were between 92.71% and 97.64%. The long-term stability and freeze-thaw stability were satisfying at each level. The present method provides a modern, rapid and robust tool for pharmacokinetic studies of cilnidipine.

Lee Heon-Woo et al.,\(^3\) worked on a simple method using a one-step liquid–liquid extraction with methyl-\(t\)-butyl ether followed by HPLC with ESI-MS/MS detection was developed for the determination of cilnidipine in human plasma using benidipine as an IS. Acquisition was performed in multiple reactions monitoring mode, by monitoring the transitions: \(m/z\) 491.1 > 121.8 for cilnidipine and \(m/z\) 504.2 > 122.1 for IS, respectively. Analytes were chromatographed on a CN column by isocratic elution using 10mM ammonium acetate buffer-methanol (30:70, v/v; adjusted with acetic acid to pH 5.0). Results were linear over the studied range (0.1–20 ng/mL) with a total LC–MS/MS analysis time per run of 3 min. The developed method was validated and
successfully applied to a cilnidipine bioequivalence study in 24 healthy male volunteers.

**He Ling-yun et al.**, developed and validated RP-HPLC method for the determination of cilnidipine and its related substances. The separation was performed on a Hypersil C18 column (5 μm, 4.6 mm×250 mm). The mobile phase was composed of acetonitrile-water (70:30, v/v), and the flow rate was 1.0 mL/min. The detection was carried out with UV detector at 240 nm. The calibration curves were linear within 0.8-200.0 μg/mL.

**Wang Shi-gang et al.**, established and validated RP-HPLC method for the determination of cilnidipine related substances. The separation was performed on octadecylsilane chemically boned silica column. The mobile phase was composed of acetonitrile:0.025 mol/l ammonium dihydrogen phosphate solution: cyclohexane (60:39:01, v/v/v) with 1.5 mL/min flow rate and 240 nm detection wavelength. Through intensity destructive test under this detection, main peak and impurity peak were well separated, with not being disturbed. Specificity calibration curves were linear within 1–16 mg/l.

**Karmalkar HS et al.**, worked on HPTLC method validated for the determination of cilnidipine from pharmaceutical formulation using nifedipine as an IS. The analyte and IS were resolved on silica gel 60F254 HPTLC plate by using a two component mobile phase system comprising toluene-ethyl acetate (6.0:4.0, v/v) with a chamber saturation of 8 min. The plate was developed up to 8cm and air-dried. The plate then was scanned and quantified at 238nm. The linear dynamic range of cilnidipine was found to be 5µg/mL to 45µg/mL. The LOD and LOQ for cilnidipine was found to be 3µg/mL and 5µg/mL respectively.

### 2.1.2 Review of work done on telmisartan drug

**Li PF et al.**, described Q-trap TM LC–MS/MS method for the determination of telmisartan, in human plasma using IS, diphenhydramine that requires only 50µL of plasma and allows high sample throughput due to a simple sample preparation procedure and short run time. Samples were extracted from plasma using diethyl ether–dichloromethane (60:40, v/v), and separated on a Zorbax extend C18 column using methanol–10mM ammonium acetate (85:15, v/v) adjusted to pH 4.5 after mixing with formic acid as mobile phase. The assay was linear over the range 0.5–600.0 ng/mL with a LOQ of 0.5 ng/mL and a LOD of 0.05 ng/mL. Intra- and inter-
day precision were <6.7% and <8.1%, respectively, and the accuracy was in the range 88.9–111.0%.

**Karst Uwe et al.**, presented LC/APCI-MS/MS with on-line sample clean-up and compare this method to previous method. The comparison of method is as follows. LC/MS/MS method provides a linear calibration curve, while the immunoassay calibration typically results in a sigmoidal function. The ratio between concentrations obtained by ELISA and LC/MS (internal or external calibration) were calculated for any of the individual samples. By comparing these results, it can be observed that the deviation between ELISA and LC/MS with internal calibration is usually higher than for ELISA and LC/MS with external calibration.

**Zhu XY et al.**, described a rapid and sensitive HPLC-ESI–MS/MS detection for the simultaneous determination of multiple angiotensin type 1 receptor antagonist WX472, WX581, 1b and telmisartan in rat plasma for the purpose of high-throughput pharmacokinetic screening. The method was operated under selected reaction monitoring mode in the positive ion mode. This method has been successfully applied to the high-throughput pharmacokinetic screening study for both cassette dosing and cassette analysis of four compounds to rats. The study suggested that cassette analysis of pooled samples would be a better choice for the high-throughput pharmacokinetic screening of angiotensin type 1 receptor antagonists. In spite of the complex matrix, acceptable values of precision and accuracy were obtained by this method. Although the high-throughput method of cassette analysis needed more rats, it could avoid the drug–drug interactions and get the more exact pharmacokinetic data. Therefore, cassette analysis was suitable for the future research of in vitro assay such as Caco-2 permeability, metabolic stability, protein stability and so on.

**Yana T et al.**, developed and applied LC–MS/MS method for pharmacokinetic study of telmisartan and hydrochlorothiazide in human plasma. Sample preparation involved liquid–liquid extraction with diethyl ether–dichloromethane (60:40, v/v). The analytes and IS, probenecid were separated on a Venusil XBP-C8 column using gradient elution with acetonitrile–10mM ammonium acetate–formic acid at a flow rate of 1.2 mL/min. Detection was by electro spray negative ionization mass spectrometry using multiple reaction monitoring.

**Singh Saranji et al.**, reported specific SIAM HPLC-MS method under ICH prescribed conditions of hydrolysis (acidic, neutral and basic), photolysis, oxidation and thermal stress. The drug showed labiality under only photo-acidic condition by
forming a single degradation product. HPLC separation of the drug and the
degradation product was achieved on C₈ column using gradient method.
Subsequently, the degradation product peak was subjected to LC–MS/TOF and on-
line H/D exchange mass studies. Based on these studies, a tentative structure was
assigned to the product as 3-((1,7-dimethyl-2-propyl- 1H,3H-2,5-bibeno(d)imidazol-
3-yl)methyl)-6H-benzo(c)chromen-6-one, which was verified through 1H LC–NMR
experiments.

