3.1 Drug and Analytical Profile

A white or almost white powder, practically insoluble in water, very slightly soluble in alcohol, freely soluble in dichloromethane, sparingly soluble in tetrahydrofuran. SPORANOX is the brand name for Itraconazole, a synthetic triazole antifungal agent. Itraconazole is a 1:1:1:1 racemic mixture of four diastereomers (two enantiomeric pairs), each possessing three chiral centers. It may be represented by the following structural formula and nomenclature:

![Structure of Itraconazole]

\[
(\pm)-1-[(R*)-\text{sec-butyl}]-4-[p-\{4-[p-\{2(R^*,4S^*)-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl] methoxy\} phenyl]-1-piperazinyl]phenyl]-2,1,2,4-triazol-5-one\]

Itraconazole has a molecular formula of C\(_{35}\)H\(_{38}\)Cl\(_2\)N\(_8\)O\(_4\) and a molecular weight of 705.64. It is a white to slightly yellowish powder. It is insoluble in water, very slightly soluble in alcohols, and freely soluble in dichloromethane. It has a pKa of 3.70 (based on extrapolation of values obtained from methanolic solutions) and a log (n-octanol/water) partition coefficient of 5.66 at pH 8.1.
3.1.1 Innovator (SPORANOX) Formulation Guidelines

I. Pharmacology

*In vitro* studies have demonstrated that Itraconazole inhibits the cytochrome P450-dependent synthesis of ergosterol, which is a vital component of fungal cell membranes.

a) Pharmacokinetics

The oral bioavailability of SPORANOX capsules is maximal and appears to be more consistent when they are taken immediately after a meal. However, there is a marked inter-subject variability. The observed absolute oral bioavailability of Itraconazole was 55%. If administered in the fasting state, \( C_{\text{max}} \) and AUC are about 30-40% lower than after meal. Peak plasma levels are reached 3 to 5 hours following an oral dose. Elimination from plasma is biphasic with a terminal half-life of 1.5 to 2 days. During chronic administration, steady state is reached after 10-14 days. Mean steady state plasma concentrations of Itraconazole 3-4 hours after drug intake are 0.4 microgram/ml (100 mg o.d.), 1.1 micrograms/ml (200 mg o.d.) and 2.0 micrograms/ml (200 mg b.i.d.). The plasma protein binding of Itraconazole is 99.8%. Concentrations of Itraconazole in whole blood are 60% of those in plasma. Steady state Itraconazole levels in the skin vary according to the distribution of sebaceous glands, ranging from one third of plasma levels in the skin of the palms to double plasma levels in the skin of the back. Itraconazole is eliminated from keratinous tissues by the shedding of cells during normal regeneration. Itraconazole is undetectable in the plasma within 7 days of stopping therapy, but levels at or above the MIC90 for dermatophytes persist in the skin for one or two weeks after discontinuation of a 4-week treatment. Itraconazole is present at high concentrations in sebum but levels in sweat are negligible. Itraconazole is extensively distributed into most tissues that are prone to fungal invasion but only minimally into CSF or ocular fluid. Concentrations in lung, kidney, liver, bone, stomach, spleen and muscle were found to be two to three times higher than the corresponding plasma concentration. Itraconazole is extensively metabolised by the liver into a large number of metabolites. One of the metabolites is hydroxy-Itraconazole, which has a comparable antifungal activity *in vitro* to Itraconazole. Serum antifungal drug levels measured by bioassay were about 3 times those of Itraconazole assayed by high performance liquid chromatography. Faecal excretion of the parent drug varies
between 3-18% of the dose. Renal excretion of the parent drug is less than 0.03% of the dose. About 35% of a dose is excreted as metabolites in the urine within 1 week.

![Sporanox Capsules](image)

**Fig 3.2 Sporanox Capsules (Janssen Pharmaceuticals)**

i) Hepatic Impairment:

A pharmacokinetic study using a single 100mg dose of Itraconazole (one 100mg capsule) was conducted in 6 healthy and 12 cirrhotic subjects. No statistically significant differences in AUC were seen between these two groups. A statistically significant reduction in mean \( C_{\text{max}} \) (47%) and a twofold increase in the elimination half-life (37 ± 17 hours) of Itraconazole were noted in cirrhotic subjects compared with healthy subjects. Patients with impaired hepatic functions should be carefully monitored when taking Itraconazole. The prolonged elimination half-life of Itraconazole observed in hepatic impairment patients (37.2 ± 17 h) should be considered when deciding to initiate therapy with other medications metabolised by CYP3A4.

ii) Renal Impairment

A pharmacokinetic study using a single 200mg dose of Itraconazole (four 50mg capsules) was conducted in three groups of patients with renal impairment (uremic: n=7; hemodialysis: n=7, and continuous ambulatory peritoneal dialysis: n=5). In uremic / hemodialysis and continuous ambulatory peritoneal dialysis subjects, \( C_{\text{max}} \) were reduced compared with normal population parameters and listed below.

\[
C_{\text{max}} 132-417 \text{ (normal)} / 50.9-505 \text{ ng.h/ml (uremic)}
\]

\[
C_{\text{max}} 18.2-341 \text{ (hemodialysis) / 51.7-111 ng.h/ml (continuous ambulatory peritoneal dialysis)}
\]
Plasma concentration-versus-time profiles showed wide inter-subject variation in all three groups.

