3.1. **INTRODUCTION TO SALT FORMATION:**

As previously discussed, due to the progress in medicinal chemistry and introduction of combinatorial chemistry and HTS in identifying new chemical entities, the solubility of new drug molecules has decreased sharply. This is the primary reason for the increased interest in solubility enhancement techniques including the salt formation. As estimated, approximately 50% of all drug molecules used in medicinal therapy are administered as salt forms. This fact indicates that the saltification or salt formation of a drug substance is a significant and useful step in the drug development. A drug substance often has certain suboptimal physicochemical or biopharmaceutic properties that can be overcome by pairing a basic or acidic drug molecule with a counter ion to create a salt. The process gives an enhanced solubility of drug which causes a remarkable change in the biopharmaceutic characteristics of the poorly soluble and poorly bioavailable drugs. The salt formation method is a simple way to modify the properties of a drug with ionizable functional groups to overcome undesirable features of the parent drug. This fact underlines the importance of salt formation for drugs that are designed, developed and marketed after a rigorous research and development program.

3.1.1 **BASIC CONCEPTS IN SALT FORMATION:**

When a compound that is ionized in solution undergoes a strong ionic interaction with an oppositely charged counter ion, leads to crystallization of the salt form. In the aqueous or organic phase, the drug and counter ion are ionized according to the dielectric constant of the liquid medium. The charged groups in the structure of drug and the counter ion are attracted by an intermolecular columbic force. During favorable conditions, this force of attraction crystallizes the salt form out of the
medium (Fig. 3.1). All acidic and basic compounds can participate in salt formation\textsuperscript{[165]}. However, the success and stability of salt formation depend upon the relative strength of acid or base or the acidity or basicity constants of the species involved\textsuperscript{[167]}.

The salt form is separated into individual entities (i.e. the ionized drug and the counter ion) in liquid medium and its solubility depends upon the solvation energy in the solvent. The solvent must overcome the crystal lattice energy of the solid salt and create space for the solute. Thus, the solubility of a salt depends on its polarity, lipophilicity, ionization potential and molecular size. The solubility of salt also depends on the properties of solvent and solid such as the crystal packing and presence of solvates\textsuperscript{[168]}.

Fig. 3.1: Diagrammatic Representation of Salt Formation

3.1.2 FORMATION OF SALT FORM:

Salts can be prepared on a small scale using various methods. Forming salts from free acid or base is the most common method. The free acid or base of the drug substance is combined with the counter ion base or acid in specific molar ratios in a
suitable solvent system. The salt form is then recrystallized and isolated under favorable conditions (Fig. 3.1).

A less common method is to form salts through salt exchange. In this method, a counter ion salt is treated with a free acid or a free base in a specific molar concentration in a suitable solvent. The solid is then isolated and recrystallized. For example, the sulfate salt of methyl pyridinium-2-aldoxime can be prepared using silver sulfate as a counter ion. The unwanted silver ions were removed as insoluble iodide salt and the desired sulfate salt was precipitated by adding an antisolvent\[169]\.

A wide range of salts are generally prepared for each new substance. Their properties are compared during a preformulation program improving the chances of selecting the optimal salt form\[170\]. However, a balanced approach should be adopted because of limited availability of resources at this early stage of drug development. Commonly used salts such as hydrochloride and sodium have advantages over other salt forming moieties like low molecular weight and low toxicity. However, other salt forms such as mesylate may sometimes offer advantages like higher solubility and bioavailability\[171\].

**3.1.3. ADVANTAGES OF SALT FORMATION:**

Salt forms of drugs have a large effect on quality, safety and performance of drugs. The properties of salt-forming species (i.e. counter ions) significantly affect the pharmaceutical properties of a salt and can greatly benefit chemists and formulators in various aspects of drug discovery and development\[167\]. The numerous advantages of salt formation can be listed as following:

1. Altered solubility or dissolution rate\[172, 173\]
2. Controlled release dosage form\[174\]
3. Targeted drug delivery\[175, 176\]
4. Improved thermal stability \cite{167, 176}

5. Improved hydrolytic properties \cite{176}

6. Improved photo stability \cite{177}

7. Decreased hygroscopicity \cite{163, 178}

8. Improved permeability \cite{167, 173}

9. Improved organoleptic properties (e.g. increased taste acceptability) \cite{163}

10. Improved drug efficacy \cite{179}

11. Decreased pain on injection \cite{167}

12. Altered melting point resulting in opportunities for more formulation approaches and processes \cite{173, 180}

13. Ease of purification, handling and processing \cite{163, 181}

14. Improved compressibility \cite{182}

15. Extended patent protection \cite{162, 183}

16. Absolute configuration of chiral s through single crystal X-ray diffraction and racemic resolution of diastereomeric salt and rotamers \cite{184}

### 3.1.4. Disadvantages of Salt Formation:

In spite of several advantages of salt formation, if a due care is not taken in salt selection process, approach of salt formation can lead to some unwanted effects.

The demerits of salt approach can be listed out as follows:

1. Decreased percentage of active drug content in the formulation leading to increased powder volume causes problems for tableting and capsule filling \cite{185}

2. Increased formation of hydrates and polymorphs, resulting in greater variability of the pharmaceutical properties of drug \cite{14, 185}

3. Reduced dissolution rate for hydrochloride salts in gastric fluid resulting from precipitated free acid of base at the surface of the solid dosage form \cite{186}
4. Increased chance of solid state stability at the micro-environment pH of the salt\textsuperscript{186}

5. Corrosiveness of salts resulting in tableting problems. e.g. highly acidic hydrochloride salts damage punch tooling\textsuperscript{173}

6. Possible disproportionation (dissociation) of hydrochloride or hydrobromide, resulting in the release of hydrochloride gas or reaction with excipients or process-related chemicals\textsuperscript{167}

7. Additional steps in the synthesis of a medicinal compound\textsuperscript{187}

3.1.5 SALT SELECTION STRATEGY:

Pharmaceutical researchers previously used to select salts at various stages in drug development. However, now the salt selection process to the research phase is used to make the process more reliable\textsuperscript{188}. Ideally, the salt form should be chosen before long-term toxicology studies (i.e. at the start of phase-I clinical trials)\textsuperscript{189}. Changing the salt form at a later stage may force a repetition of toxicological, formulation and stability studies, thus increasing development time and cost\textsuperscript{189}. A new salt form introduced at a late stage must also be evaluated for potential impurity changes. Hence, its bioequivalence (bio-bridge), pharmacokinetic equivalence (PK-bridge) and toxicity equivalence (tox-bridge) to the previous salt form must be proven.