Jain RR et al.,¹² introduced and validated rapid and sensitive liquid chromatography
tandem mass spectrometry method has been for the simultaneous determination of
ramipril, ramiprilat and telmisartan in human plasma. The solid-phase extraction
technique was used for the extraction of ramipril, ramiprilat and telmisartan from
human plasma. trandolaprilat and hydrochlorothiazide were used as the IS.
Chromatography was performed on a Hypurity C₁₈, 5µm, 50mm×4.6mm column,
with the mobile phase consisting of ammonium acetate and acetonitrile (20:80, v/v),
followed by detection using mass spectrometry. The method involves a simple
reversed isocratic chromatography condition and mass spectrometry detection, which
enables detection at sub-nanogram levels. The method was validated and the LLOQ
for ramipril, ramiprilat and telmisartan was found to be 0.1ng/mL, 0.1ng/mL and 2
ng/mL, respectively. The mean recovery for ramipril, ramiprilat and telmisartan
ranged from 90.1 to 104.1%. This method increased the sensitivity and selectivity;
resulting in high-throughput analysis of ramipril, ramiprilat and telmisartan using two
different ISs in a single experiment for bioequivalence studies, with a
chromatographic run time of 1.5 min only.

Torrealday N et al.,¹³ described HPLC with fluorimetric detection by applying
statistical analysis using central composite design. Chemometric approach allowed us
to reduce the number of experiments needed for chromatographic optimization, as
well as the attainment of a true optimum set of conditions. Chromatographic variables
were optimized by means of experimental design. A clean-up step was used for urine
samples consisting of a solid-phase extraction procedure with C₈ cartridges and
methanol as eluent. This procedure is very simple, effective and provided no
interference peaks for endogenous components, so method was proved to be selective.
Nie J et al.,¹⁴ worked on in-tube SPME coupled to HPLC method using a
(methacrylic acid-ethylene glycol dimethacrylate) polymer monolithic capillary as the
extraction medium for the direct determination of ARA-IIs (candesartan, losartan,
irbesartan, valsartan, telmisartan) in urine and plasma samples. Under the optimized extraction condition, the protein component of the biological sample was flushed through the monolithic capillary, while the analytes were successfully trapped. In comparison with existing extraction procedures for the determination of the ARA-IIs in the biological samples, the method established in the present study is simple in preparation procedure, and was relatively easy and accurate with also characters of on-line analysis.

**Ferreiros N et al.,** 15 described SPE-HPLC method coupled to photodiode array detection in human urine matrix, in order to monitor four antihypertensive angiotensin II receptor antagonist ARA-IIs drugs using experimental design. No interferences from other endogenous compounds or co-administered drugs were found.

**Nie J et al.,** 16 established simple HPLC method by using poly (methacrylic acid-ethylene glycol dimethacrylate) monolithic capillary. An advantage of said method was that it was possible direct and on-line extraction of telmisartan from rat tissue (heart, kidney, and liver) homogenates. The determination of telmisartan in treated rat tissues was achieved by using the proposed method.

**Brunetto DM et al.,** 17 developed column-switching HPLC for losartan, telmisartan, and valsartan in human urine. The use of a dual column LC system with restricted access pre column packing allows efficient and rapid clean-up of urine sample, thus reducing the concentration of interferences and minimizing matrix-induced effects. The overlap of sample cleanup, analysis and recondition of the pre column increases the sample throughput to 12 samples/h, increasing the usefulness of the method for routine analysis. It allows measuring the analytes in 20µl of human urine sample. The method permits simultaneous trace analysis of drugs, in less than 4min., which is a significant improvement in comparison to the methods reported previously.

**Rao Nageswara et al.,** 18 established LC method for related substances of telmisartan relate to a novel synthetic route and different from those A-H impurities reported by European Pharmacopeia. The modified synthetic process of telmisartan followed in the present investigation. It could be seen from that there are 8 steps and 7 intermediates are produced during the process. Thus the 7 intermediates except starting material were considered for method development. This developed method is suitable for quality assurance of telmisartan during synthesis.

**Prabhu Chitra et al.,** 19 developed a stability indicating HPTLC for telmisartan. This method is suitable for quantification for telmisartan in presence of degradation
product. Establishment of a stability indicating assay is mandatory for study of degradation pathways of drug.

**Patil UP et al.,** developed a simple, specific, accurate and precise HPTLC for analysis of telmisartan and atorvastatin calcium in fixed dose combination has been developed. The method uses aluminium plates coated with silica gel 60 F254 as stationary phase and toluene: methanol (7: 3, v/v) as mobile phase. Densitometric evaluation of the separated bands was performed at 280 nm. The two drugs were satisfactorily resolved with Rf values 0.50 ± 0.01 and 0.29 ± 0.00 for telmisartan and atorvastatin calcium, respectively. The respective calibration plots were found to be linear over the range 200-1000 and 200-700 ng/band for telmisartan and atorvastatin calcium, respectively. This method was successfully validated and applied for the analysis of drugs in pharmaceutical formulation.

**Sivasubramanian S et al.,** established HPTLC method for the quantitative determination of telmisartan and ramipril in combination using pre coated with silica gel 60F254 on aluminium sheets, toluene: acetonitrile: formic acid: water (5:5:0.3:1, v/v/v/v) as a mobile phase. Densiometric analysis of both the drugs was carried out in the absorbance mode at 212 nm. Both the drugs were subjected to acid-alkali hydrolysis, oxidation and photolytic degradation and both of them were found to be susceptible to acid-alkali hydrolysis, oxidation and photolytic degradation. Linearity of telmisartan was found to be within the range of 500-2500 ng/spot and for ramipril the range was found to be 250-1250 ng/spot, with significant high values of correlation coefficient for both the drugs.

**Palled MS et al.,** reported difference spectrophotometric method for estimation of telmisartan in bulk drug and in pharmaceutical formulations. Telmisartan exists in two different forms in acidic and basic mediums that differ in their UV spectra. Difference spectrum, obtained by keeping telmisartan in 0.01 N NaOH in reference cell and telmisartan in 0.01 N HNO$_3$ in sample cell, showed two characteristic peaks at 295 nm and 327 nm with positive and negative absorbance respectively. Difference of absorbance between these two maxima was calculated to find out the amplitude, which was plotted against concentration. The method was found to be linear in the range of 2-12 μg/mL.