II. Microbiology

a) *In vitro* Susceptibility Tests, Dilution or diffusion techniques:

Either quantitative (MIC) or breakpoint, should be used following a regulatory updated, recognised and standardised method (eg, Clinical and Laboratory Standard Institute [CLSI formerly NCCLS]). Standardised susceptibility test procedures require the use of laboratory control microorganisms to control the technical aspects of the laboratory procedures.

For Itraconazole, breakpoints have only been established for *Candida* spp. from superficial mycotic infections (CLSI M27-A2, using laboratory controlled *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258). The proposed MIC breakpoints are as follows:

Susceptible: A report of “Susceptible” indicates that the pathogen is likely to be inhibited if the antifungal compound in the blood reaches the concentrations usually achievable.

Susceptibility that is “dose or delivery-dependent” (S-DD): This category implies possible clinical applicability in body sites where the medicine is physiologically concentrated or in situations where high dosage of medicine can be used.

Itraconazole MIC values for *Aspergillus flavus*, *Aspergillus fumigatus* *Trichosporon* species, *Fonsecaea pedrosoi*, and *Trichophyton* species were reported as ≤ 1 μg/ml, although interpretive breakpoints have not been established for the filamentous fungi.

Resistant: A report of “Resistant” indicates that the pathogen is not likely to be inhibited if the antifungal compound in the blood reaches the concentrations usually achievable; other therapy should be select.

*Candida krusei*, *Candida glabrata* and *Candida tropicalis* are generally the least susceptible *Candida* species, with some isolates showing unequivocal resistance to Itraconazole *in vitro*. 
The principal fungus types that are not inhibited by Itraconazole are Zygomycetes (e.g. Rhizopus spp., Rhizomucor spp., Mucor spp. and Absidia spp.), Fusarium spp., Scedosporium spp. and Scopulariopsis spp.

Azole resistance appears to develop slowly and is often the result of several genetic mutations. Mechanisms that have been described are overexpression of ERG11, which encodes the target enzyme 14α-demethylase, point mutations in ERG11 that lead to decreased target affinity and/or transporter overexpression resulting in increased efflux. Cross-resistance between members of the azole class has been observed within Candida spp., although resistance to one member of the class does not necessarily confer resistance to other azoles. Itraconazole-resistant strains of Aspergillus fumigatus have been reported.

b) Correlation between in vitro MIC results and clinical outcomes:

Susceptibility of a microorganism in vitro does not predict successful therapy. Host factors are often more important than susceptibility test results in determining clinical outcomes, and resistance in vitro should often predict therapeutic failure. Correlation between minimum inhibitory concentration (MIC) results in vitro and clinical outcome has yet to be established for azole antifungal agents.

III. Toxicology

In three toxicology studies using rats, Itraconazole induced bone defects at dosage levels as low as 20 mg/kg/day. The induced defects included reduced bone plate activity, thinning of the zona compacta of the large bones and increased bone fragility. At a dosage level of 80 mg/kg/day over one year or 160 mg/kg/day for six months, Itraconazole induced small tooth pulp with hypocellular appearance in some rats. Increased relative adrenal weights and swollen adrenals (reversible) were seen in rats and dogs where plasma levels were comparable to those of human therapeutic doses. Adrenocortical function was not affected in studies in humans after the recommended daily doses; with higher doses (600 mg/day for 3 months), adrenal cortex response to ACTH stimulation was reduced in 1 of 8 patients, but returned to normal when the dosage was reduced.
IV. Clinical trials

**Histoplasmosis:** In five open-label, non-comparative studies in patients (n = 136) with histoplasmosis exposed to treatment and maintenance therapy with Itraconazole: sixty-one patients (45%) were HIV infected and 8 patients (6%) had other causes of immunosuppression. Ninety-eight patients (72%) had disseminated disease and 42 patients (31%) had other forms of histoplasmosis. Overall, 135 of the 136 patients (approx. 100%) responded. Five patients (4%) relapsed while on treatment. Efficacy was demonstrated for the oral treatment and maintenance therapy of histoplasmosis, both in immunocompromised and non-immunocompromised patients at the recommended dose of 200 - 400 mg/day for 8 months.