3.1.5.1 Objectives of Salt Selection:

As innumerable choice of salt forms is available to pharmaceutical scientists, the selection process must be rational and streamlined. A lack of proper strategy may lead to the synthesis of many salt forms of the drug candidate for preformulation testing. Moreover, this haphazard approach may result in failures and cause the loss of test substance, time and money as well. These considerations emphasize the need for a well formatted decision tree to choose a suitable salt form in an efficient and timely manner, depending upon the intended use and with reduced possibilities of failures.
The main objective of a salt-selection study is to identify the salt form most suitable for development. The following parameters are often considered as primary or essential criteria for the selection of a particular form:

- Aqueous solubility measured at various pH values, depending upon the intended pharmaceutical profile
- High degree of crystallinity
- Low hygroscopicity (i.e. water absorption versus relative humidity), which gives consistent performance
- Optimal chemical and solid-state stability under accelerated conditions (i.e. minimal chemical degradation or solid-state changes at 40°C and 75% RH).

A significant deficiency in any of these characteristics should exclude the molecule from further development. In addition to these essential criteria, the following desirable criteria also influence salt form selection:\[190\]:

- Limited number of polymorphs or absence of polymorphic variability
- Ease of synthesis, handling or formulation development

A single salt form generally can’t satisfy all the necessary requirements for developing drug dosage forms. However, introducing a second or third salt form consumes additional developmental resources and increases the cost of manufacturing, handling, storage and characterization. Therefore, the dosage form is developed with a single salt form whenever possible\[173\]. The major drug development issues are resolved by choosing the appropriate salt form while minor issues can be addressed using other development tools like formulation design.

Salt selection is a critical step in the preformulation stage of drug development. As stated by Gould, the balance required in assessing the correct salt form to progress into drug development makes it a difficult semi-empirical exercise\[172\]. This statement
emphasizes the need to prioritize the salt selection process so that various development issues are addressed as early as possible.

3.1.5.2 Potential Candidate for Salt Formation:

The decision about whether salt or free acid or base should be developed depends on relative pharmaceutical and commercial merits of these forms. If a free acid or a base is a water-soluble solid with a high melting point, preparing a salt form is generally unnecessary\(^{[167]}\).

In spite of the numerous advantages associated with salt forms, developing them is not always feasible. The preparation of a stable salt may not be possible for some drugs. The salt may have certain undesirable properties compared with the free acid or base and it would thus be appropriate to proceed with the free acid or base\(^{[191]}\).

Pharmacological indications also help to determine whether the salt form or the free acid or base should be pursued. For example, when a slow onset or a constant plasma level is required, a highly ionized salt form may be inappropriate if the free acid or base provides a sufficient plasma level\(^{[170]}\).

3.1.5.3 Pharmaceutical Considerations:

The choice of salt is governed largely by the acidity or basicity of the ionizable group, the safety of the counter ion, the drug indications, the route of administration and the intended dosage form. The expectations of the salt form must be outlined as a desirable pharmaceutical profile that guides the synthesis of the salt forms.

1. Intended Formulation:

Depending on the type of dosage form required

- Solid dosage forms- hydrochloride and maleate
- Solution or injectable- hydrochloride and mesylate
• Suspension- salts with large, relatively insoluble counter ions e.g. embonate, esteolate and tosylate

• Suppositories- free base

2. **Dose Consideration:**

For low dose drugs, molecular weight of counter ion weight is not an issue. But the high molecular weight counter ions such as tosylate may complicate formulation development for high dose drugs.

3. **Desired Release Kinetics and Bioavailability:**

• Immediate release- hydrochloride and sodium.

• Slow release- salts with large and relatively insoluble counter ions

4. **Pharmacological Indications:**

Counter ion may affect some physiological processes resulting in the complications in the therapy e.g. sodium salt is not useful in anti-hypertensive and anti-diabetic drugs due to restricted intake of sodium.

5. **Regulatory Considerations:**

• The salt form should have no adverse safety implications.

• Counter ions with known or potentially undesirable pharmacological implications or toxicity are restricted.

6. **Intellectual Property Considerations:**

The unauthorized commercial use of patented salt forms is restricted.

3.1.5.4 **Ionic Considerations:**

The degree of ionization is a critical parameter for the performance of the drug and for its formulation development\[^{12, 188}\]. The pK\(_a\) of the drug and counter ion is important for successful salt formation. For the preparation of salt forms of basic drugs, the pK\(_a\) of the counter ion should be at least 2 pH scale lower than the pK\(_a\) of
the drug\textsuperscript{192}. Similarly, for the preparation of salt forms of acidic drugs, the pK\textsubscript{a} of the counter ion should be at least 2 pH scales higher than the pK\textsubscript{a} of the drug. These specifications are required because the counter ion must bring the pH of solution to a level lower than the pH\textsubscript{max} (Fig. 3.2) to reach the salt plateau, at which the solubility of salt prevails over the solubility of free acid or base\textsuperscript{166}. The generalized rule of difference in pK\textsubscript{a} units of the ionizable group in the drug and in the acid or base is meant to make the proton transfer energetically favorable. When the pK\textsubscript{a} of the drug and counter ion are not significantly different, a solid complex may form but may rapidly disproportionate (i.e. break down into the individual entities of drug and counter ion) in an aqueous environment\textsuperscript{193}.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{pH-solubility-profile.png}
\caption{pH-solubility profile for a compound with a single, basic pKa value of 5}
\end{figure}
3.1.6. SALT FORM SELECTION:

The generated salt forms are compared for the desired physicochemical and biopharmaceutic properties, which guide the final selection of an optimal salt form. As the salt form and the nature of the counter ion critically affects the properties of salt, the characterization of salts and the selection process must be systematically channelized.

3.1.6.1. Techniques for the Characterization of Salts:

After synthesis, salt formation must be confirmed, followed by assessment of their pharmaceutical properties. Several analytical techniques provide valuable information for salt screening. These techniques and information derived are as follows:

1. **Elemental Analysis**: Salt formation, stoichiometry
2. **FTIR Spectroscopy**: Salt formation, intactness of the structural features of parent drug required for pharmacological activity (characteristic peak shift)
3. **Differential Scanning Calorimetry & Thermal Gravimetric Analysis**: Melting point, polymorphism, phase purity, solid-state stability, crystallinity
4. **Optical Polarized Microscopy**: Crystallinity, particle morphology, polymorphism
5. **Hot Stage Microscopy**: Thermal behavior, polymorphic transitions
6. **Scanning Electron Microscopy (SEM)**: Particle morphology
7. **Powder X-Ray Diffractometry**: Phase purity, crystallinity
8. **Single crystal PXRD**: Polymorphic structure, crystallographic information
9. **In vitro Dissolution Study**: Intrinsic dissolution rate at different pH
10. **Saturated Salt Solutions**: Hygroscopicity at different relative humidity during transportation and handling
11. **Dynamic Vapor Sorption Study**: Hygroscopicity, hysteresis effect
12. **Raman Spectroscopy**: Salt formation, polymorphs

13. **Thermo-Gravimetric Mass Spectroscopy**: Elaboration of degradation mechanism, identification of solvates

14. **Thermo-Gravimetric Infra-Red Spectroscopy**: Thermal decomposition, kinetics of decomposition

15. **High Performance Liquid Chromatography**: Salt formation, stoichiometry, purity, polarity

16. **Nuclear Magnetic Resonance**: Salt formation, stoichiometry, purity.

