Chapter 3

Objective and work plan
3.1 OBJECTIVE OF THE PRESENT STUDY

There are a number of drugs in the allopathic system of medicine which have problems of low aqueous solubility and/or low intestinal permeability which often limits their bioavailability. The Biopharmaceutical Classification System has provided a scientific basis for classifying drugs into four classes based on their solubility and intestinal permeability as:

Class I: High Solubility - High Permeability

Class II: Low Solubility - High Permeability

Class III: High Solubility - Low Permeability

Class IV: Low Solubility - Low Permeability

The Biopharmaceutical Classification System

Drugs belonging to the Classes II, III and IV are usually the ones which present a significant challenge to the pharmaceutical scientist to formulate them into highly bioavailable and therapeutically useful compositions.

Although a number of approaches have been used in the past to overcome the problems of low bioavailability, the use of these approaches have often been limited by practical factors such as toxicity, irritancy, non-selectivity, poor drug stability, manufacturing problems and high cost. Thus, there exists a definite need for identifying newer and novel approaches to overcome the problems of solubility and permeability of problematic drug candidates so as to augment their bioavailability.

The use of fulvic acids and humic acids extracted from shilajit as carrier for such problematic drugs can be a potential approach for increasing the bioavailability of such drugs.

The objective of the present work was thus to evaluate the drug complexing, solubility/permeability enhancing and surface active properties of fulvic and humic acids extracted from shilajit to see if such an interaction between the drug and
extracted fulvic or humic acids can lead to an improved bioavailability and better pharmacodynamic profile. It is possible that such problematic drugs can be entrapped or complexed with these humic substances in order to advantageously increase their solubility, permeability and hence bioavailability.

The specific objectives of the study were:

1. To authenticate and characterize shilajit samples obtained from different sources.

2. To standardize the reported method for the extraction of humic and fulvic acids from shilajit and if required, to develop an improved method for extraction of these humic substances.

3. Physico-chemical and spectral characterization of humic and fulvic acids extracted from shilajit.

4. To evaluate the surfactant properties of humic and fulvic acids extracted from shilajit.

5. To evaluate the complexing and solubility / permeability enhancing properties of humic substances using model drug candidates, itraconazole and acyclovir.

6. To determine the safety of the prepared complexes of itraconazole with humic / fulvic acids extracted from shilajit.

7. To develop an oral formulation of itraconazole with improved bioavailability by complexation with humic or fulvic acids.

8. To give the proof of concept by determining the oral bioavailability of developed formulation in healthy human volunteers and comparing it with the innovator product.
3.2 SELECTION OF MODEL DRUG CANDIDATES

3.2.1 Itraconazole

Itraconazole, a newer, broad spectrum, triazole antifungal drug was identified as a suitable drug candidate for studying the effect of bioavailability enhancers from natural sources. Itraconazole has very poor aqueous solubility and a poor bioavailability. It is generally classified as a BCS Class IV drug. Currently, itraconazole is available in the Indian market as 100 mg capsule. In the United States, it is available as 100 mg capsule, 10mg/ml oral solution and 10mg/ml injection. The capsule that is available is prepared by a lengthy and tedious process which involves coating of a solution of itraconazole and hydroxypropyl methylcellulose on sugar beads followed by a coating of polyethylene glycol 20000. Even this formulation results in poor and highly variable bioavailability of itraconazole. An oral solution and injectable preparations have been formulated by complexing the drug with hydroxypropyl β-cyclodextrin. Although complexation of itraconazole with hydroxypropyl-β-cyclodextrin has resulted in some increase in its bioavailability, the quantity of hydroxypropyl-β-cyclodextrin required to solubilize itraconazole is so large that it precludes formulation of a solid dosage form using this approach.

Itraconazole thus seems to be an ideal drug candidate for studying the complexation behaviour of humic and fulvic acids from shilajit and to study their effect on enhancement of its bioavailability.

3.2.2 Acyclovir:

Acyclovir is a synthetic nucleoside analogue having antiviral activity against herpes viruses. It is available for human administration in the form of injections as well as tablets. The absorption of orally administered acyclovir is slow, variable and incomplete with an oral bioavailability of only 15 – 30%. The poor absorption has mainly been attributed to its site dependent and poor permeability across intestinal tissue. It is generally classified as a BCS Class III drug. Thus, acyclovir seems to be a good candidate for evaluating the permeability enhancing properties of humic and fulvic acids extracted from shilajit.
triazole moiety and a long hydrophobic non-ligating portion form the basis for itraconazole's potency and selectivity.

