CHAPTER III

LITERATURE ON DRUG INVESTIGATED

RITONAVIR – PROFILE

Fig: 3.1 Structure of Ritonavir

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

Molecular Weight: 720.9

Chemical Name: 5-Thiazolylmethyl (αS)-α-[(1S, 3S)-1-hydroxy-3-(2S)-2-{3-[2-isopropyl-4-thiazolyl] methyl}-methylureido] 3-methylbutyramido)-4-Phenylbutyl] phenethyl} carbamate.

Melting Point: 120 – 125°C

Description: Ritonavir\textsuperscript{1} is a white to light tan powder and has a bitter metallic taste. It is freely soluble in methanol and ethanol, soluble in isopropanol and practically insoluble in water.
Mechanism of Action:

Ritonavir is a peptidomimetic inhibitor of both the HIV-1 and HIV-2 proteases. Inhibition of HIV protease renders the enzyme incapable of processing the gag-pol polyprotein precursor which leads to production of non-infectious immature HIV particles.

Pharmacokinetics:\(^2-^6\):

Absorption: The absolute bioavailability of ritonavir has not been determined. After a 600mg dose of oral solution, peak concentrations of ritonavir were achieved approximately 2 hours and 4 hours after dosing under fasting and non-fasting (514 KCal; 9% fat, 12% protein, and 79% carbohydrate) conditions, respectively.

When the oral solution was given under non fasting conditions, peak ritonavir concentrations decreased 23% and the extent of absorption decreased 7% relative to fasting conditions. After a single 600 mg dose under non fasting conditions, in two separate studies, the soft gelatin capsule and oral solution formulations yielded mean ±SD areas under the plasma concentration-time curve (AUC)\(\alpha\) of 121.7 ± 53.8 and 129.0 ± 39.3 µg.h/ml, respectively. Relative to fasting conditions, the extent of absorption of ritonavir from the soft gelatin capsule formulation was 13% higher when administered with a meal (615 Kcal, 14.5% fat, 9% protein and 76% carbohydrate).

Metabolism: Nearly the entire plasma radioactivity after a single 600 mg oral dose of \(^{14}\)C-ritonavir oral solution was attributed to unchanged ritonavir. Five ritonavir metabolites have been identified in human urine and faeces. The isopropylthiazole
oxidation metabolite 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 iso-form involved in ritonavir metabolism, although CYP2D6 also contributes to the formation of M-2.

**Elimination:** 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 faeces with 33.8 ± 10.8% of the dose excreted as unchanged parent drug. Upon multiple dosing, ritonavir accumulation is less than predicted from a single dose possibly due to a time and dose-related increase in clearance.

**Dosage and Administration:**

**Adults:** The recommended dosage of ritonavir is 600 mg twice daily by mouth. Use of a dose titration schedule may help to reduce treatment-emergent adverse events while maintaining appropriate ritonavir plasma levels. Ritonavir should be started at no less than 300 mg twice daily and increased at 2 to 3 day intervals by 100 mg twice daily.

**Pediatric Patients:** Ritonavir should be used in combination with other antiretroviral agents. The recommended dosage of ritonavir is 400 mg/m$^2$ twice daily by mouth and should not exceed 600 mg twice daily. Ritonavir should be started at 250 mg/m$^2$ and increased at 2 to 3 day intervals by 50 mg/m$^2$ twice daily. If patients do not tolerate 400 mg/m$^2$ twice daily due to adverse events, the highest
tolerated dose may be used for maintenance therapy in combination with other antiretroviral agents, however, alternative therapy should be considered. When possible, dose should be administered using a calibrated dosing syringe.

**Recent Past Work on Enhancement of Solubility, Dissolution rate and Bioavailability of Ritonavir.**

Musle *et al.*⁷ studied and reported solubility enhancement of poorly water soluble drug (ritonavir) using hot melt extrusion. The enhancement of oral bioavailability of poorly water soluble drugs remains one of the most challenging aspects of drug development. Complexation, precipitation, adsorption, salt information, particle size reduction, etc. have commonly been used in industry to solubility of the drug, there are practical limitation with these techniques the desired bioavailability enhancement may not always be achieved. Therefore formulation approaches are being explored to enhance bioavailability of poorly water-soluble drugs. One such formulation approach that has been shown to significantly enhance absorption of such drugs is to formulate prepare solid dispersion using hot melt extrusion. Ritonavir is anti HIV drug (BCS class ii), which is often administered orally. Ritonavir exhibits very slightly soluble and as a consequence it exhibits low bioavailibility after oral administration. Therefore the improvement of ritonavir dissolution from its oral solid dosage forms is an important issue for enhancing its therapeutic efficiency. The present study was enhancement of dissolution rate of poorly water soluble drug. The solid dispersion was using soluplus as carrier where leutrol f 68, leutol 127, tpgs was selected as plasticizer by hot melt extrusion the
resultant complexes were evaluated for drug content, dissolution rate, XRD, FTIR, DSC and SEM.

