Chapter-II
Two poorly soluble drugs saquinavir and ritonavir belonging to the category of antiretroviral (ARV) agents were selected in the present investigation to evaluate the effect of CDs and method of preparation in the improvement of solubility and dissolution rate. The objective of this work is to improve the oral bioavailability of saquinavir and ritonavir by modifying the solubility and dissolution patterns of these drugs by means of preparing inclusion complexes with the above selected carriers. In this chapter, physicochemical, pharmacological and therapeutic profiles of saquinavir and ritonavir are described. A brief profile of each of the cyclodextrin is also given. The analytical methods used in the present investigation for the selected drugs are also described.

2.1. Saquinavir\(^1\)\(^-\)\(^8\)

Saquinavir is an HIV protease inhibitor which acts as an analog of an HIV protease cleavage site. It is a highly specific inhibitor of HIV-1 and HIV-2 proteases.
2.1.1. Structure

Molecular formula: \( \text{C}_{38}\text{H}_{50}\text{N}_{6}\text{O}_{5} \)

Molecular weight: 670.84

CAS Registry: 127779-20-8

Synonyms: SQV, saquinavir mesylate, Invirase.

Generic Name: Saquinavir.

Chemical Name: \((2S)-\text{N-}[(2S,3R)-4-\text{[(3S)-3-(\text{tert-butylcarbamoyl})-}
\text{decahydroisoquinolin-2-yl]}-3\text{-hydroxy-1-phenylbutan-2-yl]}-2\text{-}(\text{quinolin-}
2\text{-yl formamido})\text{butanedi(amide).} \)

2.1.2. Physicochemical properties

Saquinavir is a white or almost white powder. It is practically insoluble in water and soluble in methanol. Saquinavir is a weak base and is having two pK\(_a\) values 1.1 and 7.1 with log P value of 4.5. Its melting point is 248-250°C.
2.1.3. Mechanism of action

Saquinavir is an inhibitor of HIV protease. HIV protease is an enzyme required for the proteolytic cleavage of viral polyprotein precursors into individual functional proteins found in infectious HIV. Saquinavir is a peptide like substrate analogue that binds to the protease active site and inhibits the activity of the enzyme. Saquinavir inhibition prevents cleavage of the viral poly proteins resulting in the formation of immature non infectious virus particles.\(^9\)

2.1.4. Pharmacokinetics

2.1.4.1. Absorption and bioavailability

Bioavailability of saquinavir mesylate from hard gelatin capsules is low, averaging 4%, due to a combination of incomplete absorption and extensive first pass metabolism.

2.1.4.2. Distribution

Distribution of the drug into body tissues and fluids (such as cerebrospinal fluid) has not been fully characterized. Saquinavir is about 98% bound to plasma proteins and extensively distributed into the tissues.

2.1.4.3. Metabolism

The drug is metabolized in the liver to several monohydroxylated and dihydroxylated inactive metabolites. Metabolism is mediated by cytochrome P450 (CYP), the isoenzyme CYP3A4 is involved in more than 90% of this metabolism.
2.1.4.4. Elimination

Systemic clearance is rapid. Saquinavir is excreted primarily in the feces, both as unchanged drug and as metabolites. Its terminal half life is about 13.2 hrs.

2.1.5. Indications and usage

Saquinavir is used in combination with other medications to treat human immunodeficiency virus (HIV) infection in patients with or without acquired immunodeficiency syndrome (AIDS). Saquinavir is in a class of antiviral medications called protease inhibitors. It works by slowing the spread of HIV infection in the body. It is not a cure and may not decrease the number of HIV related illnesses. It does not prevent the spread of HIV to other people.

Saquinavir is also used (as an alternative to indinavir) to treat health care workers and other individuals exposed to HIV infection after accidental contact with HIV-contaminated blood, tissues, or other body fluids.

2.1.6. Dosage and administration\textsuperscript{10-12}

In adults, in case of HIV infection, orally saquinavir is given 1 g twice daily with other antiretrovirals who are at the age of above 16, with ritonavir 100 mg twice daily dose. Alternatively, 400 mg of saquinavir with ritonavir 400 mg twice daily with meals is recommended.
In adults, as post exposure prophylaxis during occupational exposure to HIV, 1 g of saquinavir with ritonavir 100 mg twice daily, combined with other antiretrovirals is given. The therapy should be started as soon as possible and continued for 4 weeks if tolerated and the recommended dose should be taken with meals.

2.1.7. Adverse reactions

The most frequent adverse reactions with saquinavir administration are mild gastrointestinal symptoms including diarrhoea, nausea, loose stools and abdominal discomfort and tiredness. Some of serious but rare side effects include increased thirst or urination, excessive hunger, fruity breath odour, cough with yellow mucus and fever.

2.1.8. Drug interactions\textsuperscript{13-24}

Some products that may interact with this drug include: certain antiarrhythmics (amiodarone, flecainide, propafenone, quinidine), certain benzodiazepines (midazolam, triazolam), ergot alkaloids (such as dihydroergotamine, ergonovine, ergotamine, methylergonovine), garlic supplements, pimozide, ranolazine, rifampin, certain "statin" cholesterol drugs (simvastatin, lovastatin), St. John’s wort. Other medications can affect the removal of saquinavir from the body, which may affect how saquinavir works. Examples include rifabutin, certain anti-seizure drugs (carbamazepine, phenobarbital, phenytoin), certain drugs to lower acid in the stomach (proton pump inhibitors such as omeprazole), other HIV drugs (such as indinavir, nelfinavir), among
others. Saquinavir with ritonavir can slow down or speed up the removal of other medications from the body, which may affect how they work. Examples of affected drugs include digoxin, warfarin, calcium channel blockers (such as nifedipine, felodipine), certain drugs that weaken the immune system (cyclosporine, tacrolimus, sirolimus), drugs for male sexual function problems (such as sildenafil, vardenafil), among others. This medication may decrease the effectiveness of hormonal birth control products (such as pills, patch, and ring). This effect can result in pregnancy.

2.1.9. Food interactions

The rate of absorption is enhanced when administered with food, especially if the fat content is high in food.

