CHAPTER NO.: 3 LITERATURE REVIEW

The details of the drug profile of the selected drugs were shown below along with the current literature review and the references.

3.1 Drug profile

3.1.1 Profile of atazanavir\textsuperscript{87,88}

Category: Protease inhibitor

Chemical name: methyl N-[(1S)-1-{[(2S,3S)-3-hydroxy-4-{[(2S)-2-[(methoxycarbonyl)amino]-3,3-dimethyl-N'-{[4-(pyridin-2-yl)phenyl] methyl} butane hydrazido]-1-phenylbutan-2-yl] carbamoyl}-2,2-dimethylpropyl] carbamate

Molecular formula: C\textsubscript{38}H\textsubscript{52}N\textsubscript{6}O\textsubscript{7}

Chemical structure:

![Chemical Structure of Atazanavir](image)

Figure No. 19 Chemical Structure of Atazanavir

Physical properties:

Molecular weight: 704.87 g/mol

Appearance and color: Atazanavir sulfate is a white to pale-yellow crystalline powder.

Solubility: It is slightly soluble in water (4-5 mg/mL, free base equivalent) with the pH of a saturated solution in water being about 1.9 at 24 ± 3°C.

Dosage: Capsule

General pharmacology:

Atazanavir selectively inhibits the virus-specific processing of viral Gag and Gag-Pol polyproteins in HIV-1 infected cells by binding to the active site of HIV-1 protease, thus preventing the formation of mature virions. Atazanavir is not active against HIV-2.

Indications and usage:

Used in combination with other antiretroviral agents for the treatment of HIV-1 infection, as well as postexposure prophylaxis of HIV infection in individuals who have
had occupational or nonoccupational exposure to potentially infectious body fluids of a person known to be infected with HIV when that exposure represents a substantial risk for HIV transmission.

**Pharmacokinetics:**

**Dosage:** 50-300mg

**Cmax:** Approx. 3200ng/mL for 300mg

Atazanavir is rapidly absorbed with a Tmax of approximately 2.5 hours. Atazanavir demonstrates nonlinear pharmacokinetics with greater than dose-proportional increases in AUC and Cmax values over the dose range of 200 to 800 mg once daily. Atazanavir is 86% bound to human serum proteins and protein binding is independent of concentration. Atazanavir binds to both alpha-1-acidglycoprotein (AAG) and albumin to a similar extent (89% and 86%, respectively). Atazanavir is extensively metabolized in humans. The major biotransformation pathways of atazanavir in humans consisted of mono-oxygenation and di-oxygenation. Two minor metabolites of atazanavir in plasma have been characterized. Neither metabolite demonstrated *in vitro* antiviral activity. *In vitro* studies using human liver microsomes suggested that atazanavir is metabolized by CYP3A. Following a single 400-mg dose of $^{14}$C-atazanavir, 79% and 13% of the total radioactivity was recovered in the feces and urine, respectively. Unchanged drug accounted for approximately 20% and 7% of the administered dose in the feces and urine, respectively. The mean elimination half-life of atazanavir in healthy volunteers (n=214) and HIV-infected adult patients (n=13) was approximately 7 hours at steady state following a dose of 400 mg daily with a light meal.
3.1.2 Profile of darunavir

**Category:** Protease inhibitor

**Chemical name:** (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl N-[(2S,3R)-3-hydroxy-4-[N-(2-methylpropyl)4-aminobenzenesulfonamido]-1-phenylbutan-2-yl]carbamate

**Molecular formula:** C_{27}H_{37}N_{3}O_{7}S

**Chemical structure:**

![Chemical Structure of Darunavir](image)

**Figure No. 20 Chemical Structure of Darunavir**

**Physical properties:**

**Molecular weight:** 547.67 g/mol

**Appearance and color:** Darunavir is a white to off-white powder.

**Solubility:** Darunavir is soluble in water (approximately 0.15 mg/mL) at 20°C.

**Dosage:** Tablet, Suspension

**General pharmacology:**
Darunavir is a HIV protease inhibitor which prevents HIV replication by binding to the enzyme's active site, thereby preventing the dimerization and the catalytic activity of the HIV-1 protease. Darunavir selectively inhibits the cleavage of HIV encoded Gag-Pol polyproteins in virus-infected cells, which prevents the formation of mature infectious virus particles. Darunavir has with the main chains of the protease active site amino acids (Asp-29 and Asp-30) is an important contributing factor to its potency and wide spectrum of activity against multi-protease inhibitor resistant HIV-1 variants. Darunavir can also adapt to the changing shape of a protease enzyme because of its molecular flexibility. Darunavir is known to bind to two distinct sites on the enzyme: the active site cavity and the surface of one of the flexible flaps in the protease dimer.