**Onychomycosis:** In three double-blind, placebo-controlled studies (n = 214 total), conducted in the US, patients with onychomycosis of the toenails received 200 mg once daily for 12 consecutive weeks. Results of these studies demonstrated mycological cure in 54% of patients, defined as simultaneous occurrence of negative KOH plus negative culture. Thirty-five (35) percent of patients were considered an overall success (mycological cure plus clear or minimal nail involvement with significantly decreased signs); 14% of patients demonstrated mycological cure plus clinical cure (clearance of all signs, with or without residual nail deformity). The mean time to overall success was approximately 10 months. Twenty-one (21) percent of the overall success group has a relapse (worsening of the global score or conversion of KOH or culture from negative to positive).

**Intermittent (pulse) treatment of onychomycosis:** *Onychomycosis of the toe nail:* In a double-blind study (n= 129 total) there was no significant difference in clinical and mycological success and overall response between Itraconazole 200 mg b.i.d. one week per month (pulse) for 3 months and continuous treatment of Itraconazole 200 mg o.d. for 3 months. In an open study (n = 50 total) there was no significant difference in clinical and mycological success and overall response between a 3 pulse and 4 pulse regimen.

*Onychomycosis of the fingernail:* In a double-blind, placebo controlled study (n = 71 total) a treatment of Itraconazole 200 mg b.i.d. one week per month was more effective than placebo. The clinical and mycological success for Itraconazole pulse treatment in
compliant patients was 77% and 73% respectively and for placebo was nil and 12%. In an open study 84% of patients receiving 2 pulse treatments (n = 48) and 91% receiving 3 pulse treatments (n = 68) showed a clinical success and 77% and 85% respectively showed a mycological cure at endpoint.

**Aspergillosis:** In nine open-label studies of patients (n = 719) with systemic aspergillosis and treated with Itraconazole, an overall response rate of 63% was observed. This varied according to the clinical syndrome, e.g. pulmonary aspergilloma (60%), bronchopulmonary (78%), invasive (62%) and extra-pulmonary (62%). In eight patients with cerebral aspergillosis the response rate was 13%. In a randomised, double-blind, comparator trial against amphotericin B in patients with proven or highly suspected aspergillosis, 6 of 8 patients receiving Itraconazole responded and 2 of 5 patients responded on amphotericin B. The numbers are too small to assert any difference between treatments. The recommended dose for systemic aspergillosis is 200 mg/day for 2 - 5 months, with a dose of 200 mg twice daily for invasive or disseminated disease.

**Sporotrichosis:** In four open-label, non-comparative studies of patients (n = 124) with sporotrichosis, 115 of 124 patients (93%) treated with Itraconazole demonstrated a complete or marked remission rate. The recommended dosage is 100 - 200 mg/day for 3 months. Treatment duration may be longer in patients with lymphatic/lymphocutaneous and extracutaneous sporotrichosis.

**Candidiasis:** In three open-label studies of patients (n = 143) with systemic candidiasis and treated with Itraconazole, patients with urinary and pulmonary candidiasis responded with high efficacy, although the numbers with these conditions were small. An 85% response rate was observed in patients with oral and oesophageal candidiasis who had underlying cancer and were receiving chemotherapy and/or antibiotics or who had HIV/AIDS. In non-neutropenic patients with non-invasive candidiasis the response rate was 76%. The recommended dose is 100 - 200 mg/day for 3 weeks to 7 months.

**V. CONTRAINDICATIONS**

Co-administration of the following drugs is contraindicated with SPORANOX capsule: terfenadine, astemizole, bepridil, nisoldipine, mizolastine, cisapride, doftetilide, levacetylmethadol (levomethadyl), quinidine, pimozide, sertindole, CYP3A4-
metabolised HMG-CoA reductase inhibitors such as simvastatin and lovastatin, oral midazolam, triazolam and ergot alkaloids such as dihydroergotamine, ergometrine (ergonovine), ergotamine and methylergometrine.

Serious cardiovascular adverse events, including death, ventricular tachycardia and torsades de pointes have been observed in patients taking Itraconazole concomitantly with terfenadine, due to increased terfenadine concentrations induced by Itraconazole.

Pharmacokinetic data indicates that another oral antifungal, ketoconazole, inhibits the metabolism of astemizole, resulting in elevated plasma levels of astemizole and its active metabolite desmethylastemizole, which may prolong QT intervals. In vitro data suggests that Itraconazole, when compared to ketoconazole, has a less pronounced effect on the biotransformation system responsible for the metabolism of astemizole. Based on the chemical resemblance of Itraconazole and ketoconazole, co-administration of astemizole with Itraconazole is contraindicated.

Pharmacokinetic data indicates that oral ketoconazole potently inhibits the metabolism of cisapride resulting in an eight-fold increase in the mean AUC of cisapride. Data suggest that co-administration of oral ketoconazole and cisapride can result in prolongation of the QT interval on the ECG. In vitro data suggest that Itraconazole also markedly inhibits the biotransformation system mainly responsible for the metabolism of cisapride; therefore concomitant administration of Itraconazole with cisapride is contraindicated.

