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Formulation and evaluation of self-emulsifying formulations of itraconazole using pharmaceutically accepted lipid-based excipients
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Formulation and evaluation of self-emulsifying formulations of itraconazole using pharmaceutically accepted lipid-based excipients

Abstract
Poorly water-soluble drug, itraconazole offers a challenge in developing a drug product with adequate bioavailability. The potential for lipidic self-emulsifying drug delivery system to improve the oral bioavailability of itraconazole was investigated in fasted rats. A series of itraconazole SEDDS were prepared in three groups based on oil:S/CoS mixture ratios (1:9, 1:6 and 1:4) using Capryol 90 (oil), a mixture of Labrasol and Tween 20 (surfactants) and Transcutol P (co-surfactant). The multi-component delivery systems were optimised by evaluating their ability to self-emulsify when introduced in simulated gastric fluid, without pepsin, under gentle agitation and by determination of droplet size and visual observation. Three formulations (F5, F21 and F27), one from each group were selected based on droplet size and visual observation. Pseudo ternary phase diagram was constructed to identify the efficient self-emulsification region. The effect of oil and surfactants content on mean globule size of resulting emulsions was studied. The optimized formulations were assessed for dissolution, in vitro diffusion, ex vivo stomach and intestinal permeability along with the marketed capsule. From the data obtained in this work, it was clear that oil to surfactant and co-surfactant ratio has the main impact on the droplet size of the emulsion formed after dilution. However, the relation between droplet size and in vivo absorption of itraconazole was futile. The order of AUC was 1:4 > 1:6 > 1.9 for oil:S/CoS mixture ratios. Following an oral administration of developed SEDDS and marketed capsule to rats, a 1.7, 2.0 and 2.33-fold increase in bioavailability was observed for F5, F21 and F27 respectively, compared with marketed capsule. The results from this study demonstrate the potential use of SEDDS to provide an effective way of improving oral absorption of lipophilic drugs.
5.1. Introduction

In recent years, major advances in healthcare have led to an unwelcome increase in the number of life-threatening infections due to true pathogenic and opportunistic fungi (Richardson, 2005). Recently, there have been an increasing number of profound fungal infections caused by fungi such as those belonging to the genus Candida, the genus Aspergillus and the genus Cryptococcus. This is a particular complication encountered in transplant patients, those administered a large quantity of antibiotics, anticancer drugs (carcinostatic) or a steroidal agents over a long period, AIDS patients, or those suffering from cancer in the terminal stage, and those with hematological malignancies undergoing intensive chemotherapy and/or bone marrow transplantation (Bodey, 1992). Invasive fungal infections are a major cause of morbidity and mortality in patients receiving bone marrow transplants for leukemia as well as in immunocompromised cancer patients (Anaissie, 1992).

Itraconazole (ITZ) is a broad spectrum antifungal agent and belongs to trizole group indicated in the treatment of local and systemic fungal infections (Grant and Clissold, 1989).

Itraconazole is a synthetic anti-fungal drug, which is composed of a 1:1:1:1 racemic mixture of four diastereomers (two enantiomeric pairs). Three chiral centers are present in each diastereomer which possess a molecular formula of C_{35}H_{38}C_{12}N_{8}O_{4} and molecular weight of 705.64 g/mol (Grant and Clissold, 1989; Jain and Sehgal, 2001). Itraconazole is poorly soluble in aqueous media (less than 1 µg/mL in aqueous solutions at pHs of 1-12.7) with a partition coefficient greater than 5 in octanol/water at pH 6. Thus, in spite of the high antifungal activity, the bioavailability of unformulated crystalline itraconazole is extremely low (Jung et al., 1999; Verreck et al., 2003). In order to enhance its solubility and dissolution rate and thereby bioavailability, various formulations have been developed and are briefly reviewed in Section II.

Histoplasmosis is a serious opportunistic infection in patients with AIDS and itraconazole has demonstrated activity against Histoplasmosis capsulatum (Vyas and Bradsher, 2011).

Itraconazole is a weak base (pKa = 3.7) with high lipophilicity (log D > 5) (Peeters et al., 2002) Its aqueous solubility is estimated at approximately 1 ng/mL at neutral pH and approximately 4 µg/mL at pH 1, (Peeters et al., 2002) and thus it is ionized and water soluble only at low pHs, as in gastric juice.
Itraconazole is available as capsules or an oral solution, and doses of 200 mg daily or 200 mg twice a day are used therapeutically (Barone et al., 1993; Physician Desk Reference, 2000). Because it has relatively low bioavailability after oral administration, especially when given in capsule form (Barone et al., 1998; Heykant et al., 1989), gastric acidity is required for drug dissolution and adequate absorption with capsule formulations. Decreased absorption has been observed in the fasting state and in patients with low gastric acidity (Barone et al., 1993). Furthermore, in persons with AIDS, the absorption of itraconazole from capsules was reduced by 50% compared with absorption in healthy persons (Smith et al., 1992). Hypochlorhydria and thus a relatively high gastric pH, a frequent complication of human immunodeficiency virus (HIV) infection, may be responsible for this phenomenon (Welage et al., 1995).

Co-administration of acidic beverage with itraconazole may be an effective approach in improving the bioavailability of itraconazole in patients with who are hypochlorhydric, as AIDS patients (Lange et al., 1997a) or who are taking gastric acid suppressant (Lange et al., 1997b). Higher consumption of sugar-sweetened beverages (like coca cola) is associated with weight gain and increased risk for the development of type 2 diabetes mellitus, and caffeine is a psychoactive substance. In such situation, co-administration of vitamin C beverage and itraconazole may be useful for achieving maximum absorption of itraconazole in patients with drug- or disease-related gastric hypochlorhydria (pH > 4) (Bae et al., 2011). In addition, itraconazole has been also found to be a substrate of P-glycoprotein (P-gp) (Balayssac et al., 2005; Wang et al., 2002). P-gp activity is an important mechanism that decreases the oral bioavailability of drugs by limiting intestinal absorption (Greiner et al., 1999; Yamaguchi et al., 2002).