Pranjali et al.\textsuperscript{8} prepared and characterized Amorphous Nanoparticles for Solubility Enhancement of Ritonavir. Ritonavir is an antiretroviral drug characterized by low solubility and high permeability which corresponds to BCS class II drug. The purpose of the study was to develop amorphous nanoparticles by sonoprecipitation method in order to enhance its solubility. Ritonavir amorphous nanoparticles were produced by sonoprecipitation method. HPMC and SDS are use as a surfactant. The effect of process variables on particle size and physical state of ritonavir was investigated. The physicochemical properties of pure drug and amorphous nanoparticles were characterized by X-ray powder diffraction (XRPD), Fourier transform infrared (FT-IR) spectroscopy, differential scanning calorimetric (DSC), as well as, measuring the particle size. The DSC and XRPD results indicated that the sonoprecipitation process led to the formation of amorphous nanoparticles. Ritonavir nanoparticles completely dissolved in the dissolution medium of 0.1 N HCl within 60 min, while there was only 28.21\% of raw Ritonavir dissolved. The process by combining the antisolvent precipitation under sonication was a promising method to produce small, uniform and stable ritonavir nanoparticles with markedly enhanced dissolution rate due to an increased solubility.

Josephine et al.\textsuperscript{9} formulated and characterized ritonavir loaded Ethyl cellulose microspheres for oral delivery. The objective of the present study was to prepare and evaluate microparticles for the controlled release of Ritonavir using
cellulose polymer. The micro particles were prepared by the solvent evaporation method (O/O) using ethyl cellulose as wall materials. In order to increase the encapsulation efficiency, a mixed solvent system comprising 1:1 proportions of ethanol and dichloromethane were used as a dispersed phase. The prepared micro particles were characterized for the percent drug content, entrapment efficiency, FTIR, DSC, scanning electron microscopy (SEM) and in vitro dissolution studies. The prepared micro particles were white, free-flowing, and almost spherical in shape. The drug-loaded micro particles showed 86-111% drug entrapment, mean particle size was in the range of 36-40µm. In vitro drug release studies were carried out up to 10h in two different pH media, i.e., acidic buffer (pH 1.2) and SLS solution (0.7%). FTIR and DSC thermo grams showed the stable character of Ritonavir in the micro particles. SEM showed that the micro particles were porous in nature. The release kinetics study revealed that the prepared micro particles were best fitted to the zero order. The release kinetics data and characterization studies indicated that drug release from microcapsules was diffusion-controlled and that the micro particles were stable.

Gauravl et al.\textsuperscript{10} investigated and reported Complexation approach for fixed dose tablet formulation of lopinavir and ritonavir: an anomalous relationship between stability constant, dissolution rate and saturation solubility. In the present investigation, cyclodextrin complexation process was explored for development of tablet formulation of WHO approved fixed dose combination of lopinavir and ritonavir with reduced tablet size, shorter dis-integration time and higher bioavailability in comparison to reference product. In preliminary studies, we found that
lopinavir solubility and dissolution rate is poor into the dis-solution medium recommended by FDA, whereas ritonavir solubilized fairly into dissolution medium with adequate dissolution rate. Solid-state cyclodextrin complexation technology was used for enhancement of dissolution rate of lopinavir into dissolution medium. Various cyclodextrins were screened by comparison on basis of enhancement of dissolution rate of lopinavir (LPV) and the order was found as gamma cyclodextrin (γ-CD) [ hydroxypropyl beta-cyclodextrin (HP-β-CD) [ methyl beta-cyclodextrin (M-β-CD) [ beta-cyclodextrin (β-CD), with Q120 values (i.e. percentage of dissolved drug at 120 min.) were 10.1 for the pure LPV and 56.3, 51.3, 30.3 and 10.3 for LPV/γ-CD, LPV/HP-β-CD, LPV/M-β-CD and LPV/β-CD, respectively. Anomalous results were found between stability constant, dissolution rate and saturation solubility. It was found that cyclodextrin having higher stability constant value with LPV, provides higher saturated solubility of LPV in aqueous media but at slow dissolution rate and vice versa. The γ-CD was selected for complexation with lopinavir in the stoichiometric ratio 1:1.5 M of LPV to γ-CD. Various processes such as kneading method, milling technique, sonication, freeze drying and autoclaving were tried, from which kneading method was found to give best dissolution results. The corresponding solid complexes were characterized by differential scanning calorimetric, X-ray powder diffraction and scanning electron microscopy studies. Based on various studies, the complexation phenomenon between LPV and γ-CD was found to follow non-inclusion behavior. Pharmacokinetic studies were carried out in Sprague-Dawley rats using cross over design
with a 3 day wash out period. The bioavailability of lopinavir was found to be enhanced significantly using cyclodextrin complex tablet formulation.