**Indications and usage:**
Darunavir, co-administered with ritonavir, and with other antiretroviral agents, is indicated for the treatment of human immunodeficiency virus (HIV) infection in
antiretroviral treatment-experienced adult patients, such as those with HIV-1 strains resistant to more than one protease inhibitor.

Pharmacokinetics:

**Dosage:** 75-600mg

**Cmax:** Approx. 3000ng/mL for 300mg

Darunavir, co-administered with 100 mg ritonavir twice daily, was absorbed following oral administration with a Tmax of approximately 2.5-4 hours. The absolute oral bioavailability of a single 600 mg dose of darunavir alone and after co-administration with 100 mg ritonavir twice daily was 37% and 82%, respectively. In vivo data suggest that darunavir/ritonavir is an inhibitor of the p-glycoprotein (p-gp) transporters. Darunavir is approximately 95% bound to plasma proteins. Darunavir binds primarily to plasma alpha 1-acid glycoprotein (AAG). In vitro experiments with human liver microsomes (HLMs) indicate that darunavir primarily undergoes oxidative metabolism. Darunavir is extensively metabolized by CYP enzymes, primarily by CYP3A. A mass balance study in healthy volunteers showed that after a single dose administration of 400 mg $^{14}$C-darunavir, co-administered with 100 mg ritonavir, the majority of the radioactivity in the plasma was due to darunavir. At least 3 oxidative metabolites of darunavir have been identified in humans; all showed activity that was at least 90% less than the activity of darunavir against wild-type HIV-1. A mass balance study in healthy volunteers showed that after single dose administration of 400 mg $^{14}$C-darunavir, co-administered with 100 mg ritonavir, approximately 79.5% and 13.9% of the administered dose of $^{14}$C-darunavir was recovered in the feces and urine, respectively. Unchanged darunavir accounted for approximately 41.2% and 7.7% of the administered dose in feces and urine, respectively. The terminal elimination half-life of darunavir was approximately 15 hours when co-administered with ritonavir.
3.1.3 Profile of ritonavir\textsuperscript{91-92}

Category: Protease inhibitor

Chemical name: 1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2-\{methyl{[[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl}carbamoyl]amino}butanamido]-1,6-diphenylhexan-2-yl]carbamate

Molecular formula: $C_{37}H_{48}N_{6}O_{5}S_{2}$

Chemical structure:

![Figure No. 21 Chemical Structure of Ritonavir](image)

Physical properties:

Molecular weight: 720.94g/mol

Appearance and color: Ritonavir is a white-to-light-tan powder. Ritonavir has a bitter metallic taste.

Solubility: It is freely soluble in methanol and ethanol, soluble in isopropanol and practically insoluble in water.

Dosage: Tablet, Capsule, Solution

General pharmacology:

Ritonavir is a peptidomimetic inhibitor of the HIV-1 protease. Inhibition of HIV protease renders the enzyme incapable of processing the gag-pol polyprotein precursor which leads to production of non-infectious immature HIV-1 particles.

Indications and usage:

Indicated in combination with other antiretroviral agents for the treatment of HIV-infection for the treatment of human immunodeficiency virus (HIV) infection in antiretroviral treatment-experienced adult patients, such as those with HIV-1 strains resistant to more than one protease inhibitor.
Pharmacokinetics:

Dosage: 100-600mg

Cmax: Approx. 850ng/mL for 100mg

It is co-administered as alone or in combination with other antiretroviral drugs. It is absorbed following oral administration. The absolute bioavailability of ritonavir has not been determined. Five ritonavir metabolites have been identified in human urine and feces. The isopropylthiazole oxidation metabolite (M-2) is the major metabolite and has antiviral activity similar to that of parent drug; however, the concentrations of this metabolite in plasma are low. In vitro studies utilizing human liver microsomes have demonstrated that cytochrome P450 3A (CYP3A) is the major isoform involved in ritonavir metabolism, although CYP2D6 also contributes to the formation of M-2. In a study of five subjects receiving a 600 mg dose of $^{14}$C-ritonavir oral solution, 11.3 ± 2.8% of the dose was excreted into the urine, with 3.5 ± 1.8% of the dose excreted as unchanged parent drug. In that study, 86.4 ± 2.9% of the dose was excreted in the feces with 33.8 ± 10.8% of the dose excreted as unchanged parent drug.
3.1.4 Profile of lopinavir\textsuperscript{93-94}