3.3.4 Biopharmaceutics

Itraconazole is a representative of a class of triazole antifungals characterized by a high lipophilicity which makes it virtually insoluble in water. It is an extremely weak base (pK_a =3.7) that is ionized only at low pH, such as that found in gastric fluid. Therefore, one of the initial problems associated with the oral administration of itraconazole is that of insufficient dissolution in the stomach before the drug is delivered to the intestinal lumen for absorption (Fig. 3.1). Dissolution of itraconazole is optimal at a pH 1-4, with impaired absorption occurring above these pH values (Lange et al., 1997). Gastric emptying rate also plays an important role in the absorption of solid itraconazole dosage forms. Generally, slower emptying rates are associated with greater itraconazole dissolution and absorption. For this reason, it is recommended that itraconazole capsules be taken after a full meal for optimal dissolution.

Fig.3.1: Schematic representation of gastrointestinal dissolution and absorption of itraconazole
3.3 ITRACONAZOLE: A DRUG PROFILE

Itraconazole is an orally active triazole drug with a broad spectrum of antifungal activity.

![Chemical Structure of Itraconazole]

3.3.1 Physicochemical Properties

**Description**: White or almost white powder, odourless and having a bitter taste. It is optically active, the racemate being used clinically.

**Chemical Name**: 4-[4-[4-[4-[cis-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl][phenyl]-2-[(1RS)-1-methylpropyl]-2,4-dihydro-3H-1,2,4-triazol-3-one

**CAS #**: 36322-90-4

**Molecular Formula**: C₃₅H₃₈Cl₂N₈O₄

**Molecular Weight**: 705.64

**pKa**: 3.7

**Partition coefficient (K<sub>o/w</sub>)**: 46000 (pH 8.1)

**Solubility**: Practically insoluble in water. Very slightly soluble in alcohol. Freely soluble in dichloromethane
Itraconazole is active against a large number of fungal species including dermatophytes, *Candida* spp., *Cryptococcus* spp., *Pityrosporum* spp., *Aspergillus* spp., *Penicillium* spp., *Sporothrix* spp., dimorphic fungi, pheophyphomycetes, agent of eufungal mycetoma, and various phycomycetes and yeasts and a variety of other fungi (Van Cauteren et al., 1989). Itraconazole also has some antiprotozoal activity against Leishmania spp. (Dogra et al., 1994) and *Trypanosoma cruzi* (McCable et al., 1986; Goad et al., 1989).

3.3.3 Pharmacology

Azole and triazole antifungal agents primarily inhibit the biosynthesis of ergosterol, a major component of the cell membrane of yeast and fungal cells (Thomas et al., 1986), by replacing the precursor lanosterol as a substrate for the fungal cytochrome P450 enzyme lanosterol 14α-demethylase which catalyzes the conversion of lanosterol to ergosterol in three oxidative steps (Vanden Bosch et al., 1983; Vanden Bosch et al., 1990). One of the unsubstituted nitrogen atoms (N-3 or N-4) of the azole ring binds to the heme iron of the cytochrome P450 thereby inactivating it and inhibiting its enzyme function. Being integral to the proper functioning of the cell membrane, inhibition of the synthesis of ergosterol leads to a cascade of abnormalities in permeability, membrane bound enzyme activity, and coordination of chitin synthesis leading to inhibition of growth, abnormal cell wall formation, and accumulation of intracellular lipids and membrane vesicles (Vanden Bosch et al., 1989). The presence of
3.3.5 Pharmacokinetics

Following oral administration, itraconazole is variably and incompletely absorbed into the systemic circulation with an absolute systemic bioavailability of 55%. The mean and standard deviation of half life after intravenous administration is 25±5 h and the plasma clearance is 5.1 ml.min⁻¹.kg which is 44% of normal liver blood flow suggesting that the low bioavailability is also the result of appreciable presystemic metabolism. Two mechanisms have been identified as important modulators of presystemic clearance/metabolism of itraconazole, P-glycoprotein (P-gp) and Cytochrome P450 3A4 isoenzyme (CYP 3A4). Itraconazole is both an inhibitor and substrate of P-gp and CYP 3A4 (Gubbins, et. al., 2001). Hence, the ultimate effects of these on intestinal absorption of itraconazole can be mixed, or change over a period of prolonged exposure to itraconazole.

Itraconazole that is absorbed into the systemic circulation is highly bound to red blood cells and plasma proteins (>99%). Therefore, unbound concentrations of itraconazole in the body fluids (CSF, eye, saliva) are low in relation to plasma concentrations. However, tissue concentrations of itraconazole are generally 2-10 fold higher than concurrent plasma concentrations (Heykants et. al., 1989). Especially high concentrations of itraconazole can be found in vaginal tissue and the horny layer of the nails, where drug levels remain elevated more than 10 days after drug is cleared from the plasma (Heykants et. al., 1989).