In light of the aforementioned discussion, we explored the potential application of lipid-based drug delivery system in the development of a self-emulsifying drug delivery system (SEDDS) of itraconazole. SEDDS formulations containing itraconazole were developed using different proportions of oil and surfactants (GRAS excipients) for oral administration. Systems were evaluated for the quality of emulsion produced and mean droplet size. Optimized formulations were further evaluated for dissolution, \textit{ex vivo} stomach and intestinal permeability across rat tissues and \textit{in vivo} performance in rats. Physicochemical properties of SEDDS and pharmacokinetic parameters were evaluated in comparison to Itaspor capsules.
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The objective of the investigation was to develop and characterize the self-emulsifying drug delivery systems of poorly water soluble drug, itraconazole with a view to improve its solubility, dissolution rate, *ex vivo* permeation and *in vivo* absorption in animal model. The formulations were developed by employing excipients with established long term acceptability. The impact of excipients ratios and droplet size on bioavailability was also studies for all the developed formulations.

5.2. Materials and methods

5.2.1. Materials

Itraconazole (ITZ) was a generous gift from Matrix Laboratories Ltd. (Hyderabad, India); Isopropyl myristate (IPM), Propylene Glycol Dicaprylate/Dicaprate (Captex® 200) and Glycerol Caprylate Caprate (Captex® 355) were obtained from Subhash Chemical Industries (Pune, India); propylene glycol monocaprylate (Capmul MCM®), Capmul MCM (8) were obtained from Abitec Corp., Janesville, WI. Propylene glycol monocaprylate (CapryolTM 90), PEG-8 glycol caprylate (Labrasol®) and diethylene glycol monoethyl ether (Transcutol P®) were provided by Gattefosse, France. PEG-35 castor oil (Cremopho EL®) was obtained from BASF Corp., Germany. Glycerol caprylate (Imwitor® 988) was provided by Sasol GmbH, Witten, Germany. PEG-20 sorbitan monolaurate (Tween 20) and Tocopherol acetate were purchased from Merck (Mumbai, India). Propylene glycol was purchased from Spectrochem (Mumbai, India). Capsule shells were provided by Capsugel, Mumbai. Ketoconazole was purchased from HIMEDIA. All other chemicals and solvents used were of analytical grade.

5.2.2. Solubility studies

The solubility of itraconazole in various oils, surfactants and co-surfactants was determined. An excess amount of itraconazole was added to 5 ml of each selected oils and surfactants and was shaken reciprocally at 25°C for 24 hrs. The supernatant portion of the supersaturated solution was carefully withdrawn and suitably diluted with methanol, and solubility of itraconazole was determined using HPLC (Jasco, Japan) at 263 nm (Shim et al., 2006; Yang et al., 2008; Yoo et al., 2000). The HPLC system consisted of RP column (LCGC Qualisil BDS C18; 5 µm 250 mm x 4.6 mm i.d) and acetonitrile: water (90:10) as a mobile phase. A 40µL volume was injected into the column at a flow rate of 0.7mL/min.
5.2.3. Pseudo-ternary Phase Diagrams

The pseudo-ternary phase diagrams were constructed using water dilution method (Djordjevic et al., 2004). Capryol 90 was used as the oil phase, mixture of Labrasol and Tween 20 as the surfactant and Transcutol P as the co-surfactant. Phase diagrams were constructed by a technique well described (Kommuru et al., 2001; Nazzal et al., 2002; Taha et al., 2004) with ratios as mentioned in Table 5 and all preparations were diluted 100 times with 0.1N HCl. For each phase diagram at specific oil:S<sub>mix</sub> ratios (1:9, 1:6 and 1:4), mixtures of the oil, the surfactant and the co-surfactant were prepared, and the mixture was diluted with 0.1N HCl, maintained at 37°C. Dilution medium was added drop by drop while mixing on a cyclomixer, and the samples were marked as being optically clear or turbid. Phase diagrams were then constructed using Tri plot v1-4 software (David Graham and Nicholas Midgley, Loughborough, Leicestershire, UK).

5.2.4. Preparation of itraconazole self-emulsifying formulations

A series of itraconazole SEDDS were prepared with fixed concentration of itraconazole (25 mg) and varying concentrations of Capryol 90, Labrasol, Tween 20 and Transcutol. Itraconazole was dissolved in Capryol 90 and then mixture of Labrasol, Tween 20 and Transcutol were accurately weighed and added to itraconazole oily solution. The mixture was slightly heated on water bath for 5-10 mins at 60°C until a transparent preparation was obtained.

5.2.5. Characterization of Self emulsifying formulation

5.2.5.1. Visual observation and droplet size determination

0.05 mL formulations were diluted in 5 mL of dilution media (0.1N HCl) maintained at 37°C, 100 times dilution. Visual observation of the samples was recorded as previously reported (Craig et al., 1995; Khoo et al., 1998). Globule Size of the same diluted samples was measured by Zetasizer ZS 90 and measurements are shown in Table 5.3.

5.2.5.2. Characterization of selected formulations

Droplet size, polydispersity index and zeta potential were measured on Zetasizer ZS 90 after 100 times dilution in different media viz. Distilled water (DW), 0.1N HCl and phosphate buffer saline pH 6.8 (PBS) maintained at 37°C. Drug content was measured by HPLC.
Formulations were also evaluated for dissolution study, in vitro diffusion study, ex vivo stomach and ex vivo intestinal permeability study in rats. In vivo study was also carried out for developed itraconazole SEDDS and extemporaneous suspension of market capsule.

5.2.5.3. Drug content

SEDDS formulation equivalent to 25 mg of itraconazole was taken and diluted in methanol. Volume was made up to 25 mL with methanol (1mg/mL). 0.2 mL was withdrawn from the above solution and to it 4.8 mL of methanol was added and mixed well. 5 mL of 0.1 N HCl was added to above mixture and mixed well (20 µg/mL). Samples were prepared in triplicate and absorbances were taken at 258 nm on UV visible spectrophotometer (Shimadzu UV-2450, Japan) keeping equal mixture of methanol and 0.1N HCl (1:1) as a reference. Placebo was also treated in the same way to check interference, if any.

5.2.5.4. Spectroscopic characterization of optical clarity

The optical clarity of aqueous dispersions of SEDDS formulation was measured spectrophotometrically. Composition was prepared according to the design and diluted to 100 times with 0.1N HCl (USP 30) (USP 30/NF25). The % transmittance of solution was measured at 650 nm, using distilled water as a reference (Cirri et al., 2007; Subramanian et al., 2004; Zhang et al., 2008).

5.2.5.5. Morphological characterization

The morphology of self emulsifying formulation was observed by using a transmission electron microscope (TEM) (Phillips Tecnai 20, Holland) at an acceleration voltage of 200 kV and typically viewed at a magnification of 43,000x. The size of the colloidal structures was determined using AnalySIS® software (Soft Imaging Systems, Reutlingen, Germany). Formulations were diluted with distilled water 1:25 and shaken. Carbon-coated copper grids were glow-discharged (Edwards E306A Vacuum Coater, England) and 10 µL of sample adsorbed on to these holey film grid and observed after drying.

5.2.5.6. Determination of droplet size and zeta potential

Droplet size and zeta potential of the formed emulsion were determined by photon correlation spectroscopy that analyzes the fluctuations in light scattering due to Brownian motion of the particles, using a Zetasizer ZS 90 (Malvern Instruments, UK). Light scattering was monitored at 25°C at a 90° angle.
5.2.5.7. Dissolution study

Dissolution studies of itraconazole capsule and itraconazole SEDDS filled in hard gelatine capsule (size # “000”) was carried out using USP XXIII Dissolution apparatus I (basket type) at speed of 50 rpm in 900mL for 100 mg marketed capsule and 225 mL for 25 mg SEDDS 0.1 N HCl at 37 ± 0.5 °C. Aliquots of 5 mL were removed at 2, 5, 10, 15, 30, 45, 60, 75 and 120 min. Volume of aliquots was replaced with fresh dialyzing medium each time. These samples were analyzed quantitatively for amount of itraconazole released at corresponding time by using UV-visible spectrophotometer (Shimadzu UV-2450, Japan) at 255 nm.

5.2.5.8. In vitro diffusion study

In vitro diffusion studies were performed for the formulations developed (F5, F21 and F27), using a dialysis technique (Dixit et al., 2010; Kang et al., 2004; Patil et al., 2004). The dialyzing medium was 0.1N HCl. One end of dialysis tubing (Dialysis membrane 70, HIMEDIA; MWCO 12,000-14,000 daltons; pore size: 2.4 nm) (7 cm in length) was clamped and then 1.1 mL (=25 mg of drug) of self-emulsifying formulation was placed in it. The other end of the tubing was also secured with dialysis closure clip (HIMEDIA, Mumbai) and was allowed to rotate freely in 225 mL of dialyzing medium and stirred continuously with magnetic bead on magnetic plate at 37°C. Aliquots of 5 mL were removed at different time intervals. Volume of aliquots was replaced with fresh dialyzing medium each time. These samples were analyzed quantitatively for itraconazole dialyzed across the membrane at corresponding time by using UV-visible spectrophotometer at 255 nm.

For marketed formulation (Itaspor®-INTAS, pellets in capsule), capsules were crushed in mortar with pestle. From the crushed pellets, blend equivalent to 25 mg (113.975 mg blend) was weighed and made suspension by adding 3 mL of DW and was filled in dialysis membrane. The membrane was sealed from both the end with the help of dialysis closure clips and was placed in 225 mL of 0.1N HCl

5.2.5.9. Ex vivo stomach permeability study

Male Sprague-Dawley rats (250-300 g) were euthanized in carbon-dioxide vacuum chamber. All experiments and protocols described in this animal study were approved by the Institutional Animal Ethics Committee of B V Patel PERD Centre, Ahmedabad, India and were in accordance with the guidelines of the Committee for Purpose of Control and
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Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. To check the stomach permeability, the stomach part was isolated carefully and taken for the *ex vivo* permeation study. Then this tissue was washed with physiological acid solution containing 100 mM HCl and 54 mM NaCl to remove the mucous and gastric contents. Then the tissue was rinsed with 100 mM HCl. The method employed was modified from experimental procedures well described in the literature (Gharzouli et al., 1995; Hung and Neu, 1997; Shah and Khan, 2004).

SEDDS formulations, 0.4 mL (equivalent to 10 mg of drug) were injected into the stomach and tissues were tightly closed with the help of threads. Similarly 2 mL extemporaneous suspension of market capsule (Itaspor®-INTAS) equivalent to 10 mg of drug was filled in stomach tissue and was placed in a beaker with constant stirring and temperature of 37°C on a magnetic stirrer. The receiver compartment was filled with 30 mL of 100 mM HCl. The absorbance was measured at 255 nm, keeping the respective blank. The percent permeation of drug was calculated against time and plotted on a graph.

5.2.5.10. *Ex vivo* intestinal permeability study

This was carried out by the method well described in the literature (Araya et al., 2006; Ghosh et al., 2006). Male Sprague-Dawley rats (250-300 g) were euthanized in carbon-dioxide vacuum chamber. All experiments and protocols described in this animal study were approved by the Institutional Animal Ethics Committee of B V Patel PERD Centre, Ahmedabad, India and were in accordance with the guidelines of the Committee for Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. To check the intestinal permeability, small portion of small intestine was isolated and used for the *ex vivo* permeability study. The tissue was thoroughly washed with pH 6.8 phosphate-buffered saline (USP 30) (USP 30/NF25) to remove any mucous and lumen contents.

One mL SEDDS formulations were diluted to 5 mL with distilled water (outside mixing for 1 minute by vortex mixer), the resultant sample (5 mg/mL) was injected into the lumen of the duodenum using a syringe, and the two sides of the intestine were tightly closed with the help of threads. Extemporaneously suspension was prepared from market capsule (Itaspor®-INTAS), and 1 mL equivalent to 5 mg of drug was filled in stomach tissue similar to that of SEDDS formulations. Then the tissue was placed in a beaker with constant stirring and temperature of 37°C on magnetic stirrer. The receiver compartment
was filled with 30 mL of phosphate-buffered saline containing 20% PEG 400. The absorbance was measured at 258 nm, keeping the respective blank. The percent diffusion of drug was calculated against time and plotted on a graph.

5.2.6. HPLC analysis

See Chapter 4 Sec. 4.2.6. HPLC method development and validation

5.2.7. Bioavailability studies

Bioavailability studies were performed in male Wistar rats weighing 280 to 350 g. All experiments and protocols described in this study were approved by the Institutional Animal Ethics Committee of Sri Dhanvantary Pharmaceutical Analysis and Research Centre, Surat, India and were in accordance with guidelines of the Committee for Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. Two groups were made for the study, and four rats were kept in each group. The animals were kept under standard laboratory conditions, temperature at 25 ± 2°C and relative humidity (55 ± 5%). The animals were housed in polypropylene cages, four per cage, with free access to standard laboratory diet (Lipton feed, Mumbai, India) and water *ad libitum*. The animals were fasted overnight prior to the experiment but had free access to water.

The formulations (SEDDS and extemporaneous suspension prepared from marketed capsule (Itaspor®)) were given orally using feeding snode. Dose for the rats was selected as reported (Shin et al., 2004; Van Cauteren et al., 1987) and calculated based on the weight of the rats (30mg/kg body weight) according to the surface area ratio (Akhila et al., 2007; Paget and Barnes, 1964). The animals were anesthetized using ether and blood samples (approximately 500 μL) were collected from the retro-orbital vein using a heparinized needle (18-20 size) at 0 (pre-dose), 1, 2, 4, 8, 12, 16 and 24 hours after oral administration of formulations. The blood samples were collected into a vacutainer tube (13 x 75mm, 2mL, Accuvac, BD, NJ), mixed and centrifuged on a laboratory centrifuge at 5000 rpm for 20 min at ambient temperature. The supernatant plasma was carefully separated and kept at –20°C (Thermo Scientific, USA) until analysis was carried out using validated HPLC.

5.2.7.1. Plasma analysis

Frozen plasma samples were thawed just prior to extraction. A 100 μL of plasma sample was transferred in 2 mL centrifuge tube (Tarson, Kolkata, India). To that 300 μL of
acetonitrile containing Ketoconazole as an internal standard (Vaughn et al., 2006) (200 ng/mL in acetonitrile) was added and vortex-mixed for 1 min. The tube was centrifuged at 15,000 rpm for 20 min at 4° C. The organic layer was carefully decanted and was transferred to clean tube and dried under nitrogen stream at ambient temperature. The residue was reconstituted with a 100 µL aliquot of mobile phase, consisted of a mixture of 5 mM sodium phosphate buffer pH 5.7 and ACN (38:62 v/v) and 50 µL was injected directly onto the HPLC column at a flow rate of 1.0 mL/min.

Plasma concentration versus time data of itraconazole for rats was analyzed using standard non-compartment analysis. The area under the plasma concentration-time curve (\( AUC_{0\to t} \)) from zero to 24 hour was estimated by the linear trapezoidal method (Han and Lee, 1999; Lee and Ku, 1999). The relative bioavailability \( (F) \) of SMEDDS to the suspensions was calculated using the following equation:

\[
F = \frac{AUC_{\text{test}}}{AUC_{\text{reference}}} \times 100\%
\]

5.2.8. Statistical analysis

Statistical analysis for the determination of differences in diffusion, permeability and in vivo absorption profiles of itraconazole SEDDS and the marketed preparation was assessed by the use of Student’s \( t \)-test. The pharmacokinetic data between different formulations were compared for statistical significance by Student’s \( t \)-test. Statistical probability \( (P) \) values less than 0.05 were considered significantly different.

5.2.9. Stability studies

Chemical and physical stability of itraconazole SEDDS (F5, F21 and F27) were assessed under various storage conditions namely room temperature (RT), 30±2°C/65±5% RH and 40±2°C/75±5% RH as per ICH guidelines (ICH Q1A(R2)) in ICH certified stability chambers (Humidity Chamber, EIE Instruments Ltd., Ahmedabad, India).

Itraconazole SEDDS equivalent to 25 mg was filled in a glass vial with a rubber closure and aluminium-crimped tops. Eight such glass vials of each F5, F21 and F27 were filled and stored at various aforementioned storage conditions up to 3 months. Samples were removed at 0, 1, 2 and 3 months of interval and checked for itraconazole content (by HPLC), droplet size, polydispersity index and zeta potential after diluting formulation to 100 times with three different media viz. Distilled water (DW), 0.1N HCl and phosphate buffer saline pH 6.8 (PBS) maintained at 37°C.
5.3. Results and discussion

5.3.1. Screening of oil and surfactants

The solubilities of itraconazole in various oils and surfactants are presented in Table 5.1. Among the selected oils that were screened, maximum solubility of itraconazole was found in Capryol 90 followed by Captex 200, IPM and Captex 355. Among the surfactants (Table 5.1), Transcutol P followed by Tween 20 showed reasonable solubilizing potential for itraconazole. Transcutol P proved to be the best solublizer for itraconazole.

Before selecting Transcutol P as a co-surfactant, propylene glycol was also screened for its efficiency in preparation of a good self-emulsifying system. During the formulation development of Nevirapine, it was found out that Labrasol has poor water uptake capacity which was improved by adding Tween 80. In the case of itraconazole, Tween 20 was tried for the development of self-emulsifying system.

A series of trials were taken with three different ratios of Oil: S_{mix} (1:9, 1:6 and 1:4). Capryol 90 was selected as an oil phase, mixture of Labrasol and Tween 20 as a surfactant and Transcutol P as a co-surfactant. The ratios 9:1, 1:1, 2:1 and 4:1 of surfactant to co-surfactant were tried with 1:1, 1:2 and 1:3 ratios of Labrasol:Tween 20 as a surfactant (Table 5.2).