**Category:** Protease inhibitor

**Chemical name:** (2S)-N-[(2S,4S,5S)-5-[2-(6-dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-y1]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl)butanamide

**Molecular formula:** C\textsubscript{37}H\textsubscript{48}N\textsubscript{4}O\textsubscript{5}

**Chemical structure:**

![Chemical Structure of Lopinavir](image)

**Physical properties:**

**Molecular weight:** 628.81 g/mol

**Appearance and color:** Lopinavir is a white-to-light-tan powder.

**Solubility:** It is freely soluble in methanol and ethanol, soluble in isopropanol and practically insoluble in water.

**Dosage:** Tablet, Capsule, Solution

**General pharmacology:**

Lopinavir inhibits the HIV viral protease enzyme. This prevents cleavage of the gag-pol polyprotein and, therefore, improper viral assembly results. This subsequently results in non-infectious, immature viral particles.

**Indications and usage:**

Indicated in combination with other antiretroviral agents for the treatment of HIV-infection.

**Pharmacokinetics:**

**Dosage:** 100-800mg

**Cmax:** Approx. 4000ng/mL for 200mg

In a pharmacokinetic study in HIV-1 positive subjects, multiple dosing with 400/100 mg twice daily with food for 3 weeks produced a mean±SD lopinavir peak plasma concentration (Cmax) of 9.8±3.7 μg/mL, occurring approximately 4 hours after
administration. Lopinavir AUC over a 12 hour dosing interval averaged 92.6 ± 36.7 μg•h/mL. The absolute bioavailability of lopinavir co-formulated with ritonavir in humans has not been established. At steady state, lopinavir is approximately 98-99% bound to plasma proteins. Lopinavir binds to both alpha-1-acid glycoprotein (AAG) and albumin; however, it has a higher affinity for AAG. At steady state, lopinavir protein binding remains constant and is similar between healthy volunteers and HIV-1 positive patients. In vitro experiments with human hepatic microsomes indicate that lopinavir primarily undergoes oxidative metabolism. Lopinavir is extensively metabolized by the hepatic cytochrome P450 system, almost exclusively by the CYP3A isozyme. Ritonavir is a potent CYP3A inhibitor which inhibits the metabolism of lopinavir, and therefore increases plasma levels of lopinavir. At least 13 lopinavir oxidative metabolites have been identified in man. Ritonavir has been shown to induce metabolic enzymes, resulting in the induction of its own metabolism. Following a 400/100 mg $^{14}$C-lopinavir/ritonavir dose, approximately 10.4 ± 2.3% and 82.6 ± 2.5% of an administered dose of $^{14}$C-lopinavir can be accounted for in urine and feces, respectively, after 8 days. Unchanged lopinavir accounted for approximately 2.2 and 19.8% of the administered dose in urine and feces, respectively.
3.1.5 Profile of tenofovir\textsuperscript{95-96}

**Category:** Nucleoside reverse transcriptase inhibitor

**Chemical name:** \(((2R)-1-(6\text{-amino}-9H\text{-purin}-9\text{-yl})\text{ propan-2-yl oxy}\} \text{methyl})\) phosphonic acid

**Molecular formula:** \(\text{C}_{9}\text{H}_{14}\text{N}_{5}\text{O}_{4}\text{P}\)

**Chemical structure:**

![Chemical Structure of Tenofovir](image)

**Figure No. 23 Chemical Structure of Tenofovir**

**Physical properties:**

**Molecular weight:** 287.21g/mol

**Appearance and color:** Tenofovir is a white to off-white crystalline powder.

**Solubility:** It is soluble in water (solubility of 13.4 mg/mL) at 25 °C.

**Dosage:** Tablet, Powder

**General pharmacology:**

Tenofovir inhibits the activity of HIV reverse transcriptase by competing with the natural substrate deoxyadenosine 5’-triphosphate and, after incorporation into DNA, by DNA chain termination. Specifically, the drugs are analogues of the naturally occurring deoxynucleotides needed to synthesize the viral DNA and they compete with the natural deoxynucleotides for incorporation into the growing viral DNA chain. However, unlike the natural deoxynucleotides substrates, NRTIs and NtRTIs (nucleoside/tide reverse transcriptase inhibitors) lack a 3’-hydroxyl group on the deoxyribose moiety. All NRTIs and NtRTIs are classified as competitive substrate inhibitors.