**Pandey Ajit et al.,** developed and validated UV spectrophotometric method for the estimation of telmisartan in bulk and tablet dosage form. The zero order spectra of
telmisartan in 0.1N NaOH shows \( \lambda_{\text{max}} \) at 234.0 nm and estimation was carried out by \( A(1\% \ 1\text{cm}) \) and by comparison with standard. Calibration graph was found to be linear over the concentration range of 4-24 μg/mL.

2.1.3 Review of work done on cilnidipine and telmisartan combination

Deshpande Padmanabh et al., developed and validated HPTLC method for simultaneous determination of cilnidipine and telmisartan in combined tablet dosage form. The method uses aluminium plates coated with silica gel 60 F254 as stationary phase and toluene: methanol: ethyl acetate (7: 2:1 v/v/v) as mobile phase and detection was carried out at 260 nm. Result was linear in the range of 200-1200 ng/band for cilnidipine and 800-4800 ng/band for telmisartan. The recovery of method was in the range of 98-102 %. So this method was sensitive, selective and successfully applied for routine analysis.
2.2 Review of work done on azelnidipine and olmesartan

2.2.1 Review of work done on azelnidipine

Kawabata Kiyoshi et al.,\textsuperscript{25} developed and validated LC/ESI-MS/MS method by a single step liquid–liquid extraction using a mixture of ethyl acetate and hexane (1:1, v/v), for the simultaneous determination of azelnidipine and its two metabolites, M-1 (aromatized form) and M-2 (hydroxylated form), in human plasma. Three analytes were separated by isocratic elution on a C\textsubscript{18} column, and ionized using a positive ion electrospray ionization source. The ion transitions were monitored in selected reaction monitoring mode. The chromatographic run time was 11 min per injection, with retention time of 3.6, 10.2 and 6.8 min for azelnidipine, M-1 and M-2, respectively. The calibration curves for azelnidipine, M-1 and M-2 well fitted to equations by a weighted quadratic regression over the range of 0.5–40.0 ng/mL. The overall recoveries for azelnidipine, M-1 and M-2 were 68.8–78.6%, 54.3–62.9% and 80.4–89.7%, respectively. Furthermore, the method developed was successfully applied to pharmacokinetic studies on azelnidipine, M-1 and M-2 after an oral dose of 16 mg CALBLOCK® tablets (2 mgx8mg tablets) to healthy volunteers.

Kawabata Kiyoshi et al.,\textsuperscript{26} developed positive ion atmospheric pressure chemical ionization tandem mass spectrometry method by a solid phase extraction in a 96-well plate format, for the simultaneous determination of the enantiomers of azelnidipine, (R)-(−)-azelnidipine and (S)-(+) azelnidipine in human plasma. The azelnidipine enantiomers were separated on a chiral column under isocratic mobile phase conditions. The standard curve was linear over the studied range 0.05–20 ng/mL with run time of 5.0 min/injection. The intra- and inter-assay precision, calculated from the assay data of the quality control samples, was 1.2–8.2% and 2.4–5.8% for (R)-(−)-azelnidipine and (S)-(+) azelnidipine, respectively. The accuracy was 101.2–117.0% for (R)-(−)-azelnidipine and 100.0–107.0% for (S)-(+) azelnidipine. The overall recoveries for (R)-(−)-azelnidipine and (S)-(+) azelnidipine were 71.4–79.7% and 71.7–84.2%, respectively. The LLOQ for both enantiomers was 0.05ng/mL using 1.0mL of plasma. Furthermore, the method described above was used to separately measure the concentrations of the azelnidipine enantiomers in plasma samples collected from healthy subjects who had received a single oral dose of 16 mg of azelnidipine.
Ding L et al.,\textsuperscript{27} established and validated LC–ESI-MS method for the determination of azelnidipine in human plasma. Nicardipine was used as the IS. After adjustment to a basic pH with sodium hydroxide solution (0.1 M), plasma samples were extracted with cyclohexane–diethyl ether (1:1, v/v) and separated on a C\textsubscript{18} column with a mobile phase of 20mM ammonium acetate solution–methanol–formic acid (25:75:0.5, v/v/v). The electrospray ionization was employed in a single quadrupole mass spectrometer for the determination. The method was linear over the concentration range of 0.05–40 ng/mL. The LLOQ was 0.05 ng/mL. The intra- and inter-run standard deviations were less than 9.5% and 11.0%, respectively. The method was successfully applied to study the pharmacokinetics of azelnidipine in healthy Chinese volunteers.

Zou Jian-Jun et al.,\textsuperscript{28} applied a simple, rapid and sensitive HPLC-ESI-MS assay for determination of azelnidipine in human plasma using Perospirone as the IS was established. After adjustment to a basic pH with sodium hydroxide solution, plasma samples were extracted with diethyl ether and separated on a C\textsubscript{18} column with a mobile phase of methanol-5 mM ammonium acetate solution (90:10, v/v). The LLOQ was 0.20 ng/mL.

Jing J et al.,\textsuperscript{29} established HPLC-MS-MS method for determination of azelnidipine in human plasma and to study its pharmacokinetics. HPLC-MS-MS system consisted of SIL-H\textsuperscript{Tc} automatic HPLC instrument, Shiseido Capcell Pak C\textsubscript{18} column (100 mm×4.6 mm,5 μm),and API 3000 triple quadrupole tandem mass spectrometer, The mobile phase was methanol-water-formic acid (80:20:0.1),the flow rate was 0.3 mL/min. The plasma samples were extracted by liquid-liquid extraction with ether, the analyses were detected by the multiple reaction monitoring modes, Venlafaxine were used as IS. After that, pharmacokinetic study of azelnidipine was done after single and multiple administration of 8 mg and 16 mg azelnidipine tablets. The linear range was 25-40 μg/l. The LOD was 25 μg/l. The methodology recovery was 96.9%-102.5%, extraction recovery is 55.0%-63.4%, intra-and inter-assay precision is 2.71%-3.36% and 0.80%-2.87%. It is success to be used on pharmacokinetics study of azelnidipine and achieves good results.