2.1.10. Contraindications

Saquinavir is contraindicated in the following conditions:

- Lactation
- Hypersensitivity
- Hepatic impairment

2.1.11. Available dosage form

Saquinavir is available as hard gelatin capsules, film coated tablets and tablets and is given along with ritonavir to increase bioavailability.
2.2 Ritonavir\textsuperscript{25-27}

2.2.1 Structure

![Molecular structure of Ritonavir](image)

Molecular formula: $\text{C}_{37}\text{H}_{48}\text{N}_{6}\text{O}_{5}\text{S}_{2}$

Molecular weight: 721.0

CAS Registry: 155213-67-5

Generic Name: Ritonavir

Chemical name: 5-Thiazolylmethyl (αS)-α-[(1S, 3S)-1-hydroxy-3-[(2S)-2-[3-[(2-isopropyl-4-thiazolyl)methyl]-3-methylureido]-3-methylbutyramido]-4-phenylbutyl]phenethylcarbamate.

2.2.2. Physicochemical properties

Ritonavir is a white or almost white powder. It is practically insoluble in water, freely soluble in methanol, sparingly soluble in acetone and very slightly soluble in acetonitrile. Ritonavir is a weak base and its $pK_a$ value is 2.8. Its melting point is 119-123°C.
2.3. Mechanism of action

Ritonavir is a peptidomimetic inhibitor of both the HIV-1 and HIV-2 proteases. HIV protease is classified as an aspartic protease and is essential for the production of infectious virions. HIV virus is composed of two copies of positive single stranded RNA that codes for the virus’s nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein. The RNA genome consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS) and nine genes (\textit{gag}, \textit{pol}, and \textit{env}, \textit{tat}, \textit{rev}, \textit{nef}, \textit{vif}, \textit{vpr}, \textit{vpu}, and sometimes a tenth \textit{tev}, which is a fusion of \textit{tat}, \textit{env} and \textit{rev}), encoding 19 proteins. Three of these genes, \textit{gag}, \textit{pol}, and \textit{env}, contain information needed to make the structural proteins for new virus particles. During the late stages of the HIV replication cycle, the viral \textit{gag} and \textit{gag-pol} polypeptides combine with two molecules of viral RNA and envelope proteins to form immature virus particles. Viral protease then cleaves the \textit{gag} and \textit{gag-pol} polypeptides to form mature virus particles that can recognize and infect other target cells. Inhibiting viral protease leads to the release of immature, non-infectious, virions that halt the spread of virus to the other infected cells. Inhibitors of HIV protease do not effect mammalian proteases due to dissimilarity of HIV protease and human aspartic protease (e.g. renin).\textsuperscript{28-31}
2.2.4. Pharmacokinetics

2.2.4.1. Absorption

Ritonavir is well absorbed from GI tract (60-70%). But the absorption is highly variable peak plasma concentrations are attained within 2–4 hours (fasting). Bioavailability following administration of ritonavir capsules is the same as that following administration of the oral solution.

2.2.4.2. Distribution

Ritonavir is more lipophilic with >98-99% bound to plasma proteins. Its volume of distribution is 20-40 L and CSF: plasma ratio is 0.0 to 0.52.

2.2.4.3. Metabolism

Ritonavir is majorly metabolized in liver. Five metabolites have been identified. The isopropylthiazole oxidation metabolite (M-2) is the major metabolite and has antiviral activity similar to that of ritonavir, however, plasma concentrations are low. The cytochrome P450 enzymes CYP3A and CYP2D6 are primarily involved in the metabolism of ritonavir.\textsuperscript{32}

2.2.4.4. Excretion

Approximately 86% of the dose is excreted in the feces, with approximately 34% excreted as unchanged drug. 11% of the dose is excreted in urine with approximately 4% excreted as unchanged drug.
2.2.5. Indications and usage

Ritonavir is indicated in combination with nucleoside analogs or as monotherapy for the treatment of HIV infection or AIDS.

2.2.6. Dosage and administration

The recommended dosage of ritonavir is 600 mg twice daily by mouth to be taken with meals. 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 not less than 300 mg twice daily and increased at 2 to 3 day intervals by 100 mg twice daily. The maximum dose of 600 mg twice daily should not be exceeded upon completion of the titration.

The recommended dosage of ritonavir in children > 1 month is 350 to 400 mg/m² twice daily by mouth to be taken with meals and should not exceed 600 mg/m² twice daily. Ritonavir should be started at 250 mg/m² and increased at 2 to 3 day intervals by 50 mg/m² twice daily. If patients do not tolerate 400 mg/m² 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.

2.2.7. Adverse reactions

Ritonavir can cause severe side effects. These include liver problems, pancreatitis (inflammation of the pancreas), heart rhythm problems, severe allergic reactions, and life threatening drug
interactions. Other possible side effects of ritonavir include increase in cholesterol and triglycerides, diabetes and high blood sugar, changes in the immune system, changes in body fat, increased bleeding in people with haemophilia.

2.2.8. Drug interactions\textsuperscript{26, 27, 33}

Ritonavir increases clarithromycin, desipramine, ketoconazole, sildenafil concentrations, so reduction in the dose of these drugs is recommended. Ritonavir may result in decreased didanosine, ethinyl estradiol, methadone, rifampin, theophylline concentrations. Dosing of these drugs is to be adjusted when administered with ritonavir. Ritonavir may result in a disulfiram reaction due to ethanol in formulation of soft gelatin capsules and oral solution. Co-administration of ritonavir with saquinavir improves the bioavailability of saquinavir.

2.2.9. Food interactions

Administration with food delays time to peak plasma concentration by 2 hrs. Compared with administration in the fasting state, extent of absorption was increased 13\% when ritonavir capsules were administered with a meal (615 Kcal, 14.5\% fat, 9\% protein, 76\% carbohydrate).

Compared with administration in the fasting state, extent of absorption was decreased 7\% when ritonavir oral solution was administered with a meal.
2.2.10. Contraindications

Ritonavir is contraindicated in patients with known hypersensitivity (e.g. toxic epidermal necrolysis (TEN) or Stevens-Johnson syndrome) to ritonavir or any of its ingredients. Co-administration of ritonavir with several classes of drugs (including sedative hypnotics, antiarrhythmics, or ergot alkaloid preparations) is contraindicated and may result in potentially serious and/or life-threatening adverse events due to possible effects of ritonavir on the hepatic metabolism of these drugs.

2.2.11. Available dosage forms

Ritonavir is available in the form of tablets, capsules (100 mg) and also in the form of oral solution (80 mg/mL).\(^3\)

2.3. Past work carried out on saquinavir and ritonavir for the improvement of its solubility, dissolution rate and bioavailability

Boudad et al. aimed to prepare and characterize hydroxy propyl \(\beta\) cyclodextrin and saquinavir (SQV) inclusion complex with the purpose of incorporating this complex into poly (alkylcyanoacrylate) nanoparticles in order to increase the drug loading. HP\(\beta\)CD and saquinavir complex was characterized by thermal (differential scanning calorimetry), crystallographic (X-ray diffractography) and spectroscopic methods (circular dichroism, \(H^1\)-NMR). Nanoparticles were prepared by polymerization of alkylcyanoacrylate monomers (isobutyl and isohexyl cyanoacrylate) in a water solution of the complex and further
characterized. This study found that large amounts of cyclodextrins remained associated with the particles, resulting in a 20 fold increase in saquinavir loading compared to nanoparticles prepared in absence of cyclodextrins. Finally, it was suggested that preparing saquinavir-loaded nanoparticles in the presence of drug cyclodextrin complex would be considered as a valuable tool for improving the delivery of saquinavir. They concluded that, the present formulation would be having a potential for improving saquinavir bioavailability and simultaneously reducing the oral dosing of saquinavir in HIV infected patients.\textsuperscript{34}

Vyas \textit{et al.} developed nanoemulsion formulations to improve the oral bioavailability and brain transport of saquinavir. The mixture of oil (1 mL) and drug (saquinavir 400 µg/mL) was stirred homogenously to evaporate ethanol completely. The oil phase was added to aqueous phase (deionised water containing 120 mg egg phosphatidyl choline and 40 mg deoxycholic acid) with further sonication. They evaluated the enhancement in oral bioavailability and brain distribution of SQV, an anti-HIV protease inhibitor, using poly unsaturated fatty acid (PUFA-rich) oil containing nanoemulsion formulations. Both flax seed and safflower oil containing nanoemulsions, formulated with deoxycholic acid, improved the oral bioavailability and brain uptake of SQV as compared to the aqueous suspension formulation. Overall, the results of this study showed tremendous promise of nanoemulsions, made with (PUFA-rich) oils, for enhancing oral bioavailability and
efficient brain delivery of anti-HIV drugs. They concluded that this strategy would be helpful in reducing viral load.\textsuperscript{35}

Buchnan \textit{et al.} evaluated the ability of hydroxyl butenyl \(\beta\) cyclodextrin (HBen\(\beta\)CD) to enhance saquinavir \textit{in vitro} solubility and \textit{in vivo} oral bioavailability, both the base and mesylate salt forms of saquinavir were investigated. In this study, they had shown that saquinavir solubility in aqueous media can be significantly increased by formulating the base or salt form with HBen\(\beta\)CD. Complexation of saquinavir base with HBen\(\beta\)CD in the presence of pH 3.0 tartarate or citrate buffers provided the greatest drug solubility increase. Dissolution of saquinavir and HBen\(\beta\)CD formulations was found to be very rapid in the pH range of 1.2–6.8, and the solubility of the drug in these media was maintained over the time course of the experiments. \textit{In vivo} studies showed rapid absorption of saquinavir but rapidly eliminated. They also observed rapid saquinavir absorption and elimination after oral administration of saquinavir base HBen\(\beta\)CD powder filled capsules. Saquinavir transport assays in Caco-2 cells showed that there was no inhibition of P-gp or influenced influx pathways by HBen\(\beta\)CD. The experimental results from these studies are very promising for the potential to improve protease inhibitor therapy. Saquinavir over all oral bioavailability was increased in rats with capsules containing solid saquinavir base and HBen\(\beta\)CD complex than with saquinavir mesylate capsules alone.\textsuperscript{36}
Pathak et al. investigated in depth the combined effect of pH control and cyclodextrin complexation on saquinavir solubilization by formulating saquinavir complex with methyl β cyclodextrin (MβCD) and evaluated in vitro and in vivo oral absorption. Optimized formulation of saquinavir and MβCD inclusion complex was studied in Wistar rats after intravenous and oral administrations. Significant increase in oral bioavailability with reduced variation was observed along with increased AUC and $C_{\text{max}}$. The solubilization ability and P-gp inhibitory activity of MβCD resulted in significant improvement in pharmacokinetic profile of SQV. The results suggested the potential use of MβCD for improving the gastrointestinal absorption of saquinavir as oral preparations.

Sinha et al. has carried out study on solid dispersion as an approach for bioavailability enhancement of poorly water soluble drug ritonavir. 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 was characterized for differential scanning calorimetry (DSC), X ray diffractometry, scanning electron microscopy, and FTIR. Oral bioavailability of 10 mg of ritonavir in solid dispersion prepared by solvent evaporation and melt method 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 performed in 0.1 N HCl and biorelevant media showed enhanced dissolution rate as compared to pure drug in both FeSSIF media and 0.1N HCl. In vivo studies showed enhanced absorption with solvent evaporated solid dispersion than prepared with melt method and pure drug. 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.