Systemically-available itraconazole is extensively metabolized in the liver to both active and inactive metabolites, which are subsequently cleared by the kidneys. The drug excreted in urine accounted for 35% of the dose given to three volunteers and faecal excretion accounted for further 54%. The main site of metabolism is the liver and involves oxidative degradation of the dioxolane, piperazine, triazolone rings and oxidation or N-alkylation of the 1-methylpropyl substituent. The principal route of excretion is hepatobiliary. One metabolite, hydroxyitraconazole, has similar antifungal activity in vitro to the parent drug (Mikami et. al., 1994).
Over a dosing range of 100-400 mg/day, itraconazole displays dose-dependent pharmacokinetics in healthy adults. However, at doses >400 mg/day plasma concentrations are higher than might be expected from dosing because of saturation of itraconazole CYP 3A4 metabolism in the gut and/or liver (Poirier and Cheymol, 1998). Plasma AUCs are generally 4-5x higher after 2-3 weeks of therapy compared to those achieved after a single dose (Heykants et al., 1989).

3.3.6 Pharmacokinetic differences of itraconazole formulations

3.3.6.1 Capsules

As mentioned previously, one of the major drawbacks to administering itraconazole via the oral route lies in the poor solubility (dissolution) of the antifungal. The first strategy used to overcome this poor solubility involved the synthesis of antifungal-coated sugar spheres in a capsule formulation to maximize the available surface area for dissolution of itraconazole. This approach had limited success, however, in patients with drug or disease-related gastric hypochlorhydria (pH > 4); a common condition in seriously-ill patients. In some cases, capsule absorption could be improved if the capsules were administered with an acidic beverage (i.e., cola or juice) in divided doses (Lange et al., 1997).

3.3.6.2 Oral Solution

A more successful approach towards the improvement of itraconazole absorption involved the incorporation of itraconazole into a 40% hydroxypropyl-β-cyclodextrin vehicle. With the incorporation of itraconazole into the cyclodextrin vehicle, the problems of incomplete dissolution at high gastric pH are avoided. This was evident from pharmacokinetic studies performed in normal volunteers, which demonstrated 30% higher AUC\(_{(0-24)}\) for the oral solution under fed conditions, and 60% higher AUC\(_{(0-24)}\) when the cyclodextrin/itraconazole solution is administered under fasting conditions (Van de Velde et al., 1996). Therefore, unlike the capsule formulation, itraconazole solution is generally taken on an empty stomach.
3.3.6.3 Intravenous Formulation
Administration of itraconazole by intra-venous route by-passes problems associated with oral absorption, as well as mechanisms of pre-systemic clearance/metabolism in the gut and liver. Not surprisingly, the IV formulation achieves effective levels of itraconazole in the plasma much faster and with fewer side effects than oral administration (De Beule and Van Gestel, 2001).

3.3.7 Concentration-effect relationship
Itraconazole shows wide subject to subject variation in its plasma concentration – time curve profile (Pierard et al., 1995). While there is no clear concentration-effect relationship for itraconazole, patients whose plasma level of itraconazole is low are more likely to have therapeutic failure (Thomas et al., 1986). Similarly therapeutic response (Thomas et al., 1986; Tricot et al., 1987; Boogaerts et al., 1989) was better in patients whose plasma itraconazole concentration were higher, usually in excess of 0.25 mg.l⁻¹.

3.3.8 Toxicology
Acute oral toxicity is low and it was not possible to calculate an LD₅₀ after administering a dose of 160 mg.kg⁻¹. In chronic toxicity studies of 3 months duration in rats and dogs, the no effect level was 10 mg.kg⁻¹. At 40 mg.kg⁻¹ rats showed behavioural changes and some foamy cells consisting of proteinaceous deposit in macrophages were seen in histological sections. These changes were more marked at 160 mg.kg⁻¹ and some of the animals dosed at this level died (Van Cauteren et al., 1989).

3.3.9 Indications
3.3.9.1 Dermatophytosis
Itraconazole is active in dermatophytes infections at the recommended dose of 100mg daily for 15-30 days.
3.3.9.2 Superficial candidiasis
Vulvovaginal candidiasis caused by *Candida albicans* responds to a course of 200mg itraconazole given on 3 consecutive days attaining a remission rate of 75% with a relapse rate of 15%. (Sanz et. al., 1988).

3.3.9.3 Pityriasis versicolor
In pityriasis versicolor itraconazole has been shown to produce mycological and clinical response within 30 days. However, the length of treatment may be considerably shorter and 7 days, therapy with 200mg daily may produce cures in 95% of those treated (Estrada et. al., 1987).

3.3.9.4 Occulomycoses
The study in India showed a moderate to excellent response was obtained in 69% of 110 patients treated for keratomycosis with itraconazole administered either orally or topically or by both routes.

3.3.9.5 Subcutaneous mycosis
Itraconazole is the first oral antifungal agent to produce good response in sporotrichosis within 6 weeks of starting therapy with 100mg itraconazole daily.

3.3.9.6 Systemic mycoses
Itraconazole is used in systemic infections owing to pathogenic fungi (Paracoccidioidomycosis, Cryptococcal meningitis, Histoplasmosis, Coccidioidomycosis, Blastomycosis, etc.) and Systemic opportunistic mycoses.

3.3.9.7 Antifungal prophylaxis
Itraconazole has been used prophylactically to prevent systemic fungal infection in severely neutropenic patients in doses of 100mg daily.

3.3.10 Adverse reactions
The most common adverse effects associated with itraconazole include dyspepsia, abdominal pain, nausea, constipation and diarrhoea (with the oral liquid). Other reported adverse effects include peripheral oedema, congestive heart failure and pulmonary oedema, headache, dizziness, peripheral neuropathy, menstrual
disorders, reversible increase in hepatic enzymes, hepatitis, liver failure, hyperaemia, hypertriglyceridemia, alopecia, allergic reactions such as pruritis, rash, urticaria and angioedema and anaphylaxis, Stevens-Johnson syndrome and neutropenia.

3.3.11 Contraindications
Use of itraconazole has been contraindicated in pregnancy, liver disease and history of congestive heart failure. Co-administration of cisapride, pimozide, quinidine, or dofetilide with itraconazole is also contraindicated since itraconazole is a potent cytochrome P450 3A4 isoenzyme system inhibitor and co-administration of drugs metabolized by this pathway may increase their plasma concentrations.

3.3.12 Use and administration
Dose used in clinical trials have ranged from 50 to 600 mg daily but the current recommended dosage ranges from 100 mg to a maximum of 400 mg daily, depending on the nature of infection. The normal duration of therapy is 15 to 30 days but the drug has been administered for up to 6 months.

3.3.13 Analytical methodology
Various microbiological and chromatographic methods have been reported for the determination of itraconazole in pharmaceuticals and in plasma during pharmacokinetic investigations as well as for therapeutic drug monitoring purposes (Koks, et. al., 2002). Microbiological assays have the disadvantage that they are non-specific and unable to distinguish the metabolite from the parent compound. Another disadvantage is that these methods lack adequate precision and sensitivity for therapeutic drug monitoring. HPLC methods (Table 3.1) utilizing either UV or fluorescence detection have been used more successfully.
### Table 3.1: Reported HPLC methods for analysis of Itraconazole

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Column</th>
<th>Temp.</th>
<th>Mobile Phase</th>
<th>Flow Rate</th>
<th>Detection (UV/Fluorescence)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>RSiC&lt;sub&gt;18&lt;/sub&gt;HL 150 × 2.1 mm, 5μm</td>
<td>R.T.</td>
<td>Water-acetonitrile-diethyl amine (40:60:0.05 v/v)</td>
<td>0.5 ml/min</td>
<td>UV 263 nm</td>
<td>Woestenborghs, et. al., 1987</td>
</tr>
<tr>
<td>2.</td>
<td>Hypersil ODS 100 × 3 mm</td>
<td>R.T.</td>
<td>Water-acetonitrile (40:60) + 30μl/l diethyl amine, pH adjusted to 7.8 with orthophosphoric acid</td>
<td>0.5 ml/min</td>
<td>UV 263 nm</td>
<td>Warnock, et. al., 1988</td>
</tr>
<tr>
<td>3.</td>
<td>Ultrasphere ODS 150 × 4.6 mm, 5μm</td>
<td>R.T.</td>
<td>Water-acetonitrile-diethyl amine (40:60:0.05 v/v)</td>
<td>1.5 ml/min</td>
<td>UV 261 nm</td>
<td>Badcock, 1990</td>
</tr>
<tr>
<td>4.</td>
<td>RSiC&lt;sub&gt;18&lt;/sub&gt;HL 150 × 2.1 mm, 5μm</td>
<td>R.T.</td>
<td>Water-acetonitrile-diethyl amine (40:60:0.05 v/v)</td>
<td>0.5 ml/min</td>
<td>UV 293 nm</td>
<td>Hardin ct. al., 1988</td>
</tr>
<tr>
<td>5.</td>
<td>Hypersil ODS-5 150 × 4.6 mm</td>
<td>R.T.</td>
<td>Water-acetonitrile (40:60) + 0.02% diethyl amine</td>
<td>0.5 ml/min</td>
<td>UV 254 nm</td>
<td>Miyake, et. al., 1999</td>
</tr>
<tr>
<td>6.</td>
<td>Lichrospher 100 RP8 250 × 4 mm, 7μm</td>
<td>R.T.</td>
<td>Water containing 0.05% diethyl amine-ace tonitrile (40:60) pH adjusted to 6.0 with 30% acetic acid</td>
<td>2.0 ml/min</td>
<td>UV 263 nm</td>
<td>Yoo, et. al., 2000a and 2000b</td>
</tr>
<tr>
<td>7.</td>
<td>C&lt;sub&gt;18&lt;/sub&gt; Microsorb MV 250 mm, 5μm</td>
<td>R.T.</td>
<td>Water containing 0.05% diethyl amine-ace tonitrile (40:60) pH adjusted to 8.0 with 30% acetic acid.</td>
<td>1.2 ml/min</td>
<td>UV 254 nm</td>
<td>Kapai &amp; Ayres, 2001</td>
</tr>
<tr>
<td>8.</td>
<td>Lichrospher RP8 250 × 4 mm, 5μm</td>
<td>R.T.</td>
<td>Acetonitrile-water (55:45)</td>
<td>1.5 ml/min</td>
<td>UV 263 nm</td>
<td>Ciciglio, et. al., 1997</td>
</tr>
<tr>
<td>9.</td>
<td>Symmetry C&lt;sub&gt;18&lt;/sub&gt; column 100 × 4.6 mm, 3.5 μm</td>
<td>R.T.</td>
<td>Acetonitrile-water containing 0.1% triethyl amine with pH adjusted to 3.0 with 85%w/v phosphoric acid. (55:45)</td>
<td>1.0 ml/min</td>
<td>Fluor. Ex 265nm Em 363nm</td>
<td>Koks, et. al., 2002</td>
</tr>
<tr>
<td>10.</td>
<td>C&lt;sub&gt;8&lt;/sub&gt; Kromasil 250 × 4.6 mm, 5μm</td>
<td>30°C</td>
<td>Acetonitrile, 2-propanol and 0.01% triethylamine buffer, pH 2.8 (variable ratios)</td>
<td>1.0 ml/min</td>
<td>Fluor. Ex 264n Em 380nm</td>
<td>Srivatsan, et. al., 2004</td>
</tr>
</tbody>
</table>
3.3.14 Strategies employed for solubility/dissolution enhancement of itraconazole