**Table 5.1:** Solubility of itraconazole in various oils and surfactants at 25°C (n =3)

<table>
<thead>
<tr>
<th>Oil /Surfactant</th>
<th>Solubility (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropyl myristate</td>
<td>0.206 ± 2.07</td>
</tr>
<tr>
<td>Captex 200</td>
<td>1.008 ± 2.08</td>
</tr>
<tr>
<td>Captex 355</td>
<td>2.127 ± 1.97</td>
</tr>
<tr>
<td>Tocopherol acetate</td>
<td>5.22 ± 1.87</td>
</tr>
<tr>
<td>Capryol 90</td>
<td>22.132 ± 1.58</td>
</tr>
<tr>
<td>Tween 20</td>
<td>3.709 ± 1.04</td>
</tr>
<tr>
<td>Labrasol</td>
<td>7.147 ± 1.65</td>
</tr>
<tr>
<td>Transcutol</td>
<td>4.6 ± 1.48</td>
</tr>
<tr>
<td>Capmul MCM</td>
<td>7.4533 ± 1.15</td>
</tr>
<tr>
<td>Capmul MCM (8)</td>
<td>5.8483 ± 1.43</td>
</tr>
<tr>
<td>Imwitor 988</td>
<td>6.5708 ± 1.25</td>
</tr>
<tr>
<td>Cremophor EL</td>
<td>4.0015 ± 1.21</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>1.1804 ± 0.55</td>
</tr>
</tbody>
</table>
Obviously, as the proportion of oil phase increases, the optical clarity of the formulations was poor on dilution. When the ratio of oil:S\text{mix} was 1:2, prepared systems exhibited turbidity and they were dropped from the study.

Table 5.2: Formulation development trials

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Size (nm)</th>
<th>PdI</th>
<th>Visual Observation</th>
<th>Trial No.</th>
<th>Size (nm)</th>
<th>PdI</th>
<th>Visual Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>149.2</td>
<td>0.159</td>
<td>Bluish Tinge</td>
<td>19</td>
<td>185.0</td>
<td>0.293</td>
<td>Slight Turbid</td>
</tr>
<tr>
<td>2</td>
<td>56.87</td>
<td>0.151</td>
<td>Poor quality</td>
<td>20</td>
<td>141.0</td>
<td>0.277</td>
<td>Bluish Tinge</td>
</tr>
<tr>
<td>3</td>
<td>98.77</td>
<td>0.309</td>
<td>Poor quality</td>
<td>21</td>
<td>95.39</td>
<td>0.4</td>
<td>Slight Bluish Tinge</td>
</tr>
<tr>
<td>4</td>
<td>162.6</td>
<td>0.248</td>
<td>Bluish Tinge</td>
<td>22</td>
<td>182.4</td>
<td>0.264</td>
<td>Bluish Tinge</td>
</tr>
<tr>
<td>5</td>
<td>118.2</td>
<td>0.172</td>
<td>Slight Bluish Tinge</td>
<td>23</td>
<td>121.0</td>
<td>0.208</td>
<td>Bluish Tinge</td>
</tr>
<tr>
<td>6</td>
<td>38.85</td>
<td>0.639</td>
<td>Poor quality</td>
<td>24</td>
<td>147.1</td>
<td>0.976</td>
<td>Poor quality</td>
</tr>
<tr>
<td>7</td>
<td>163.7</td>
<td>0.275</td>
<td>Bluish Tinge</td>
<td>25</td>
<td>200.3</td>
<td>0.401</td>
<td>Turbid</td>
</tr>
<tr>
<td>8</td>
<td>56.89</td>
<td>0.56</td>
<td>Poor quality</td>
<td>26</td>
<td>167.5</td>
<td>0.252</td>
<td>Bluish Tinge</td>
</tr>
<tr>
<td>9</td>
<td>18.15</td>
<td>0.6</td>
<td>Poor quality</td>
<td>27</td>
<td>130.2</td>
<td>0.156</td>
<td>Bluish Tinge</td>
</tr>
<tr>
<td>10</td>
<td>142.5</td>
<td>0.206</td>
<td>Bluish Tinge</td>
<td>28</td>
<td>546.1</td>
<td>0.392</td>
<td>Turbid</td>
</tr>
<tr>
<td>11</td>
<td>25.33</td>
<td>0.205</td>
<td>Poor quality</td>
<td>29</td>
<td>320.3</td>
<td>0.116</td>
<td>Turbid</td>
</tr>
<tr>
<td>12</td>
<td>53.14</td>
<td>0.403</td>
<td>Poor quality</td>
<td>30</td>
<td>255.0</td>
<td>0.265</td>
<td>Turbid</td>
</tr>
<tr>
<td>13</td>
<td>158.8</td>
<td>0.199</td>
<td>Bluish Tinge</td>
<td>31</td>
<td>452.6</td>
<td>0.136</td>
<td>Turbid</td>
</tr>
<tr>
<td>14</td>
<td>105.0</td>
<td>0.278</td>
<td>Slight Bluish Tinge</td>
<td>32</td>
<td>189.3</td>
<td>0.278</td>
<td>Bluish Tinge</td>
</tr>
<tr>
<td>15</td>
<td>40.68</td>
<td>0.256</td>
<td>Poor quality</td>
<td>33</td>
<td>180.0</td>
<td>0.239</td>
<td>Bluish Tinge</td>
</tr>
<tr>
<td>16</td>
<td>251.7</td>
<td>0.142</td>
<td>Turbid</td>
<td>34</td>
<td>360.9</td>
<td>0.206</td>
<td>Turbid</td>
</tr>
<tr>
<td>17</td>
<td>159.9</td>
<td>0.264</td>
<td>Bluish Tinge</td>
<td>35</td>
<td>205.5</td>
<td>0.341</td>
<td>Bluish Tinge</td>
</tr>
<tr>
<td>18</td>
<td>155.6</td>
<td>0.259</td>
<td>Bluish Tinge</td>
<td>36</td>
<td>203.1</td>
<td>0.331</td>
<td>Bluish Tinge</td>
</tr>
</tbody>
</table>

S\text{mix} = Mixture of surfactants and co-surfactant; Lab = Labrasol; T20 = Tween 20; ITZ = itraconazole

5.3.2. Characterisation of formulation development trials

5.3.2.1. Visual observation: The prepared self emulsifying systems of itraconazole were diluted 100 folds with 0.1N HCl and checked for optical clarity. When the ratio of oil:S\text{mix} was 1:2, prepared systems exhibited turbidity and they were dropped from the study. Obviously, as the proportion of oil phase increases, the optical clarity of the formulations becomes poor on dilution.