Co-administration of Itraconazole with oral midazolam or triazolam has resulted in elevated plasma concentrations of the latter two drugs. This may potentiate and prolong hypnotic and sedative effects. These agents should not be used in patients treated with Itraconazole. If midazolam is administered parenterally, special precaution is required since the sedative effects may be prolonged.

SPORANOX capsules are contraindicated in patients with a known hypersensitivity to the drug or its excipients. There is no information regarding cross hypersensitivity between Itraconazole and otherazole antifungal agents. Caution should be used in prescribing Itraconazole to patients with hypersensitivity to other azoles.
SPORANOX capsules should not be administered to patients with evidence of ventricular dysfunction such as congestive heart failure (CHF) or a history of CHF except for the treatment of life-threatenning or other serious infections.

Itraconazole is contraindicated in pregnant women except for the treatment of life-threatening cases of systemic mycoses, where the potential benefits outweigh the potential harm to the foetus. Adequate contraceptive precautions should be taken by women of childbearing potential throughout Itraconazole therapy, and continued until the next menstrual period following the completion of Itraconazole therapy.

VI. PRECAUTIONS

Peripheral neuropathy:

Isolated cases of peripheral neuropathy have also been reported, predominantly during long-term treatment with Itraconazole. If neuropathy occurs that may be attributable to Itraconazole, the treatment should be discontinued.

Decreased gastric acidity:

Absorption of Itraconazole from SPORANOX capsules is impaired when the gastric acidity is decreased. In patients also receiving acid neutralising medicines (e.g. aluminium hydroxide), these should be administered at least 2 hours after the intake of Itraconazole. In patients with achlorhydria, such as certain AIDS patients and patients on acid secretion suppressors (e.g. H2-antagonists, proton-pump inhibitors), it is advisable to administer SPORANOX capsules with a cola beverage.

Other azole antifungal agents:

There is no information regarding cross hypersensitivity between Itraconazole and other azole antifungal agents. Caution should be used in prescribing SPORANOX capsules to patients with hypersensitivity to other azoles.

Use in patients with congestive heart failure

In a study with SPORANOX IV in healthy volunteers a transient asymptomatic decrease of the left ventricular ejection fraction, which resolved before the next infusion, was observed. The clinical relevance of these findings to the oral formulations is not known.
Itraconazole has been shown to have a negative inotropic effect. SPORANOX has been associated with reports of congestive heart failure. Heart failure was more frequently reported among spontaneous reports of 400 mg total daily dose than among those of lower total daily doses, suggesting that the risk of heart failure might increase with the total daily dose of Itraconazole.

SPORANOX should not be used in patients with congestive heart failure or with a history of congestive heart failure unless the benefit clearly outweighs the risk. The risk benefit assessment should consider factors such as the severity of the indication, the dosing regimen (e.g. total daily dose) and individual risk factors for congestive heart failure. Risk factors include cardiac disease, such as ischaemic and valvular disease; significant pulmonary disease, such as chronic obstructive pulmonary disease; and renal failure and other oedematous disorders. Patients with these risk factors, who are being treated with SPORANOX, should be informed of the signs and symptoms of congestive heart failure. Caution should be exercised and the patient monitored for the signs and symptoms of congestive heart failure. SPORANOX should be discontinued if such symptoms occur during treatment.

Calcium channel blockers can have negative inotropic effects which may be additive to those of Itraconazole. In addition, Itraconazole can inhibit the metabolism of calcium channel blockers. Therefore, caution should be used when co-administering Itraconazole and calcium channel blockers due to an increased risk of CHF.

Use in patients with hepatic impairment

Itraconazole is predominantly metabolised in the liver. Patient with impaired hepatic function should be carefully monitored when taking Itraconazole and when deciding to initiate therapy with other medication a metabolised by CYP3A4. Dose adjustments may be considered in these patients.

Patients with pre-existing abnormalities of hepatic function (raised liver enzymes, an active liver disease, or patients who have experienced liver toxicity with other drugs) who require Itraconazole should be monitored, regardless of the duration of therapy.

Rare cases of cholestatic jaundice and very rare cases of hepatitis have been reported. Very rare cases of serious hepatotoxicity, including some cases of fatal acute liver failure, have occurred with the use of SPORANOX. Most of these cases involved
patients who had pre-existing liver disease, were treated for systemic indications, had significant other medical conditions and/or were taking other hepatotoxic drugs. Some patients had no obvious risk factors for liver disease. Some of these cases have been observed within the first month of treatment, including some within the first week. Liver function monitoring should be considered in patients receiving SPORANOX treatment. Patients should be instructed to promptly report to their physician signs and symptoms suggestive of hepatitis such as anorexia, nausea, vomiting, fatigue, abdominal pain or dark urine. In these patients treatment should be stopped immediately and liver function testing should be conducted.