**Indications and usage:**

Tenofovir is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in adults and pediatric patients 2 years of age and older. It is also indicated for the treatment of chronic hepatitis B in adults and pediatric patients 12 years of age and older.
Pharmacokinetics:

**Dosage:** 150-300mg

**Cmax:** Approx. 300ng/mL for 300mg

Following oral administration, maximum tenofovir serum concentrations are achieved in 1.0 ± 0.4 hour. Less than 0.7% of tenofovir binds to human plasma proteins *in vitro* and the binding is independent of concentration over the range of 0.01–25 μg/mL. Approximately 70–80% of the intravenous dose of tenofovir is recovered as unchanged drug in the urine. Tenofovir is eliminated by a combination of glomerular filtration and active tubular secretion. Following a single oral dose, the terminal elimination half-life of tenofovir is approximately 17 hours.
3.1.6 Profile of emtricitabine\textsuperscript{97-98}

**Category:** Nucleoside reverse transcriptase inhibitor

**Chemical name:** 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one

**Molecular formula:** C$_8$H$_{10}$FN$_3$O$_3$S

**Chemical structure:**

![Chemical Structure of Emtricitabine](image)

**Physical properties:**

**Molecular weight:** 247.25 g/mol

**Appearance and color:** Tenofovir is a white to off-white crystalline powder.

**Solubility:** It is soluble in water (solubility of 112 mg/mL) at 25 °C.

**Dosage:** Capsule, Solution

**General Pharmacology:**

Emtricitabine works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. Emtricitabine is a synthetic nucleoside analogue of cytidine. It is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate, which is responsible for the inhibition of HIV-1 reverse transcriptase. It competes with the natural substrate deoxycytidine 5'-triphosphate and incorporates into nascent viral DNA, resulting in early chain termination. Therefore emtricitabine inhibits the activity of HIV-1 reverse transcriptase (RT) both by competing with the natural substrate deoxycytidine 5'-triphosphate and by its incorporation into viral DNA. By inhibiting HIV-1 reverse transcriptase, emtricitabine can help to lower the amount of HIV, or "viral load", in a patient's body and can indirectly increase the number of immune system cells (called T cells or CD4+ T-cells).

**Indications and usage:**

Indicated, in combination with other antiretroviral agents, for the treatment of HIV-1 infection in adults and for post-exposure prophylaxis of HIV infection in health care workers and others exposed occupationally or non-occupationally via percutaneous
injury or mucous membrane or non-intact skin contact with blood, tissues, or other body fluids associated with risk for transmission of the virus.

**Pharmacokinetics:**

**Dosage:** 60-240mg

**Cmax:** Approx. 1800ng/mL for 200mg

Following oral administration, emtricitabine is rapidly absorbed with peak plasma concentrations occurring at 1–2 hours post-dose. Less than 4% of emtricitabine binds to human plasma proteins *in vitro* and the binding is independent of concentration over the range of 0.02–200 μg/mL. Following administration of radiolabelled emtricitabine, approximately 86% is recovered in the urine and 13% is recovered as metabolites. The metabolites of emtricitabine include 3'-sulfoxide diastereomers and their glucuronic acid conjugate. Emtricitabine is eliminated by a combination of glomerular filtration and active tubular secretion. Following a single oral dose, the plasma emtricitabine half-life is approximately 10 hours.
3.1.7 Profile of raltegravir\textsuperscript{99-100}

**Category:** Integrase inhibitor

**Chemical name:** N-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-2-[(5-methyl-1,3,4-oxadiazol-2-yl)formamido]propan-2-yl]-6-oxo-1,6-dihydropyrimidine-4-carboxamide

**Molecular formula:** C\textsubscript{20}H\textsubscript{21}FN\textsubscript{6}O\textsubscript{5}

**Chemical structure:**

![Chemical Structure of Raltegravir](image)

**Physical properties:**

**Molecular weight:** 444.42 g/mol

**Appearance and color:** Raltegravir is a white to off-white powder.

**Solubility:** It is soluble in water, slightly soluble in methanol, very slightly soluble in ethanol and acetonitrile and insoluble in isopropanol.

**Dosage:** Tablet, Suspension

**General pharmacology:**

Raltegravir inhibits the catalytic activity of HIV-1 integrase, an HIV-1 encoded enzyme that is required for viral replication. Inhibition of integrase prevents the covalent insertion, or integration, of unintegrated linear HIV-1 DNA into the host cell genome preventing the formation of the HIV-1 provirus. The provirus is required to direct the production of progeny virus, so inhibiting integration prevents propagation of the viral infection.

**Indications and usage:**

For the treatment of HIV-1 infection alone or in conjunction with other antiretrovirals.