Pan Ying Feng et al.,\textsuperscript{30} described to establish an HPLC method for determination of content in azelnidipine Tablets. The HPLC system consisted of a Diamonsil C\textsubscript{18} column(250mm×4.6mm,5μm).The mobile phase was phosphate buffer(0.005 mol/l disodium dihydrogen phosphate,0.004 mol/l disodium hydrogen phosphate:
acetonitrile (30:70, v/v). The flow rate was 1.0 mL/min. The detection wavelength was 256nm. The linear range of azelnidipine was 2.50–40.0μg/mL. It can be used for quality control of content in azelnidipine tablets.

**An Hua Min et al.,**[^31] established an HPLC method for determination of content and related substances in azelnidipine tablets and capsules. The HPLC system consisted of a Diamonsil (TM) C\textsubscript{18} column (250 mm×4.6 mm, 5 μm), a mixture of acetonitrile and solution of ammonium dihydrogen phosphate (10 mmol/l, adjusting pH to 6.5 with triethylamine) (82:18) as mobile phase; the flow rate was 1.0 mmol/l. The detection wavelength was set at 254 nm. The column temperature was 30°C. Azelnidipine could be completely separated from other impurities. The linear range of azelnidipine was 16.84–336.80 μg/mL. The method is simple, accurate. It is suitable for determination of content and related substances in azelnidipine tablets and capsules.

### 2.2.2 Review of work done on olmesartan drug

**Vaidya Vikas et al.,**[^32] reported LC-MS-MS method in human plasma for olmesartan using zidovudine as IS. Sample preparation involved a simple solid-phase extraction procedure. Chromatography was performed isocratically on a 5 μm C\textsubscript{18} analytical column (50 mm×4.6 mm i.d.) with water–acetonitrile–formic acid (20:80:0.1, v/v/v) as mobile phase. The response to olmesartan was a linear function of concentration over the range 4.82–1,928 ng/mL. The LLOQ in plasma was 4.82 ng/mL. The method was successfully applied in a bioequivalence study of an olmesartan formulation after administration as a single oral dose.

**Patil Kiran et al.,**[^33] developed SIAM LC method for estimation of olmesartan and ramipril in presence of degradation product. A chromatographic separation was achieved with YMC Pack ODS A (250 x 4.6 mm) analytical column. The mobile phase composed of buffer, acetonitrile and methanol (50:40:10, v/v/v). The buffer used in the mobile phase is 0.1M sodium perchlorate monohydrate in double distilled water and pH adjusted 3.0 with trifluoroacetic acid. The instrumental settings are flow rate of 1.0 mL/min, column oven temperature at 30°C and detector wavelength of 210 nm using a photodiode array detector. olmesartan, ramipril and combination drug product were exposed to thermal, light, hydrolytic and oxidative stress conditions, and the stressed samples were analysed by the proposed method. The proposed method was found to be suitable and accurate for quantitative determination and stability study of olmesartan and Ramipril in pharmaceutical preparations.
Patil Pournima et al.,\textsuperscript{34} described one spectrophotometric method for determination of olmesartan medoxomil and amlodipine besylate in formulation by multi wavelength method. For olmesartan, the interference due to amlodipine was eliminated by absorbance difference at 265 and 324 nm whereas quantification of Amlodipine 360 nm. The system obeyed Beers law over the concentration of 2 to 32 μg/mL for olmesartan and 2 to 20 μg/mL for Amlodipine. The recoveries from 97.99 to 101.23 % and 98.46 to 102.31 % for olmesartan and amlodipine which do not differ from 100% showed that there was no interference from common excipients indicates accuracy and reliability of the method. LOD for olmesartan and amlodipine was found to be 0.0116 μg/mL and 0.049 μg/mL. LOQ was found to be 0.0335 μg/mL and 0.093 μg/mL Standard deviation for tablet analysis by using methanol was ranging from 99.1 to 101.9 % for olmesatan and 98.8 to 101.98 % for amlodipine which proves the ability of the method remains unaffected by small but deliberate changes in the conditions of analysis.

Hamrapurkar Purnima et al.,\textsuperscript{35} established SIAM HPLC method for olmesartan drug and formulation with degrading impurities. The drug was subjected to stress condition of hydrolysis, oxidation, photolysis, thermal degradation. Extensive degradation was found in acid medium and alkaline medium. Minimum degradation was found in thermal degradation while there was no degradation found in photolytic condition. Successful separation of a drug from degradation product formed under stress condition was achieved on C18 column using methanol: water (60:40, v/v), pH 3.75 adjusted with 10mM o-phosphoric acid mobile phase. Flow rate was 1 mL/ min and the detector was set at wavelength of 270 nm. Stability indicating provides the evidence of quality during storage condition.

Raveendra Ganduri et al.,\textsuperscript{36} worked on simple HPLC to assay olmesartan medoxomil in tablet dosage form. The HPLC analysis used a reversed phase Hypersil BDS C 8 (250X4.6mm, 5μm) column and a mobile phase constituted of buffer and acetonitrile (55:45, v/v). The buffer is composed of 3 g of sodium perchlorate and 3 mL of tri ethyl amine in 1000 mL of water and the pH of the solution was adjusted to 3.0 with orthophosphoric acid. The wave length of the detection is 250 nm. The method is linear from10 μg/mL to120 μg/mL. The accuracy of the method was found to be 99.54%. The proposed method provided an accurate and precise analysis of olmesartan in its pharmaceutical dosage form.
Sharma Ritesh et al.,\textsuperscript{37} published a simple, sensitive and precise RP-HPLC-DAD method for the determination of olmesartan medoxomil in the presence of its degradation products. Olmesartan medoxomil and all the degradation products were resolved on a C18 column with the mobile phase composed of methanol, acetonitrile and water (60:15:25, v/v/v, pH 3.5 by o-phosphoric acid) at 260 nm using a photodiode array detector. The method was linear over the concentration range of 1-18 μg/mL. Excellent recoveries of 99.3 ± 0.9 to 100.8 ± 1.2% proved the accuracy of the method. Developed method was specific, as indicated by chromatographic resolution > 2.0 for each peak and sensitive with LOD 0.03 μg/mL and LOQ 0.1 μg/mL. Four degradation products (DP-I, II, III, IV) were formed during the degradation study in 0.1 mol/l HCl whereas only DP-I, II and III were formed in water, 0.01 mol/l NaOH and 3% H2O2. No significant thermal or photolytic degradation was observed in solid drug. The method was applied successfully for the assay of olmesartan medoxomil in the tablet dosage form.

Dubey Nitin et al.,\textsuperscript{38} developed one RP- HPLC method for the simultaneous estimation of olmesartan medoxomil, amlodipine besilate, and hydrochlorothiazide in commercially available tablet formulations. The chromatographic separation was achieved on instrument Shimadzu LC 10 AT VP, Japan, equipped with photodiode array detector SPD-10 AVP, attached with a class M 10 A software, (version 1.6) and Phenomenex Luna C8 (25 cm × 4.6 mm i.d. × 5 μg) column using acetonitrile: phosphate buffer (pH 4 ± 0.1) (40:60 % v/v) as mobile phase at a flow rate of 1.0 mL/min. Quantitation was carried out at 258, 237 and 270 nm for olmesartan medoxomil, amlodipine besilate and hydrochlorothiazide respectively. The method was validated as per ICH guidelines. The method may be applied for the routine simultaneous laboratory analysis of the above mentioned drugs in tablet dosage form.

Pai Nandini et al.,\textsuperscript{39} worked on impurity profiling of olmesartan in drug and tablet formulation using RP-HPLC method. The HPLC analysis used a reversed phase Kromasil C18 (150 x 4.6mm, 5μm) column and a mobile phase constituted of buffer and acetonitrile (60:40, v/v). The buffer was composed of 4.7 g of sodium dihydrogenorthophosphate and 1mL of triethyl amine in 1000 mL of water and the pH of the solution was adjusted to 4.0± 0.05 with ortho-phosphoric acid. The wave length of the detection was 225 nm. The method was found to be linear from2 μg/mL to 7μg/mL for olmesartan medoxomil and from 0.25μg/mL to 7μg/mL for olmesartan acid impurity. The accuracy of the method was found to be 100.73% for olmesartan
acid impurity. Inter and intraday assay RSD was less than 0.71% in drug form and 1.10% in tablet dosage form for olmesartan acid impurity. The proposed method provided an accurate and precise analysis of olmesartan acid impurity in olmesartan medoxomil drug form as well as in pharmaceutical dosage form.

Patil Pournima et al., developed and validated spectrophotometric method for quantitation of olmesartan medoxomil and amlodipine besylate in tablet dosage form. The method is based upon determination of olmesartan at 265 nm and amlodipine at 360 nm in acetonitrile: water. The system obeyed Beers law over the concentration of 2 to 32 μg/mL for olmesartan and 2 to 20 μg/mL for amlodipine. Interday and intraday studies showed repeatability of the method. Results of tablet analysis in the range of 98.15 to 100.15% and 99.67 to 100.88% for olmesartan and amlodipine, which indicate repeatability of the method. The recoveries from 101.99 to 100.25% and 99.66 to 101.15% for olmesartan and amlodipine which do not differ from 100% showed that there was no interference from common excipients indicates accuracy and reliability of the method.

Qi Wenyuan et al., established UPLC-MS-MS method for quantification of olmesartan and amlodipine in human plasma and urine simultaneously. Chromatographic separation was carried out on an Acquity UPLC BEH C18 column and mass spectrometric analysis was performed using a QTrap5500 mass spectrometer coupled with an ESI source in the positive ion mode. The linearity of this method was found to be within the concentration range of 0.2–500 ng/mL and 4–5000 ng/mL for olmesartan in human plasma and urine and 0.1–50 ng/mL and 2–1000 ng/mL for amlodipine in human plasma and urine. Only 2 min were needed for an analytical run. This assay was used to support a clinical study where multiple oral doses were administered to healthy Chinese subjects to investigate the pharmacokinetics of olmesartan and amlodipine.

Bajerski Lisiane et al., developed stability-indicating MEKC method for the analysis of olmesartan medoxomil in tablets. Successful separation was achieved using a fused silica capillary (40 cm x 50 μm i.d.); background electrolyte consisted of a combination of 10 mmol/l borate buffer and 5 mmol/l anionic detergent sodium dodecyl sulfate (95:5; v/v) pH 6.5 and column temperature 25 °C with detection at 257 nm. The proposed method, validated following ICH guidelines, was applied to the determination of this antihypertensive with good results compared with an LC method.
Amudhavalli V et al.,43 worked on HPLC method for the determination of olmesartan and Hydrochlorothiazide on a Shimadzu Class VP series HPLC system with a phenomnex C18 column (150 x 4.6 mm, 5 μ) using a mobile phase mixture containing acetonitrile and ammonium acetate buffer, pH-3.5 (55:45, v/v). The flow rate was 1.0 mL/min and effluents were monitored at 252 nm and eluted at 2.14 min and 4.62 min. Calibration curve was plotted with a range from 2.0-20 μg/mL for olmesartan and 1.0 -10 μg/mL for hydrochlorothiazide. The results were found to be satisfactory and the method can be adapted for the routine quality control of the drugs in formulations.

Patil KR et al.,44 worked on stability indicating LC method simple for the quantitative simultaneous estimation of amlodipine and olmesartan in combined pharmaceutical dosage form. A chromatographic separation of the two drugs was achieved with an ACE 5 C18 25-cm analytical column using buffer-acetonitrile (60:40, v/v). Theoretical plates for olmesartan and amlodipine were 6970 and 11,841, respectively. Tailing factor for olmesartan and amlodipine was 0.90 and 0.98, respectively. Olmesartan, amlodipine, and combination drug product were exposed to thermal, photolytic, hydrolytic, and oxidative stress conditions, and the stressed samples were analyzed by the proposed method. Peak homogeneity data of olmesartan and amlodipine is obtained by photodiode array detector in the stressed sample chromatograms, demonstrating the specificity of the method for their estimation in presence of degradation product. The described method shows excellent linearity over a range of 20-400 μg/mL for olmesartan and 5-100 μg/mL for amlodipine. The relative standard deviation for six measurements in two sets of each drug in tablets is always less than 2%. The proposed method was found to be suitable and accurate for quantitative determination and stability study of olmesartan and amlodipine in pharmaceutical preparations.

Singh Sumita et al.,45 developed spectrophotometric method for quantification of olmesartan medoxomil in pharmaceutical formulations. The absorption maximum in ethanol solvent was found to be 257.8. Linearity was obtained in the concentration range 5 to 30μg/mL for olmesartan medoxomil with a correlation coefficient of 0.9982. The precision (intra-day and inter-day) of method was found within limits. The result of the analysis was validated statistically and recovery studies confirmed the accuracy and precision of the proposed method. The developed methods were
applied successfully to the determination of this drug in various pharmaceutical formulations.

**Farouk M et al.** established isocratic RP-HPLC method for the analysis of torasemide (I), irbesartan (II) and olmesartan medoxomil (III) at ambient temperature, using Atlantis 4.6 mm x 250 mm RP-C18 Column, with a flow rate of 1.5 mL/min, and UV detector at 288 nm and 260 nm for (I) and (II and III), respectively. By adopting the mentioned chromatographic technique, (I) and (III) were determined in the presence of their acidic and alkaline-degradates separately as stability-indicating methods utilizing phosphate buffer pH 3:acetonitrile (60:40, v/v), phosphate buffer pH 3.2:acetonitrile (60:40, v/v) as a mobile phase, respectively, while (II) was determined in presence of Hydrochlorothiazide, using phosphate buffer pH 4:acetonitrile (70 :30, v/v). All the proposed methods were validated according to the ICH guidelines and successfully applied to determine the mentioned studied drugs in pure form, in laboratory prepared mixtures and in pharmaceutical preparations. The obtained results were statistically compared to the reference methods of analysis.

**Wankhede SB et al.** described two UV spectrophotometric and one RP-HPLC methods for the simultaneous estimation of amlodipine besilate and olmesartan medoxomil in tablet dosage form. First UV spectrophotometric method was a determination using the simultaneous equation method at 237.5 nm and 255.5 nm over the concentration range 10-50 μg/mL and 10-50 μg/mL, for amlodipine besilate and olmesartan medoxomil with accuracy 100.09%, and 100.22% respectively. Second UV spectrophotometric method was a determination using the area under curve method at 242.5-232.5 nm and 260.5-250.5 nm over the concentration range of 10-50 μg/mL and 10-50 μg/mL, for Amlodipine besilate and olmesartan medoxomil with accuracy 100.10%, and 100.48%, respectively. In reverse phase high performance liquid chromatography analysis carried out using 0.05M potassium dihydrogen phosphate buffer: acetonitrile (50:50 v/v) as the mobile phase and Kromasil C18 (4.6 mm i.d.×250 mm) column as the stationary phase with detection wavelength of 238 nm. Flow rate was 1.0 mL/min. Retention time for Amlodipine besilate and olmesartan medoxomil were 3.69 and 5.36 min, respectively. Linearity was obtained in the concentration range of 4-20 μg/mL and 10-50 μg/mL for amlodipine besilate and olmesartan medoxomil, respectively. Proposed methods can be used for the estimation of amlodipine besilate and olmesartan medoxomil in tablet dosage form provided all the validation parameters are met.
Kachave Raman et al.,\textsuperscript{48} established spectrophotometric methods for the simultaneous estimation of olmesartan medoxomil and hydrochlorothiazide in tablet formulation. In dual wavelength method absorbance difference at 254.8 nm and 284 nm were considered for estimation of olmesartan while hydrochlorothiazide was estimated as single component at 322 nm. In simultaneous equation method estimation of olmesartan and hydrochlorothiazide was carried out at 249 nm and 273.5 nm respectively. Regression analysis of beers plots showed good correlation in concentration range of 0-25 µg/mL for olmesartan and hydrochlorothiazide respectively. Accuracy was determined by recovery studies from tablet dosages form and ranges from 98-101%. Precision of method was find out as repeatability, day to day and analyst to analyst variation and shows the values within limit.

Patil Pournima et al.,\textsuperscript{49} developed the present work describes RP-HPLC method for simultaneous estimation of Amlodipine besylate and olmesartan medoxomil from tablet. Best resolution of two drugs was achieved with the mobile phase having composition of acetonitrile and water in the ratio 60:40, v/v. The linearity response of the HPLC system for both olmesartan and amlodipine was obtained over the range of 5-35 µg/mL. Optimum retention time with greater resolution of the two drugs and internal standard eluting within six min was achieved with a flow rate of 1 mL/min. After recording the spectra of the two drugs and internal standard, 248 nm was selected as suitable wavelength for estimation. The result of analysis showed excellent recoveries for both the drugs ranging from 99.75 % to 100.62 % for olmesartan and 98.91 % to 102.05 % for amlodipine. The results of analysis of tablet indicated that no interference due to common tablet excipients was observed with the developed method.

Prasad Chaitanya et al.,\textsuperscript{50} established RP-HPLC for estimation of olmesartan medoxomil in bulk and formulation. Selected mobile phase was a combination of phosphate buffer with pH adjusted at 2.8 and acetonitrile (35:65% v/v) and wavelength selected was 250 nm. Retention time of olmesartan medoxomil was 2.591 min. Linearity of the method was found to be 50-150 µg/mL, with the regression coefficient of 0.9993. Quantification was done by calculating area of the peak and the detection limit and quantitation limit ware 0.02 µg/mL and 0.09 µg/mL, respectively. Present method can be applied for the determination of olmesartan medoxomil in quality control of formulation without interference of the excipients.
2.2.3 Review of work done on azelnidipine and olmesartan combination

Shimada Kazuyuki et al.,\textsuperscript{51} reported the results from an a priori planned analysis and post hoc analyses of the diurnal BP and pulse rate profiles of olmesartan/azelnidipine versus monotherapy with either agent from the REZALT study. In this study in these Japanese patients with essential hypertension, combination treatment with olmesartan/azelnidipine was associated with significantly greater reductions in daytime, nighttime, and early-morning BP, as assessed using 24 hr ABPM, compared with either monotherapy, regardless of dipping pattern at baseline. A total of 867 patients were enrolled, and 862 randomized patients were included in the full analysis set (590 men, 272 women; mean age, 56.6 years). A total of 839 patients had assessable ABPM data (213, 211, 206, and 209 patients in the olmesartan/azelnidipine 10/8 mg, olmesartan/azelnidipine 20/16 mg, olmesartan, and azelnidipine groups, respectively). No clinically significant between-group differences were observed in baseline demographic and clinical characteristics. Combination therapy was associated with significantly greater antihypertensive effects on 24 hr ABPM compared with either monotherapy in all of the time periods. The antihypertensive effects associated with olmesartan/azelnidipine 10/8 mg or 20/16 mg were significantly greater than those with monotherapies regardless of dipping pattern at baseline (all, \( P < 0.05 \)) in all of the time periods, with the exception of nighttime reduction with olmesartan/azelnidipine 10/8 mg versus olmesartan in dippers.
2.3 Review of work done on ambrisentan and tadalafil