SunilKumar et al. prepared, characterized and reported PGS - PVP co-processed excipient as directly compressible vehicle in the formulation development of antiretroviral drugs. Direct compression is the preferred method for the preparation of tablets. Co-processing is the one of the most widely explored and commercially utilized method for the preparation of directly compressible vehicles. The objective of the present study is to prepare and characterize pregelatinized starch-poly vinyl pyrrolidone (PGS-PVP) co-processed excipient and to evaluate its application as directly compressible vehicle in the tablet formulations of three anti-retroviral drugs namely efavirenz, ritonavir and stavudine. PGS-PVP co-processed excipient was prepared by gelatinizing potato starch in the presence of PVP and drying the resulting mass. The co-processed excipient prepared was characterized by determining melting point, solubility, swelling index in water, pH, and micromeritic characters namely particle size, bulk density, tapped density, angle of repose and compressibility index and evaluated for its application in tablet formulations. PGS-PVP co-processed excipient prepared by gelatinizing potato starch (49 parts) in the presence of PVP (1 part) is a crystalline, discrete and free flowing powder. It is insoluble in water and aqueous fluids of pH 1.2, 4.5 and 7.4 and in several organic solvents. It exhibited high swelling (284 %) in water. PGS-PVP co-processed excipient has excellent flow properties alone and as blends with selected drugs it exhibited excellent to good flow properties. Tablets of (i) efavirenz (100 mg) (ii)
ritonavir (100 mg) and (iii) stavudine (30 mg) prepared by direct compression method employing PGS-PVP co-processed excipient as DCV were of good quality with regard to drug content, hardness, friability and disintegration time. All the tablets formulated disintegrating rapidly within 3.5 min. With all the three drugs, the tablets prepared gave rapid dissolution of the contained drug, 100 % within 20 min and fulfilled the official (IP/USP) dissolution rate test specification prescribed in each case.

Chowdary et al.\textsuperscript{12} studied and reported enhancement of dissolution rate and formulation development of ritonavir tablets employing starch phosphate- a new modified starch. The objective of the study is to prepare, characterize and evaluate starch phosphate, a new modified starch as a carrier in solid dispersions for enhancing the dissolution rate of ritonavir. The feasibility of formulating solid dispersions of ritonavir in starch phosphate into compressed tablets with enhanced dissolution rate was also investigated. Starch phosphate was prepared by reacting starch with di-sodium hydrogen orthophosphate anhydrous at elevated temperatures. It was insoluble in water and has good swelling (400%) property without pasting or gelling when heated in water. Solid dispersions of ritonavir in starch phosphate were prepared by solvent evaporation method employing various weight ratios of drug: starch phosphate such as 2:1(SD-1), 1:1(SD-2), 1:2(SD-3), 1:3(SD-4) and 1:9(SD-5) and were evaluated for dissolution rate and efficiency. All the solid dispersions prepared gave rapid and higher dissolution of ritonavir when compared to pure drug. A 58.34 and 94.41 fold increase in the dissolution rate (K\textsubscript{1}) of ritonavir was observed with solid dispersions SD-4 and SD-5 respectively. The DE\textsubscript{30} was also increased from 6.80\% in the case of
Ritonavir pure drug to 76.25% and 84.05% in the case of these solid dispersions. Ritonavir (50 mg) tablets were prepared employing ritonavir alone and its solid dispersions SD-3 and SD-4 by wet granulation method and were evaluated. Ritonavir tablets formulated employing its solid dispersions in starch phosphate gave rapid and higher dissolution rate and $DE_{30}$ when compared to plain and commercial tablets. A 9.95 and 28.14 fold increase in the dissolution rate ($K_1$) was observed with tablet formulations containing solid dispersions SD-3 and SD-4 respectively when compared to plain tablets.