Because of its inherent poor solubility and dissolution characteristics, itraconazole has been a formulator’s delight for evaluating various techniques to increase its solubility/dissolution characteristics. Table 3.2 lists some of these strategies employed.

Table 3.2: Strategies employed for solubility/dissolution enhancement of itraconazole

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Formulation developed</th>
<th>Strategy employed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Itraconazole capsule (Innovator product)</td>
<td>A solution of itraconazole and HPMC 2910 in methylene chloride and ethanol (60:40) was coated on to sugar beads in a GLATT coater. The coated beads were dried and further coated with a 10% solution of PEG 20000 in methylene chloride and ethanol (60:40)</td>
<td>Gilis et. al., 1997</td>
</tr>
<tr>
<td>2</td>
<td>Itraconazole tablet</td>
<td>A solid dispersion of itraconazole was prepared by blending it with HPMC 2910 in the ratio of 2:3 and melt extruding the blend at 120-130°C. The resulting solid dispersion was pulverized and formulated into a tablet</td>
<td>Baert, et. al., 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Verreck, et. al., 2003a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Six, et. al., 2003</td>
</tr>
<tr>
<td>3</td>
<td>Itraconazole capsule</td>
<td>A solid solution containing itraconazole, PEG 20000, glycerol, sodium starch glycolate and HPMC K15M in the ratio 1:2.75:0.75:0.25:0.25 was prepared by heating at 120°C. The solution was cooled, pulverized and filled into capsules</td>
<td>Kapsi &amp; Ayres, 2001</td>
</tr>
<tr>
<td>4</td>
<td>Itraconazole oral solution</td>
<td>An oral solution containing 1% itraconazole, 40% w/v hydroxypropyl β-cyclodextrin, 10% v/v propylene glycol, 19% sorbitol and sufficient quantity of hydrochloric acid to adjust the pH to 2.0 was prepared by dissolving itraconazole in a mixture of acid and propylene glycol and adding it to a solution of cyclodextrin in water followed by addition of sorbitol and mixing.</td>
<td>Fran.cedilla.ois, &amp; Dries, 1998</td>
</tr>
<tr>
<td>5</td>
<td>Itraconazole microspheres</td>
<td>A solution of itraconazole was prepared by dissolving in glacial acetic acid to which was added sufficient quantity of proteinoid. The resulting solution was spray dried to yield the microspheres.</td>
<td>Kantor, M.L., 2000</td>
</tr>
<tr>
<td>6</td>
<td>Itraconazole capsules</td>
<td>Complexes of itraconazole with succinic acid, Pluronic P127 and HPMC E5 were prepared by dissolving in a mixture of methanol and methylene chloride. Potassium dihydrogen phosphate and lactose were added to the solution and the mixture was evaporated to dryness. The resulting powder was filled into capsules.</td>
<td>Bharatirjan, et. al., 2001</td>
</tr>
<tr>
<td>7</td>
<td>Itraconazole tablet</td>
<td>Solid dispersions of itraconazole were prepared by dissolving it in methylene chloride and pouring the solution onto superdisintegrants like AcDiSol. The wet solid mixture was mixed thoroughly and dried at 60°C. The resulting mass was granulated using starch paste and compressed into tablets.</td>
<td>Chowdhary and Srinivasa Rao, 2000</td>
</tr>
<tr>
<td>8</td>
<td>Itraconazole complexes</td>
<td>Complexes of itraconazole with β-cyclodextrin or hydroxypropyl β-cyclodextrin were prepared by kneading and co-evaporation methods</td>
<td>Chowdhary and Srinivasa Rao, 2001 and 2002</td>
</tr>
<tr>
<td>9</td>
<td>Itraconazole electrospun nanofibres</td>
<td>Polyurethane fibres loaded with 10% or 40% itraconazole were obtained by dissolving itraconazole and polymer in DMF and feeding it to an electrostatic spinner where a high voltage was applied to yield the fibres.</td>
<td>Verreck, et. al., 2003b and 2003c</td>
</tr>
<tr>
<td>10</td>
<td>Itraconazole solid dispersion</td>
<td>A solid dispersion of itraconazole, HPMC and hydroxy propyl β-cyclodextrin in the ratio 40:45:15 was prepared by melt extruding the physical mixture in a melt extruder.</td>
<td>Rambali, et. al., 2003</td>
</tr>
</tbody>
</table>
3.4 ACYCLOVIR: A DRUG PROFILE