Table 5.3: Visual observation and droplet size of formulation development trials as shown in Table 5.2
When oil:S\textsubscript{mix} ratio was 1:9, S/CoS ratio of 9:1 with Labrasol to Tween 20 ratio of 1:1 gave bluish tinge. The effect of various ratios of surfactant to co-surfactant along with different ratios of Labrasol to Tween 20 has been shown in the Table 5.3.

5.3.2.2. **Droplet size measurement:** The droplet size of formulations developed with Oil:S\textsubscript{mix} ratios were in the range of 18.13 nm – 149.2 nm, 40.68 nm – 251.7 nm, 180 nm – 546.1 nm for 1:9, 1:6 and 1:4, respectively.

A comparative study of data obtained from the droplet size measurements and visual observations were made. Those formulations which gave bluish tinge and good optical clarity were selected. In the first group (Formulation trials 1-12), formulations 1, 2, 5, 7 and 10 gave bluish tinge with droplet size in the range of 118.2 nm – 149.2 nm. The formulation 5 with lowest droplet size (118.2 nm) was selected. Similarly, in the second group 8 formulations were compared for their droplet size and the formulation 21 with lowest droplet size of 95.39 nm was selected. Formulation 27 with droplet size of 130.2 nm was selected among the 5 formulation with good optical clarity.

5.3.3. **Construction of pseudo-ternary phase diagrams**

The regions which showed bluish tinge were identified as transparent and isotropic mixtures. The percentage of three different phases i.e. oil, water and the mixture of surfactant and co-surfactant were calculated and plotted with the help of Tri plot v1.4 software for fabrication of ternary plot. The points (formulation trials) which showed bluish tinge were joined with the line and were shaded (Figure 5.1). It can be seen that, the formulations which showed bluish tinge in 1:9, 1:6 and 1:4 ratios of Oil:S\textsubscript{mix} were not more than 203.1 nm. The formulation prepared within these regions (dark shaded) may give droplet size in micron size.

![Figure 5.1: Psuedoternary phase diagram of Oil to S\textsubscript{mix} ratios of 1:9, 1:6 and 1:4](image-url)
5.3.4. Characterization of selected formulations

The droplet size, polydispersity index and zeta potential of SEDDS formulation after 100 times dilution in different media is shown in Table 5.4. Size distribution by intensity in various media for F5 is shown in Figure 5.2(a), (b) and (c). Data shows that there is not wide variation in size of droplets in distilled water and 0.1N HCl. The droplet size increases in phosphate buffer. The size of droplets increases as the percentage of oil increases. The droplet size follows the order of F27 > F21 > F5.

The polydispersity index is most poor in phosphate buffer. Zeta potential was also measured of the same diluted samples and shown in the Table 5.4. Drug content of SEDDS formulations was measured and is shown in Table 5.4.

<table>
<thead>
<tr>
<th>Droplet size D (nm)</th>
<th>Polydispersity Index (PdI)</th>
<th>Zeta potential (mV)</th>
<th>Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F5</td>
<td>F21</td>
<td>F27</td>
</tr>
<tr>
<td>DW</td>
<td>417.3</td>
<td>201.0</td>
<td>194.4</td>
</tr>
<tr>
<td>0.1NHCl</td>
<td>152.1</td>
<td>175.2</td>
<td>179.0</td>
</tr>
<tr>
<td>PBS</td>
<td>501.3</td>
<td>539.6</td>
<td>742.2</td>
</tr>
</tbody>
</table>

Figure 5.2(a): Droplet size distribution of itraconazole SEDDS F5 after dilution in distilled water
Figure 5.2(b): Droplet size distribution of itraconazole SEDDS F5 after dilution in 0.1N HCl

Figure 5.2(c): Droplet size distribution of itraconazole SEDDS F5 after dilution in phosphate buffer saline pH 6.8
Size distribution by intensity in various media for F21 are shown in Figure 5.3 (a), (b) and (c).

**Figure 5.3(a):** Droplet size distribution of itraconazole SEDDS F21 after dilution in distilled water

**Figure 5.3(b):** Droplet size distribution of itraconazole SEDDS F21 after dilution in 0.1N HCl
Figure 5.3(c): Droplet size distribution of itraconazole SEDDS F21 after dilution in phosphate buffer saline pH 6.8

Size distribution by intensity in various media for F27 are shown in Figure 5.4 (a), (b) and (c).

Figure 5.4(a): Droplet size distribution of itraconazole SEDDS F27 after dilution in distilled water
Figure 5.4(b): Droplet size distribution of itraconazole SEDDS F27 after dilution in 0.1N HCl

Figure 5.4(c): Droplet size distribution of itraconazole SEDDS F27 after dilution in phosphate buffer saline pH 6.8

The TEM picture is shown in Figure 5.5. The microemulsion droplets were observed to be spherical and homogenous with large population of the smaller droplet in the size range of less than 200 nm. The droplet size of formed emulsion determined by TEM is in correlation with that of measured by photon correlation spectroscopy (PCS).
Figure 5.5: Transmission electron microscopic image of itraconazole self-emulsifying systems after dilution showing size of some oil globules. (Scale: 500 nm). (a) SEDDS F5, (b) SEDDS F21 and (c) SEDDS F27

Statistically there was significant difference in the dissolution profiles of all developed formulations and market formulation at $P < 0.05$ and can be seen in Figure 5.6.
In vitro diffusion profiles (Figure 5.7) shows that there was no significant difference between the formulations with F5 & F21. For all other formulations there was a significant difference in their diffusion profiles at $P < 0.05$
There was significant difference in stomach permeability between all other formulations at $P < 0.05$, except F5 & F21 and F21 & F27 (Figure 5.8).

**Figure 5.8:** *Ex vivo* stomach permeability of itraconazole SEDDS and extemporaneous suspension of marketed capsule in 100 mM HCl ($n=3$)

Significant difference ($P < 0.05$) was also seen in *ex vivo* intestinal permeability of developed SEDDS and marketed formulation except for formulation F5 and F21 (Figure 5.9).