2.3.1 Review of work done on ambrisentan drug

**Kaja Ravi et al.,**\(^52\) worked on development and validation of a new and accurate chiral liquid chromatographic method for the separation of ambrisentan and its (R)-enantiomer in bulk drugs and pharmaceutical dosage forms. Normal phase chromatographic separation was performed on an immobilized cellulose based chiral stationary phase (chiralpak-ADH) with n-hexane: ethanol (85:15, v/v) as mobile phase at a flow rate of 1.0 mL/min. The resolution (Rs) between the enantiomers was greater than 3.0. The LOD and LOQ for the (R)-enantiomer were 0.03 µg and 0.1 mg respectively. The linearity of the method for the (R)-enantiomer was excellent over the range from LOQ to 0.3%. Percentage recovery of the (R)-enantiomer from bulk drug samples and pharmaceutical dosage forms ranged from 98.5-101.2% indicative of the high accuracy of the method.

**Zhou Fu-gang et al.,**\(^53\) used chiral stationary phase and developed HPLC method for the enantiomeric separation of racemic ambrisentan. Effects of isopropanol modifier concentration, temperature and flow rate with polysaccharide-based chiral column as a stationary phase were investigated. The enantiomers of ambrisentan were separated on a chiralpak AD-H column (250 mm×4.6 mm, 5 µm) with a mobile phase consisted of n-hexane-isopropanol (17.5: 82.5) at a flow rate of 0.4 mL/min. The wavelength detector was set at 263 nm. It was recommended that this sample and selected method can be used for determination and quality control of ambrisentan and its enantiomer.

**Nigori Ramkrushn et al.,**\(^54\) investigated HPLC-ESI-MS method for the quantification of ambrisentan in rat plasma. The analyte and the IS (armodafinil) were extracted from plasma by acetonitrile precipitation and they were separated on a reversed-phase C\textsubscript{18} column with a gradient program. The assay exhibited a linear dynamic range of 1-2000 ng/mL for ambrisentan in plasma. Acceptable precision (<10%) and accuracy (100 ± 8%) were obtained for concentrations over the standard curve range. The method was successfully applied to quantify ambrisentan concentrations in a rodent pharmacokinetic study after a single oral administration of ambrisentan at 2.5 mg/kg to rats. Therefore, development of such a simple and sensitive method in rat plasma should translate into a method for ambrisentan in human plasma for clinical trials.
2.3.2 Review of work done on tadalafil drug

Flores Rodríguez et al.,\textsuperscript{55} investigated MEKC chromatography methods for simultaneous estimation of sildenafil, vardenafil and tadalafil. A background electrolyte solution consisting of 10 mM phosphate buffer adjusted to pH 12.0, sodium dodecyl sulfate 25 mM, hydrodynamic injection, and 25 KV as separation voltage were used. Relative standard deviations were 1.0, 1.0, 0.4% and 2.9, 2.9, 1.9% for migration time and corrected peak area \((n = 9)\) for sildenafil, vardenafil and tadalafil, respectively. Detection limits obtained for the three drugs ranged from 0.19 to 0.61 mg/l. A linear concentration range between 1 and 20 mg/l was obtained. A ruggedness test of this method was checked using the fractional factorial model of Plackett–Burman, in which the influence of six factors at three different levels was tested on different electrophoretic results: resolution and corrected peak area. The statistical evaluation of the electrophoretic results was achieved by Youden and Steiner method. The described method was rapid, sensitive and rugged and it was tested in the pharmaceutical formulations analysis obtaining recoveries between 98 and 107% respect to the nominal content.

Ramakrishna NVS et al.,\textsuperscript{56} developed and validated simple, rapid, sensitive and specific liquid chromatography–tandem mass spectrometry method for quantitation of tadalafil (I) in human plasma, a new selective, reversible phosphodiesterase 5 inhibitor. The analyte and IS (Sildenafil, II) were extracted by liquid–liquid extraction with diethyl ether/dichloromethane (70/30, v/v) using a Glas-col multi-pulse vortexer. The chromatographic separation was performed on reverse phase Xterra MS C18 column with a mobile phase of 10 mM ammonium formate/acetonitrile (10/90, v/v, pH adjusted to 3.0 with formic acid). The protonate of analyte was quantitated in positive ionization by multiple reactions monitoring with a mass spectrometer. The assay exhibited a linear dynamic range of 10–1000 ng/mL for tadalafil in human plasma. The LLOQ was 10 ng/mL with a relative standard deviation of less than 15%. Acceptable precision and accuracy were obtained for concentrations over the standard curve ranges. Run time of 1.2 min for each sample made it possible to analyze a throughput of more than 400 human plasma samples per day. The validated method has been successfully used to analyze human plasma samples for application in pharmacokinetic, bioavailability or bioequivalence studies.

Khabbaz LR et al.,\textsuperscript{57} worked on development and validation of simple, rapid, and sensitive high-performance liquid chromatographic method was developed and
validated for determination of tadalafl, a selective and reversible phosphodiesterase inhibitor, in human serum. Methylparaben was used as the internal standard. Optimum conditions for tadalafl assay were investigated. The analyte and internal standard were extracted by a single-step liquid–liquid extraction with dichloromethane in alkaline serum. The chromatographic separation was performed on reverse phase Li Chrospher 100, C18 column (Agilent Technologies, Palo Alto, CA) with a mobile phase consisting of 35% acetonitrile-65% water containing 0.1 mM glacial acetic acid (pH 2.5-2.7). Ultraviolet detection was performed at 280 nm. Detection limit was 1.5 ng/mL, and limit of quantification was less than 10 ng/mL for tadalafl. The calibration curves were linear over the concentration range tested (10-800 ng/mL). Accuracy, precision, and stability studies were satisfactory. This method can be used to determine serum tadalafl concentrations in drug monitoring or in pharmacokinetic investigations.