Acyclovir is a synthetic nucleoside analogue having antiviral activity against herpes viruses.

![Chemical Structure of Acyclovir]

3.4.1 Physicochemical Properties

<table>
<thead>
<tr>
<th>Description</th>
<th>A white to off-white crystalline powder.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Name</td>
<td>2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6H-purin-6-one</td>
</tr>
<tr>
<td>CAS #</td>
<td>59277-89-3</td>
</tr>
<tr>
<td>Molecular Formula</td>
<td>C_{9}H_{11}N_{5}O_{3}</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>225.226</td>
</tr>
<tr>
<td>pKa</td>
<td>2.27 and 9.25</td>
</tr>
<tr>
<td>Solubility</td>
<td>Slightly soluble in water; insoluble in alcohol; soluble in dilute hydrochloric acid.</td>
</tr>
<tr>
<td>Melting point</td>
<td>166°C to 170°C</td>
</tr>
<tr>
<td>Category</td>
<td>Broad spectrum antifungal drug</td>
</tr>
<tr>
<td>Pharmacopoeial Status</td>
<td>Official in European Pharmacopoeia and British Pharmacopoeia</td>
</tr>
<tr>
<td>Formulations available</td>
<td>Capsules, Oral liquid and Injection</td>
</tr>
<tr>
<td>Innovator Product</td>
<td>Zovirax (GlaxoSmithKline)</td>
</tr>
</tbody>
</table>
3.4.2 Pharmacology

Acyclovir is a synthetic purine nucleoside analogue with in vitro and in vivo inhibitory activity against herpes simplex virus types 1 (HSV-1), 2 (HSV-2), and varicella-zoster virus (VZV) (Richards et al., 1983).

The inhibitory activity of acyclovir is highly selective due to its affinity for the enzyme thymidine kinase (TK) encoded by HSV and VZV. This viral enzyme converts acyclovir into acyclovir monophosphate, a nucleotide analogue. The monophosphate is further converted into diphosphate by cellular guanylate kinase and into triphosphate by a number of cellular enzymes (Elion, 1982). In vitro, acyclovir triphosphate stops replication of herpes viral DNA. This is accomplished in 3 ways: 1) competitive inhibition of viral DNA polymerase, 2) incorporation into and termination of the growing viral DNA chain, and 3) inactivation of the viral DNA polymerase. The greater antiviral activity of acyclovir against HSV compared to VZV is due to its more efficient phosphorylation by the viral TK.

3.4.3 Pharmacokinetics

Acyclovir is slowly and poorly absorbed from the gastrointestinal tract and the time to reach peak concentration is 1.5-2 h (de Miranda, et al., 1983). Absorption across the small intestine appears to be passive and is incomplete, resulting in 15 – 30% bioavailability. Following oral administration in adult volunteers and patients with normal renal function the plasma half-life ranges from 2.5 to 3.3 h. Food does not affect acyclovir absorption.

Acyclovir is widely distributed in tissues and body fluids including brain, kidney, lung, liver, muscle, spleen, uterus, vaginal mucosa, vaginal secretions, CSF, and herpetic vesicular fluid. Renal excretion is the major route of elimination in individuals with normal renal functions: 75-80% of intravenous dose and 9-20% of an oral dose may be recovered unchanged from the urine.

The pharmacokinetics of intravenous acyclovir best fit a two-compartment open model. Dose independent pharmacokinetics are observed in the range 0.5 to 15 mg.kg⁻¹, whether after single doses or at steady state after multiple dosing (Laskin,
1983). The mean steady-state peak and trough plasma concentrations of acyclovir after intravenous administration of 5 mg.kg⁻¹ to adults (or after 250 mg.kg⁻¹ to children by 1 h infusions every 8 h) are 9.8 mg.kg⁻¹ and 0.7 mg.kg⁻¹ respectively. The mean peak and trough plasma concentrations after 10 mg.kg⁻¹ (or 500 mg.kg⁻¹ in children) are 22.9 mg.kg⁻¹ (14.1-44.1 mg.kg⁻¹) and 1.9 mg.kg⁻¹, respectively. The mean plasma half-life of intravenous acyclovir is 2.93 h with a range of 1.5-6.3 h.