3.1.6.2 **Salt Selection Study**:

Morris et al. [194] adopted a multilevel approach to screen salts for their optimal physical forms. In this approach, physicochemical tests are conducted in several tiers and decision about proceeding of selection or rejection process is made after each test level. Only appropriate salts, free acids or bases are tested further. Thus generation of extensive data about each salt form synthesized is avoided.

The study can be planned mainly in two stages. In the first phase, the least time-consuming experiments that could still prompt a decision process are conducted. Experiments that are time-consuming and labor intensive can be conducted at later stages. In this way, many salt forms can be screened with a minimum of experimental efforts. If the tiered approach eliminates all the candidates, additional salts must be considered before re-evaluating any salt rejected in an earlier tier.

For a rational approach to salt screening, the tiered approach should be combined with a goal-oriented approach in which the main problems associated with the free acid or base are handled first, followed by secondary problems.

The final salt form selected should have a fine balance of the optimal physicochemical and biopharmaceutic properties. Each stage of salt selection is
relevant and contributes to selection of the optimal salt form. However, salt selection can be a difficult task because each salt imparts unique properties to the parent drug.

3.1.6.3 Stages of Salt Selection:

As shown in Fig. 3.3, salt screening starts with the characterization of free acid or base, followed by the identification of possible counter ion. The acid or base characterization provides information for selection of potential counter ion and for planning relevant crystallization experiments. This stage is followed by a screening of crystallization conditions for the desired salts, salt formation and its confirmation and finally the preformulation of generated salts^{184}.

During salt form selection, the determination of pK_a and corresponding ionizable groups give an idea of the feasibility of salt formation. This information is the basis for selecting suitable counter ions and a preliminary synthesis of salt forms, preferably at the micro-level, coupled with characterization for salt formation. After the confirmation of salt formation, the prepared salts are screened for various biopharmaceutic properties to select the optimal salt form.

A. Assessment of crystallinity:

Assessment of crystallinity is the first stage of salt selection. The salt form should preferably be crystalline so that its properties remain constant during pharmaceutical handling and transportation as crystalline structure is a low energy and comparatively stable form. However, the amorphous form may have advantages (e.g. solubility) that can be tackled by proper formulation strategy. While, stabilizing the amorphous form for devitrification to crystalline form may lead to loss of these advantages^{195}.

B. Hygroscopicity & Solubility Profile:

The hygroscopicity profile of salt form is assessed to select a salt form that retains its properties in the varying humidity conditions during pharmaceutical operations.
This assessment can be performed using methods such as traditional saturated salt solutions in a desiccator or more advanced dynamic vapor sorption methods. The salt forms with acceptable hygroscopicity profiles are then evaluated for their intrinsic solubility at various pH conditions.

C. Stability:

The salts with adequate solubility are tested for their physicochemical stability, including polymorphic stability and excipient compatibility. These tests are especially relevant in combination formulations\textsuperscript{[172]}.  

D. Polymorphic Characterization

Salt forms having adequate stability are assessed for variability in their properties resulting from polymorphism. Compounds with a limited number of polymorphs are preferred because their performance during pharmaceutical operations and performance remains predictable.

E. Process Control & Feasibility Study:

The salt forms that qualify the stage of polymorphism are tested for process control, economic feasibility and processability (including parameters such as corrosiveness, taste, wettability and flowability).

F. Pharmacodynamic Evaluation of Selected Salt Form:

The selected salt form is subjected to pharmacological testing for drug release as per the requirements of onset and the duration of activity. Pharmacological safety studies are also performed\textsuperscript{[196]}.

G. Clinical Trials:

The selected salt form may then undergo to extensive long-term toxicology studies in phase-I clinical trials of drug development.
Fig. 3.3: Decision Tree for Selection of Optimal Salt Form
3.1.7 PATENT ASPECTS OF SALT FORMS:

Salt selection studies provide a viable extension of a drug patent as the salts with superior properties can be patent protected. New salt forms often have novel and improved physical properties related to processability (e.g. crystallization, morphology and filtration) and formulation (e.g. stability and solubility) \(^{197}\). They may also result in the detection of new polymorphs\(^{162}\).

A new salt form may have a profile that makes it suitable for a new route of administration. For example, diclofenac sodium salt (Ciba-Geigy) was marketed as Voltaren. Before the Voltaren patent expired, other salts (e.g. diclofenac diethylamine) with substantially better skin penetration properties were discovered and patented. These salts, in corresponding formulations, were particularly suitable for topical applications. Patenting new salts, therefore secures an exclusive position in the market\(^{184}\).
3.2. REVIEW OF LITERATURE:

Serajuddin Abu TM\textsuperscript{[13]} reviewed physicochemical principles of salt solubility with special reference to the influence of pH-solubility profiles of acidic and basic drugs on salt formation and dissolution. Non-ideality of salt solubility due to self-association in solution was also discussed. The author emphasized on salt formation of certain acidic or basic drugs and dissociation mechanism into free acid or base forms. The inter-relationships of several factors e.g. intrinsic solubility, pH, pKa, $K_{sp}$ (solubility product) and pH$_{max}$ with dissociation was also studied which elaborated their influence on salt screening and the selection of optimal salt forms.

Tao Tao et al.\textsuperscript{[198]} explored the feasibility of preparing itraconazole dihydrochloride to improve the solubility and dissolution rate. Itraconazole dihydrochloride was synthesized by bubbling anhydrous hydrogen chloride gas into the acetonic suspension of itraconazole. The FTIR and elemental analysis was employed for confirmation of salt formation. XRD study revealed that a new crystalline form of the salt was formed. The morphology and mean size distribution study by scanning electron microscopy (SEM) and dynamic light scattering (DLS) confirmed that the salt was in form of dispersible nanoparticles aggregation. Salt was also evaluated in its inclusion complex with β-cyclodextrin. The solubility and dissolution rate as well as bioavailability of itraconazole salt and inclusion complex of salt- β-CD was found to be highly increased.

Sevukarajan et al.\textsuperscript{[199]} synthesized and characterized the aceclofenac salt by using triethanolamine as alkalyzing agent. Aceclofenac is an orally effective non-steroidal anti-inflammatory drug which exhibits very low aqueous solubility. Aceclofenac salt showed improved solubility. The micromeritic study showed poor flow and hence, the effect of addition of glidant was also studied.
Huang et al.\textsuperscript{[177]} reviewed the effect of solid state properties on the developability of drug candidates. An emphasis was given on effect of salt formation on solubilization of BCS Class II drugs. The salt screening process was also discussed.

Connor et al.\textsuperscript{[180]} synthesized a range of diclofenac salts using eight different counter ions and including five novel salts, obtained with the bases 2-amino-2-methyl-1,3-propanediol, 2-amino-2-methylpropanol, tert-butylamine, benzylamine and deanol. The aqueous solubilities of the salts showed a 113-fold difference in solubility. The solubility of diclofenac deanol was higher than the solubilities of diclofenac salts reported earlier. Correlation was established between the inverse of the salt melting point and the logarithm of salt solubility. A log-log relationship was observed between salt solubility and hydrogen ion concentration in the salt solution. Relationships between the properties of the counter ions and those of the resulting diclofenac salts were explored. Reasonable correlation was also found between the free base melting point and the salt melting point.

Nandgude et al.\textsuperscript{[200]} characterized tannate salt and hydrochloride salt of diphenhydramine for enhancement of crystallinity, flow properties, intrinsic solubility and dissolution rate of diphenhydramine from its salt form.

Rubino et al.\textsuperscript{[201]} correlated solubility, melting point and salting-out behavior in a group of secondary amine hydrochloride salts.

Serajuddin et al.\textsuperscript{[202]} mentioned that the salt gets converted to its free base form in the micro-environment of the tablet formulation and identified the need of stabilization. They attempted to stabilize the maleate salt of a basic drug by adjustment of micro-environmental pH in solid dosage form.

Balata et al.\textsuperscript{[203]} prepared solid dispersion of KTZ for solubility enhancement. From solubility study, the need of solubility enhancement of KTZ was demonstrated.
3.3. OBJECTIVES:

Solubility of a drug plays a very important role in dissolution and hence absorption and bioavailability of drug. Poorly soluble drugs particularly belonging to BCS Class II represent a problem for their scarce availability.

Ketoconazole (C_{26}H_{28}Cl_{2}N_{4}O_{4}) is 1-Acetyl-4-[[2RS, 4SR]-2-(2, 4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl) 1, 3-dioxolan-4-yl] methoxy] phenyl] piperazine\(^{[204-206]}\). Ketoconazole (KTZ) is an active triazole antifungal agent used against variety of fungal strains. It inhibits growth of dermatophytes and yeast species such as *Candida albicans*\(^{[207, 208]}\). Because of the highly hydrophobic characteristics and extremely weak basicity, it is having very poor aqueous solubility which is a major rate limiting drawback for little use of KTZ in oral administration\(^{[203, 209, 210]}\). It is also used in the anti-dandruff shampoo preparations. But due to poor solubility of KTZ, high amount of solubilizers is needed in topical dosage forms.

Thus, improvement of KTZ solubility can assist the oral use of it as well as can enhance the applicability of KTZ in anti-dandruff shampoo preparations to make them cost effective as well as translucent and elegant.

Ketoconazole is a dibasic compound (pKa = 6.51 and 2.94). Thus the difference in pKa between KTZ and hydrochloride counter ion makes KTZ a suitable and potential candidate for hydrochloride formation.

Considering the factors affecting the solubility of a weak acid or weak base and methods of solubility enhancement, attempts are been made to apply the principles of salt formation to enhance the KTZ solubility with following objectives:

1. Preparation of salt form by simple and industrially feasible method
2. Confirmation of salt formation by various analytical techniques
3. Evaluation of salt based on salt selection strategy for physicochemical properties like crystallinity, thermal characteristics, morphological characteristics, saturation solubility, dissolution rate, hygroscopicity and stability

4. Comparison of antifungal activity of drug and salt to confirm effectiveness after salt formation
3.4. PLAN OF WORK:

1. Review of literature

2. Procurement of materials

3. Experimental
   
   3.1. Drug authentication
   
   3.2. Calibration curve of Ketoconazole
   
   3.3. Preparation of Ketoconazole Salt
   
   3.4. Confirmation of Salt formation by various analytical techniques
      
      3.4.1. Melting point
      
      3.4.2. Elemental analysis
      
      3.4.3. Fourier Transform Infra-Red Spectroscopy
      
      3.4.4. Gas Chromatography- Mass spectroscopy
      
      3.4.5. Differential Scanning Calorimetry
      
      3.4.6. UV Spectrophotometric study
   
   3.5. Physicochemical Characterization of Salt
      
      3.5.1. Calibration curve of Salt
      
      3.5.2. Saturation solubility study
      
      3.5.3. Morphological study by SEM
      
      3.5.4. In vitro dissolution study
   
   3.6. In vitro antifungal activity
   
   3.7. Stability Study
3.5. DRUG PROFILE:

KETOCONAZOLE\textsuperscript{[203-210]}

Ketoconazole, a synthetic antifungal drug was the first oral azole introduced into clinical use for the treatment of systemic mycoses and to prevent and treat fungal infections, especially in immuno-compromised patients such as those with Acquired Immuno-Deficiency Syndrome. Ketoconazole is structurally similar to imidazole and interferes with the fungal synthesis of ergosterol, a constituent of fungal cell membranes.

**Chemical Name:**

1-acetyl-4-[4-[[2RS,4SR]-2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine

**Chemical Structure:**

![Chemical Structure of Ketoconazole](image)

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**Molecular Formula:** \( C_{26}H_{28}Cl_2N_4O_4 \)

**Molecular Weight:** 531.4

**Description:** White to off white crystalline powder.

**pKa:** 6.51 and 2.94

**Solubility:**

Practically insoluble in water, freely soluble in methylene chloride, soluble in methanol, sparingly soluble in alcohol and soluble in dimethyl sulfoxide
Pharmacodynamics:

Ketoconazole interacts with 14-α-demethylase, a Cytochrome P-450 enzyme necessary for the conversion of lanosterol to ergosterol. This results in inhibition of ergosterol synthesis causing increased fungal cellular permeability. Other mechanisms include the inhibition of endogenous respiration, interaction with membrane phospholipids, inhibition of yeast transformation to mycelial forms, purine uptake and impairment of triglyceride and/or phospholipid biosynthesis. KTZ can also inhibit the synthesis of thromboxane and sterols such as aldosterone, cortisol and testosterone.

Pharmacokinetics:

KTZ is a weak dibasic agent and thus requires acidity for dissolution and absorption. Mean peak plasma level of approximately 3.5 μg/ml is reached within 1 to 2 hr, following oral administration of a single 200 mg dose taken with a meal. Subsequent plasma elimination is biphasic with a half-life of 2 hr during the first 10 hr and 8 hr thereafter. In vitro, the plasma protein binding is about 99% mainly to the albumin fraction. Only a negligible proportion of KTZ reaches the cerebrospinal fluid. Following absorption from the gastrointestinal tract, KTZ is converted into several inactive metabolites. The major identified metabolic pathways are oxidation and degradation of the imidazole and piperazine rings, oxidative O-dealkylation and aromatic hydroxylation. About 13% of the dose is excreted in the urine, of which 2-4% is unchanged drug. The major route of excretion is through the bile.

Dosage and Administration:

KTZ is given 200 to 400 mg once daily, preferably after food. The duration of the therapy ranges between 1 to 4 weeks. Treatment is given for a minimum of 6 months in systemic mycosis.
**Indication:**

KTZ is primarily indicated in conditions like candidal chronic paronychia, chronic vaginal candidosis, cutaneous candidiasis, fungal prophylaxis, liver transplant, pityriasis capitis, pityriasis versicolor, recalcitrant cutaneous dermatophyte infection, renal transplant, seborrheic dermatitis, serious chronic resistant mucocutaneous candidiasis, severe chronic mucocutaneous candidiasis, social anxiety disorder, systemic mycoses, ventricular arrhythmias, paroxysmal supraventricular tachyarrhythmias and can also be given in adjunctive therapy as an alternative drug of choice in prophylaxis of organ rejection in kidney allograft recipients.

**Contraindication:**

KTZ is contraindicated in the cases of hypersensitivity to KTZ and CNS fungal infections (due to poor CNS penetration). Co-administration of KTZ with ergot derivatives, astemizole or cisapride is contraindicated due to risk of potentially fatal cardiac arrhythmias.

**Drug Interactions:**

Ketoconazole is known to interact with other drugs like alcohol, aliskiren, alprazolam, carbamazepine, cimetidine, cisapride, indinavir, isoniazid, lovastatin, paclitaxel, pantoprazole, phenytoin, prednisolone, quinidine, ranitidine, repaglinide, rifampicin, ritonavir, salmeterol, simvastatin, tacrolimus, terfenadine, theophylline, tolbutamide, tolterodine, torasemide, warfarin, zidovudine etc.

**Adverse Reactions:**

The severe or irreversible adverse effects of KTZ give rise to further complications including impotence, papilloedema, androgen suppression, oligospermia, gynecomastia etc.
Its potentially life-threatening effects are hepatitis and anaphylaxis which are responsible for the discontinuation of KTZ therapy.

The symptomatic adverse reactions produced by KTZ are more or less tolerable and if they become severe, they can be treated symptomatically; these include dizziness, headache, nausea, vomiting, alopecia, diarrhea, abdominal pain, urticaria, pruritus, itching, thrombocytopenia, photophobia, paresthesias and local burning sensation.
3.6. EXPERIMENTAL:

3.6.1. Materials

Ketoconazole was kindly gifted by Ajanta Pharma, Mumbai. All other chemicals were of analytical grade. Samples of isolated strains of *Candida albicans* and *Aspergillus niger* were provided by Dept. of Microbiology, V.M. Medical College, Solapur.

3.6.2. Authentication of Drug:

3.6.2.1. Melting Point

Melting point of KTZ was checked by conventional capillary method and reported uncorrected.

3.6.2.2. FTIR Spectra

FTIR absorption spectrum of KTZ was recorded using a spectrometer (Nexus 670, Nicolet Instrument Co.) equipped with a DTGS detector. KBr disks were and scanned over a range of 400-4000 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\).

3.6.3. Preparation of Ketoconazole Dihydrochloride:

Preparation of salt form of ketoconazole was carried out by gas bubbling method\(^{[198]}\). Ketoconazole (10 g) was suspended in 200 ml of acetone. Into the suspension heated under reflux, the anhydrous hydrogen chloride gas was slowly bubbled. The suspension turned into a clear solution after about 30 min. A pale yellow to orange recrystallized product was observed in another 5 to 10 min. The passage of hydrogen chloride gas lasted for 30 min and the mixture was allowed to stand overnight at room temperature. The product was collected by filtration, washed with acetone and dried to remove the residual solvent. The proposed scheme for salt preparation of ketoconazole by HCl bubbling method is depicted in Fig. 3.5.
Fig. 3.5: Schematic Representation of preparation of KTZ Dihydrochloride

3.6.4. CONFIRMATION OF SALT FORMATION:

3.6.4.1. Melting Point Determination:

Primary assessment of structural changes after the chemical treatment was done by determination of melting point of treated KTZ and compared with melting point of KTZ. Melting point of prepared salt was checked by conventional capillary method and are reported uncorrected.

3.6.4.2. Elemental Analysis:

Elemental Analysis of treated KTZ was performed on Analytischer Funktionstest Vario EL Fab-Nr.11975059 for the calculation of contribution by carbon, hydrogen and nitrogen atoms.

3.6.4.3. Fourier Transform Infra-Red Spectroscopy:

FTIR absorption spectrum of treated KTZ was recorded using a spectrometer (Nexus 670, Nicolet Instrument Co.) equipped with a DTGS detector. KBr disks were prepared (2 mg sample in 200 mg KCl) and scanned over a range of 400-4000 cm$^{-1}$ with a resolution of 4 cm$^{-1}$.

3.6.4.4. Thermal Analysis:

Thermal behavior of KTZ and treated KTZ were investigated using Differential Scanning Calorimeter (DSC-7, PerkinElmer Inc.) with nitrogen flow rate of 40 ml/min and a heating rate of 10°C/min from 25 to 400°C.
3.6.4.5 Gas Chromatography- Mass spectroscopy (GC-MS):

GC-MS analysis of KTZ and salt was performed using a Shimadzu GC/MS (GC-17A) equipped with a ZB-1 MS fused silica capillary column (30 m × 0.25 mm internal diameter, film thickness 0.25 mm). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as the carrier gas at a constant flow rate of 1 ml/min. Injector and MS transfer line temperature were set at 260°C and 320°C respectively. The oven temperature was programmed from 60-320°C at 3°C/min, increase then held isothermal for 11 min and finally raised to 320°C at 10°C/min. Diluted samples (1/100 v/v in methanol) were injected manually in the split less mode. Software adopted to handle mass spectra and chromatograms was a ChemStation.

3.6.4.6 UV Spectroscopy:

The drug and salt were analyzed for the UV spectra (Systronics Double Beam Spectrophotometer- 2201). Determination of absorption maxima was performed for KTZ and its salt in 0.1 N HCl. Different spectrophotometric parameters of drug and salt like absorption maxima, absorption spectra, Sandel’s sensitivity and molar extinction coefficients were estimated and compared. Molar absorptivities were calculated by preparing 0.01mM solutions in 0.1N HCl.

3.6.5 CHARACTERIZATION OF SALT

3.6.5.1 Scanning Electron Microscopy (SEM):

Particle morphology as well as size is the important factor affecting the solubility and dissolution rate of drug. SEM photomicrographs were taken to compare the crystal morphology as well as micromeritic properties of KTZ and KTZ salt. SEM photomicrographs were taken using scanning electron microscope (XL30, Philips Analytical Inc.). Samples were coated with gold before examination.
3.6.5.2 Solubility Study:

Solubility determination of KTZ and salt was done by Higuchi-Connor’s method\cite{154}. An excess amount of sample was added to 10 ml of distilled water. The suspensions were shaken on a rotary shaker at constant temperature (25±0.5°C) for 48 hr. The vials were allowed to stand for 24 hr for equilibration. The samples were filtered through 0.45 µm membrane filter paper and appropriately diluted with distilled water. The amounts of dissolved solutes were quantified by UV spectroscopy at 227.2 nm. The results were presented as mean± standard deviation of three sets of experiments.

3.6.5.3 In vitro Dissolution Study:

Dissolution profiles of KTZ and KTZ salt were determined in 900 ml of 0.1 N HCl (pH 1.2) as the dissolution medium at 37±1°C using USP-XXIV Type-II paddle dissolution test apparatus (Electrolab TDT-06P, India)\cite{203,209,210}. The accurately weighed samples equivalent to 100 mg of KTZ were filled in size 0 gelatin capsules. The dissolution media was stirred at 100 rpm in the paddle method. 5ml aliquots were collected periodically and filtered through 0.45µm membrane filters maintaining the sink condition. Suitably diluted samples were analyzed by UV spectroscopy at 227.2 nm. All the readings were taken in triplicates.

3.6.5.4 In vitro antifungal Study:

An agar diffusion method\cite{207,208} was used for the determination of antifungal activity of KTZ and KTZ salt. The antifungal activity was determined against fungal strains viz. Candida albicans and Aspergillus niger. Standard Petri dishes (9 cm diameter) containing medium to a depth of 0.5 cm were used. The sterility of the lots was controlled before use. Inocula were prepared by suspending 1 or 2 colonies from 24 hr cultures in Sabouraud medium into tubes containing 10 ml of sterile saline. The
tubes were diluted 1/2 with saline. The inoculum (0.5 ml) was spread over the surface of agar and the plates were dried at 35°C for 15 min prior to placing the antifungal samples. The bores of 0.5 cm diameter were prepared and 20 µl samples of antifungal agents (2% w/v) were added in the bores. After incubation at 35°C for 24 hr, the halos of inhibition were measured. Experiments were performed in triplicates.

### 3.6.5.5 Stability Study:

Stability testing was conducted under the storage conditions of 30±2°C, RH 65±5% and 40±2°C, RH 75±5%. Capsules filled with KTZ dihydrochloride were packaged in closed aluminum-polyethylene laminated bags. Physical examination and assay were evaluated at fixed time intervals at 0, 1, 2, 3 and 6 months.
3.7. RESULTS & DISCUSSION:

3.7.1 AUTHENTICATION OF DRUG:

3.7.1.1 Melting Point:

Melting point of KTZ was found to be 143-145°C, which is found in agreement with the literature\cite{206}.

3.7.1.2 FTIR Spectroscopy:

As shown in Fig. 3.6, FTIR spectrum of KTZ shows strong absorption bands at 1480 cm$^{-1}$ corresponding to C-N aromatic stretch. A strong peak at 1650 cm$^{-1}$ indicates the presence of amide functionality. Characteristic peaks of C-O stretch in phenyl alkyl ether are observed at 1050 cm$^{-1}$ and 1230 cm$^{-1}$. The absorption band at 1110 cm$^{-1}$ is due to C-Cl vibration corresponding to aryl chloride. The absorption bands at 700 cm$^{-1}$ and 800 cm$^{-1}$ suggest 1, 2, 4- substitution on benzene ring. Also the positions and intensities of the characteristic peaks in the FTIR spectrum of the ketoconazole resembles with the standard reference spectrum of ketoconazole. Thus it can be concluded that the drug is authentic.

3.7.2 CONFIRMATION OF SALT FORMATION:

3.7.2.1 Percentage Yield and Appearance:

The product was obtained as a pale yellowish to slightly orange colored powder with a good yield of about 96%.

3.7.2.2 Melting Point:

Melting points were determined for primary assessment of the chemical changes. Processed KTZ showed melting point range at 235-238°C. The shift in the melting point suggests the changes in the structure due to completion of reaction.
3.7.2.3 Elemental Analysis:

Elemental analysis (% found/calculated) based on $\text{C}_{26}\text{H}_{28}\text{Cl}_{2}\text{N}_{4}\text{O}_{4}\cdot 2\text{HCl}$ shows C: 51.56/51.62; H: 5.327/4.963; N: 9.225/9.265. The results of elemental analysis suggest the evidence of dihydrochloride salt formation.

3.7.2.4 FTIR Spectra:

Fig. 3.6 demonstrates overlay of FTIR spectra of KTZ and treated KTZ. The absorption peaks of hydrochloride salt were observed at 2366.7 cm$^{-1}$ and 3416 cm$^{-1}$ might be attributed to O-H bond stretching vibration, implying a small amount of adsorbed water in the sample.

Other characteristic absorptions in the fingerprint region of FTIR spectra of KTZ dihydrochloride were similar to those of KTZ. The overlap and coinciding of fingerprint regions of drug and product suggests that all other basic structural features required for the antifungal activity are not affected by the reaction.

![Fig. 3.6: FTIR spectra of KTZ and salt](image-url)
3.7.2.5 Thermal Analysis:

Figure 3.7 shows the overlay of DSC curves of KTZ and KTZ salt. KTZ was characterized by a single, sharp melting endotherm at 148.12°C (\(\Delta H = -106.5\text{J/g}\)). The findings are in agreement with its melting point. The sharp melting endotherm indicates the presence of drug in a pure and crystalline form.

Thermogram of salt showed a melting endotherm peak at 229.67°C (\(\Delta H = -44.77\text{J/g}\)) supporting results of melting point. The presence of melting peak at up-field position can be attributed to change in the structural characteristics of KTZ. The increase in the melting point also indicates a possibility of higher stability profile of the drug molecule.

Fig. 3.7: DSC Analysis of KTZ and Salt
3.7.2.6 Gas Chromatography- Mass Spectroscopy:

As shown in Fig. 3.8, gas chromatogram of drug and salt demonstrates the altered elution time of KTZ and salt. The different elution time may be due to the change in the polarity and solubility characteristics of the drug and hence the affinity towards the stationary and mobile phases.

The mass spectrum (Fig. 3.9) of KTZ salt shows the presence of molecular peak at m/e = 602.71, suggesting the increment in the molecular weight of the KTZ and confirming the dihydrochloride salt formation.

![Fig. 3.8: Gas Chromatograms of KTZ and Salt](image)

![Fig. 3.9: Mass Spectra of KTZ. 2HCl](image)
3.7.2.9 UV- Spectrophotometric Analysis:

The UV- spectrophotometric absorption is a characteristic of the basic structure and absorbing groups or chromophores attached to the basic skeleton. The structural characteristics define the wavelength of maximum absorption as well as the absorption intensity or absorptivity of the compound. Depending on this fact, spectrophotometric properties like UV absorption spectra and molar absorptivity of both the compounds were compared.

It was found that UV spectra of KTZ and salt exactly overlap with each other suggesting the intactness of the KTZ structure in its salt form\(^{[21]}\) (Fig 3.10).

Moreover, the absorbance intensity of salt was found less in salt than parent form indicating the less molar content of the absorbing species i.e. KTZ.

Molar extinction coefficients of KTZ and its dihydrochloride salt were found to be similar (Table 3.1). This result suggests that 607.2 moles of KTZ salt are equivalent to 532.2 moles of KTZ, supporting the ‘dihydrochloride’ salt formation.

Fig. 3.10: UV Spectra of KTZ and Salt
3.7.3. CHARACTERIZATION OF SALT:

3.7.3.1. Standard Calibration Curve for Ketoconazole:

The UV spectrum of KTZ was performed in 0.1 N HCl. The $\lambda_{\text{max}}$ was found to be at 227.2 nm. The calibration curve showed good linearity characterized by coefficient of correlation ($R^2$) equal to 0.9997 over the Beer’s concentration range of 0-20 µg/ml.

The line equation of standard calibration curve can be expressed as:

$$Y = 0.0522X + 0.009$$  \hspace{1cm} (3.1)

3.7.3.2. Standard Calibration Curve for Ketoconazole Salt:

The $\lambda_{\text{max}}$ was found to be at 227.2 nm, which is similar to that of KTZ. As shown in Table 3.1, the calibration curve demonstrates a good linearity characterized by coefficient of correlation ($R^2$) equal to 0.9998 over the Beer’s range of 0-20 µg/ml.

The equation 3.2 represents the line characteristics for calibration curve of salt in 0.1N HCl.

$$Y = 0.0477X + 0.0013$$  \hspace{1cm} (3.2)

<table>
<thead>
<tr>
<th>Table 3.1: Optic Characteristics of KTZ and KTZ Dihydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$</td>
</tr>
<tr>
<td>$R^2$</td>
</tr>
<tr>
<td>Slope</td>
</tr>
<tr>
<td>y-Intercept</td>
</tr>
<tr>
<td>Bear’s Range (µg/ml)</td>
</tr>
<tr>
<td>Molar Absorptivity (lit/mole/cm)</td>
</tr>
<tr>
<td>Sandal’s Sensitivity (µg/cm²/0.001)</td>
</tr>
</tbody>
</table>
3.7.3.3. **Morphological Study:**

The morphological and micromeritic characteristics of KTZ and its salt form are shown in the photomicrographs (Fig. 3.11).

Particles of KTZ were observed as rod shaped in a size range from 6 to 36 µm (Fig. 3.11-A and 3.11-B). In contrast, the salt was found be in form of the clusters of aggregated nanoparticles (Fig. 3.11 C). The high magnification SEM images (Fig 3.11 D) revealed that the salt particles are present with a geometric diameter of <1000 nm.

![SEM microphotographs of KTZ and KTZ 2.HCl](image)

Fig. 3.11: SEM microphotographs of KTZ and KTZ 2.HCl
3.7.3.4. **Solubility Study:**

The solubility of KTZ was found to be $24.29\pm1.47\mu g/ml$ in distilled water and $57.607\pm3.677 \mu g/ml$ in 0.1 N HCl. The results are in good agreement with the reported value by Connors and Elder$^{212}$. The aqueous solubility of dihydrochloride salt was about $1.417\pm0.34 \text{ g/ml}$ which shows a tremendous enhancement of intrinsic solubility of salt compared to its base. Hydrochloride salt formation significantly improves the solubility of KTZ in distilled water as well as 0.1N HCl at 25°C.

The enhanced solubility can be correlated with salt formation as well as nanoparticulate structure of prepared compound giving rise to increased effective surface area as well as increased dispersibility. Thus salt particles can get easily dispersed and immediately dissolved in presence of solvent.

3.7.3.5. **In vitro Dissolution Study:**

Fig. 3.12 demonstrates the dissolution profiles KTZ and its salt. Less than 20% of KTZ was dissolved after 120 min. While dissolution profile of hydrochloride salt form of KTZ that more than 65% of KTZ was dissolved from KTZ dihydrochloride within first 15 min and a complete dissolution was observed in 30 min (Table 3.2 and 3.3). The comparison of dissolution profile can also be done from the area under curve (AUC) of both the samples. The AUC of KTZ was found to be 22.17, while that of salt was 216.58. The results show that the salt formation is successful approach to enhance the solubility and dissolution rate of KTZ.
### Table 3.2: Comparison between KTZ and KTZ salt

<table>
<thead>
<tr>
<th>Evaluation Parameter</th>
<th>KTZ</th>
<th>Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous Solubility</td>
<td>24.288±1.47µg/ml</td>
<td>1.417±0.34 g/ml</td>
</tr>
<tr>
<td>%R&lt;sub&gt;15 min&lt;/sub&gt;</td>
<td>1.203±0.36</td>
<td>65.537±2.34</td>
</tr>
<tr>
<td>%R&lt;sub&gt;30 min&lt;/sub&gt;</td>
<td>7.783±0.24</td>
<td>99.925±0.91</td>
</tr>
<tr>
<td>AUC</td>
<td>22.17</td>
<td>216.58</td>
</tr>
<tr>
<td>Molar Absorptivity (lit/mole/cm)</td>
<td>30145.57</td>
<td>29356.56</td>
</tr>
<tr>
<td>Sandals Sensitivity (µg/Sq.cm/0.001)</td>
<td>0.017628</td>
<td>0.020588</td>
</tr>
</tbody>
</table>

**Fig. 3.12:** Dissolution profiles of KTZ and Salt
Table 3.3: Comparison of Dissolution Profiles of KTZ and Salt

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>KTZ</th>
<th>SALT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AVG %R</td>
<td>AUC</td>
</tr>
<tr>
<td>3</td>
<td>0.069±0.05</td>
<td>0.001</td>
</tr>
<tr>
<td>5</td>
<td>0.07±0.05</td>
<td>0.000</td>
</tr>
<tr>
<td>7</td>
<td>0.76±0.05</td>
<td>0.238</td>
</tr>
<tr>
<td>10</td>
<td>1.253±0.17</td>
<td>0.086</td>
</tr>
<tr>
<td>15</td>
<td>1.203±0.36</td>
<td>0.004</td>
</tr>
<tr>
<td>20</td>
<td>3.021±1.13</td>
<td>0.945</td>
</tr>
<tr>
<td>25</td>
<td>5.223±0.4</td>
<td>3.149</td>
</tr>
<tr>
<td>30</td>
<td>7.783±0.24</td>
<td>3.456</td>
</tr>
<tr>
<td>45</td>
<td>11.506±0.52</td>
<td>7.316</td>
</tr>
<tr>
<td>60</td>
<td>14.532±0.86</td>
<td>5.840</td>
</tr>
<tr>
<td>75</td>
<td>14.64±0.65</td>
<td>0.004</td>
</tr>
<tr>
<td>90</td>
<td>16.589±1.03</td>
<td>1.169</td>
</tr>
<tr>
<td>105</td>
<td>16.996±0.53</td>
<td>-0.016</td>
</tr>
<tr>
<td>120</td>
<td>16.887±0.7</td>
<td>-0.024</td>
</tr>
</tbody>
</table>
3.7.3.6. *In vitro* Antifungal Activity:

*In vitro* antifungal activity on two different fungal species *viz. Candida albicans*, *Aspargillus niger* was done by agar diffusion method.