Chowdary et al.\textsuperscript{13} studied and reported enhancement of dissolution rate and formulation development of ritonavir tablets employing starch citrate. The objective of the study is to prepare, characterize and evaluate starch citrate, a new modified starch as a carrier in solid dispersions for enhancing the dissolution rate of ritonavir. The feasibility of formulating solid dispersions of ritonavir in starch citrate into compressed tablets with enhanced dissolution rate was also investigated. Starch citrate was prepared by reacting potato starch with citric acid at elevated temperatures. It was insoluble in water and has good swelling (1500%) property without pasting or gelling when heated in water. Solid dispersions of ritonavir in starch citrate were prepared by solvent evaporation method employing various weight ratios of drug: starch citrate such as 2:1(SD-1), 1:1(SD-2), 1:2(SD-3), 1:3(SD-4) and 1:9(SD-5) and were evaluated for dissolution rate and efficiency. All the solid dispersions prepared gave rapid and higher dissolution of ritonavir when compared to pure drug. A 58.34 and 94.41 fold increase in the dissolution rate ($K_1$) of ritonavir was observed with solid dispersions SD-4 and SD-5 respectively. The
DE$_{30}$ was also increased from 6.80% in the case of ritonavir pure drug to 76.25% and 84.05% in the case of these solid dispersions. Ritonavir (50 mg) tablets were prepared employing ritonavir alone and its solid dispersions SD-3 and SD-4 by wet granulation method and were evaluated. Ritonavir tablets formulated employing its solid dispersions in starch citrate gave rapid and higher dissolution rate and DE 30 when compared to plain and commercial tablets. A 9.95 and 28.14 fold increase in the dissolution rate ($K_1$) was observed with tablet formulations containing solid dispersions SD-3 and SD-4 respectively when compared to plain tablets.

Shilpi Sinha et al. prepared and studied Solid Dispersion as an Approach for Bioavailability Enhancement of Poorly Water-Soluble Drug Ritonavir. Ritonavir is an antiretroviral drug characterized by low solubility and high permeability which corresponds to BCS class II drug. The purpose of the study was to develop solid dispersion by different methods and investigate them for in vitro and in vivo performance for enhancing dissolution and bioavailability, respectively. Since the drug possesses food-related absorption, the effect of biorelevant media (FaSSIF and FeSSIF state) on dissolution behavior was also studied. The solid dispersion was prepared using Gelucire as carrier in 1:4 ratio by different methods and were characterized for differential scanning calorimetry (DSC), X-ray diffractometry, scanning electron microscopy, and FT-IR. Oral bioavailability of 10 mg of ritonavir in solid dispersion prepared by solvent evaporation (SE1) and melt method (MM1) was compared with pure drug after oral administration of solid dispersion and pure drug to Albino Wistar rats of either sex. The results suggested formation of eutectic solid dispersion. In vitro dissolution studies was performed in 0.1 N HCl and
biorelevant media showed enhanced dissolution rate as compared to pure drug in both FeSSIF media and 0.1 N HCl. The apparent rate of absorption of ritonavir from SE1 ($C_{\text{max}}$ 20221.37 ng/ml, $t_{\text{max}}$ 0.5 h) was higher than that of MM1 ($C_{\text{max}}$ 2,462.2 ng/ml, $t_{\text{max}}$ 1 h) and pure drug ($C_{\text{max}}$ 1,354.8 ng/ml, $t_{\text{max}}$ 0.5 h). On the basis of the result obtained, it was concluded that solid dispersion is a good approach to enhance solubility and bioavailability of poorly water-soluble ritonavir.