Chowdary et al. formulated solid dispersions using starch phosphate, a new modified starch as a carrier 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. 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, 1:1, 1:2, 1:3 and 1:9 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 of ritonavir was observed with solid dispersions at 1:3 and 1:9 ratios 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 prepared at 1:2 and 1:3 ratios 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 was observed with tablet formulations containing solid dispersions respectively when compared to plain tablets.$^{39}$

Ingunn Tho et al. conducted a study to characterize the aqueous dispersions of ritonavir melt extrudates. Melt extrudates with and without ritonavir were studied. The drug containing extrudate was confirmed to be molecular dispersions of drug in a polymer/surfactant matrix. Particulate dispersions were formed in water from both drug and placebo extrudates. The dispersions were investigated with respect to mean particle size and particle size distribution (photon correlation spectroscopy and optical particle counting), surface charge (zeta potential), particle composition (ultracentrifugation), tendency to form aggregates and precipitate (turbidity), in vitro dissolution rate and drug release. It was concluded that dispersion of melt extrudates in aqueous medium give rise to nano/micro-dispersions. The stability of the nano/micro dispersion is sensitive to anions and may be subjected to association/aggregation/flocculation as time proceeds after preparation of dispersion. Melt extrudate showed improved dissolution rate and drug release properties compared to crystalline raw material.$^{40}$

However there are no reports on comparative studies with different cyclodextrin derivatives and different methods of preparation of saquinavir complexes and finding out best suited method and best
cyclodextrin for improving drug dissolution and bioavailability. No reports were observed for ritonavir solubility and dissolution rate enhancement with cyclodextrins. Hence in the present investigation, this principle is implemented to find out ideal cyclodextrin and best suitable method of preparation for improving dissolution rate and bioavailability of both the drugs.

2.4. **In vitro estimation of saquinavir and ritonavir**

The methods available for the estimation of saquinavir and ritonavir reported in the literature are UV-visible spectrophotometric methods,\textsuperscript{41,42} high performance liquid chromatography methods\textsuperscript{43-48} (HPLC) and liquid chromatographic-mass spectrometric assay\textsuperscript{49-51} (LC-MS).

2.4.1. **Estimation of saquinavir and ritonavir in the present investigation**

In the present investigation, samples of saquinavir and ritonavir were estimated by UV-visible spectrophotometric method.\textsuperscript{41,42}

2.4.1.1. **Preparation of stock and standard solution of saquinavir and ritonavir**

100 mg of pure drug was accurately weighed and transferred into the 100 mL volumetric flask and dissolved with 5 ml of methanol. Saquinavir is estimated in pH 6.8 phosphate buffer and ritonavir is estimated using 0.1N HCl. The volume was made up using corresponding buffers to contain 1 mg/mL as stock solution. A series of dilutions were made from the stock solution to get 2, 4, 6, 8, 10, and 12 µg/mL solutions using pH 6.8 phosphate buffer as dilution medium.
for saquinavir. For ritonavir, series of dilutions were made to get 10, 20, 30, 40, 50, and 60 µg/mL solution using 0.1N HCl as dilution medium. The absorbance of these solutions was measured against respective buffers as blank in UV spectrophotometer (Model AX120, M/s. Shimadzu Corporation, Japan) at 240 nm. All the estimations were done in triplicate and average values are reported.

2.4.1.2. Interference study

The interference in the above method by the cyclodextrins used in the present work was studied by analyzing drug cyclodextrin mixture. For this study, an accurately weighed amount of both the drugs and CDs were mixed thoroughly (1:1 ratio). The mixture equivalent to 25 mg of drug was shaken with methanol (25 mL) for 15 minutes. The solution was then sufficiently diluted with pH 6.8 phosphate buffer for estimating absorbance against pH 6.8 phosphate buffer as blank for saquinavir and for ritonavir, 0.1N HCl was used as dilution medium and also as blank. The procedure was done in triplicate.

2.4.1.3. Results and discussion

The method obeyed Beer’s law in the concentration range of 2-12 µg/mL for saquinavir and 10-60 µg/mL for ritonavir. The ‘r’ value was found to be >0.999 for both the drugs, which indicated a positive correlation between concentration of the drugs and the corresponding absorbance values. The concentrations of both drugs and the corresponding absorbances are given in Table 2.1 and Table 2.2. The
standard curve of saquinavir is shown in Fig. 2.1 and ritonavir is shown in Fig. 2.2. Thus the methods were found to be suitable in the present investigation for the estimation of saquinavir and ritonavir contents in various products and in vitro dissolution studies.

**Table 2.1: Absorbance vs. concentration of saquinavir in pH 6.8 phosphate buffer (mean±s.d.) (n=3)**

<table>
<thead>
<tr>
<th>Concentration in µg/mL</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.139±0.001</td>
</tr>
<tr>
<td>4</td>
<td>0.268±0.002</td>
</tr>
<tr>
<td>6</td>
<td>0.409±0.001</td>
</tr>
<tr>
<td>8</td>
<td>0.524±0.002</td>
</tr>
<tr>
<td>10</td>
<td>0.658±0.001</td>
</tr>
<tr>
<td>12</td>
<td>0.802±0.0009</td>
</tr>
</tbody>
</table>

\[ y = 0.0661x + 0.0036 \]
\[ r = 0.9995 \]

**Figure 2.1: Calibration curve of saquinavir in pH 6.8 phosphate buffer**
Table 2.2: Absorbance vs. concentration of ritonavir in 0.1N HCl (mean±s.d.) (n=3)

<table>
<thead>
<tr>
<th>Concentration in µg/ml</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.170±0.001</td>
</tr>
<tr>
<td>20</td>
<td>0.302±0.004</td>
</tr>
<tr>
<td>30</td>
<td>0.460±0.002</td>
</tr>
<tr>
<td>40</td>
<td>0.614±0.001</td>
</tr>
<tr>
<td>50</td>
<td>0.764±0.001</td>
</tr>
<tr>
<td>60</td>
<td>0.958±0.0009</td>
</tr>
</tbody>
</table>

Figure 2.2: Calibration curve of ritonavir in 0.1N HCl

2.4.1.4 Interference studies

Interference studies showed that there was no interference between each drug and CDs as the %CV was found to be very low (<0.5) and results are mentioned in Table 2.3 and Table 2.4.
Table 2.3: Amount of SQV estimated in interference studies (mean±s.d.) (n=3)

<table>
<thead>
<tr>
<th>Cyclodextrins</th>
<th>Amount of SQV added (mg)</th>
<th>Amount of SQV estimated (mg)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>βCD</td>
<td>25</td>
<td>24.89±0.05</td>
<td>0.2</td>
</tr>
<tr>
<td>HPβCD</td>
<td>25</td>
<td>24.90±0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>RMβCD</td>
<td>25</td>
<td>24.85±0.023</td>
<td>0.09</td>
</tr>
<tr>
<td>SBE7βCD</td>
<td>25</td>
<td>24.92±0.01</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Table 2.4: Amount of RTV estimated in interference studies (mean±s.d.) (n=3)

<table>
<thead>
<tr>
<th>Cyclodextrins</th>
<th>Amount of RTV added (mg)</th>
<th>Amount of SQV estimated (mg)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPβCD</td>
<td>25</td>
<td>24.78±0.11</td>
<td>0.443</td>
</tr>
<tr>
<td>RMβCD</td>
<td>25</td>
<td>24.74±0.12</td>
<td>0.485</td>
</tr>
</tbody>
</table>

2.5. In vivo estimation of saquinavir and ritonavir

Several methods have been reported to determine a number of HIV protease inhibitors (PIs) in human plasma by liquid chromatography coupled to mass spectrometry. In the present investigation, saquinavir and ritonavir were determined by using LC-MS/MS.