In patients with raised liver enzymes or active liver disease, or who have experienced liver toxicity with other drugs, treatment should not be started unless the expected benefit exceeds the risk of hepatic injury. In such cases liver enzyme monitoring is necessary.

**Use in patients with renal impairment**

Limited data are available on the use of oral Itraconazole in patients with renal impairment. Caution should be exercised when this drug is administered in this patient population.

**Immunocompromised patients**

In some immunocompromised patients (e.g. neutropenic, AIDS or organ transplant patients) the oral bioavailability of SPORANOX capsules may be decreased.

**Patients with immediately life-threatening systemic fungal infections**

Due to the pharmacokinetic properties SPORANOX capsules are not recommended for initiation of treatment in patients with immediately life-threatening systemic fungal infections.

**Patients with AIDS**

In patients with AIDS having received treatment for a systemic fungal infection such as sporotrichosis, blastomycosis, histoplasmosis or cryptococcosis (meningeal and non-meningeal) and who are considered at risk for relapse, the treating physician should evaluate the need for a maintenance treatment.
**Hearing loss**

Transient or permanent hearing loss has been reported in patients receiving treatment with Itraconazole. Several of these reports included concurrent administration of quinidine which is contraindicated. The hearing loss usually resolves when treatment is stopped, but can persist in some patients.

**Use in the elderly**

Clinical data on the use of SPORANOX capsules in elderly patients is limited. Use SPORANOX capsules in these patients only if the potential benefits outweigh the potential risks.

**Use in children**

The efficacy and safety of Itraconazole have not been established in children. Since clinical data on the use of Itraconazole in children is limited, SPORANOX capsules should not be used in these patients unless the potential benefit outweighs the potential risks.

Toxicological studies have shown that Itraconazole, when administered to rats, can produce bone toxicity. While such toxicity has not been reported in adult patients, the long-term effect of Itraconazole in children is unknown.

**Carcinogenicity, mutagenicity, impairment of fertility**

Itraconazole showed no evidence of carcinogenicity potential in mice treated orally for 23 months at dosage levels of up to 80 mg/kg/day. Male rats treated with 25 mg/kg/day had a slightly increased incidence of soft tissue sarcoma. These sarcomas may have been a consequence of hypercholesterolaemia, which is a response of rats, but not dogs or humans to chronic Itraconazole administration.

Female rats treated with 50 mg/kg/day had an increased incidence of squamous cell carcinoma of the lung (2/50) as compared to the untreated group. Although the occurrence of squamous cell carcinoma in the lung is extremely uncommon in untreated rats, the increase in this study was not statistically significant.

Itraconazole produced no mutagenic effects when assayed in appropriate bacterial, non-mammalian and mammalian test systems.
Effects of Itraconazole on other drugs

Itraconazole can inhibit the metabolism of drugs metabolised by the cytochrome 3A family. This can result in an increase and/or a prolongation of their effects, including side effects. When using concomitant medication, the corresponding label should be consulted for information on the route of metabolism. After stopping treatment, the plasma levels of Itraconazole decline gradually, depending on the dose and duration of treatment. This should be taken into consideration when other drugs are co-administered.

In addition to possible pharmacokinetic interactions involving the drug metabolising enzyme CYP3A4, calcium channel blockers can have negative inotropic effects which may be additive to those of Itraconazole. Itraconazole can inhibit the metabolism of calcium channel blockers. Caution should be used when co-administering Itraconazole and calcium channel blockers due to an increased risk of CHF.

VII. DOSAGE AND ADMINISTRATION

It is essential that SPORANOX capsules are taken immediately after a meal for maximal absorption. Treatment schedules are as follows:

**Superficial dermatomycoses:**
- Tinea corporis, tinea cruris: 1 capsule (100 mg) daily for 2 weeks.

**Fungal keratitis:**
- 2 capsules (200 mg) once daily for 3 weeks.

**Pityriasis versicolor:**
- 2 capsules (200 mg) once daily for 1 week

**Vulvovaginal candidiasis:**
- 2 capsules (200 mg) morning and evening for 1 day or 2 capsules (200 mg) once daily for 3 days.