Shakya Ashok et al.,58 worked on HPLC method to quantify tadalafl in human plasma. The tadalafl and internal standard (loratadine) were extracted by liquid–liquid extraction technique followed by an aqueous back-extraction allowing injection of an aqueous solvent in the HPLC system. The chromatographic separation was performed on a reverse phase BDS Hypersil C-18 column (250 mm × 4.6 mm, 5 μm, Thermo Separation Co., USA) with a mobile phase of acetonitrile and aqueous solution containing 0.012 M triethylamine and 0.020 M orthophosphoric acid (50/50, v/v). The analytes were detected at 225 nm. The assay exhibited a linear range of 5–600 ng/mL for tadalafl in human plasma. The LLOQ was 5 ng/mL. The within- and between batch precision did not exceed 10.3% and the accuracy was within 7.6% deviation of the nominal concentration. The recovery of tadalafl from plasma was greater than 66.1%. Stability of tadalafl in plasma was excellent with no evidence of degradation during sample processing (auto-sampler) and 30 days storage in a freezer. This validated method is applied for the clinical study of the tadalafl in human volunteers.

Rao Subba et al.,59 determined tadalafl in bulk samples and in pharmaceutical dosage forms in the presence of the degradation products by stability-indicating HPLC. It involved a 250 mm × 4.6 mm, 5 μm C-18 column. The gradient LC method employs solution A and B as mobile phase. Solution A contains a mixture of buffer (phosphate buffer and tetra-n-butyl ammonium hydrogen sulfate) pH 2.5: acetonitrile (80:20, v/v) and solution B contains a mixture of water: acetonitrile (20:80, v/v). The
flow rate was 1.0 mL/min and the detection wavelength was 220 nm. The retention time of tadalafil is about 17 min. Degradation was found to occur in hydrolytic and to some extent in oxidative stress conditions, while the drug was stable to photolytic and thermal stress. The drug was particularly labile under alkaline hydrolytic conditions. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. The assay of stress samples was calculated against a qualified reference standard and the mass balance was close to 99.5%. The developed RP-LC method was validated with respect to linearity, accuracy, precision and ruggedness. **Madhavi G et al.,** used simple isocratic chiral RP-LC method for separation and quantification of the enantiomer of \((R,R)\)-tadalafil in bulk drugs and dosage forms with an elution time of about 20 min. Chromatographic separation of \((R,R)\)-tadalafil and its enantiomer was achieved on a bonded macro cyclic glycopeptide stationary phase. The method resolves the \((R, R)\) - tadalafil and its enantiomer with a resolution greater than 2.4 in the developed chiral RP-LC. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. There was no interference of degradants with \((R, R)\) - tadalafil and its enantiomer in the developed method. The developed chiral RP-LC method was validated with respect to linearity, accuracy, precision and robustness. The percentage recovery for the enantiomer of \((R, R)\) - tadalafil in bulk drug samples and in dosage forms ranged from 97.0 to 102.5%. The test solution was found to be stable in the mobile phase for 48 h after preparation. **Yang YJ et al.,** estimated sildenafil, vardenafil, and tadalafil by RP-HPLC DAD method in pharmaceutical preparations and counterfeit drugs. An agilent zorbax SB C8 column (50 × 4.6 mm i.d., 1.8 μm particle size) was used. The mobile phase consisted of a mixture of 0.030 M of ammonium formate (adjusted to pH 3.0 with formic acid) and acetonitrile in the ratio 70:30 at 230 nm. These three drugs were eluted at the retention times of 1.654, 2.032, and 5.067 min for vardenafil, sildenafil, and tadalafil, respectively. Calibration curves were linear over the concentration ranges of 0.2–200 μg/mL for sildenafil, vardenafil, and tadalafil. The LOD values were 1.0, 1.1, and 1.0 ng and the LOQ values were 2.0, 2.1, and 2.0 ng for sildenafil, vardenafil, and tadalafil, respectively. The method is rapid both for routine quantitative analysis of sildenafil, vardenafil, and tadalafil in pharmaceutical preparations and screening their suspected counterfeit drugs. **Adlin Jino et al.,** worked on determination of tadalafil drug by developed extractive spectrophotometric methods in both pure and tablet dosage form. Methods A and B
are based on the formation of ion-pair complexes of the drug with dyes such as bromothymol blue and bromocresol green in acidic buffer solution followed by their extraction with chloroform to form yellow colored chromogen with absorption maxima at 420 nm and 415 nm respectively. Beer’s law is valid in the concentration range of 10-50 μg/mL for both the methods. These developed methods were validated for precision, accuracy, ruggedness and robustness. Statistical analysis proves that the methods are reproducible and selective for the routine analysis of the said drug. 

**Yunoos Mohammad et al.** investigated UV spectrophotometric method for the determination of tadalafil in bulk and tablet dosage form. Solution of tadalafil in methanol shows maximum absorbance at 284 nm. Beer’s law was obeyed in the concentration range of 2-20 μg/mL. The method was validated by determining its sensitivity, accuracy and precision which proves suitability of the developed method for the routine estimation of tadalafil in bulk and solid dosage form.

2.3.3 Review of work done on ambrisentan and tadalafil combination

**GlaxoSmithKline et al.** sponsored clinical trial on a Study of first-line ambrisentan and tadalafil combination therapy in subjects with pulmonary arterial hypertension (PAH) (AMBITION). The purpose of this study is to compare the two treatment strategies; first-line combination therapy (ambrisentan and tadalafil) versus first-line monotherapy (ambrisentan or tadalafil) in subjects with pulmonary arterial hypertension. This will be assessed by time to the first clinical failure event. This study is to test how well the drugs ambrisentan and tadalafil work together to treat pulmonary arterial hypertension (PAH) (a narrowing of the arteries connecting the lungs to the heart that leads to an increase in blood pressure) compared to ambrisentan or tadalafil alone. We want to find out what effects, good or bad, they have on people with PAH. We also want to see how safe these drugs are when used together. These drugs have been approved by the United States (U.S.) Food and Drug Administration (FDA) for the treatment of PAH. However, the combination of ambrisentan and tadalafil has not been previously studied and approved by the FDA.
2.4 References


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