**Pharmacokinetics:**

**Dosage:** 25-400mg

**Cmax:** Approx. 2250ng/mL for 400mg

Raltegravir is absorbed with a Tmax of approximately 3 hours postdose in the fasted state. Raltegravir AUC and Cmax increase dose proportionally over the dose range 100 mg to 1600 mg. The absolute bioavailability of raltegravir has not been established. Based on a formulation comparison study in healthy adult volunteers, the chewable
tablet and oral suspension have higher oral bioavailability compared to the 400 mg film-coated tablet. Raltegravir is approximately 83% bound to human plasma protein over the concentration range of 2 to 10 μM. Raltegravir was also measured in the cerebrospinal fluid. Cerebrospinal fluid concentration was 5.8% (range 1 to 53.5%) of the corresponding plasma concentration. This proportion was approximately 3-fold lower than the free fraction of raltegravir in plasma. The apparent terminal half-life of raltegravir is approximately 9 hours. Following administration of an oral dose of radiolabeled raltegravir, approximately 51 and 32% of the dose was excreted in feces and urine, respectively. The major circulating entity was raltegravir and represented approximately 70% of the total radioactivity.
3.2 Reported bioanalytical methods

3.2.1 Atazanavir\textsuperscript{101-108}

1. Comparison of extraction procedures for assessment of matrix effect for selective and reliable determination of atazanavir in human plasma by LC–ESI-MS/MS.
2. Simultaneous quantification of the new HIV protease inhibitors atazanavir and tipranavir in human plasma by high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry.
3. Quantitative determination of the HIV protease inhibitor atazanavir (BMS-232632) in human plasma by liquid chromatography–tandem mass spectrometry following automated solid-phase extraction.
4. Fast and simultaneous determination of darunavir and eleven other antiretroviral drugs for therapeutic drug monitoring: method development and validation for the determination of all currently approved HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors in human plasma by liquid chromatography coupled with electrospray ionization tandem mass spectrometry.
5. Simple determination of the HIV protease inhibitor atazanavir in human plasma by high-performance liquid chromatography with UV detection.
7. Validation of a rapid and sensitive high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds.
8. Determination of the new HIV-protease inhibitor atazanavir by liquid chromatography after solid-phase extraction.

3.2.2 Darunavir & ritonavir\textsuperscript{109-110, 107}

1. A LC–tandem MS assay for the simultaneous measurement of new antiretroviral agents: raltegravir, maraviroc, darunavir, and etravirine.
2. Quantification of seven nucleoside/nucleotide reverse transcriptase inhibitors in human plasma by high-performance liquid chromatography with tandem mass-spectrometry.
3. Validation of a rapid and sensitive high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds.
3.2.3 Ritonavir\textsuperscript{111-114,107}

4. Quantification of antiretroviral drugs in dried blood spot samples by means of liquid chromatography/tandem mass spectrometry.
5. Validation of a rapid and sensitive high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds.

3.2.4 Lopinavir & ritonavir\textsuperscript{115-119,107}

1. Rapid, simultaneous determination of lopinavir and ritonavir in human plasma by stacking protein precipitations and salting-out assisted liquid/liquid extraction, and ultrafast LC–MS/MS.
4. Application of a rapid and selective method for the simultaneous determination of protease inhibitors, lopinavir and ritonavir in human plasma by UPLC–ESI-MS/MS for bioequivalence study in Indian subjects.
5. Simultaneous determination of ritonavir and lopinavir in human plasma after protein precipitation and LC-MS-MS.
6. Validation of a rapid and sensitive high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds.
3.2.5 Tenofovir & emtricitabine\textsuperscript{120-124}


2. The simultaneous assay of tenofovir and emtricitabine in plasma using LC/MS/MS and isotopically labeled internal standards.

3. Selective determination of antiretroviral agents tenofovir, emtricitabine, and lamivudine in human plasma by a LC-MS-MS method for a bioequivalence study in healthy Indian subjects.

4. A new assay based on solid-phase extraction procedure with LC-MS to measure plasmatic concentrations of tenofovir and emtricitabine in HIV infected patients.

5. Quantification of seven nucleoside/nucleotide reverse transcriptase inhibitors in human plasma by high-performance liquid chromatography with tandem mass-spectrometry.

3.2.6 Raltegravir\textsuperscript{125-128, 107}

1. A LC–tandem MS assay for the simultaneous measurement of new antiretroviral agents: raltegravir, maraviroc, darunavir, and etravirine.


5. Validation of a rapid and sensitive high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds.