Novel and Sustainable Processes for Amorphous Solid Dispersions of Drugs to Enhance their Dissolution and Bioavailability

THESIS

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By

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CERTIFICATE

This is to certify that the thesis entitled **Novel sustainable amorphous solid dispersion for enhancement of drug dissolution and bioavailability of poorly water soluble drugs** submitted by **Rashmi Nair** ID No **2010PHXF0702H** for award of Ph.D. of the Institute embodies original work done by her under my supervision.

Signature of the Supervisor	Signature of the Co-supervisor
Name in capital letters	Name in capital letters
Designation	Designation
Date:	Date:

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An opportunity to test a thought, convert it to a hypothesis and analyse how thought could translate into a small scientific advancement is a privilege. This beautiful journey of discovering the unknown or studying the less known and creating tangible knowledge that could build deeper insights into this field has been immensely enriching, both personally and professionally. Days where all work went as planned and days where no plans worked have been part of this journey. Few exhilarating moments, some moments of anxiety, many moments of satisfaction and many more of hope, all this was part of me for last few years. Today in retrospection, it is satisfying that this work has contributed to the field of pharmaceutical sciences.

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List of abbreviations and symbols	
%RSD	Percentage relative standard deviation
°C	Degree Celsius
µg	Micro gram
µL	Micro litre
µm	Micro meter
µM	Micro molar
2θ	X-ray scattering angle
A	Area
Å	Angstrom
ASD	Amorphous solid dispersion
DSC	Differential scanning calorimetry
FTIR	Fourier transform infrared spectroscopy
PLM	Polarised light microscopy
HSM	Hot stage microscopy
ADME	Absorption, distribution, metabolism and elimination
AUC	Area under the curve
$AUC_{0\text{-}6h}$	Area under the curve for 0 to 6 hours
AUC_{Total}	Total area under the curve
cm	Centimetre
C_{max}	Maximum plasma concentration
DCM	Dichloromethane
g	Gram
h	Hours
HPLC	High-performance liquid chromatography
ICH	International council of harmonization
kg	Kilogram
L	Litre

List of abbreviations and symbols	
LOD	Limit of detection
Log P	Logarithm of partition coefficient
LOQ	Limit of quantitation
mg	Milligram
min	Minute
mL	Millilitre
mM	Millimolar
mm	Millimetre
mol	Moles
ms	Millisecond
pH	Potential of hydrogen
pXRD	Powder X-ray diffraction
R^2	Regression coefficient
RH	Relative humidity
T_{max}	Time taken to reach maximum plasma concentration
USP	United States Pharmacopoeia
UV	Ultraviolet
v/v	Volume by volume
w/w	Weight by weight

Abstract

This research is an attempt to understand alternatives to conventional methods of product design for amorphous solid dispersions considering environmental sustainability. Various scientific aspects like amorphization, polymer supported stabilization, drug-polymer miscibility, advanced analytical characterizations etc., have been researched, however, studies on sustainable manufacturing methods & green processes has been scant. This is an important gap that needs to be addressed because as a process amorphous solid dispersions are either solvent intensive (spray drying) or energy intensive (hot melt extrusion) and both of these are threats to the concept of sustainability.

In this work two case scenarios were evaluated, one each for solvent based amorphous solid dispersion & thermal energy based amorphous solid dispersion. For first case scenario, a poorly water soluble drug, Itraconazole, was selected as model drug. This class of drugs, triazole antifungals, have been a subject of research because they are not amenable to most solubility & bioavailability enhancement techniques. Conventionally hazardous solvents like dichloromethane have been used for production of amorphous Itraconazole. In this research, a retrospective analysis of drug in discovery stage was done. What would have been the stages for a first formulation? A salt screening exercise that selects crystalline salts, automatically eliminates non-crystalline or amorphous salts/drug-counter ion mixtures. With this as a starting point, literature was analysed to see reasons for no major salts of Itraconazole. Avoiding salt isolation and stabilization of amorphous form with polymer(s) was considered as a approach. In salt screening, aqueous content (water) is known to cause drug crystallization, therefore, a non-aqueous solvent system that would also be as per the recommendations for a green solvents was researched.

Acidified methanol was found to be a suitable solvent that could be utilised to design a benign process. With evidence of good physico-chemical characterization data and promising stability studies, in vivo evaluation was performed for bioavailability in Wistar rats. This study proved that in-situ salification in polar protic solvents could be a viable alternative manufacturing method for amorphous solid dispersions. Similar, study was conducted with another triazole antifungal, Posaconazole. Unlike Itraconazole that was stabilised with immediate release polymer hydroxypropyl methyl cellulose (HPMC), Posaconazole was stabilised with a delayed release polymer Hypromellose Acetate Succinate (HPMCAS). This proved the larger application of the proposed approach. Additionally, an alternative to spray drying was evaluated in agitated thin film drying (ATFD). Later process is capable of drying solutions/slurries/suspensions with higher solid contents and offers the advantage of solvent recycling. This process was found to be very useful alternative to the popular spray drying method.

For the second case scenario, Efavirenz was selected as model drug. In recent years, there has been a surge in the number of marketed amorphous solid dispersions of antiretroviral drugs. This is majorly because most of these drugs are high dose, have poor solubility in organic solvents and are thermolabile or prone to polymorphic conversions caused by heat. Extensive preformulation studies revealed that a lipophilic or amphiphilic surfactant that could solubilize Efavirenz and convert the drug into a non-crystallizing melt would prevent the drug's undue exposure to heat. Through detailed literature review and experimental screening with hot stage microscopy (HSM), Poloxamer 407 was selected as a suitable surfactant. It was observed that when poloxamer is melted and held isothermal for sometime, it gains & retains heat. At this stage when Efavirenz is added and mixed with hot poloxamer, it

readily dissolves into poloxamer. This observation is an indicator of melting point depression for drug. On cooling the drug does not re-crystallise until its surface or bulk is disturbed by physical intervention like scratching/mixing with a spatula. Neusilin® was found to stabilise this melt against re-crystallization. Unlike popular confinement theory of amorphous form stabilization in mesoporous materials, this study observed surface adsorption that had no detectable chemical interactions with the drug or poloxamer but possible stabilization by dilution of drug molecules in a matrix of poloxamer & Neusilin® that prevented close interactions required for nucleation & crystal growth. In vivo study in rats confirmed enhanced drug bioavailability.

These preliminary studies are evidence that processes can be designed to be benign.

Chapter 1

Introduction

1.0 Background

All natural resources are based on a balanced equation of use and replenish. Man-made interventions are adversely affecting this equation by using more than what is getting replenished, disturbing our ecosystem. In case of process waste this equation is between generation and remediation or safe disposal. Process intensive industries like pharmaceuticals, petroleum, mining etc. are major contributors to these undesired environmental changes including climatic changes, water pollution, soil pollution, air pollution, harmful chemicals in food chain of ecosystem etc. [1]. Concept of environmental sustainability emphasises the importance of considering action vs effect scenarios wherein how an action could impact environment and what could be done to minimise any adverse impact is considered and made an integral part of product and process design [2- 4].

With particular reference to pharmaceutical industry, environmental sustainability has been around for over three decades and the twelve principles of green chemistry laid out by Anastas and Warner continue to be the guiding philosophy [5]. Pharmaceutical industry is a manufacturing oriented sector that is focused on priority areas like efficient manufacturing processes and optimum time-to-market. While it is entrusted with creating products (drug substances and drug products) that could treat/manage/mitigate diseases in human beings and animals, sometimes its methods to develop these products conflict this purpose. Pharmaceutical industry is a major contributor to environmental waste generation [6, 7]. Various types of waste get generated [8] and the control of each waste needs a different strategy, implementation and monitoring plan. Pharmaceutical waste are mainly chemicals as disposal waste or

emissions [9]. Chemical substances are involved as key starting materials, intermediates, drug substances, excipients, analytical reagents, solvents, packaging materials etc. and therefore constitute major part of the total waste. Other than this, there is generation of carbon dioxide during waste incineration, water pollution by effluent release and land pollution by landfills [10].

Toxics Release Inventory (TRI) report by Environment Protection Agency (EPA), USA [11] presents some interesting data points on production related waste managed by different industries as shown in Figure 1.1 and how pharmaceutical industry is managing disposal through various approaches, Figure 1.2. Hereby it is evident that pharmaceutical industry is a contributor to the environmental concerns and needs to earnestly consider inculcation of sustainability in all its functions.

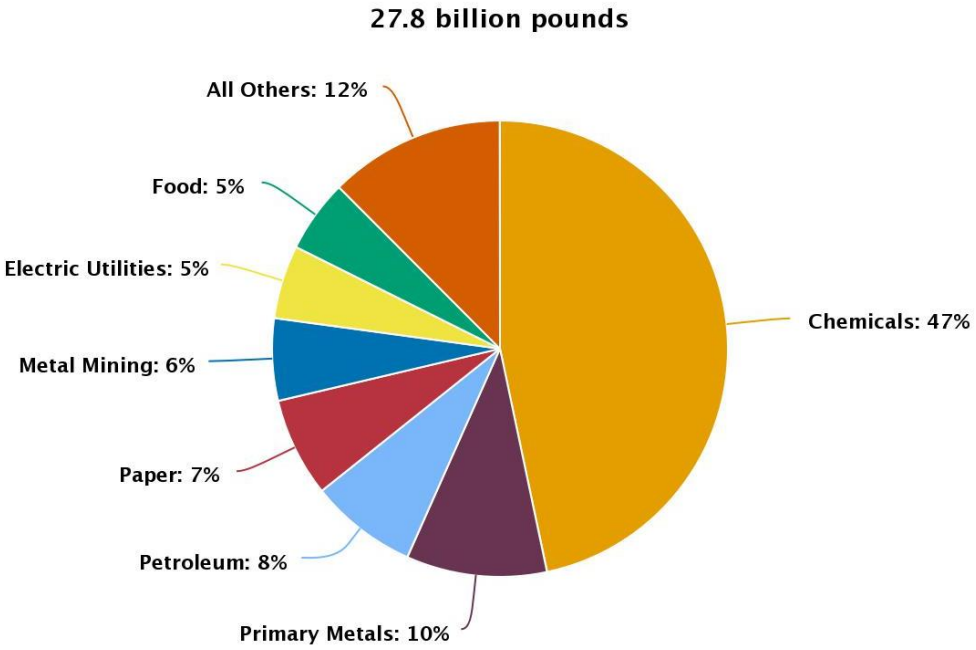

Figure 1.1 Production-related waste managed by industry, 2016

(Source: Toxics Release Inventory report, United States)