**Oral candidiasis in immunocompromised patients:**
- 1 capsule (100 mg) or 2 capsules (200 mg) daily for 4 weeks.

**Onychomycosis:**
- 2 capsules (200 mg) once daily for 3 months.
3.1.2 Pharmacokinetics:

Itraconazole is absorbed from the gastrointestinal tract when administered by mouth either as capsules containing Itraconazole onto sugar spheres or as an oral liquid formulated with hydroxyl propyl β cyclodextrin. Absorption from the capsule formulation is enhanced by acidic gastric environment and is greatest when doses are taken with food; absorption from oral liquid is not dependent on acidic environment, and absorption is greatest in the fasting state. Peak plasma concentrations are achieved within 1.5 and 5 hrs after a dose of either formulation, or steady state is reached within 15 days during daily dosing. Bioavailability increases with doses of 100 to 400mg in such a manner as to suggest that Itraconazole undergoes saturable metabolism. Itraconazole is highly protein bound; only 0.2% circulates as free drug. Itraconazole is widely distributed but only small amounts diffuse into CSF. Therapeutic concentrations of Itraconazole remain in the skin and mucous membranes for 1 to 4 weeks after the drug is discontinued. Small amounts are distributed in breast milk.

a) Definition: Itraconazole contains not less than 98.5% and not more than the equivalent of 101.5% of 4-[4-[4-[4-[[cis-2- (2,4-dichlorophenyl) -2- (1H-1, 2, 4-triazol -1- ylmethyl) -1, 3-dioxolan-4-yl] methoxyl] phenyl] piperazin-1-yl] phenyl]-2- [(1RS)-1-methylpropyl]-2,4-dihydro-3H-1,2,4-triazol-3-one, calculated with reference to the dried substance.

3.2. Identification:

A. Melting point: 166°C to 170°C

B. Examined by infrared spectroscopy comparing with the spectrum obtained with Itraconazole CRS. Examine the substance prepared as discs.

C. Examine by thin layer chromatography, using a suitable octadecylsilyl silica gel as the coating substance.

Test solution: Dissolve 30 mg of the substance to be examined in a mixture of equal volumes of methanol R and methylene chloride R and dilute to 5 ml with the same mixture of solvents.
Reference solution (a): Dissolve 30 mg of Itraconazole CRS in a mixture of equal volumes of methanol R and methylene chloride R and dilute to 5 ml with the same mixture of solvents.

Reference solution (b): Dissolve 30 mg of Itraconazole CRS and 30 mg of ketoconazole CRS in a mixture of equal volumes of methanol R and methylene chloride R and dilute to 5 ml with the same mixture of solvents. Apply to the plate 5µl of each solution. Develop in an unsaturated tank over a path of 10 cm using a mixture of 20 volumes of ammonium acetate solution R, 40 volumes of dioxin R and 40 volumes of methanol R. Dry the plate in a current of warm air for 15 min. and expose it to iodine vapor until the spots appear. Examine in daylight. The principal spot in the chromatogram obtained with the test solution is similar in position, color and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Identification by HPLC

Mobile phase composition: Buffer solution : Acetonitrile = 50 : 50 (v/v)

Buffer Solution preparation: Weight 27.2 gm of tetra butyl ammonium hydrogen sulphate and dissolve it in 1000 ml of mq. Water, filter through 0.45µ filter and degass.

Diluent: (1) Mixture of Methanol and Tetrahydrofuran (1:1)

(2) Mobile phase

Chromatographic condition

Column: Inertsil ODS 3-V, (150 x 4.6) mm, 5µ

Flow rate: 1.5 ml/min.

Wavelength: 225 nm

Column oven temperature: 30°C

Run time: 8.0 min.

Injection volume: 10 µl
Standard preparation: Weigh 50 mg WS into 50 ml volumetric flask, add some amount of dil (1) and sonicate to dissolve make up the volume to the mark with diluent further dilute 5 ml to 50 ml with mobile phase.

Sample preparation: Take eq. to 100 mg of crushed pellets into 100 ml volumetric flask, add 75 ml (dil. (1) and sonicate for 30 min. then make volume up to mark with dil. (1). Further take 5.0 ml of this solution and dilute it upto 50.0 ml with mobile phase.

3.3. Physico-Chemical studies:

Preformulation commences when a newly synthesized drug shows sufficient pharmacologic promise in animal models to warrant evaluation in man. These studies should focus on those physic-chemical properties of the new compound that could affect drug performance and development of an efficacious dosage form. A thorough understanding of these properties may ultimately provide a rationale for formulation design, or support the need for molecular modification. Itraconazole identification tests carried out includes chemical tests, Infrared spectroscopy and melting point determination.

a) Solubility studies: Drug absorption requires that molecules be in solution at the absorption site. Dissolution of solid dosage forms in gastrointestinal fluids is a prerequisite to the delivery of a drug to the systemic circulation following oral administration. Dissolution depends in part on the solubility of the drug substance in the surrounding medium (Table 3.1).