**Figure 5.9:** *Ex vivo* intestinal permeability of itraconazole SEDDS and extemporaneous suspension of marketed capsule in pH 6.8 PBS ($n=3$)
5.3.5. Bioavailability Studies

Typical chromatograms obtained showing peaks for ITZ at 1 h after administration of marketed suspension and SEDDS F5, F21 and F27 are shown in Figure 5.10 (a) 5.10(b), 5.10(c) and 5.10(d), respectively. Table 5.5 summarises mean pharmacokinetic parameters for various formulations.

![Typical chromatogram obtained while analyzing plasma for ITZ level after administration of the extemporaneous suspension of marketed capsules at 1 h](Figure 5.10(a))
Figure 5.10(b): Typical chromatogram obtained while analyzing plasma for ITZ level after administration of the SEDDS F5 at 1 h

Figure 5.10(c): Typical chromatogram obtained while analyzing plasma for ITZ level after administration of the SEDDS F21 at 1 h
Figure 5.10(d): Typical chromatogram obtained while analyzing plasma for ITZ level after administration of the SEDDS F27 at 1 h

Figure 5.11 depicts the plasma concentration profile of itraconazole after oral administration of SEDDS and extemporaneous suspension of marketed capsule to rats.

Figure 5.11: Plasma concentration profile of itraconazole after oral administration of SEDDS and extemporaneous suspension in rats (n=4 and 30 mg/kg)
There was no significant difference in $AUC_{0\rightarrow t}$ for extemporaneous suspension with F5 and F21, but F27 was statistically significant different from extemporaneous suspension. $AUC_{0\rightarrow \infty}$ of F5 and F27 was significantly different from extemporaneous suspension, but there was no significant difference between extemporaneous suspension and F21. $C_{\text{max}}$ of extemporaneous suspension and F5 was not significant different, but there was a significant difference in $C_{\text{max}}$ of extemporaneous suspension with F21 and F27.

The relative bioavailabilities of itraconazole SEDDS F5, F21 and F27 were 170.56%, 204.55% and 233.91%, respectively when compared to extemporaneous suspension. It was found that there was no significant difference between F5, F21 and F27 with regard to $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow \infty}$ and $C_{\text{max}}$.

The three self emulsifying formulations were prepared, containing varying amounts of oil and surfactants and as mentioned previously the droplet size followed the order F27 > F21 > F5. However, the formulations developed in this study contained varying concentrations of oil and surfactants. The observed bioavailability indicated that there was little correlation of AUC and $C_{\text{max}}$ with respect to droplet size of formed emulsions. The observed results are in agreement with few researchers groups in different laboratories. Yap & Yuen (2004) observed that droplet size difference (1.5 and 10.6 µm) between two SEDDS containing varying amounts of soybean oil, Tween 80 and Labrasol did not affect the bioavailabilities of tocotrienols.

In several cases avoidance of drug precipitation could be the predominant factor governing improvement of oral bioavailability from lipid vehicles than the size of the droplet size. It has been well documented that the influence of droplet size on bioavailability has been observed for several molecules for e.g. Vitamin (Julianto et al., 2000), cyclosporine (Trull et al., 1995), and halofantrine (Khoo et al., 1998); while it is limited for some others, e.g., atovaquone (Sek et al., 2006), danazol (Porter et al., 2004), and ontazolast (Hauss et al., 1998). A very limited number of studies have focused on the influence of the particle/droplet size of comparable formulations on the absorption and bioavailability.

The formulation F5 showed least particle size having maximum amount of surfactant. Contrary to expectations that smaller particle size increases absorption, this did not lead to higher bioavailability. Formulation F21 with slightly bigger droplet size compared to F5 showed higher bioavailability (204.55%). The maximum bioavailability (233.91%) was shown by F27 with least concentration of surfactant and maximum concentration of oil.
The study revealed that the bioavailability decreased when concentration of oil was decreased from 17.27% to 8.77%.

Formulation F5 exhibited faster absorption and higher $C_{\text{max}}$ but lower bioavailability (170.56%) compared to F21 (204.55%) and F27 (233.91%). This indicates that having a highly dispersed system improves absorption rate which is due to higher amount of surfactants, but is not enough to ensure higher bioavailability (Nielsen et al., 2008).

It has been observed and reported that lipid systems which contain more amount of surfactant or hydrophilic excipients are more prone to drug precipitation. Using such excipients may improve solvent capacity, drug loading and in vitro performance but may decrease in vivo absorption due to precipitation of drug on oral administration (Pouton, 2006).

The role of droplet size in the performance of the formulation in vivo is generally less important than the assumption. The possible reason for this is that as soon as the dispersed formulation leaves the stomach it encounters digestive power of the small intestine. The fate of the drug after the formulation has been digested is more important than the initial particle size. Esters can be rapidly hydrolyzed in the presence of pancreatic lipase and most commonly used surfactants are ethoxylated esters that are rapidly hydrolyzed. The physical state of the degradation products will be changed significantly by contact with the mixed bile salt micelles and the drug will partition between the various phases in the gut lumen, or could precipitate out if the total solvent capacity is reduced as a result of lipolysis (Pouton, 2006).

| Table 5.5: Relative bioavailability and pharmacokinetic parameters of itraconazole after oral administration of itraconazole SEDDS and extemporaneous suspension to the rats ($n=4$) |
|---------------------------------|----------------|----------------|----------------|----------------|
|                                | MKT            | SEDDS (F5)     | SEDDS (F21)    | SEDDS (F27)    |
| $AUC_{0-\infty}$ (ng.h/mL)     | 3037.268 ± 198.86 | 4111.651 ± 1567.14 | 3521.983 ± 1030.00 | 4664.412 ± 228.138 |
| $AUC_{0-\infty}$ (ng.h/mL)     | 5264.334 ± 1705.79 | 8978.953 ± 3191.02 | 10768.64 ± 8767.13 | 12314.27 ± 5551.154 |
| $C_{\text{max}}$ (ng/mL)       | 171.956 ± 5.715 | 276.80 ± 138.645 | 243.499 ± 44.90 | 304.848 ± 78.942 |
| $T_{\text{max}}$ (h)           | 1.0 ± 0.0 | 1.5 ± 0.577 | 1.5 ± 0.577 | 1.5 ± 0.577 |
| Relative bioavailability (%)   | --- | 170.56 | 204.55 | 233.91 |
5.3.6. Stability studies

No change in the physical parameters such as homogeneity and clarity was observed during the stability studies. Droplet size, polydispersity index and zeta potential remained unchanged, as compared to initial characterization at 0 month (Table 5.4), even after keeping the formulations at different environmental storage conditions for 3 months, indicating the physical stability of developed formulations (Table 5.6, 5.7 and 5.8). No decline in the drug content was observed at the end of 3 months indicating that drug remained chemically stable in SEDDS.