Radio labelled disposition studies in individuals with normal renal function show that almost all of an administered dose is recovered from the urine. About 80% is unchanged and a further 8.5-14% is the only significant metabolite, the oxidized, 9-carboxymethoxymethyl guanine (de Miranda et. al., 1983).

3.4.4 Pharmaceutics

Preparations containing acyclovir are available for oral, topical, and ophthalmic administration and containing acyclovir sodium for intravenous administration.

Oral forms
1. Zovirax capsules (Glaxo Wellcome, USA)
2. Zovirax tablets (Glaxo Wellcome, USA)
3. Zovirax dispersible tablets (Wellcome, UK)
4. Zovirax suspension (Glaxo Wellcome, USA; Wellcome, UK)
5. Zovirax Double Strength suspension (Wellcome, UK)

Parenteral forms
1. Zovirax sterile powder (Glaxo Wellcome, USA)
2. Zovirax IV (Wellcome, UK)

Topical forms
1. Zovirax ointment (Glaxo Wellcome, USA)

Ophthalmic forms
1. Zovirax eye ointment (Wellcome, UK)
3.4.5 Toxicology

Rapid administration of parenteral doses may cause the solubility to be exceeded and may lead to crystalluria. Renal lesions resulting from obstruction of renal tubules by precipitated acyclovir crystals have been observed in several species, for example after 20-80mg.kg\(^{-1}\) for 3 weeks in rats by rapid bolus intravenous dosing.

3.4.6 Clinical Pharmacology

The concentration of drug required to inhibit by 50% the growth of virus in cell culture (ID\(_{50}\)) varies greatly depending upon the particular assay used, the cell type employed, and the laboratory performing the test (O'Brien, et. al., 1989). For HSV type 1 the ID\(_{50}\) of acyclovir ranges from 0.02 mg.l\(^{-1}\) to 5.9-13.5 mg.l\(^{-1}\). For HSV type 2 the respective concentrations are 0.01 mg.l\(^{-1}\) and 9.9 mg.l\(^{-1}\). The ID\(_{50}\) for varicella-zoster virus ranges from 0.17-1.53 mg.l\(^{-1}\) to 1.4-10.8 mg.l\(^{-1}\).

No effects on human metabolism have been observed at clinically used doses.

3.4.7 Indications

Use of various formulations of acyclovir has been indicated for the:

1. Treatment of herpes simplex keratitis
2. Treatment and prophylaxis (suppression) of herpes simplex infections of skin and mucous membranes in immunocompetent individuals.
3. Treatment of severe and/or generalized herpes simplex infections in immunocompromized and immunocompetent individuals.
4. Treatment of varicella-zoster infections in immunocompromized and immunocompetent individuals.
5. Prophylaxis of herpes simplex, varicella-zoster and cytomegalovirus infections in the immunocompromized.

3.4.9 Adverse reactions

Potentially life-threatening effects

None has been reported
Severe or irreversible adverse effects
Rise in blood urea and/or creatinine are well described in patients on intravenous therapy

Symptomatic adverse effects
Severe local inflammatory reactions sometimes leading to breakdown of skin have occurred when intravenous acyclovir has been inadvertently infused into extravascular tissues.

3.4.10 Contraindications
Use of acyclovir is contraindicated for patients who develop hypersensitivity to acyclovir or valacyclovir.

3.4.11 Use and administration
Oral doses of acyclovir vary according to indication. For treatment of primary herpes simplex infections, including genital herpes, the usual dose by mouth is 200 mg five times daily (usually every 4 hours while awake) for 5 to 10 days. Severely immunocompromised patients or those with impaired absorption may be given 400 mg five times daily for 5 days.

3.4.12 Analytical methodology
A number of analytical methods have been reported for the determination of acyclovir in pharmaceuticals and in plasma during pharmacokinetic investigations as well as for therapeutic drug monitoring purposes in biological samples (Loregian et. al., 2001). Drug concentrations were measured either by immunological techniques or reverse-phase high-performance liquid chromatography, the latter being the preferred technique (Table 3.3).
### Table 3.3: Reported HPLC methods for analysis of acyclovir