<table>
<thead>
<tr>
<th>Media</th>
<th>Solubility (gm/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1N HCl</td>
<td>0.932</td>
</tr>
<tr>
<td>0.01N HCl</td>
<td>0.932</td>
</tr>
<tr>
<td>pH 3.0 acetate buffer</td>
<td>0.103</td>
</tr>
<tr>
<td>pH 4.5 acid base buffer</td>
<td>0.067</td>
</tr>
<tr>
<td>pH 6.8 phosphate buffer</td>
<td>0.015</td>
</tr>
<tr>
<td>Water</td>
<td>0.0024</td>
</tr>
</tbody>
</table>
b) **Partition coefficient**: A measurement of drug’s lipophilicity and an indication of its ability to cross cell membranes is the oil/water partition coefficient in systems such as n-octanol/water and chloroform/ water. The partition coefficient is defined as the ratio of un-ionized drug distributed between the organic and aqueous phases at equilibrium. (Equation 3.1)

\[
P_{\text{o/w}} = \frac{C_{\text{oil}}}{C_{\text{water}}} \text{ equilibrium}
\]

For drug delivery, the lipophilic/hydrophilic balance has been shown to be a contributing factor for the rate and extent of drug absorption. The partition coefficient of Itraconazole was found to be 5.31 which was closed to the reported value.

**3.4. Drug Polymer Interaction Studies:**

Drug polymer interaction studies were determined using equilibrium dialysis technique. 2% w/v solution of polymer was introduced in cellulose dialysis tube, which served as donor compartment along with 5 ml of 0.4% w/v solution of drug in pH 0.1 N HCl. The dialysis tube containing the drug and polymer solution was suspended into the receiver compartment containing 100 ml of 0.1N HCl. Whole assembly was stirred at 100 rpm at 37°C using magnetic stirrer and after 24, 48 and 72 hrs, 2 ml sample was withdrawn from the recipient compound, diluted to 10 ml with 0.1N HCl and drug content was estimated spectrophotometrically. The control experiment was carried out in a same manner without having the drug in the dialysis tube. Same analysis technique was carried out with another polymer (Table 3.2).

**Table 3.2 Drug polymer interaction data**

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Initial</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>100</td>
<td>99.6 ± 4.13%</td>
</tr>
<tr>
<td>48</td>
<td>100</td>
<td>99.5 ± 4.25%</td>
</tr>
<tr>
<td>72</td>
<td>100</td>
<td>99.1 ± 3.85%</td>
</tr>
</tbody>
</table>
3.5. Drug Excipients Interaction Studies:

Pre weight quantity of each additive was added separately to a series of 25 ml volumetric flask containing 5 ml of 0.1% w/v solution of Itraconazole in 0.1N HCl. Flasks were kept for 6 hrs with intermittent shaking, volume was made up to the mark with 0.1N HCl and centrifuged at 3000 rpm for 10 min. The solution was then filtered and analyzed spectrophotometrically.

![Fig. 3.3 An IR spectra of Itraconazole (Standard)](image)

3.6. Result and Discussion:

The drug, Itraconazole which was obtained as a gift sample form Zydus Cadila Healthcare Ltd, Ahmedabad, was subjected to various tests in the view of confirmation of identity and purity. The identification tests reveal that the drug complies with the official standard. Melting point determination using melting point apparatus was found to be 168°C. The infrared spectrum of drug was found to be identical to that reported literature in confirming identity and purity of drug. The solubility determined the type of method to be followed or solvent to be employed for designing and developing formulation. Selection of solvent exhibits an influential role in the performance of delivery system. Qualitative solubility was determined in various solvents which showed that drug is practically insoluble in water; very slightly soluble in alcohol; freely soluble in dichloromethane; sparingly soluble in tetrahydrofuran.

Drug partitioning in n-octanol : distilled water and n-octanol : PBS was also determined and was found to be 5.31 and 5.43 respectively which indicated lipophilic nature of drug. The absorption maxima of drug were reduced while scanning a 0.001% w/v drug solution within a range of 200 -400 nm using double beam spectrophotometer.
The value of $\lambda_{\text{max}}$ was found to be 264 nm in buffer solution of pH ranging from pH 2.0 to 6.8. This is in accordance with the reports published. Various calibration curves of drug in solution of different pH values were constructed. It was observed that drug in concentration of 2-20µg/ml obeys Beer’s Lambert law. The calibration curves data were subjected to statistical analysis and parameters like, slope, intercept, equation for straight line, correlation coefficient and standard error was calculated. The linearly regressed calibration curves were plotted and calculated correlation coefficient which was found to be in the range of 0.9996 to 0.9999 showing good linearity between concentration and absorbance within the concentration range 2-20µg/ml. The standard error value indicates good reproducibility of the data as on running experiments in triplicates.

The polymer which is used in the preparation of formulation should not show any interaction with the drug. The drug is distributed in the matrix of polymer and any interaction of the drug with the polymer can lead to anomaly in the performance of the designed delivery system. In the present investigation, the interaction of the drug with the polymer was determined by incubating the drug with polymers, which revealed that an insignificant amount (p>0.05) of drug was bound to polymer. The amount of drug remained after 24, 48 and 72 hrs was found to be 99.6, 99.5 and 99.1 respectively. Similarly interaction of drug with other excipients was performed.