Table 5.6: Stability studies of itraconazole SEDDS F5 at various storage conditions

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>25 ± 2°C (RT)</th>
<th>30 ± 2°C/65 ± 5% RH</th>
<th>40 ± 2°C/75 ± 5% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters ↓</td>
<td>Medium ↓</td>
<td>1 M</td>
<td>2 M</td>
</tr>
<tr>
<td>Droplet Size</td>
<td>DW</td>
<td>389.21</td>
<td>372.83</td>
</tr>
<tr>
<td></td>
<td>0.1N HCl</td>
<td>134.35</td>
<td>146.49</td>
</tr>
<tr>
<td></td>
<td>PBS</td>
<td>448.29</td>
<td>459.82</td>
</tr>
<tr>
<td>Polydispersity</td>
<td>DW</td>
<td>0.152</td>
<td>0.153</td>
</tr>
<tr>
<td>index (PdI)</td>
<td>0.1N HCl</td>
<td>0.219</td>
<td>0.235</td>
</tr>
<tr>
<td></td>
<td>PBS</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Zeta Potential</td>
<td>DW</td>
<td>-8.3</td>
<td>-6.42</td>
</tr>
<tr>
<td>(mV)</td>
<td>0.1N HCl</td>
<td>5.32</td>
<td>4.28</td>
</tr>
<tr>
<td></td>
<td>PBS</td>
<td>-3.29</td>
<td>-3.81</td>
</tr>
<tr>
<td>Drug Content (%)</td>
<td>104.54 ± 1.6</td>
<td>102.02 ± 1.82</td>
<td>100.70 ± 0.46</td>
</tr>
</tbody>
</table>

Table 5.7: Stability studies of itraconazole SEDDS F21 at various storage conditions

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>25 ± 2°C (RT)</th>
<th>30 ± 2°C/65 ± 5% RH</th>
<th>40 ± 2°C/75 ± 5% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters ↓</td>
<td>Medium ↓</td>
<td>1 M</td>
<td>2 M</td>
</tr>
<tr>
<td>Droplet Size</td>
<td>DW</td>
<td>194.46</td>
<td>200.37</td>
</tr>
<tr>
<td></td>
<td>0.1N HCl</td>
<td>181.2</td>
<td>169.25</td>
</tr>
<tr>
<td></td>
<td>PBS</td>
<td>492.52</td>
<td>482.46</td>
</tr>
<tr>
<td>Polydispersity</td>
<td>DW</td>
<td>0.232</td>
<td>0.321</td>
</tr>
<tr>
<td>index (PdI)</td>
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<td>0.476</td>
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</tr>
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<td>1.0</td>
</tr>
<tr>
<td>Zeta Potential</td>
<td>DW</td>
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<td>4.47</td>
</tr>
<tr>
<td>(mV)</td>
<td>0.1N HCl</td>
<td>6.43</td>
<td>6.73</td>
</tr>
<tr>
<td></td>
<td>PBS</td>
<td>-5.38</td>
<td>-6.37</td>
</tr>
<tr>
<td>Drug Content (%)</td>
<td>102.42 ± 1.84</td>
<td>101.91 ± 0.63</td>
<td>101.61 ± 0.63</td>
</tr>
</tbody>
</table>
5.4. Conclusion

In the recent years the utilization of lipid excipients for the enhancement of oral bioavailability of poorly soluble drugs has attracted attention. Self-emulsifying drug delivery systems containing itraconazole were formulated using various ratios of oil:S/CoS mixture in an attempt to increase its release rate and bioavailability. Three formulations containing itraconazole were developed through the construction of pseudo-ternary diagrams, droplet size determination and visual inspection. F5 consisted of 8.77% of Capryol 90, 13.31% Labrasol, 32.15% of Tween 20, 43.32% Transcutol; F21 consisted of 12.4% Capryol 90, 12.5% Labrasol, 45.3% Tween 20 and 27.23% Transcutol; F27 consisted of 17.27% Capryol 90, 15.73% Labrasol, 57% Tween 20 and 7.5% Transcutol. Itraconazole was 2.4% in all three SEDDS formulations. After oral administration to rats, the SEDDS F5, F21 and F27 containing itraconazole had a relative bioavailability of 170.56%, 204.55% and 233.91% compared with the extemporaneous suspension prepared from marketed capsules. The order of droplet size of formed emulsion was 1:4 (F27) > 1:6 (F21) > 1:9 (F5) for ratios of oil: S\textsubscript{mix}. Although the three self-emulsifying formulations with different ratios of oil:S\textsubscript{mix} did show differences in exposures of itraconazole in rats, the results were not in consistent with strong dependence on emulsion droplet size and oral absorption. Contrary to expectations, the droplet size of the formed emulsion played very little role in in vivo absorption of itraconazole from developed self emulsifying formulations. The possible explanation for this observation can be a very high lipophilicity of itraconazole (log \( P \) 5.66). Such drugs have more affinity toward lipophilic domain of the formulation and remain in a solubilised state for better absorption. When the drug
solubilisation capacity at interface has been increased with the use of surfactants, dilution with physiological fluid may lead to migration of surfactant form the oil/water interface and can result in reduction in drug loading capacity and precipitation. This phenomenon may be responsible for the decrease in bioavailability as the proportion of surfactant increased. In conclusion, the data overall showed little apparent correlation between emulsion droplet size and oral absorption in rats, but statistically ($P < 0.05$) there was no significant difference between all the three formulations with respect to AUC and $C_{\text{max}}$. The results obtained illustrate the magnitude of bioavailability enhancement that can be achieved through rational design of lipid-based formulations.
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Chapter 5

Itraconazole


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