This method was developed as per literature by Nano Bio Labs Pvt. Ltd., Bangalore, where the analysis of SQV and RTV in rat plasma samples was carried out.

For detection, Atmospheric pressure ionization (API) 4000 triple quadrupole mass spectrometer detector equipped with turbo ion spray source and operated in the positive mode. Analysis was performed with electrospray ionization using a turbo ion spray ionization source. The
turbo ion spray ionization source was operated at 550°C with an ionization voltage of 5500 V with ultrahigh-purity nitrogen as curtain gas 60 PSI, nebulizer gas (40 PSI) and auxiliary gas (40 PSI). Nitrogen was used as collision activated dissociation (CAD) gas and was set at 6L/hr. Quantification was performed using multiple reaction monitoring (MRM) mode based on the precursor m/z and its fragment m/z (MRM transition) for each analyte. Analyst 1.5.2 software was used for system operation and data handling. Samples were chromatographed on a Waters BEH C18 (Ethylene bridged Hybrid particles with 1.7 µm particle size), 50 x 2.1mm (length x dia) column. The column temperature was maintained at 35°C and peltier temperature was maintained at 10°C.

Formic acid buffer (0.1%) and acetonitrile in the ratio of 50:50 v/v were used as mobile phase. The mobile phase components were filtered before use through a 0.45 µm membrane filter and pumped isocratically at a flow rate of 1.0 mL/min. The volume of the sample analyzed was 10 µL. The drug concentrations (analytes) were detected by monitoring the transactions 675.5 ± 0.5 amu to 570.5 ± 0.5 amu and 721.5 ± 0.5 amu to 296.30 ± 0.5 amu with collision energy of 4 and 27 V for both the drugs and lopinavir which was used as internal standard (IS) respectively. The analytical time for each run was 3 min in total.
2.5.1. Preparation of stock and working standard solutions of SQV and RTV

A stock solution (1000 µg/mL) of both the drugs was prepared by dissolving 10 mg in 10 ml volumetric flask with methanol. 1ml of this stock solution was taken in 50 mL volumetric flask and made up to 50 mL with methanol to give 20 µg/mL (intermediate stock solution). Intermediate stock solution was subsequently diluted with methanol to obtain working standard solutions of (0.5, 1, 2, 4, 6, 10 and 15 µg/mL).

2.5.2. Preparation of stock solution of internal standard (IS)

Lopinavir was used as internal standard. 25 mg of lopinavir was dissolved in methanol in 25 mL volumetric flask to get stock solution of 1000 µg/mL. IS stock solution was further diluted with the acetonitrile and water with 0.1% formic acid to obtain 500 ng/mL.

2.5.3. Preparation of Quality Control samples (QC)

Quality control samples were prepared by using the same procedure as mentioned in Sec. 2.5.1 but from a separately weighed stock solution. Intermediate stock solution containing 20 µg/mL was diluted with methanol to obtain solutions of 1, 4, 10 µg/mL.

2.5.4. Preparation of plasma standard solutions

Plasma calibration curve standard solutions (50, 100, 200, 400, 600, 1000 and 1500 ng/mL) and quality control samples 100 ng/mL as low quality control (LQC), 400 ng/mL as medium quality control (MQC) and 1000 ng/mL as high quality control (HQC) were prepared by taking 100 µL of drug free rat plasma in vials and 10 µL of drug working
standard solutions. The samples were vortex mixed to ensure complete mixing of the contents and 100 µL of samples were distributed into empty vials, labelled and stored in deep freezer until analysis.

2.5.5. Data analysis

Data acquisition and processing was performed by Analyst 1.5.2 software. Standard curves were constructed using linear regression for saquinavir and for ritonavir using 1/concentration² weighted quadratic regression of peak area: IS ratio vs. target concentration and drug content of unknown samples were interpolated. Based on the standard curve generated, unknown concentrations were calculated using Analyst software.

In addition, coefficient of variation values were also determined using Eq.2.1.

\[
\%CV = \frac{\text{s.d.}}{\text{mean}} \times 100
\]

- Eq.2.1

2.5.6. Assessment of performance characteristics

Quality control standards were prepared and analyzed in triplicate in three independent runs. Standard curves were constructed for each drug using the ratio of the observed peak area for each antiretroviral to the IS. Unknown concentrations were computed from the linear regression equation of the peak area ratio against the concentration of each antiretroviral. To assess linearity, deviations of the mean calculated concentrations over three runs should be within
±15% from nominal concentrations for the non-zero (blank) calibration standards.

**2.5.6.1 Accuracy**

Accuracy was determined for quality control samples performed for both the drugs. Accuracy was measured as the percentage deviation from the nominal concentrations.

**2.5.6.2 Recovery**

Recovery was calculated as the extraction yield obtained from spiked drug free plasma samples. It was performed by comparing the analytical results for extracted quality control samples at three different concentrations (low, medium, high concentrations) with aqueous standards.

**2.5.6.3. Limit of quantitation (LOQ) and limit of detection (LOD)**

The LOQ was defined as the lowest concentration such as the deviation between the measured and nominal concentration was less than 20% CV, as determined in three separate analytical runs.

The LOD was the lowest concentration that the bioanalytical procedure can reliably differentiate from ratio of signal to noise (S/N) bigger than 3.0 in the chromatogram. LOQ was set was set to S/N more than 10.0.