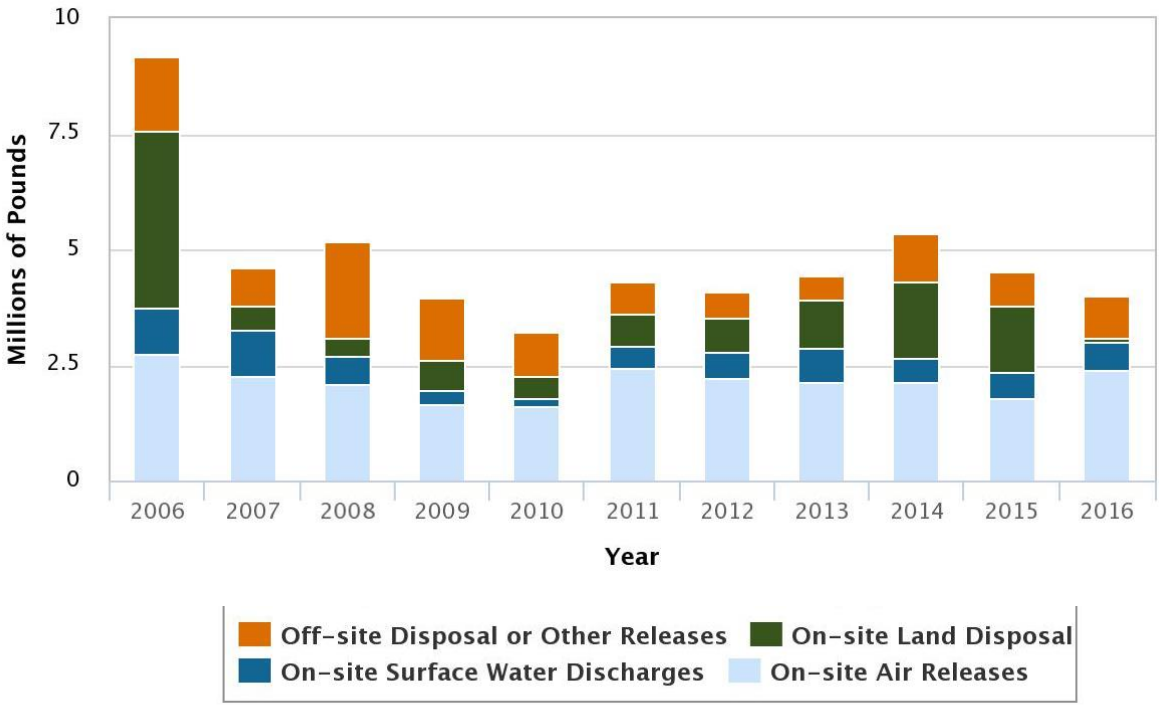

Figure 1.2 Disposal or other releases from pharmaceutical manufacturing, 2016

(Source: Toxics Release Inventory report, United States)

In the fine chemicals and pharmaceutical industry, usage of solvents and energy intensive manufacturing processes are two major impediments to sustainable process development. There are two approaches to addressing a problem, namely,

1. Innovate a new solution/remedy: Needs investment of time, cost and resources. Initially there could be regulatory uncertainties and economic feasibility challenges but these are usually root cause oriented solutions to a problem.
2. Improvise existing process: These incremental innovations are novel ideas that solve problems by implementing alternatives from existing knowledge base.

While there are various areas that need attention, usage of solvents and energy intensive processes are two major areas that could address a substantial portion of the sustainability challenge for pharmaceutical industry.

1.1 Problem analysis

Every ‘x’ kilogram of input material(s) transforms into ‘y’ desired product and ‘z’ undesired waste. This ‘z’ could be a potential threat to environment and people involved in the process. What are the options available to assess this threat and eliminate or reduce its impact? What could be done to make such a process sustainable? The response to these questions lies in the definition and metrics of sustainability. Various approaches could be adopted to address impact of ‘z’, namely:

1. Treat ’z’ so that it becomes safer or benign
2. Generate less ‘z’
3. Prevent generation of ‘z’

While approach 1 would be cost intensive [12], approach 2 may be constrained by technical feasibility and therefore, due deliberation is required on approach 3. One way to approach 3 could be to utilize alternatives in input materials that are safer or to design processes in a manner that ‘z’ is not generated. This motto of ‘design to be benign’ is the motivation for this research.

1.2 Field of idea feasibility testing

There are various fields in pharmaceutical industry where feasibility of this idea could be tested. Common reported area is of synthetic chemistry and analytical methods where green chemistry and green engineering principles are applied to design sustainable products [13-15]. During literature review it was observed that application of sustainability concepts to drug product development are very few [16, 17]. Operations like granulation, tablet coating, spray drying etc. utilise organic solvents and could be interesting field of research to see if alternatives to harmful/undesirable

organic solvents are possible without altering product performance. Scope of exploration was further focused by identifying a scope frame as in Figure 1.3.

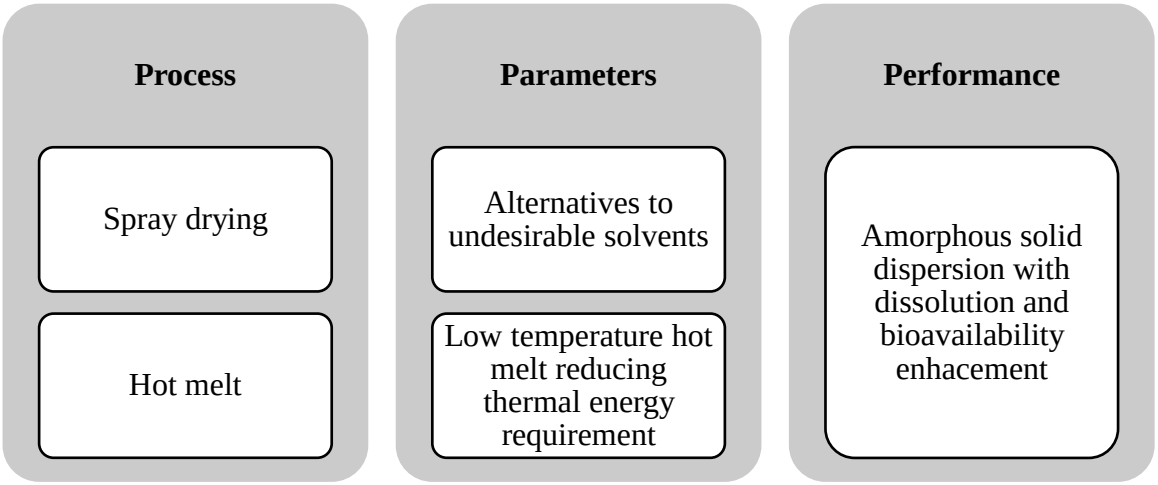

Figure 1.3 Scope frame defining area of research

1.3 Understanding drug dissolution and bioavailability

Oral drug delivery is considered as the most common route of drug administration, offering major advantages like self-administration, non-invasiveness and cost effective production. It constitutes about half of the total drug dosage forms. In 2017, US FDA approved 46 drugs, out of that 24 were oral dosage forms [18]. As a drug traverses the gut, it is encountered by various environments, enzymes, pH media, microflora etc. The drug dissolves, solubilizes and then permeates through cellular membranes to impart its action(s). This seemingly simple process is jeopardised when drug undergoes first pass metabolism, does not dissolve or has permeability issues. Such cases are not rare. About 17% of clinical attrition is attributed to pharmacokinetic and bioavailability issues of drugs [19]. Biopharmaceutical classification system was introduced in 1995 and continues to be a reference for preliminary evaluation and categorization of drugs as soluble, permeable or otherwise. In vitro and in silico tools have added advanced predictability to the drug discovery and development process

[20]. Still the challenge of poorly soluble drugs with bioavailability issues remains under resolved. Why?

One major reason that is attributable here is the way that drug development is happening. Focus of lead selection and optimization is to show pharmacological activity at target sites/receptors (biological selectivity and specificity). For this, lipophilic ligands are added to drug structures which generate highly lipophilic drugs with challenges of solubility in biological fluids. This problem is usually identified in late clinical stages because during preclinical in vivo and in silico testing either the early formulations are solutions in solvents, surfactants etc. or the drug dose is low that masks the issue [21]. To a very large extent, enabling formulation interventions can address solubility and bioavailability challenges of drugs [22]. Time to evaluate the need for such interventions is critical. When?

Ideally, during initial drug development stages itself a holistic plan to evaluate and address bioavailability challenges should be devised. It is easier to make process changes when the product is in drug substance development stage than in drug product, example modulating crystallization for small size crystals (could avoid micronization) or evaluating various solid forms (could help select more soluble forms like amorphous form). 'Formulate-ability' can be better assessed if an integrated approach is followed from drug discovery to drug product development [23]. How?

A combination of prognostic and diagnostic tools would be required for assessing solubility and bioavailability challenges of a drug. One of the first steps is to determine solubility. It is important that the solubility testing is performed in relevant media, representing the physiological environment that a drug is likely to encounter in vivo. Intrinsic dissolution testing, pH solubility profile and solubility in simulated fluids

(gastric fluid, intestinal fluid etc.) provide valuable information as to whether a drug has solubility and bioavailability challenge and if yes, then what is the cause; solvation-limited solubility (grease ball drugs that have high log P/log D values > 3) or solid state-limited solubility (brick dust drugs that have high melting point > 200°C) that needs to be addressed with enabling formulation strategies [24]. Few drugs have characteristics of both classes, i.e high log P values and high melting point like Levothyroxine (logP 4.6 and Tm 235°C) and are difficult to formulate [25]. Increasingly the role of in silico tools, in vitro tests and computational predictions is being recognised [26].

Bioavailability is an important pharmacokinetic parameter that defines the fraction of drug reaching systemic circulation. Various factors, physiological and physicochemical, affect bioavailability. While devising a strategy for enhancing bioavailability, it is important to identify the cause of low bioavailability [27]. Formulation interventions are better suited where bioavailability is a function of drug's dissolution and solubility. Permeability modulations, though possible, are not very easy to achieve because of the multiple factor that influence this property.

As per the biopharmaceutical classification systems (BCS), class II and class IV drugs are amenable to formulation interventions for solubility and bioavailability enhancement [28]. Selection of appropriate formulation strategy would depend on following considerations:

1. Stage of drug development where formulation is required.

   At early stage of drug development (preclinical and before), availability of limited drug quantities and constraint of time and money necessitate that a simple, reproducible and physico-chemically stable formulation is developed. In later stages

(phase 1 and later), more in-depth study is possible and various formulation strategies could be evaluated. However, if a solubility enhancement is applied at later stages, it calls for a bridging study between the early and late phase formulations [29]. This would require additional time and cost.

2. Purpose of formulation

   It is important to understand the purpose of a formulation development. A toxicology study requires maximum exposure of drug while a phase 1 study is for dose ranging, phase 2 requires a composition that is closer to market product etc. are few of the clear objectives that each phase has and a fit-for-purpose formulation should be designed. . Accordingly, the approach that is utilised for enabling formulation development needs to be considered. It would be appropriate to state that any enabling formulation approach needs to distinguish itself as discovery formulation [30], preclinical formulation [31] or clinical formulation [32]. Basis to contribution of melting point, partition coefficient and solubility in solvents to drug wetting, dissolution and solubilisation, few of the popular enabling formulation approaches for poorly water soluble drugs are depicted in Figure 1.4. A more commonly known classification is based on BCS, shown in Figure 1.5.

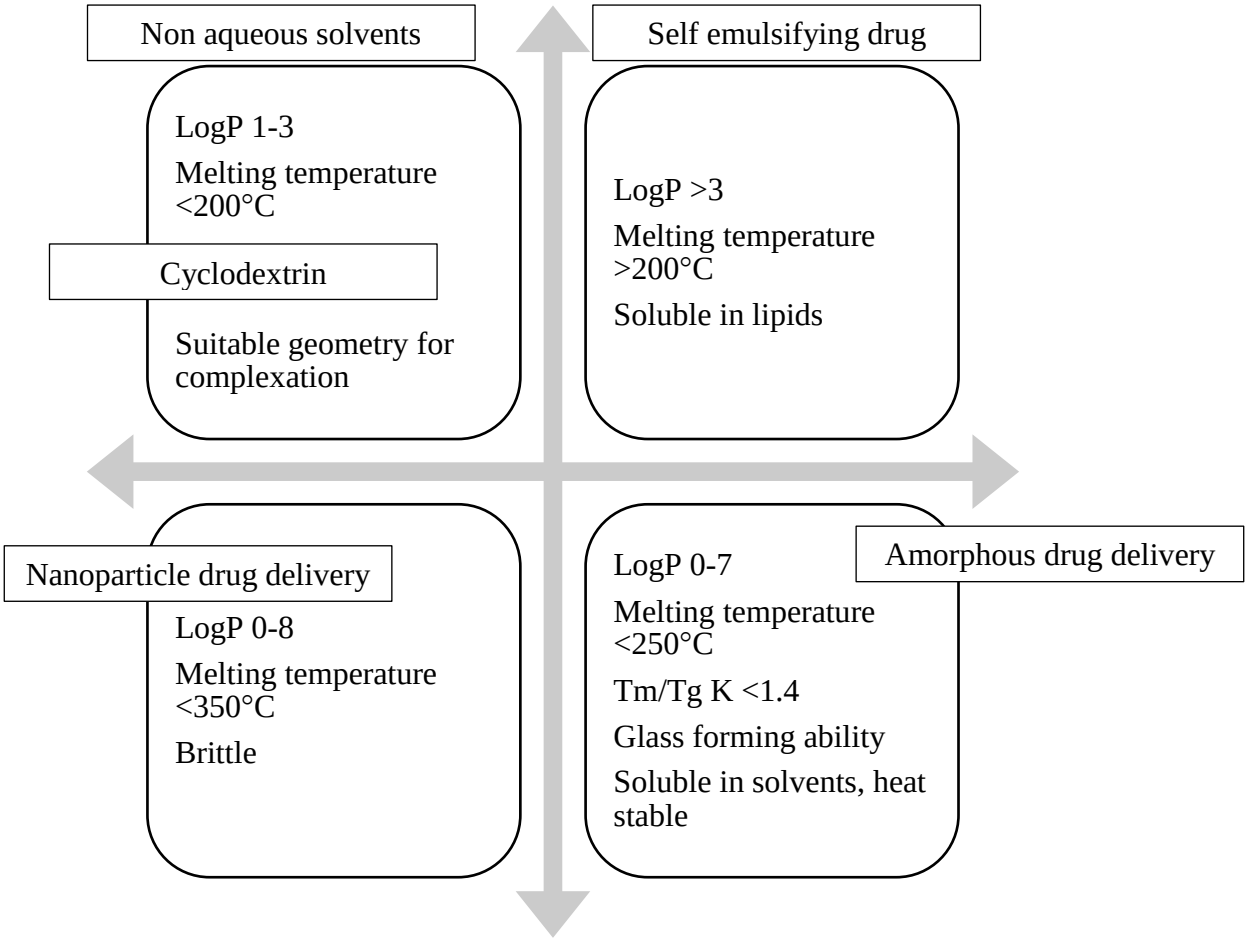

Figure 1.4 Enabling formulation approaches for poorly water soluble drugs

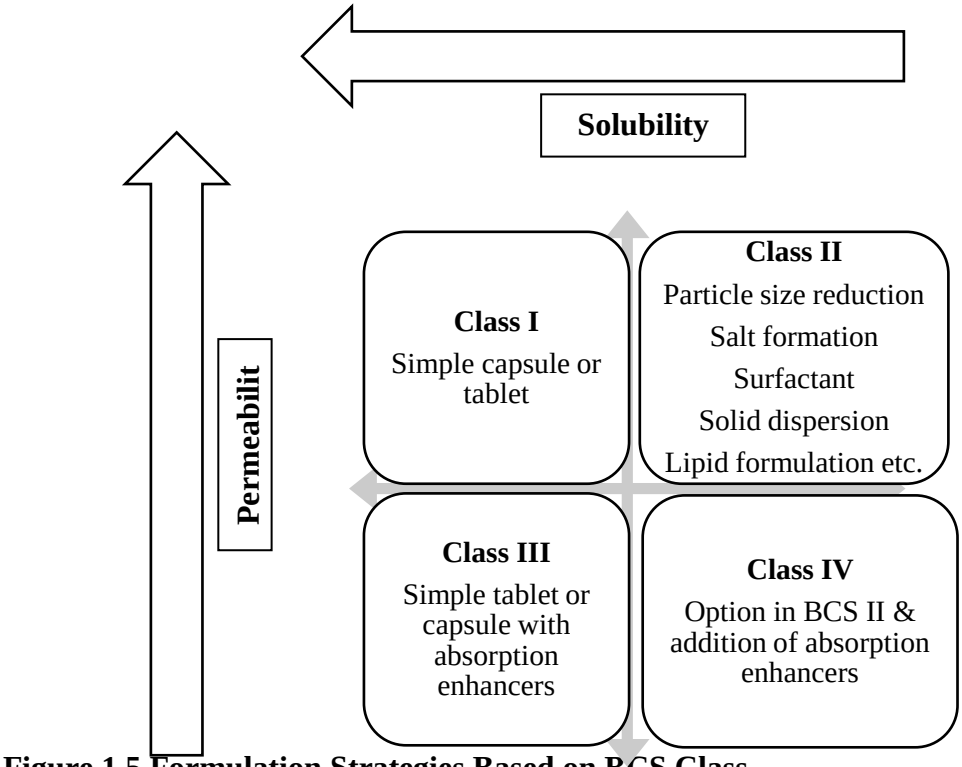

Figure 1.5 Formulation Strategies Based on BCS Class

Until late stage of clinical study, it is preferable to keep the formulation as simple as possible, mainly for below reasons:

1. Addition of many additives/excipients would require extensive drug excipient compatibility studies.
2. Complex technologies would require a lot of work on the process, its optimization, scale-up etc. This would increase the time for drug to reach dosing stage.
3. Till phase 1/2a, formulation development is an iterative process which could involve various changes in target in vivo profile of drug. Therefore, investing in sophisticated product design/process would not be appropriate.

There are various tools that are utilized to support the decision of which enabling formulation approach should be selected for a poorly water soluble drug [33]. Formulation scientists are moving away from shear empirical approach to a more structured and predictive model. Few important tools are:

1. High throughput screening of physicochemical and biological properties
2. Mini scale preparation, in vitro testing and ex-vivo studies
3. Guidance maps
4. Decisions trees
5. Computer modelling and simulations

Drug classification systems are also evolving from the Biopharmaceutical Classification System (BCS) to the Developability Classification System (DCS). Later system was devised by Butler and Dressman [34] and it subdivides class 2 into 2a (dissolution rate limited) and 2b (solubility limited), further guiding the decisions for appropriate enabling formulations. Figure 1.6 represent the DCS classifications, respectively.

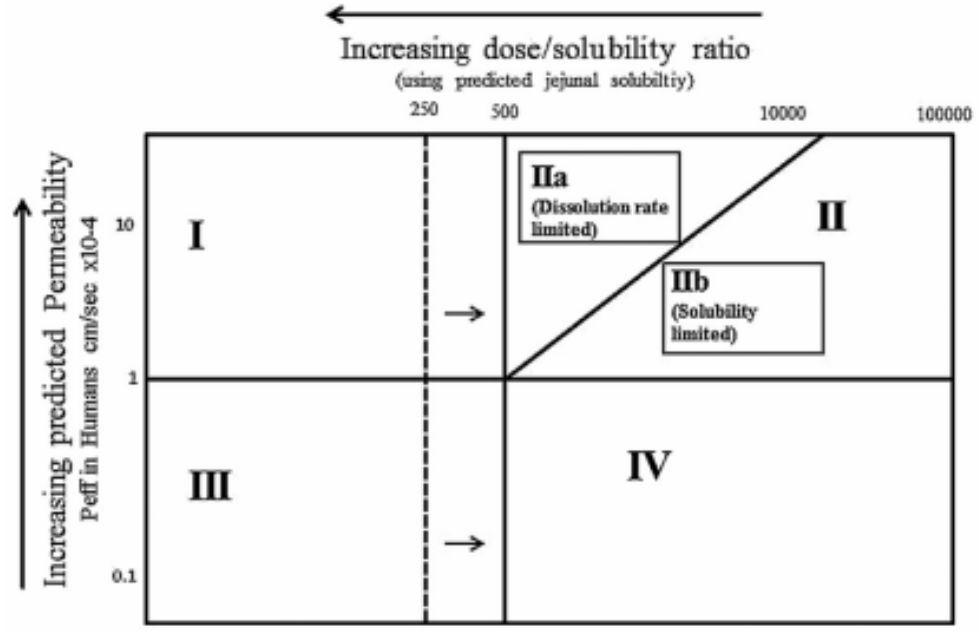

Figure 1.6 Developability Classification System. Adapted from Butler and Dressman [34]

1.4 Research methodology

Primary requirement of research is to identify a problem/concern, assess its present options for resolution, limitations of the present options and how they can be improved or sometimes invented. Often new research are based upon extensive literature reviews of a subject matter. This is not only to know what has been done in the past but also to identify what has not been done and what is still an unaddressed need. It is important that a structured approach is utilised for collection of literature from authentic sources, categorization of data points, deep deliberation of the addressed areas and understanding of the unaddressed sections that could be taken up as a research subject. For this research a data collection and analysis protocol was devised, Figure 1.7. It comprised of following elements: area of interest, literature types, sources of literature, key search words, analysis of data etc. It is important to bucket

information from primary, secondary and tertiary sources so that as per the relevance of that work, it could be referred, Figure 1.8.

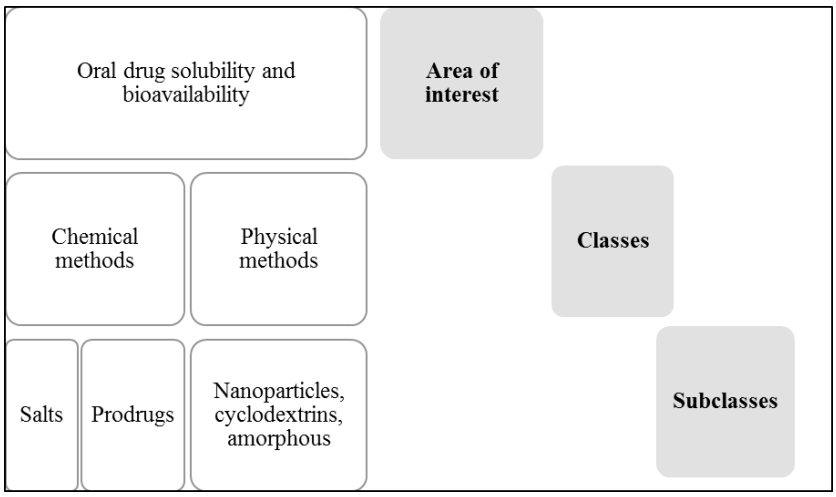

Figure 1.7 A typical data collection and analysis protocol

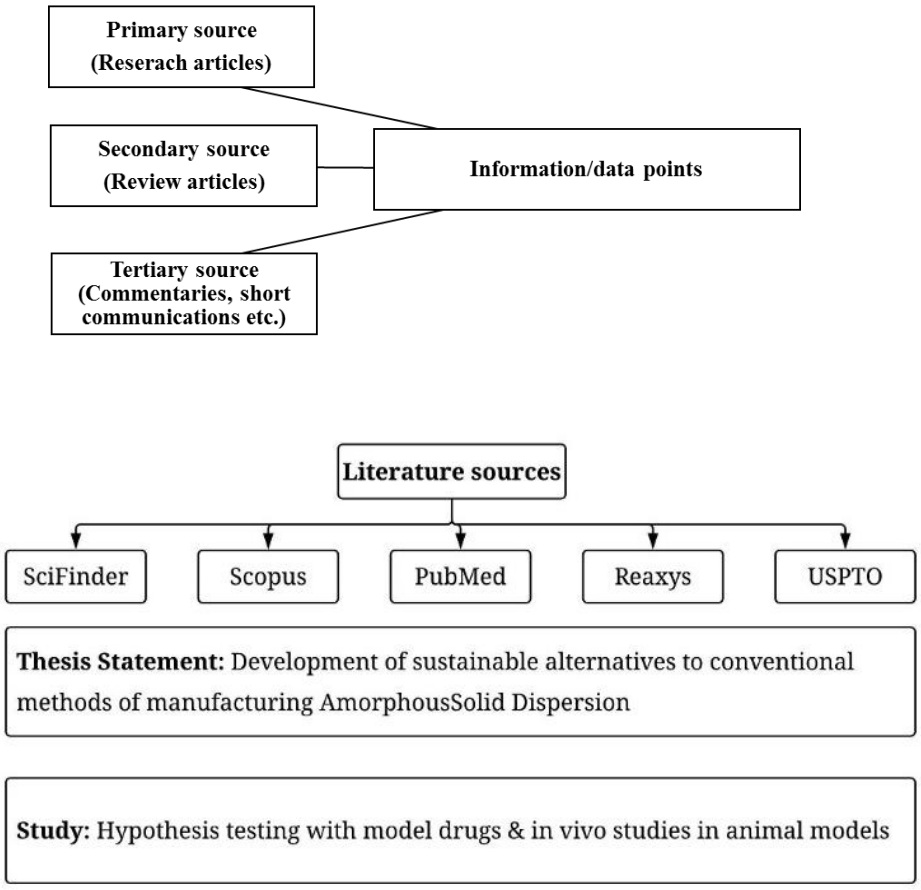

Figure 1.8 Information sources and overview of project structure

Following key words were utilised for literature search. Summary of literature is presented in Figure 1.9 and 1.10

1. Amorphous solid dispersion
2. Characterization of amorphous solid dispersion
3. Preparation methods for amorphous solid dispersions
4. New methods for amorphous solid dispersions
5. Drug-polymer screening
6. Hot melt extrusion
7. Antifungal drugs-Itraconazole and Posaconazole
8. Antiretroviral drugs-Efavirenz
9. In vitro testing for amorphous solid dispersions
10. In vivo testing for amorphous solid dispersions

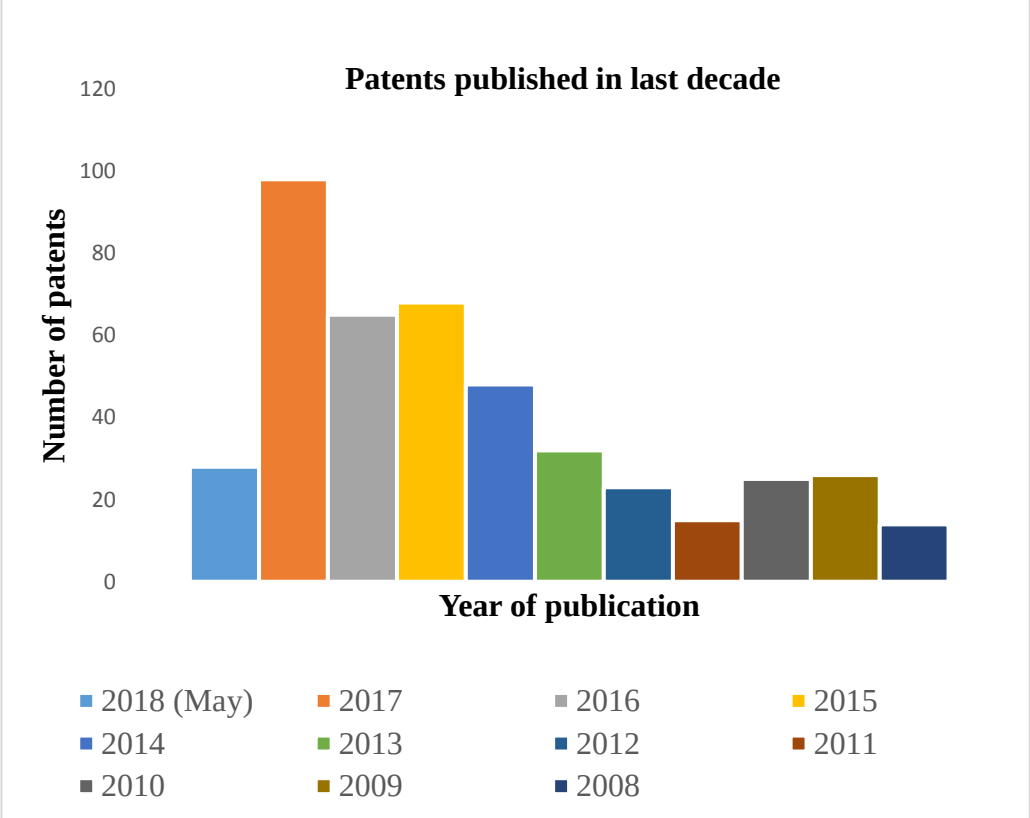

Figure 1.9 Analysis of patents in literature (Year 2008-2018, Source: SciFinder)

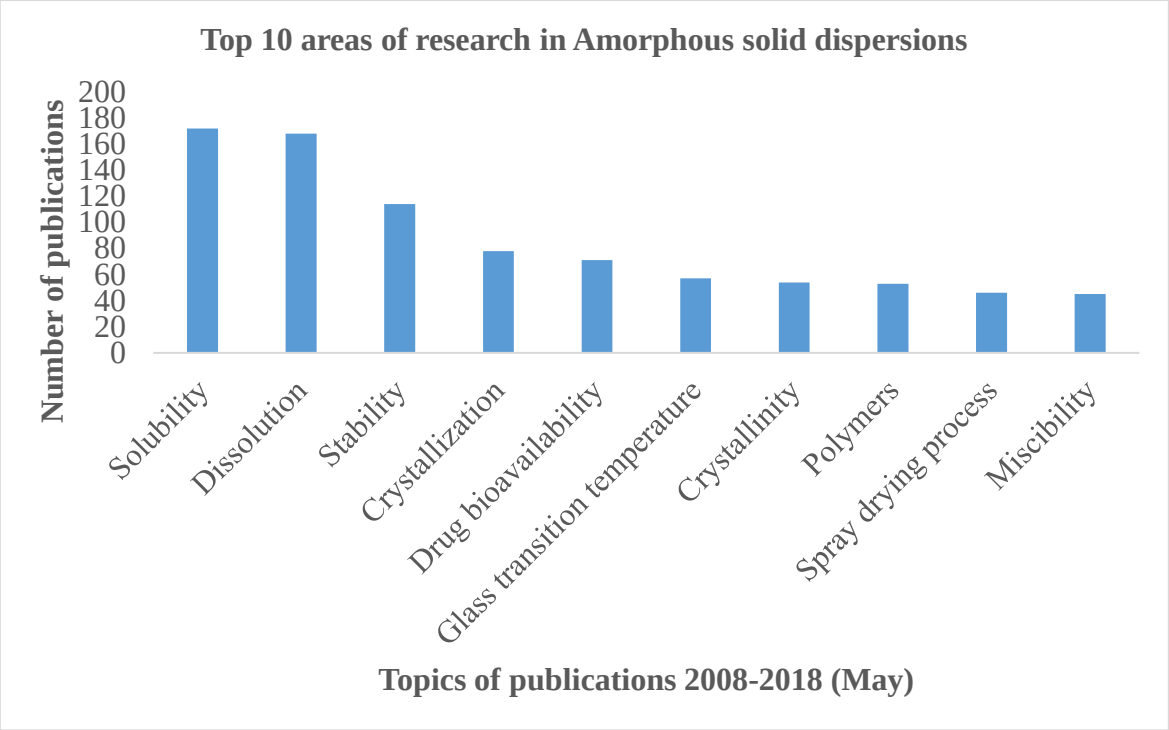

Figure 1.10 Analysis of research literature (Year 2008-2018, Source: SciFinder)

From this literature review a SWOT analysis was performed, Figure 1.11. It was evident from this exercise that proposed area of research was an important information gap.

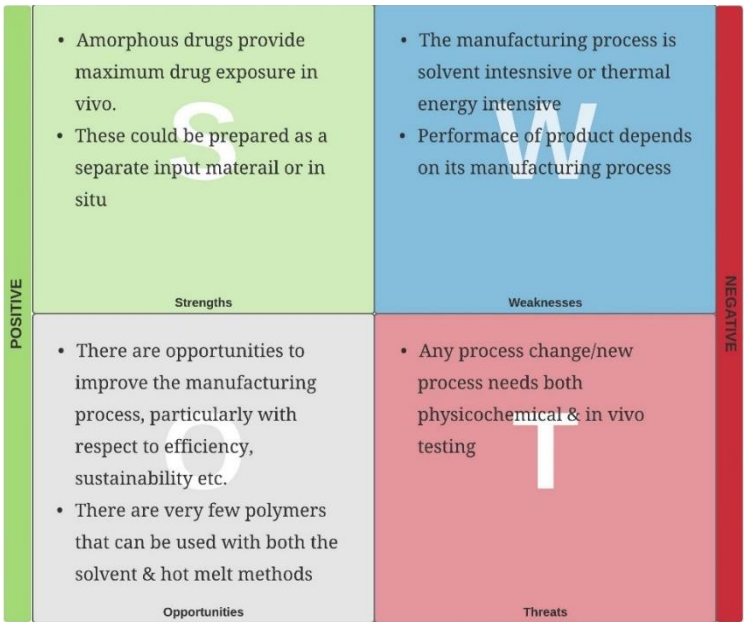

Figure 1.11 SWOT analysis of Amorphous Solid dispersion space

1.5 Plan of work

This involved stating the gap, hypothesis for research, identification of model drugs, experimental plan, observations and inferences. Broadly, the work plan is depicted below. Sustainable processes for ASDs was selected as the research area and based on literature review it was found there is insufficient information on environment friendly processing of ASDs like with alternative solvents and less energy intensive manufacturing processes, Figure 1.12. Model drug selection was based on the selected hypothesis. For salification hypothesis weakly ionisable drugs Itraconazole and Posaconazole were selected. Process intensification was evaluated with two processes: Agitated Thin Film Dryer (ATFD) and hot melt. For the later, Efavirenz was selected as model drug. Major physico-chemical properties that were considered for model drug selection are enlisted in Figure 1.13. A broad framework for prototype characterization was designed and is shown in Figure 1.14.

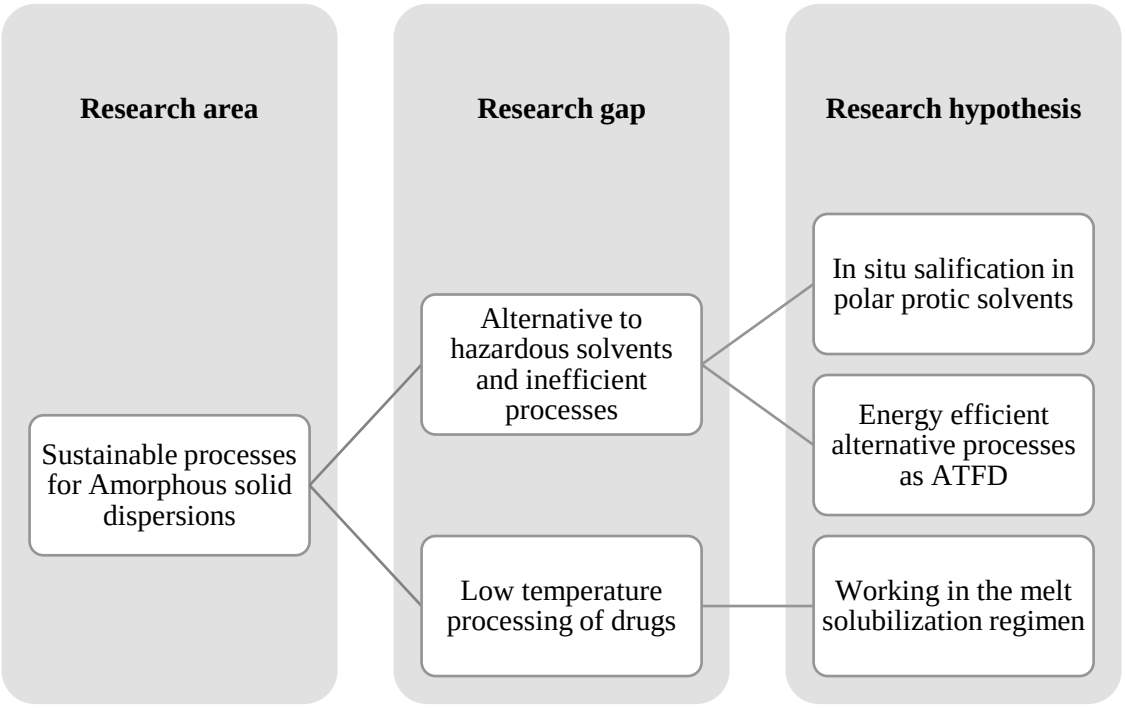

Figure 1.12 Research gap and technical approach for gap bridging

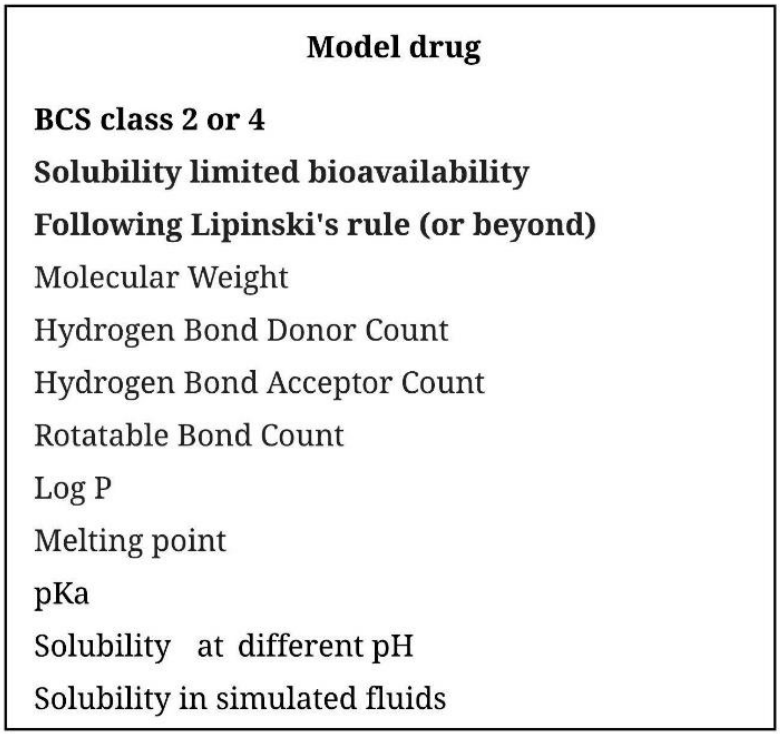

Figure 1.13 Model drug selection criteria

Parameter	Analytical method	Test information
Preliminary screening		
Glass forming ability (GFA)	DSC	Glass transition temp. Tg
		Onset temp. of crystallization Tcr
		Onset temp. of melting Tm
		Enthalpy of melt ΔH
Thermal stability	TGA/DSC	Decomposition temperature
Solid state	PLM	Amorphous/crystalline
	XRD	
Moisture sorption	DVS	Moisture sorption
Stability in aqueous pH solutions	HPLC/UV/HSM	Assay
Stability in organic solvents/co-solvents		Related substances/stability
Miscibility in polymers		
Parameter	**Analytical method**	**Test information**
Preliminary screening		
Dissolution in simulated media	HPLC/UV	Assay
		Related substances/stability
Stability (shelf life)	DSC	Amorphicity, physical stability
	XRD	
	PLM	
Advanced characterization		
Thermodynamics of drug-polymer interaction	FTIR	Chemical mapping
Relative interactions of prototypes	FTIR/NMR/Raman	Spectral imaging

Figure 1.14 Analytical characterization and testing plan for model drugs

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Chapter 2

Analytical method development

2.0 Introduction

Analytical method development is an important part of product development program which is performed to establish drug's identity, potency, purity, impurities etc. Analytical tests serve to identify the drug, quantify the drug and impurities or/and test for compliance to limits as per product specifications [1]. These include analytical testing in API bulk and in dosage forms, also in biological matrixes like blood, spinal fluids, tissues etc. [2] Depending on the stage of product development (early phase, clinical, phase III or commercial) details of analytical method development and validation vary [3] as shown in Figure 2.1.

Stage	**Drug development phase**		
	Early phase	**GMP phase (Phase I and II)**	**GMP phase (Phase III)**
Defining method objective	Focus on identification and quantification of drug	Focus on limited precision and robustness of method	Focus on extensive precision and robustness of method
Method validation	Preliminary characterization	Minimally validated methods	Fully validated methods

Figure 2.1 Analytical method evolution in the drug development program

For this research work, analytical method development and validation was carried out considering preclinical phase of product and it was performed for three model drugs: Itraconazole, Posaconazole and Efavirenz. Typical sequence of activities involved in analytical method development are depicted in Figure 2.2.

Analytical method development and validation was performed in accordance to International Conference on Harmonization (ICH) guidelines [4] and US FDA guidance for compendial [5] and non compendial methods [6]. Following parameters were validated: specificity, linearity, accuracy, precision, detection limit (DL), quantitation limit (QL).

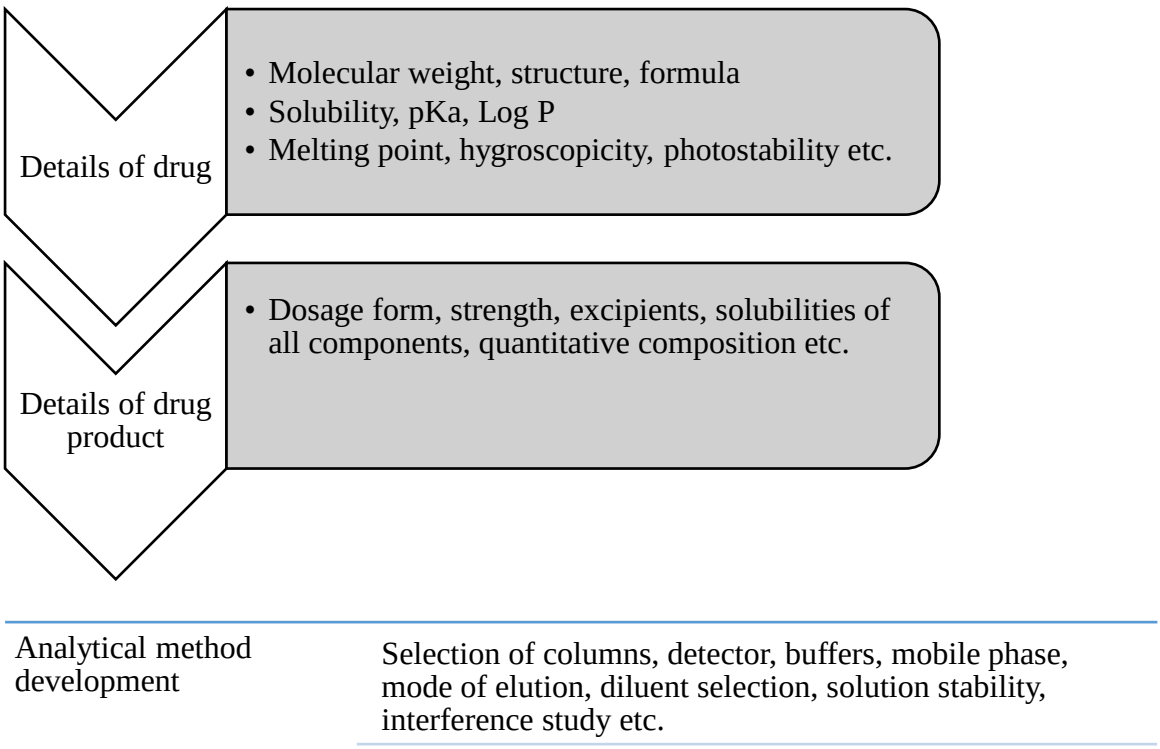


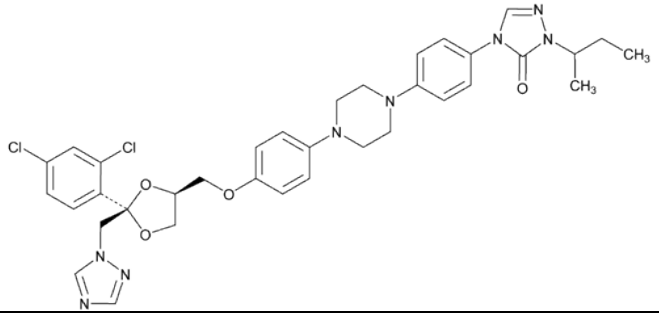
Figure 2.2 Scheme of typical analytical method development activities

2.1 Chemical analytical method development and validation for Itraconazole

Itraconazole is an azole antifungal that is indicated for blastomycosis, histoplasmosis, aspergillosis (in patients refractory/intolerant to amphotericin B therapy) and onychomycosis. It is poorly water soluble, with pKa 3.7 and a log (n-octanol/water) partition coefficient of 5.66 at pH 8.1 [7]. Physico-chemical properties of Itraconazole are provided in Table 2.1. From literature review, it was found that commonly reported methods for Itraconazole include Ultra Violet (UV) spectroscopy [9], Reverse Phase–High Performance Liquid Chromatography, RP-HPLC [10] and Liquid Chromatography-Mass Spectroscopy, LC-MS [11]. This drug is compendial with monographs in both United States Pharmacopeia (USP) [12] and European Pharmacopeia (EP) [13]. These methods were utilized as basis for analytical method development for Itraconazole amorphous solid dispersions. Diluent, mobile phase and mode of

elution were selected based on analyte solubility and solution stability studies. Ultra violet detection wavelength was determined by scanning the standard solution 10µg/ml in 200-400 nm range. Interference study was performed with blank (diluent) and placebo (excipients in diluent, also called drug matrix). All test and reference solutions were filtered with pre-wetted Fluoropore^{TM} PTFE membrane filter 0.45 µm (Millipore, Massachusetts, USA).

Table 2.1 Key properties of Itraconazole

S. No.	Parameters	Details [8]
1.	Generic name	Itraconazole
2.	Molecular Structure	
3.	Molecular formula	$C_{35}H_{38}Cl_2N_8O_4$
4.	Molecular weight	705.63
5.	CAS number	84625-61-6
6.	Melting point	166-170ºC

From literature review, it was found that commonly reported methods for Itraconazole include Ultra Violet (UV) spectroscopy [9], Reverse Phase–High Performance Liquid Chromatography, RP-HPLC [10] and Liquid Chromatography-Mass Spectroscopy, LC-MS [11]. This drug is compendial with monographs in both United States Pharmacopeia (USP) [12] and European Pharmacopeia (EP) [13]. These methods were utilized as basis for analytical method development for Itraconazole amorphous solid dispersions. Diluent, mobile phase and mode of elution were selected based on analyte solubility and solution stability studies. Ultra violet detection wavelength was determined by scanning the standard solution 10µg/ml in 200-

400 nm range. Interference study was performed with blank (diluent) and placebo (excipients in diluent, also called drug matrix). All test and reference solutions were filtered with pre-wetted Fluoropore^{TM} PTFE membrane filter 0.45 µm (Millipore, Massachusetts, USA).

Drug solubility and solution stability study of analyte was performed to select suitable solvent for stock solution, diluent and mobile phase preparation. HPLC column selection was based on analyte molecular weight, polarity, chemical characteristics like acidic/basic nature etc. [14]. With empirical approach and literature references, as mention in above sections, preliminary trials were performed that helped in selection of chromatographic components. Table 2.2 summarizes the validation parameters, procedures and acceptance criteria in this research. Various literature reference on analytical methods for Itraconazole [20-24], Posaconazole [25-28] and Efavirenz [29-30] were reviewed for understanding present status of work.

All major terms used here are defined in literature of analytical method development and validation [5, 6]. Few common terms utilized in this section and the later sections are defined below:

Analyte: A specific chemical moiety (active, metabolite, degradant etc.) that is being analyzed.

Standard: An analyte of known molecular composition and purity

Standard solution: Solution of a known concentration of standard dissolved in known concentration of solvent. It is further diluted, suitably, to obtain working or secondary solutions that are used for calibration/standard curve and quality control (QC) samples.

Test solution: Solution of the test material that is to be analyzed.

Blank: A sample to which no analyte has been added. It is the same medium in which test and standard solutions are prepared.

Placebo: A sample to which no analyte has been added. It contains the excipients and additives that would be present in test sample.

Method: All procedures used in an analysis.

Table 2.2 Major analytical validation parameters, procedures and acceptance criteria

Parameter	Procedure	Acceptance criteria [5,6]
Specificity	Triplicate samples of blank and the placebo preparation were spiked with known quantity of drug and examined to ensure that no interference occurred at the retention time of drug.	Sample and standard maxima occur at the same wavelengths and position. No interference from blank or placebo.
Linearity	Five concentrations of analyte standard, with minimum range as 50%-150% were analysed to generate a standard curve.	Linear regression coefficient of determination r^2 greater than or equal to 0.995
Limit of detection	Calculated based on the standard deviation of the response and the slope of regression line	3 times the noise level
Limit of quantitation	Calculated based on the standard deviation of the response and the slope of regression line	10 times the noise level
Accuracy	Matrix spike method. Three replicates at three concentrations of sample containing a known quantity of added analyte were analyzed. Range of sample covered was 80%-120% of expected content.	97.0% - 103.0% recovery for each spike level for APIs; 95.0% - 105.0% for finished dosage forms
Precision	Six replicate injections of standard solution of analyte at 100% of expected concentration	RSD less than or equal to 2.0%

2.1.1 Materials

Itraconazole was provided by Nosch labs (Hyderabad, India). HPLC grade acetonitrile and methanol were purchased from Rankem (Mumbai, India), Tetrabutyl ammonium hydrogen

sulphate was purchased from Merck (Mumbai, India)**.** Milli–Q water purification system EMD Millipore® (Billerica, USA) was used for obtaining high quality HPLC grade water.

2.1.2 Instruments

HPLC system consisted of solvent delivery pumps Waters Alliance® separation module 2695, photo diode array detector 2996, UV detector 2487 (Waters Corporation, Milford, USA) with data collection and integration with Empower®2 software. Electronic weighing balance (Sartorius, Germany), pH meter (Mettler-Toledo, Switzerland) and sonicator (Enertec, Mumbai, India) were utilized.

2.1.3 Chromatographic Conditions

Itraconazole concentrations were determined using isocratic elution with mobile phase consisting of 45:55 v/v aqueous solution of tetrabutyl ammonium hydrogen sulphate and acetonitrile, filtered through pre-wetted Fluoropore™ PTFE membrane 0.45µm filter (Millipore, MA, USA). Mobile phase was degassed with sonication. Prior to analysis, HPLC system was allowed to stabilize for about 30 minutes at flow rate of 1.5 mL/min.

2.1.4 Standard and test solution preparations

Stock solution of concentration 1 mg/ml was prepared by dissolving 100 mg Itraconazole standard in diluent (Acidified methanol, 4.0 mL concentrated hydrochloric acid 37.5% w/w in 1000 ml methanol). 1 ml of this stock was further diluted with diluent to obtain standard solution of 100µg/ml.

Separately, two test solutions were prepared. For each test solution 100 mg Itraconazole was dissolved in 100 ml of diluent and further 1 ml of this solution was diluted with diluent to 10 ml giving a concentration of 100 µg/ml. These solutions were labeled as test solution A and test solution B.

2.1.5 System suitability testing

System suitability testing is performed to ensure that parts of the HPLC system are operating within acceptable range. As per regulatory requirements, it should be performed before and after an analytical method is executed. It ensures that the integrity of HPLC system and samples is same throughout the analytical procedure. Five replicates of standard solution (prepared as as described in section 2.1.4) were bracketed between blank solutions. Injection sequence is listed in Table 2.3. %RSD and tailing factor were recorded.

Table 2.3 Sample sequence for system suitability testing

S.No.	Name of sample	Number of injections
1	Blank	2
2	Standard	5
3	Blank	1

2.1.6 % Assay calculation

HPLC system was equilibrated until uniform baseline was observed. Along with system suitability, test solutions A & B (section 2.1.4) were analyzed as per sequence in Table 2.4

Table 2.4 Sample sequence for % assay estimation

S.No.	Name of sample	Number of injections
1	Blank	2
2	Standard	5
3	Test solution A	1
4	Test solution B	1
5	Blank	1

2.1.7 Solution stability

Solution stability of test solution 100µg/ml (either of the test solutions, A or B, could be used. Here test solution A was taken forward for all analysis), and standard solution 100µg/ml was evaluated by storing them for two days, day-1 and day-2, at refrigerated condition of 2-8°C and protected from light. These were compared with freshly prepared test solution and standard solution.

2.1.8 Specificity

Specificity of the developed method was determined by analyzing triplicate samples of blank and placebo preparation spiked with known concentration of drug. Interference at retention time of Itraconazole was evaluated.

2.1.9 Linearity

Linearity plot was constructed for Itraconazole in the range of 50% to 150 % of the test concentration of analyte. Five concentrations 50µg/ml, 75µg.ml, 100µg/ml, 125µg.ml and 150µg/ml were used for this study. Coefficient of regression (R^2), slope and intercept were determined.

2.1.10 Limit of detection (LOD) and limit of quantitation (LOQ)

Standard deviation of the intercepts (σ) and mean of the slopes (S) of the calibration curve was utilized. These values were used to calculate the limit of detection (LOD) and limit of quantitation (LOQ) by using the below equations. LOQ is the concentration at which the signal-to-noise ratio was about 10:1. LOD is the concentration at which the signal-to-noise ratio was about 3:1.

$LOD=3.3\sigma/S$

$LOQ=10\sigma/S$

2.1.11 Accuracy

Three replicates at three concentrations of sample, 75µg/ml, 100µg/ml and 125µg/ml, were prepared by adding a known quantity of analyte to the standard solutions. Range of sample covered was 50%-150% of standard solution. Analyte recovery was estimated.

2.1.12 Precision

Three concentrations and three replicates of each concentration, 75µg/ml, 100µg/ml and 125µg/ml, were utilized for this study. Intra-day and inter-day precision were determined. Peak area % RSD was estimated.

2.1.13 Results and discussion

2.1.13.1 Analytical method development

Identification and optimization of suitable chromatographic parameters was performed. Reference to compendial methods was helpful in selecting suitable diluent and mobile phase composition. To assess the effect of organic phase type on peak properties, methanol and acetonitrile were evaluated. Sharp peak with good shape was obtained with acetonitrile. Mobile phase composition was varied from 40 to 60% of organic phase (acetonitrile) and it was observed that with higher content of organic phase, 50% and above, shorter retention time of 4 minutes was obtained, Figure 2.3 and Figure 2.4. Additionally, the peak tailing was less than 1.5. Based on these results, the optimum chromatographic conditions for analysis of Itraconazole were finalized as in Table 2.5.

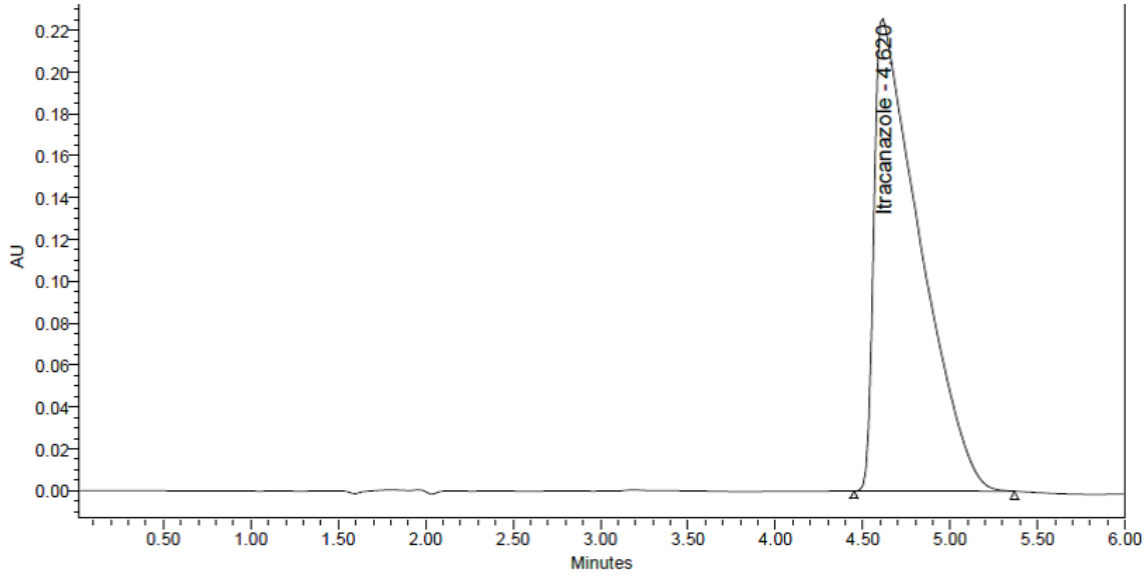


Figure 2.3 HPLC chromatogram of Itraconazole with 0.02M tetrabutylammonium hydrogen sulphate in water and acetonitrile 50:50 v/v

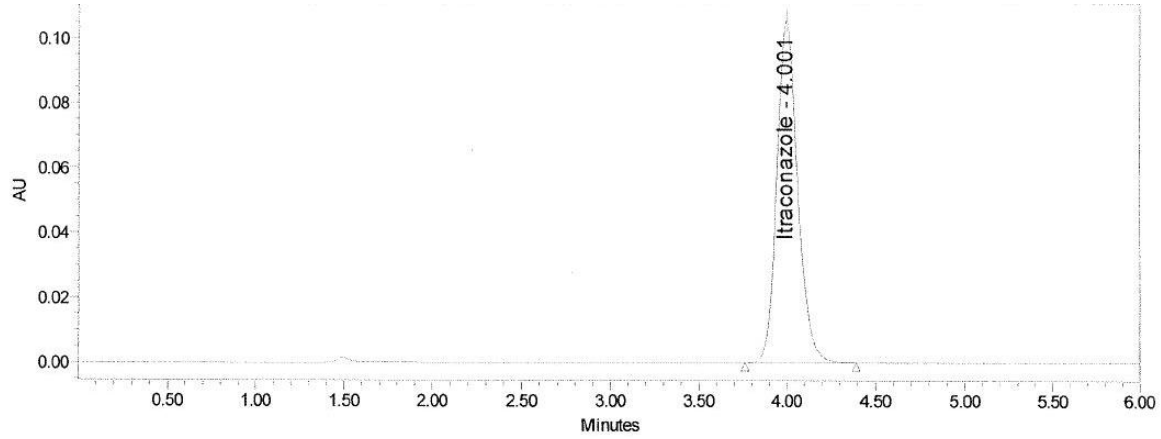


Figure 2.4 HPLC chromatogram of Itraconazole with 0.02M tetrabutylammonium hydrogen sulphate in water and acetonitrile 45:55 v/v

2.1.13.2 Analytical method validation

System suitability and % assay for a test sample

System suitability were found to be satisfactory and supported that the equipment was working to generate reliable data. Results are depicted in Table 2.6. % assay calculated for two test solutions, was found to be satisfactory as shown in Table 2.7

Table 2.5 Chromatographic conditions for Itraconazole

S.No.	Parameter	Details
1	Column	Hypersil^{TM} BDS C18 100 x 4.6 mm 3 µm
2	Flow rate	1.5 ml/min.
3	Injection volume	10 µL
4	Run time	10 minutes
5	Detector wavelength	260 nm
6	Buffer	27.2 g tetrabutylammonium hydrogen sulphate dissolved in 900 ml distilled water, volume made to 1000 ml with distilled water.
7	Mobile phase	Buffer: Acetonitrile 45:55 v/v
8	Diluent	Acidified methanol (4.0 mL concentrated hydrochloric acid 37.5% w/w in 1000 ml methanol)

Table 2.6 System suitability results for Itraconazole

S.No.	Parameter	Observation	Acceptance criteria
1	% RSD for five replicate injections of standard solution	0.7	<2.00
2	Tailing factor of Itraconazole peak	1.07	<2.00

Table 2.7 % assay of test solutions for Itraconazole

S.No.	Parameter	Assay % w/w	Acceptance criteria
1	Assay of test solution A	100.5	98-102%w/w
2	Assay of test solution B	100.4	
	Average	100.5	

Specificity

There was no interference of the excipients within retention time of drug. This indicated that the developed method was specific for Itraconazole. Chromatograms of blank and placebo, Figure 2.5 and 2.6, are presented below.

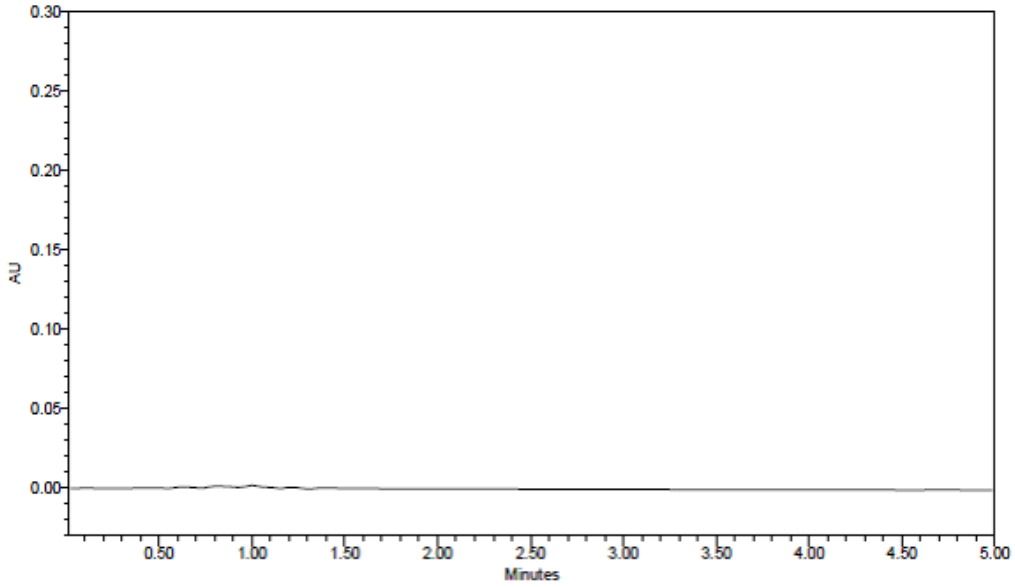


Figure 2.5 Chromatogram of blank for Itraconazole

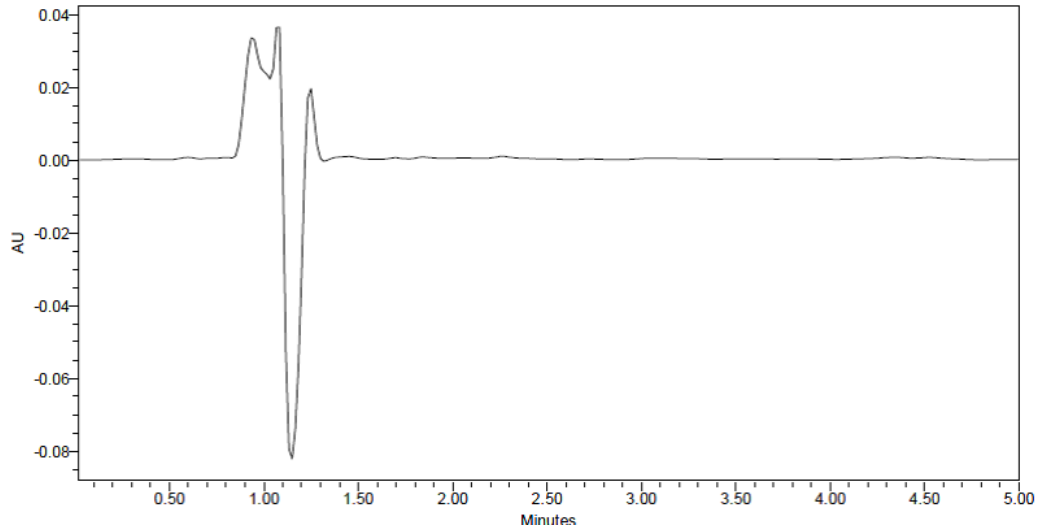


Figure 2.6 Chromatogram of placebo for Itraconazole

Solution stability

Test solution A of 100µg/ml and standard solution 100 µg/ml were found to be stable for 48 hours when stored at 2-8°C. Acceptance criteria was 98-102% w/w and difference in % assay from initial should not be more than 1%. Table 2.8 and 2.9 represent the stability data.

Table 2.8 Test solution stability study

S.No.	Parameter	Assay % w/w	Difference in % assay from initial
1	Initial	100.3	-
2	Day-1	100.2	0.1
3	Day-2	100.2	0.1

Table 2.9 Standard solution stability study

S.No.	Parameter	Assay % w/w	Difference in % assay from initial
1	Initial	100.5	-
2	Day-1	100.1	0.4
3	Day-2	100.3	0.2

Linearity

A linear response for chosen concentration range with a regression coefficient (R^2) value of 0.9996 was observed. Variation of the tested concentrations was evaluated by determining the %RSD values, presented in Table 2.10.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOQ values were found to be 0.0004 mg/ml and LOD was found to be 0.0001 mg/ml

Table 2.10 Linearity of Itraconazole in HPLC.

Nominal concentration (µg/mL)	Mean ± SD[a]	% RSD[b]
50	282428 ± 734.3	0.26
75	458272 ± 1604.0	0.35
100	614242 ± 3194.1	0.52
125	775714 ± 5507.6	0.71
150	924842 ± 20346.5	2.2

[a]Standard deviation.
[b]Percentage relative standard deviation.
Each value is represented as mean of five replicates (n=5)

Limit of detection (LOD) and limit of quantitation (LOQ)

LOQ values were found to be 0.0004 mg/ml and LOD was found to be 0.0001 mg/ml

Accuracy and precision

Accuracy of the developed method was estimated by recovery method or standard addition method. Data was found to confirm to recoveries more than 98%. Precision was carried out by evaluating the variation of the intra-day and inter-day samples. RSD was determined and found to be below 2%.

Table 2.11: Accuracy for the HPLC method of Itraconazole (*n* = 3)

Nominal concentration %	Sample amount (µg/mL)	Standard added (µg/mL)	%Recovery ± SD
50 %	50	25	98.9 ± 0.586
100%	50	50	99.4 ± 0.271
150%	50	75	99.5 ± 0.265

Table 2.12: System precision for the HPLC method of Itraconazole

Concentration (µg/ml)	Intra-day precision		Inter-day precision	
	% assay Mean[a] ± SD (n=5)	%RSD	% assay Mean[b] ± SD (n=10)	%RSD
20	100.15 ± 1.041	1.04	99.5 ± 0.676	0.68
40	101.23 ± 0.860	0.85	99.58 ± 0.822	0.83
60	100.54 ± 1.020	1.01	99.9 ± 0.892	0.89

a Mean of 5 replicates in a day± standard deviation,

b Mean of 5 replicates per day for 2 days± standard deviation

2.1.14 Conclusion: From above data it is concluded that the developed method is suitable for use in formulation development of Itraconazole. This simple method is a phase appropriate analytical method validated for quantitative estimation of Itraconazole. The validation data is satisfactory for all tested method parameters. This method is specific, precise, accurate and linear. Hence, it can be used for routine testing in development stage.

2.2 Bioanalytical method development for Itraconazole

This method was developed and qualified for fit-for-purpose use in preclinical studies.

2.2.1 Materials

Itraconazole (drug) was provided by Nosch labs (Hyderabad, India), Ketoconazole (internal standard) was bought from Sigma aldrich 9Mumbai, India) and Hydroxyitraconazole (metabolite) was provided by ClearSynth (Hyderabad, India). Agilent Zorbax Eclipse C18 column (150 x 4.6 mm, 5 µm) was procured from Agilent Technologies. Acetonitrile was procured from Lab Scan (Thailand) and Formic acid was obtained from Merck (Germany). Water was purified through a Elix®Milli-Q system (Elix®10, In-house, Hyderabad, India).

Dimethyl sulfoxide was from Sigma Aldrich (France). All the chemicals and reagents used were of the highest purity that was commercially available.

2.2.2 Methods

The LC-MS/MS analysis was performed on Applied Biosystems/MDS SCIEX API 4000 LC-MS/MS-Q trap with a liquid chromatography system consisting of Agilent 1200 infinity series (Agilent Technologies, Germany). Electrospray ionization (ESI) was used as a source of ionization. Analyst software (version 1.6.2) was used for automation control and data processing.

Chromatographic separation of Itraconazole and internal standard (IS) was achieved using Agilent Zorbax Eclipse C18 column (150 x 4.6 mm, 5 µm). The mobile phase consisted of pump A: 0.1% Formic acid in water and pump B: 0.1% Formic acid in acetonitrile in the ratio of 25:75 v/v. The seal wash solution contained acetonitrile and water in the ratio of 50:50 v/v. All separations were performed in isocratic mode at a flow rate of 1.2 µL/min and the injection volume was kept at 5 µL. The effluent was ionized by negative ion mode by ESI and detected by mass spectrometry.

Mass spectrometric data was acquired in negative ion mode with the following ESI MS parameters: curtain gas flow rate, 20 psi; collision gas flow rate, medium; ion source gases GS1 40 and GS2 45 psi, respectively; ion spray voltage, -4500 V; dwell time for each transition, 200 ms; inter channel delay, 5 ms; transfer capillary temperature, 500^0C. Data was recorded in multiple reaction monitoring (MRM) mode. Itraconazole and IS dissolved in dimethyl sulfoxide (1 mg/µL) were used for the MS/MS optimization (tuning). Quadrupole Q1 and Q3 were set on unit resolution. Data acquisition was performed with Analyst software (version 1.6.2).