Oostendorp et al.\textsuperscript{15} studied and reported Coadministration of Ritonavir Strongly Enhances the apparent Oral Bioavailability of Docetaxel in Patients with Solid Tumors Purpose: To enhance the systemic exposure to oral docetaxel by coadministration of ritonavir, an efficacious inhibitor of CYP 3A4 with minor P-glycoprotein inhibiting effects, in patients with cancer. Experimental Design: A proof-of-concept study was carried out in 12 patients with solid tumors. The first cohort of patients ($n = 4$) received 10 mg and the subsequent cohort ($n = 8$) 100 mg of oral docetaxel, coadministered with 100 mg oral ritonavir randomized simultaneously or ritonavir given 60 minutes before docetaxel on days 1 and 8. On day 15 or 22, patients received 100 mg i.v. docetaxel. The area under the plasma concentration-time curve in patients who received 10 mg oral docetaxel in combination with ritonavir was low, and the dose could safely be increased to 100 mg. The area under the plasma concentration-time curve in patients who received 100 mg oral docetaxel combined with ritonavir simultaneously or ritonavir given 60 minutes before docetaxel was 2.4 ± 1.5 and 2.8 ± 1.4 mg/h/L, respectively, compared with 1.9 ± 0.4 mg/h/L after i.v. docetaxel. The apparent oral bioavailability of docetaxel combined with ritonavir simultaneously or ritonavir
given 60 minutes before docetaxel was 131% F 90% and 161% F 91%, respectively. The oral combination of docetaxel and ritonavir was well tolerated. Coadministration of ritonavir significantly enhanced the apparent oral bioavailability of docetaxel. These data are promising and form the basis for further development of a clinically applicable oral formulation of docetaxel combined with ritonavir.

Garren et al16 studied and reported Bioavailability of Generic Ritonavir and Lopinavir/Ritonavir Tablet Products in a Dog Model. In this study, they explored the bioavailability in dogs and chemical potency of generic ritonavir and lopinavir/ritonavir tablet products manufactured by various pharmaceutical companies. Chemical potency of the products was examined by HPLC quantitation of ritonavir and lopinavir. Using a dog model, we determined point estimates for $C_{\text{max}}$ and AUC of ritonavir and lopinavir/ritonavir for eight generic products compared to Abbott’s Norvir1 capsule and Kaletra1 tablet. Chemical potencies ranged from 79.0% to 104.6%. Point estimates for AUC in the generic tablet products ranged from 0.01 to 1.11, indicating that the relative bioavailability of these formulations was in the range of 1–111% compared to the branded products. This study showed significant variability in bioavailability in a dog model amongst generic tablet products containing the protease inhibitors ritonavir or lopinavir/ritonavir. The chemical potency of the generic products was not indicative of the plasma levels of ritonavir or lopinavir that were achieved. These results reinforce the need for human bioequivalence testing of generic products containing ritonavir or lopinavir/ritonavir to assure that efficacy in patients is not compromised.
prior to these products being made available to patients. Procurement policies of funding agencies should require such quality assurance processes.

Law et al.\textsuperscript{17} studied and reported Ritonavir-PEG 8000 amorphous solid dispersions: in vitro and in vivo evaluations. Ritonavir is a large, lipophilic molecule that is practically insoluble in aqueous media and exhibits an exceedingly slow intrinsic dissolution rate. Although it has favorable lipophilicity, in vitro permeability studies have shown that ritonavir is a substrate of P-glycoprotein. Thus, the oral absorption of ritonavir could be limited by both dissolution and permeability, thereby making it a Class II compound in the Biopharmaceutics Classification System. Because formulations rarely exert direct influence on local intestinal permeability, the effect of enhanced dissolution rate on oral absorption was explored. More specifically, polyethylene glycol (PEG)-amorphous ritonavir solid dispersions were prepared with different drug loadings, and the in vitro and in vivo performances of the dispersions were evaluated. In vitro dissolution was conducted in 0.1N HCl with a USP Apparatus I. A crossover design was used to evaluate the oral bioavailability of amorphous dispersions relative to crystalline drug in beagle dogs. Intrinsic dissolution measurements of the two solid phases indicated a 10-fold improvement in intrinsic dissolution rate for amorphous ritonavir compared with the crystalline counterpart. In vitro dissolution of ritonavir depended on the solid phase as well as drug loading of the dispersion. In vivo study results indicate that amorphous solid dispersions containing 10-30\% drug exhibited significant increases in area under the curve of concentration versus time (AUC) and maximum concentration (\(C_{\text{max}}\)) over crystalline drug. For example, 10\% amorphous dispersion
exhibited increases of 22- and 13.7-fold in AUC and $C_{\text{max}}$ respectively. However, both in vitro dissolution and bioavailability decreased with increasing drug load, which led to the construction of a multiple Level C in vitro-in vivo relationship for this Class II compound. The established relationship between in vitro dissolution and in vivo absorption can help guide formulation development.
REFERENCES

1. Lea AP, Faulds D. 1996; 52; 541.