**2.5.7. Results and discussion**

The chromatographic conditions and sample preparation for the proposed method were optimized to suit the preclinical
pharmacokinetic studies. Interferences from biomatrix components like plasma proteins were observed, and therefore, separation of analytes from matrix components was a key issue during method development process. Among the several columns evaluated, a Waters BEH C18 column was chosen because a good peak shape and acceptable retention times were obtained and it gives widest usable pH range (pH 1-12), superior low pH stability for high sensitivity MS applications. Different mobile phases comprising several combinations of aqueous and organic solvents (viz. methanol and acetonitrile) were tested to provide sufficient resolution between analyte and IS. The effective separation of the bands in the chromatogram was achieved when the mobile phase composition was 50% of water containing 0.1% formic acid and 50% acetonitrile (1:1 ratio).

Lopinavir was chosen as internal standard because it showed similar chromatographic behaviour to both SQV, RTV with no interference with both saquinavir and ritonavir by admixture in rat plasma. The chromatograms of SQV, RTV were clearly distinguishable and retention times of SQV, RTV and LPV were found to be 0.73, 1.96 and 2.39 min respectively, under specified chromatographic conditions.

A summary of parent and daughter ions of all three protease inhibitors and their collision energies used to produce these ions are represented in Table 2.5. Corresponding mass spectrum of parent molecule and daughter ion are shown in Fig.2.3. The percentage recovery of the quality control samples for both SQV and RTV is shown
in Table 2.6. The percentage recoveries of SQV and RTV in plasma concentrations of 100, 400, 1000 ng/mL were 94±1, 89.08±2.55, 86.23±1.45 for SQV and 103.67±5.13, 98.5±0.25 and 99.9±3.59 for RTV.

The correlation coefficients (r) of the calibration curves greater than 0.99 for all seven analytes as determined by linear regression analysis for saquinavir and for ritonavir, with a 1/x^2 regression, where x is the concentration, over a concentration range of 50-1500 ng/mL for both the drugs. A representative calibration curve of SQV and RTV (Fig.2.4 and Fig.2.5) for resulted in the linear least squares regression equations, SQV: y=0.000368x+-0.0154, RTV: y=0.000646x+4.14e^-005, where x is the concentration of SQV and RTV (ng/mL) and y is the peak area ratio of both SQV and RTV to the IS (Table 2.7 and 2.8).

Representative spectra and chromatograms of a blank sample and a standard solution extracted from a blank plasma were respectively, illustrated in Fig.2.6 to Fig.2.9 for saquinavir and Fig.2.10 to Fig.2.13 for ritonavir. Typical chromatograms of LQC, MQC and HQC were shown in Fig.2.14 for SQV and Fig.2.15 for RTV.

Lower and upper limits of quantification (LLQ, ULQ) were set as the bottom (level 1) and top (Level 7) points of the standard curve respectively. LOD for both drugs was found to be 20 ng/mL. LLQ and ULQ were found to be 71.7 and 1570 ng/mL (SQV) and 60.9 and 1650 ng/mL (RTV).
Table 2.5: Parent and daughter ions and collision energies used to evaluate saquinavir, ritonavir and IS (lopinavir)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Parent ion (m/z)</th>
<th>Daughter ion (m/z)</th>
<th>Collision energies (CE)</th>
<th>RT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saquinavir</td>
<td>671.5</td>
<td>416.1</td>
<td>433.4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>570.5</td>
<td>1.96</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>721.5</td>
<td>268.6</td>
<td>296.5</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>426.4</td>
<td>0.73</td>
</tr>
<tr>
<td>Lopinavir(IS)</td>
<td>629.8</td>
<td>429.6</td>
<td>447.4</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>611.8</td>
<td>2.39</td>
</tr>
</tbody>
</table>

Table 2.6: Quality controls for the simultaneous determination of saquinavir and ritonavir by LC-MS/MS in rat plasma

<table>
<thead>
<tr>
<th>Drug</th>
<th>QC level</th>
<th>Concentration of drug added (ng/mL)</th>
<th>Mean Target concentration (ng/mL)</th>
<th>%CV</th>
<th>%Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saquinavir</td>
<td>Low</td>
<td>100</td>
<td>94±1</td>
<td>0.78</td>
<td>94±1</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>400</td>
<td>356.33±10.21</td>
<td>2.86</td>
<td>89.08±2.55</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>1000</td>
<td>862.33±14.57</td>
<td>1.68</td>
<td>86.23±1.457</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>Low</td>
<td>100</td>
<td>103.67±5.13</td>
<td>3.59</td>
<td>103.67±5.13</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>400</td>
<td>394±1</td>
<td>0.25</td>
<td>98.5±0.25</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>1000</td>
<td>999±35.93</td>
<td>3.59</td>
<td>99.9±3.59</td>
</tr>
</tbody>
</table>

Data are expressed as (mean±s.d.)(n=3); Percentage recovery = (peak area extracted from plasma/peak area from directly injected solution)*100.
Fig. 2.3. Parent molecule and daughter ion mass spectrum of A) ritonavir B) saquinavir C) lopinavir
**Table 2.7: Concentration vs. peak area ratio for SQV in rat plasma**

<table>
<thead>
<tr>
<th>Concentration of SQV (ng/ml)</th>
<th>Peak area</th>
<th>Ratio</th>
<th>%Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SQV</td>
<td>LPV(IS)</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>12900</td>
<td>914000</td>
<td>0.0141</td>
</tr>
<tr>
<td>100</td>
<td>25100</td>
<td>918000</td>
<td>0.0273</td>
</tr>
<tr>
<td>200</td>
<td>61200</td>
<td>947000</td>
<td>0.0646</td>
</tr>
<tr>
<td>400</td>
<td>117000</td>
<td>902000</td>
<td>0.1291</td>
</tr>
<tr>
<td>600</td>
<td>177000</td>
<td>902000</td>
<td>0.1962</td>
</tr>
<tr>
<td>1000</td>
<td>288000</td>
<td>903000</td>
<td>0.3189</td>
</tr>
<tr>
<td>1500</td>
<td>523000</td>
<td>929000</td>
<td>0.5629</td>
</tr>
</tbody>
</table>