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Column</th>
<th>Temp.</th>
<th>Mobile Phase</th>
<th>Flow Rate</th>
<th>Detection (UV/Fluorescence)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>C18 Hypersil ODS 250 x 4.6 mm, 5μm</td>
<td>R.T.</td>
<td>Acetonitrile- ammonium acetate buffer pH 4.5 (40:60)</td>
<td>0.8 ml/min</td>
<td>UV 250 nm</td>
<td>Basavaiah et al., 2003</td>
</tr>
<tr>
<td>2.</td>
<td>Chemosorb 5-ODS-H 150 x 4.6 mm, 5μm</td>
<td>R.T.</td>
<td>Methanol-phosphate buffer (20:80)</td>
<td>0.7 ml/min</td>
<td>Fluor. Ex. 270 nm Em 370nm</td>
<td>Fujoko, et al., 1991</td>
</tr>
<tr>
<td>3.</td>
<td>C18 Waters 250 x 3.9 mm, 5μm</td>
<td>R.T.</td>
<td>1% v/v acetonitrile in 10mM ammonium acetate buffer pH 5.0 containing 1.0mM sodium heptane sulfonate</td>
<td>1.0 ml/min</td>
<td>UV 254 nm</td>
<td>Park, et al., 1992</td>
</tr>
<tr>
<td>4.</td>
<td>C8 Lichrospher Select B 250 x 4 mm, 5μm</td>
<td>R.T.</td>
<td>Acetonitrile- ammonium acetate buffer (0.05M) (1:99 v/v)</td>
<td>1.0 ml/min</td>
<td>UV 252 nm</td>
<td>De Jalon et al., 2002</td>
</tr>
<tr>
<td>5.</td>
<td>C8 Nucleosil 250 x 4 mm, 5μm</td>
<td>R.T.</td>
<td>Phosphate buffer pH 7.9-methanol-acetonitrile</td>
<td>-</td>
<td>UV</td>
<td>Legen &amp; Kristi, 2001</td>
</tr>
<tr>
<td>6.</td>
<td>RP8 Lichrosorb 250 x 4 mm, 7μm</td>
<td>R.T.</td>
<td>1% acetonitrile-0.02M disodium hydrogen orthophosphate buffer pH 2.5</td>
<td>1.2 ml/min</td>
<td>Fluor. Ex. 270nm Em 380nm</td>
<td>Peh &amp; Yuen, 1997</td>
</tr>
<tr>
<td>7.</td>
<td>Hypersil ODS 150 x 4.6 mm, 3μm</td>
<td>R.T.</td>
<td>0.02M potassium dihydrogen phosphate pH 3.5</td>
<td>1.5 ml/min</td>
<td>UV 254 nm</td>
<td>Boulieu, et al., 1997</td>
</tr>
<tr>
<td>8.</td>
<td>C8 Silica 300 x 4.6 mm, 10μm</td>
<td>R.T.</td>
<td>Methanol-0.05M octane sulfonic acid buffer pH 2.5 (8:92)</td>
<td>1.5 ml/min</td>
<td>UV 254 nm</td>
<td>Bangaru, et al., 2000</td>
</tr>
<tr>
<td>9.</td>
<td>Spherisorb S5-ODS2 2μm 250 x 4.6 mm</td>
<td>40°C</td>
<td>Methanol-5mM monopotassium phosphate pH 3.0 + 7mM hexylamine (5:95 v/v)</td>
<td>1.3 ml/min</td>
<td>UV 254 nm</td>
<td>Caamano, et al., 1999</td>
</tr>
<tr>
<td>10.</td>
<td>C18 column 250 x 4.6 mm, 5μm</td>
<td>R.T.</td>
<td>Distilled water</td>
<td>1.2 ml/min</td>
<td>UV 254 nm</td>
<td>Volpato et al., 1997</td>
</tr>
</tbody>
</table>
3.5 PLAN OF WORK

3.5.1 Literature and Patent Search

3.5.2 Procurement of shilajit and model drug candidates, itraconazole and acyclovir

3.5.3 Standardization of analytical methodology for itraconazole
   3.5.3.1 Analytical method for routine analysis
   3.5.3.2 Analytical method for plasma analysis

3.5.4 Standardization of analytical methodology for acyclovir
   3.5.4.1 Analytical method for routine analysis
   3.5.4.2 Analytical method for permeability studies

3.5.5 Characterization and Identification of itraconazole and acyclovir
   3.5.5.1 Physico-chemical characterization
   3.5.5.2 Spectral characterization

3.5.6 Characterization and authentication of shilajit from different sources
   3.5.6.1 Physico-chemical characterization
   3.5.6.2 Spectral characterization
   3.5.6.3 HPTLC fingerprinting
   3.5.6.4 X-ray diffraction
   3.5.6.5 Scanning electron microscopy

3.5.7 Extraction of humic acids and fulvic acids from shilajit
   3.5.7.1 Standardization of reported method
   3.5.7.2 Development of improved method

3.5.8 Characterization of humic acids and fulvic acids
   3.5.8.1 Physico-chemical characterization
   3.5.8.2 Spectral characterization
   3.5.8.3 X-ray diffraction
   3.5.8.4 Scanning electron microscopy

3.5.9 Determination of surfactant properties of humic and fulvic acids

3.5.10 Phase solubility studies

3.5.11 Preparation of complexes by
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   3.5.11.2 Solvent evaporation
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3.5.12 Characterization of complexes by
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