The Differential scanning calorimetric measurements were performed on DSC-60 (Shimadzu Co., Japan) differential scanning calorimeter with a thermal analyzer. All accurately weighed samples (about 5 mg) were heated in hermetically sealed aluminum pans under nitrogen atmosphere at the flow rate of 20ml/min. with a scanning rate of 10C/min. from 50 to 250°C. An empty aluminum pan was used as a reference.

Differential Scanning Calorimetry studies were also carried out to determine the thermal behavior of the pure drug, and along with different excipients to check the compatibility of drug with rest of excipients. The thermogram of the pure drug, Itraconazole, is given in Fig 3.3. In the thermogram a sharp endothermic peak at 167.65°C is obtained which is a characteristic peak of Itraconazole. DSC of Itraconazole along with polymer revealed that there has been no considerable change in peak value which was found to be 167.47°C as compared to167.65°C for pure drug. This indicates
compatibility of drug with polymer. DSC studies of another polymer (HPMC) used for preparation of buccal films indicates further no change in peak of drug which was found to be 167.75°C indicating compatibility of drug with polymer (Fig 3.4 – 3.9).

Fourier Transform Infrared Spectra were acquired to draw information on the molecular state of Itraconazole and mixture of Itraconazole and Chitosan and HPMC. Chitosan is an amino glucose characterized by a small proportion of amide groups via an amide linkage with acetic acid. In FTIR spectrum chitosan exhibited a broad peak at 3431 cm⁻¹, which is assigned to the N-H and hydrogen bonded O-H stretch vibrational frequencies, while a sharp peak at 3610 cm⁻¹ is that of free O-H bond stretch of glucopyranose units. Further, in the C-H stretch region of FTIR spectrum, the higher intensity peak at 2923 cm⁻¹ is assigned to the asymmetric and the lower intensity peak at 2857 cm⁻¹ is assigned to the symmetric modes of CH₂. The peaks at 1550 and 1599 cm⁻¹ were assigned to strong N-H bending vibrations of secondary amide, which usually occur in the range of 1640 to 1550 cm⁻¹ as strong band.

The characteristic IR peak of ITCZ alone over the frequency range 500 – 4000 cm⁻¹ occurred at 3439, 3126 and 3069 cm⁻¹ due to the absorption of NH2 groups, 2964 cm⁻¹ resulted fromCH2 stretching frequency band and a sharp peak occurred at 1698 cm⁻¹ due to C=O stretching vibration. The peaks observed at 1609 cm⁻¹ and 1429 cm⁻¹ may be assigned to the C=N and C-N bonds, respectively. The characteristic peaks occurred at 1510 and 1451 cm⁻¹ owed to C-H deformation. The IR region from 600-1400 cm⁻¹ which is called the fingerprint, usually contains a large number of unassigned vibrations characteristic of the molecule. The IR spectra of the physical mixtures of ITCZ with HPMC and Chitosan did not show any significant differences in the characteristic bands of the respective spectra of the pure components and the functional groups still showing their characteristic bands indicating that there is no complex formation. FTIR spectra of HPMC gave the characteristic peaks at about 1643, 1109 and 1033 cm⁻¹ vibration region.

According to Henrikson et al. (1996), chitosan is a promising bioadhesive material at neutral or slightly alkaline pH, which is found to be advantageous for adsorption on the mucosal surface. It was suggested that, at this pH, chitosan exhibits numerous amine and hydroxyl groups that may increase the interaction of polymer with
The rheological interaction between chitosan and mucin, and/or hydrophilic additives and mucin produces strong force of attraction between polymer and mucus membrane and in turn influences mucoadhesive property of the films (Fig 3.10 to 3.16).

Fig 3.4 DSC spectra of Itraconazole.
Fig 3.5 DSC spectra of physical mixture of Itraconazole and chitosan.

Fig 3.6 DSC spectra of physical mixture of Itraconazole and HPMC.
Fig 3.7 DSC spectra of physical mixture of Itraconazole and Carboxy methyl cellulose.

Fig 3.8 DSC spectra of physical mixture of Itraconazole and Ethyl cellulose.
Fig 3.9 DSC spectra of physical mixture of Itraconazole and Eudragit.

Fig 3.10 FT-IR spectra of Itraconazole.
Fig 3.11 FT-IR spectra of physical mixture of Itraconazole and HPMC.

Fig 3.12 FT-IR spectra of physical mixture of Itraconazole and chitosan.
Fig 3.13 FT-IR spectra of final formulation.

Fig 3.14 FT-IR spectra of Itraconazole and Carboxy methyl cellulose.
Fig 3.15 FT-IR spectra of Itraconazole and Ethyl cellulose.

Fig 3.16 FT-IR spectra of Itraconazole and Eudragit polymer.