**Fig. 2.4: Calibration curve of SQV in rat plasma**

\[ y = 0.0004x - 0.0146 \ (r = 0.9954) \]
Table 2.8: Concentration vs. peak area ratio of RTV in rat plasma

<table>
<thead>
<tr>
<th>Concentration of RTV (ng/ml)</th>
<th>Peak area</th>
<th>Ratio</th>
<th>%Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RTV</td>
<td>LPV (IS)</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>36200</td>
<td>918000</td>
<td>0.0394</td>
</tr>
<tr>
<td>100</td>
<td>61400</td>
<td>915000</td>
<td>0.0671</td>
</tr>
<tr>
<td>200</td>
<td>114000</td>
<td>946000</td>
<td>0.1205</td>
</tr>
<tr>
<td>400</td>
<td>232000</td>
<td>900000</td>
<td>0.2577</td>
</tr>
<tr>
<td>600</td>
<td>336000</td>
<td>904000</td>
<td>0.3716</td>
</tr>
<tr>
<td>1000</td>
<td>568000</td>
<td>901000</td>
<td>0.6304</td>
</tr>
<tr>
<td>1500</td>
<td>993000</td>
<td>932000</td>
<td>1.0654</td>
</tr>
</tbody>
</table>

Fig. 2.5: Calibration curve of RTV in rat plasma

\[ y = 0.0007x - 0.0172 \ (r = 0.9967) \]
Fig. 2.6: LCMS Spectra of A) SQV B) LPV (IS) in aqueous blank
Fig. 2.7: LCMS spectra of A) SQV B) LPV (IS) in blank plasma
Fig. 2.8: LCMS chromatograms of SQV for standards at four different concentrations A) 50 B) 100 C) 200 D) 400 ng/mL
Fig. 2.9: LCMS chromatograms of SQV for standards at three different concentrations A) 600 B) 1000 C) 1500 ng/mL
Fig. 2.10: LCMS Spectra of A) RTV B) LPV (IS) in aqueous blank
Fig. 2.11: LCMS Spectra of A) RTV and B) LPV (IS) in blank plasma
Fig. 2.12: LCMS chromatograms of RTV for standards at four different concentrations A) 50 B) 100 C) 200 D) 400 ng/mL
Fig. 2.13: LCMS chromatograms of RTV for standards at three different concentrations A) 600 B) 1000 C) 1500 ng/mL
Fig. 2.14: LCMS chromatograms of SQV for quality controls at three different concentrations 100, 400, 1000 ng/mL A) LQC B) MQC C) HQC
Fig. 2.15: LCMS chromatograms of RTV for quality controls at three different concentrations 100, 400, 1000 ng/mL
A) LQC  B) MQC  C) HQC
2.5.8. Conclusion

The assay method developed as per old methods\textsuperscript{49-51} to determine protease inhibitors SQV and RTV by LC-MS/MS has been optimized. The results of this study indicated that the method was sensitive and accurate and this assay can be used to successfully determine PK profiles. Ranges of standard curves were chosen to encompass the broad spectrum of drug concentrations likely to be encountered in PK studies. This assay requires small volume of plasma for analysis (100µL) which is advantageous when measuring PI concentrations in PK studies. The method combines short run time of 3 min per sample with a quick and simple extraction procedure allowing a large number samples to be processed quickly and efficiently.
2.6. β-Cyclodextrin\textsuperscript{58,59}

2.6.1. Structure

![Diagram of β-Cyclodextrin structure]

Molecular weight: 1135
Molecular formula: C\(_{42}H\sb{70}O_{35}\)

2.6.2. Synonyms
Caraway, Cycloheptaamylose, Cyclomaltoheptaose, Schardinger β Dextrin, Kleptose, Betadex.

2.6.3. CAS Registry
7585-39-9

2.6.4. Properties

It is white, almost odorless, slightly sweet tasting and crystalline powder. Its water solubility is 1.8 g/L. Its melting point is 255-265°C. It is having maximum of 14% moisture content.
2.6.5. Production process

A non-reducing cyclic saccharide consisting of seven alpha-1, 4-D glucopyranosyl units manufactured by the action of cyclodextrin transglycolase on hydrolysed starch followed by purification of the β CD. Purification is done by preparation of a βCD/solvent inclusion compound followed by steam stripping of the solvent before final purification.

2.6.6. Regulatory status

It is listed in a number of pharmacopoeial sources, including the United States Pharmacopoeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP). It is also listed in the Generally Regarded as Safe (GRAS) list of the US FDA93 for use as a food additive.

2.6.7. Stability

It is stable in the solid state if protected from high humidity.

2.6.8. Safety

It is regarded as nontoxic and non irritant material. However, when administered parenterally, β cyclodextrin is not metabolized but accumulates in the kidneys as insoluble cholesterol complexes, resulting in severe nephrotoxicity.

2.6.9. Applications

β cyclodextrins are used to improve solubility, stability, compressibility, organoleptic properties and to improve bioavailability.
2.7. Hydroxy propyl β cyclodextrin\textsuperscript{58,59}

2.7.1 Structure

\begin{center}
\includegraphics[width=0.5\textwidth]{hydroxy-propyl-beta-cyclodextrin.png}
\end{center}

Molecular weight: 1380.

Molecular Formula: \((\text{C}_{6}\text{H}_{10}\text{O}_{5})_7\text{(C}_{4}\text{H}_{6}\text{O})_{4.5}\)

2.7.2. Synonym

Kleptose HPB.

2.7.3. CAS Registry

128446-35-5.

2.7.4. Properties

It is white amorphous powder. It is soluble in water up to 65% at 25°C and 80% at 50°C. It is having moisture content of maximum 5%.

2.7.5. Production Process

Starch is transformed into βCD after enzymatic hydrolysis and cyclization followed by purification. Hydroxypropylation is then performed, followed by purifications and spray drying. The number of
moles of hydroxylpropyl group per anhydrous glucose unit is called the Molar Substitution (MS) and characterizes the product.

2.7.6. Regulatory Status

Compliant with EP and USP monographs.