As depicted in table 3.4 and figure 3.13, the zones of growth inhibitions for drug and salt showed that the *in vitro* antifungal efficacy of the KTZ was not significantly affected by the salt formation, suggesting the intactness of the necessary structural characteristics for antifungal activity.

Table 3.4: Antifungal Activity of KTZ and KTZ salt

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zone of inhibition (mm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>KTZ</td>
<td>Salt</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>22.67±1.15</td>
<td>21.00±1.00</td>
<td>0</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>24.67±1.53</td>
<td>22.67±1.15</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 3.13: Antifungal Activity of KTZ and KTZ Salt
3.7.3.7. **Stability Study:**

Table 3.5 depicts the drug content and dissolution characteristics of salt expressed in terms of percent drug release at 15 min and 30 min ($Q_{15}$ and $Q_{30}$ respectively) during the accelerated stability study. The accelerated storage conditions for 6 months caused no significant change in assay. The results indicated that prepared ketoconazole dihydrochloride is chemically stable. Fig. 3.14 shows the dissolution profiles of KTZ salt under stability conditions. It can be seen that in case of storage conditions of 40°C±2°C temperature and 75%±5% relative humidity, the amount of drug dissolved at 30 minutes was decreased.

As shown in table 3.6, the antifungal activity of the prepared KTZ salt against both fungal strains under consideration was also not significantly affected by the exposure of accelerated stability conditions.

<table>
<thead>
<tr>
<th>Time (Months)</th>
<th>Stability Condition</th>
<th>% Drug Remaining</th>
<th>$Q_{15}$</th>
<th>$Q_{30}$</th>
<th>% Drug Remaining</th>
<th>$Q_{15}$</th>
<th>$Q_{30}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30°C±2°C, 65% ±5% RH</td>
<td></td>
<td></td>
<td></td>
<td>40°C±2°C, 75% ±5% RH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>99.90±3.07</td>
<td>60.68±0.14</td>
<td>98.08±2.16</td>
<td>101.17±1.32</td>
<td>60.29±1.17</td>
<td>94.97±0.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>100.64±1.68</td>
<td>62.28±1.82</td>
<td>98.31±5.42</td>
<td>99.77±1.39</td>
<td>60.82±0.18</td>
<td>95.01±1.69</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>97.67±1.04</td>
<td>62.66±1.04</td>
<td>98.88±3.96</td>
<td>99.77±1.39</td>
<td>61.42±1.59</td>
<td>93.05±3.32</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>99.24±2.09</td>
<td>63.55±1.7</td>
<td>97.39±1.46</td>
<td>98.72±2.72</td>
<td>62.26±2.35</td>
<td>94.89±0.75</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.6: Antifungal Activity During Stability Study

<table>
<thead>
<tr>
<th>Time (Months)</th>
<th>Zone of Inhibition (mm)</th>
<th>30±2°C, 65±5% RH</th>
<th>40±2°C, 75±5% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C. albicans</td>
<td>A. niger</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. albicans</td>
<td>A. niger</td>
</tr>
<tr>
<td>1</td>
<td>20.33±1.53</td>
<td>21.33±1.53</td>
<td>20.66±1.54</td>
</tr>
<tr>
<td>2</td>
<td>20.67±0.58</td>
<td>21.67±2.52</td>
<td>20.67±0.58</td>
</tr>
<tr>
<td>3</td>
<td>20.67±1.15</td>
<td>21.33±2.52</td>
<td>20.67±1.15</td>
</tr>
<tr>
<td>6</td>
<td>20.67±2.08</td>
<td>21.67±1.53</td>
<td>20.67±2.08</td>
</tr>
</tbody>
</table>

Fig. 3.14: Dissolution Profile during Accelerated Stability Study
3.8 CONCLUSION:

The aim of present investigation was to improve dissolution rate of ketoconazole by application of the approach of salt formation.

The drug was authenticated by comparison of melting point and FTIR absorption spectrum with the literature. Solubility study was performed by Higuchi-Connor’s method. The solubility of KTZ in distilled water is found to be $24.29\pm1.47 \, \mu g/ml$, while, $57.607\pm3.677 \, \mu g/ml$ was solubilized in 0.1N HCl. Dissolution of KTZ alone was very slow and incomplete up to 120 min. According to the obtained results, less than 20% of drug is dissolved after 2 hr. Hence, as the intrinsic solubility as well as rate of drug dissolution is poor, there is strong necessity to enhance its solubility and dissolution rate.

Salt of KTZ was prepared by a simple and industrially feasible technique which includes bubbling of anhydrous hydrogen chloride gas in an acetonic solution of ketoconazole. Salt formation was primarily assessed by a conventional melting point determination method, in which a significant shift in the melting points of KTZ and end product of the reaction suggesting the completion of the reaction. Structural changes were confirmed by elemental analysis, FTIR, DSC, UV spectroscopy and GC-MS. The drug and salt were also evaluated for their microscopic morphology by SEM. The solubility and dissolution rates of drug and salts were compared.

Depending on theoretical and observed values of percentages of C, H, N and O, elemental analysis suggests the formation of dihydrochloride salt. FTIR study showed overlap of fingerprint regions of KTZ and salt, which indicates the intactness of the basic skeleton of ketoconazole required for the antifungal activity. The findings of UV spectrophotometric evaluation showed similar Sandel’s sensitivity and molar absorptivity of KTZ and KTZ dihydrochloride. Astonishingly, in scanning electron
microscopy, the salt was observed as the clustures of nanoparticles with geometric diameter less than 1000 nm, whereas the drug was found in flat crystalline particular form. The particles of salt were more spherical in clustures. Drug release rate was profiled in 0.1 N HCl and found to be tremendously enhanced with salt form. Increased solubility and dissolution rates can be attributed to the salt formation as well as formation of dispersible clustures of nanoparticles, which significantly reduced particle size resulting in the increased effective surface area.

*In vitro* antifungal study reveals that the KTZ and its dihydrochloride salt forms demonstrate equivalent antifungal activity against two strains of fungi *viz.* *Candida albicans* and *Aspergillus niger*.

The stability study showed that the drug content was not affected by accelerated stability conditions of temperature and humidity, suggesting the chemical stability. The drug release rate of salt was not significantly altered upon aging. Hence the stability study shows that prepared salt form is physically and chemically stable under the tested pharmaceutical conditions.

Thus according to the salt selection strategy illustrated in figure 3.3, the prepared dihydrochloride salt fulfills the requirements as it shows crystalline characteristics, lack of hygroscopicity, increased solubility, physicochemical stability, intact pharmacological activity and industrial feasibility.

Finally it can be concluded that, dihydrochloride salt form of ketoconazole can be prepared by a simple, reproducible process which has industrial and economical feasibility. The salt was efficient to improve dissolution rate of ketoconazole without altering its pharmacologically active structural components. The salt form passes all the criteria for appropriate salt screening process including solubility, physical and chemical stability, hygroscopicity, crystallinity and industrial feasibility.