2.7.7. Stability

Powder form is stable as per ICH stability study. Solution form is stable to hydrolysis, sterilization, to freezing.

2.7.8. Safety profile

It is having reduced haemolytic potential making it suitable for oral and parenteral applications.

2.7.9. Applications

HPβCD is suitable for parenteral use, syrups, solutions and oral suspensions as well as for dry formulations (with possibility of in situ encapsulation). It is used to increase water solubility of poorly soluble drugs, to increase bioavailability and to improve organoleptic properties of bitter or unpleasant actives. It is suitable for encapsulation of volatile compounds and to increase shelf life of expensive flavours.
2.8. Randomly methylated β cyclodextrin\textsuperscript{58}

2.8.1. Structure

\begin{center}
\includegraphics[width=0.5\textwidth]{structure.png}
\end{center}

Molecular formula: C\textsubscript{54}H\textsubscript{94}O\textsubscript{35}

Molecular weight: 1312.

2.8.2. Synonyms

2-O-methyl beta cyclodextrins, Kleptose Crysmeb, Methyl β cyclodextrin, Methyl B Cyclodextrin, Methyl SS Cyclodextrin, Dimethyl β Cyclodextrin.

2.8.3. CAS Registry

128446-36-6.

2.8.4. Properties

It is an anhydrous white powder with the ability to crystallize as rod-like, colourless crystals, containing 13% water, when crystallized from water. Its aqueous solubility is 20% at 20°C and 65% at 75°C. It is easily soluble in water and its solubility increases with temperature.
2.8.5. Production process

It is produced by specific methylation of βCD, followed by filtration and purification steps. The purified product is subjected to demineralization. The final product is concentrated and dried.

2.8.6. Regulatory status

It is not yet official in compendia but efforts are being made to enter in the future.

2.8.7. Stability

Kleptose Crysmeb is very stable in neutral and alkaline solutions and is stable when exposed to oxygen of air.

2.8.8. Safety profile

If administered orally, CD derivatives remain mainly in the GIT until excreted. The oral toxicity is expected to be negligible, to be proven by formal toxicological studies. At a different level, parenteral administered CD derivatives are eventually excreted in urine with a small fraction being able to remain in the kidneys. It could provoke renal toxicity at high doses and further toxicological studies will be needed.

2.8.9. Applications

It is used to increase water solubility and rate of dissolution of poorly soluble drugs, and thus increase their bioavailability, when solubility and rate of dissolution are the limiting factors in bioavailability. This increase can be used to improve both liquid and
solid dosage forms. It is used to increase the rate of transfer of a drug from solution into tissue without damaging the tissue. This is a critical factor for sublingual formulations and suppository dosage forms that potentially replace parenteral formulations for drugs destroyed at first pass by the portal system and dosage forms of drugs for which immediate onset of effect is important. It is also used to reduce side effects of active ingredients, reduce bitterness of unpleasant odor of some drugs. It can be used in cosmetics for dissolution of water insoluble, active ingredients and their transfer into tissues, encapsulation of sensitive compounds can improve their stability, encapsulation of volatile fragrances, so that these are released gradually in contact with skin, encapsulation and solubilization of undesirable water insoluble materials from the skin so these can be washed off and the skin cleaned.
2.9. Sulfo butyl ether β cyclodextrin\textsuperscript{59-61}

2.9.1. Structure

Molecular weight: 2163.
Molecular formula: C\textsubscript{80}H\textsubscript{84}Na\textsubscript{2}O\textsubscript{4}S\textsubscript{2}

2.9.2. Synonyms

β cyclodextrin sulfo butyl ether, sodium salt, Captisol, (SBE)m-beta-CD, SBEC\textsubscript{D}, SBE7βCD.

2.9.3. CAS Registry

182410-00-0

2.9.4. Properties

It is an anionic βCD derivative with a sodium sulfonate salt which is separated from the hydrophobic cavity by a butyl spacer
group. It is a white amorphous powder. It is soluble 1 in less than 2 parts of water, 1 in 30-40 parts of methanol at 25°C. It is hygroscopic solid. It will reversibly take up moisture without any effect on the appearance of the material at humidities upto 60% RH. Equilibration above 60% will result in deliquescence.

2.9.5. Production process

It is prepared by alkylation of β cyclodextrin using 1, 4-butane sultone under basic conditions. Degree of substitution in β cyclodextrin is controlled by the stoichiometric ratio of β cyclodextrin to sultone used in the process.

2.9.6. Regulatory status

It is included in IV and IM injectable products currently approved and marketed in the USA, Europe and Japan. It is included in FDA inactive ingredients database for IM and IV use.

2.9.7. Stability

It is stable in solid state and should be protected from high humidity. It is stable in aqueous solutions at and above pH 1. It can degrade in highly acidic solutions at elevated temperatures.

2.9.8. Safety profile

It is derived from β cyclodextrin, which is nephrotoxic when administered parenterally. However, studies have shown that it is well tolerated at high doses, when administered via i.v bolus injections, oral
and by inhalations. Up to 9 g/day may be administered by i.v. infusion in a licensed voricozole formulation.\textsuperscript{61}

### 2.9.9. Applications

It is widely used for solubilization, dissolution and to improve absorption. It can form non covalent complexes with many types of compounds including small organic molecules, peptides\textsuperscript{62} and proteins.\textsuperscript{63} It can also enhance their solubility\textsuperscript{64,65} and stability\textsuperscript{66-68} in water. The first application of captisol was in injectable preparation.\textsuperscript{69} It can also be used in oral solid,\textsuperscript{70,71} liquid dosage forms,\textsuperscript{72} ophthalmic dosage forms,\textsuperscript{73,74} inhalations and intra nasal formulations. It can function as an osmotic agent and a solubilizer for controlled release delivery\textsuperscript{70} and has anti microbial properties at sufficient concentrations.

Although extensive work was done on βCD and HPβCD, the concept of using different CD derivatives, mainly with RMβCD and SBE7βCD and different methods of preparation for improving the solubility of saquinavir was not reported earlier. On ritonavir, utilization of cyclodextrins in improving dissolution rate was not reported earlier. So, in the present investigation, these derivatives of CDs were tried in enhancing solubility and dissolution rate.