2.2.2.1 Calibration curve

Working standard solution of Itraconazole was added to 10 µL drug-free plasma to obtain concentration levels of 2, 6, 30, 80, 160, 268, 382, 516, and 600 ng/ml. Quality control samples were prepared in pool at concentrations of 1.5, 10, 181, and 452 ng/ml and stored in the freezer at 5ºC until the time of analysis.

2.2.2.2 Preparation of stock and standard solutions

50 µL each of calibration standards, quality control (QC) samples, and study samples and 200 µL of rat plasma and 50 µL of 480 ng/mL internal standard (IS) solutions were added to a polypropylene tube and vortexed for about 30 sec. To this, 2 mL of acetonitrile was added and shaken for 15 min on a platform shaker. Then, it was centrifuged for approximately 5 minutes at 4000 rpm at 10ºC. The supernatant was collected in another glass tube and evaporated in a low volume evaporator at 40ºC under nitrogen. The residue was then reconstituted with 200 ml of mobile phase and 10 ml of the aliquot was injected into the LC/MS system for analysis.

2.2.2.3 Study

Itraconazole standard was weighed and prepared using dimethyl sulfoxide to yield primary standard stock solutions of a concentration of 1 mg/µL. Working stock solutions for calibration curve and the quality control (QC) working stock solutions were prepared from primary stock solution. The IS was weighed and dissoved in dimethyl sulfoxide to make 1 mg/µL concentration. The working concentration of 100 ng/µL was prepared in acetonitrile.

50 µL each of calibration standards, quality control (QC) samples, and study samples and 200 µL of rat plasma and 50 µL of 480 ng/m/ internal standard (IS) solutions were added to a polypropylene tube and vortexed for about 30 sec. To this, 2 µL of acetonitrile was added and shaken for 15 min on a platform shaker. Then, it was centrifuged for approximately 5 minutes at 4000 rpm at 10ºC. The supernatant was collected in another glass tube and evaporated in a

low volume evaporator at 40ºC under nitrogen. The residue was then reconstituted with 200 µL of mobile phase and 10 µL of the aliquot was injected into the LC/MS system for analysis.

Male Wistar rats (8–10 weeks of age) weighing 230–250 g were procured from Aurigene Discovery Technologies Limited animal house and were group housed in polypropylene cages for one week prior to the experiment. Animals had free access to normal laboratory diet (Envigo Teklad, 2014C, Madison, WI, USA) and potable water. The animal room was maintained at 23–25 °C and 40–70% humidity with a 12 h light/dark cycle with lights on at 7:00 a.m. All experimental protocols were approved by the Institutional Animal Ethical Committee.

Prior to the day of study, animals (n = 3) were fasted overnight (12 h) with free access to water. On the day of the study, Itraconazole API (F1), Itraconazole salt (F2) and amorphous solid dispersion of drug and polymer (F3) were formulated as an oral suspension using 0.5% Tween 80 and 0.5% methyl cellulose. Itraconazole formulation was administered orally at 10 mg/kg dose, and blood samples (150 µL at each time point) were collected by retro-orbital puncture at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h post dose in K_2EDTA tubes placed on crushed ice. Plasma was harvested by centrifuging the blood samples at 13000 rpm for 5 min at 4°C, transferred to labelled tubes and stored below -20°C until analysis.

2.2.3 Results and discussions

Data acquisition and peak integration were performed using Analyst software (version 1.6.2) and the peak area ratios of Itraconazole to the IS were plotted against Itraconazole nominal concentrations in plasma. Linear regression with a weighting factor of 1/x2 was used for the calibration curve. Itraconazole concentrations in rat plasma were quantified using the corresponding matrix-spiked calibration curves. The oral pharmacokinetic parameters of Itraconazole were calculated by noncompartmental analysis using Phoenix WinNonlin software (version 8.0, Pharsight Corporation, St Louis, MO, USA). Itraconazole plasma area under the plasma concentration–time curve (AUC) was calculated by linear trapezoidal method

from time zero to the last detectable time point, post dose. Mean plasma concentration–time profile of Itraconazole after single oral administration at 10 mg/kg dose in male Wistar rats. Rat were fasted overnight (12 h) and fed 4 h post dose with free access to water.

Plasma matrices were obtained from different sources and assayed to evaluate the selectivity of the method and the detection of interference at the analyte and IS retention times. Itraconazole and IS were well separated from the rat plasma matrix components under the described chromatographic conditions No endogenous peak from rat plasma was found to interfere with the elution of either the drug or the IS. These data indicate that the developed method is highly specific and selective for the analysis of Itraconazole in rat plasma samples. Mean accuracy data is presented in Table 13. Mean oral pharmacokinetic parameters are summarized in Table 14. Following single oral administration of Itraconazole API (F1), Itraconazole salt (F2) and amorphous solid dispersion of drug and polymer (F3) mean plasma concentrations reached Cmax values 40, 195, 1006 ng/µL and systemic exposure $AUC_{(0\text{-}inf)}$ were 326, 2199, 14690ng*h/µL following administration of F1, F2 and F3 formulation at 10 mg/kg dose in male Wistar rats, respectively. The systemic exposure of F3 was 6.7 and 45 fold higher as compared to F1 and F2. In the present investigation we observed that Itraconazole ASD enhance bioavailability in rats.

2.2.3 Conclusion

Simple, specific, reproducible, precise and cost-effective methods were developed for the estimation of analytes. The processing technique was efficient and repeatable, as, nearly complete recovery was obtained throughout the study. Further, the analytes were stable under the processing and storage conditions used for analysis. Method was found to be satisfactory and usable for pharmacokinetic studies in rat. Application of this method is illustrated in chapter 3.

Table 2.13: Back calculated concentrations and accuracy for Itraconazole in calibration standards (CS) and quality control (QC) samples (n=6)

Sample name	Nominal concentration (ng/mL)	Back Calculated concentration (ng/mL)	Mean % Accuracy
CS1	5.10	5.01	98.2
CS2	10.2	9.86	96.7
CS3	20.4	22.7	111
CS4	51.0	51.2	100
CS5	102	112	110
CS6	204	221	108
CS7	510	448	87.9
CS8	1020	977	95.7
CS9	2040	1830	89.5
CS10	5100	5200	102
HQC1	4080	3760	92.1
MQC1	2200	1960	88.9
LQC1	15.3	16.0	105
HQC2	4080	3150	77.1
MQC2	2200	1780	80.9
LQC2	15.3	15.9	104

Table 2.14. Mean oral pharmacokinetic parameters of Itraconazole following single oral administration at 10 mg/kg dose in male Wistar rats (n=3)

Parameters	Units	F-1		F-2		F-3	
		Mean	SD	Mean	SD	Mean	SD
C_{max}	ng/µL	40.0	15.1	195	66.7	1006	263
T_{max}	h	7.33	1.15	5.33	2.31	8.00	0.00
$AUC_{(0\text{-}last)}$	(h*ng/µL	326	373	2199	498	14690	3680

SD, Standard deviation; $AUC_{(0\text{-}inf)}$, area under the curve with extrapolation to infinity; C_{max}, peak plasma concentration; T_{max}, time to reach peak plasma concentrations

2.3 Chemical analytical method development and validation for Posaconazole

Posaconazole is an azole antifungal that is indicated for prophylaxis of invasive Aspergillus and Candida infections and treatment of oropharyngeal candidiasis (OPC), including OPC refractory (rOPC) to Itraconazole and/or fluconazole. It is poorly water soluble, with pKa 3.6 and 4.6 and a log (n-octanol/water) partition coefficient of 5.4 at pH 8.1 [17]. Physico-chemical properties of Posaconazole are provided in Table 2.15

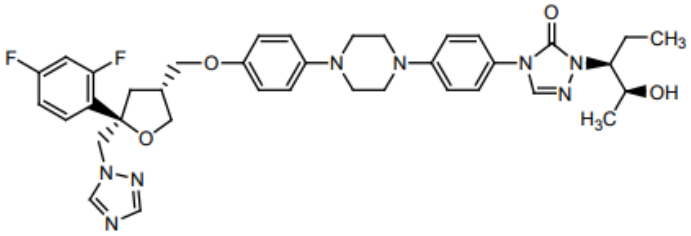
2.3.1 Materials

Posaconazole was obtained from Dr.Reddy's Laboratories Ltd. (Hyderabad, India). HPLC grade acetonitrile and methanol were purchased from Rankem (Mumbai, India), potassium dihydrogen orthophosphate was purchased from Merck (Mumbai, India). Milli–Q water purification system (EMD Millipore, Billerica, USA) was used for obtaining high quality HPLC grade water.

2.3.2 Instruments

High performance liquid chromatography (HPLC) system consisted of Waters Alliance® separation module 2695, photo diode array detector 2996, UV detector 2487 (Waters Corporation, Milford, USA) with Empower 2 software. Electronic weighing balance (Sartorius, Germany), pH meter (Mettler-Toledo, Switzerland) and sonicator (Enertec, Mumbai, India) were utilised.

Table 2.15 Key properties of Posaconazole

S.	Parameters	Details [8]
1.	Generic name	Posaconazole
2.	Molecular Structure	
3.	Molecular formula	$C_{37}H_{42F2}Cl_2N_8O_4$
4.	Molecular weight	700.68
5.	CAS number	171228-49-2
6.	Melting point	170-172ºC

All activities were similar to that performed with Itraconazole in above sections.

2.3.3 Chromatographic Conditions

Based on the drug supplier analytical methods for drug substance, these methods were developed. Posaconazole concentrations were determined using gradient elution with mobile phase A composition of aqueous solution of buffer (2.72 g in 1000 ml water, pH 5.5±0.5), acetonitrile and methanol 75:15:10 v/v and mobile phase B 22:60:18 v/v, filtered through pre-

wetted Fluoropore^{TM} PTFE membrane filter 0.45 µm (Millipore, USA). Mobile phases were degassed with sonication. 1:1 v/v mixture of water and acetonitrile was used as diluent. Prior to analysis, HPLC system was allowed to stabilize for about 30 minutes at flow rate of 0.8 ml/min. Chromatographic conditions are summarized in Table 2.16, mobile phase and diluent compositions are detailed.

2.3.4 Solution preparations

Stock solution of concentration 1 mg/ml was prepared by dissolving 100 mg Posaconazole standard in diluent (Acidified methanol, 4.0 mL concentrated hydrochloric acid 37.5% w/w in 1000 ml methanol). 1 ml of this stock was further diluted with diluent to obtain standard solution of 100µg/ml. Separately, two test solutions were prepared. For each test solution 100 mg Posaconazole was dissolved in 100 ml of diluent and further 1 ml of this solution was diluted with diluent to 10 ml giving a concentration of 100 µg/ml. These solutions were labeled as test solution A and test solution B.

System suitability testing is performed to ensure that parts of the HPLC system are operating within acceptable range. As per regulatory requirements, it should be performed before and after an analytical method is executed. It ensures that the integrity of HPLC system and samples is same throughout the analytical procedure. Five replicates of standard solution (prepared as as described in section 2.1.4) were bracketed between blank solutions. Injection sequence is listed in Table 2.3. %RSD and tailing factor were recorded.

2.3.5 Results and discussion

After iterations, finalized chromatographic conditions are listed in Table 2.16. Elution/retention time of 3.9 minutes was obtained with this method.

Table 2.16 Chromatographic conditions for Posaconazole

S.No.	Parameter	Details
1	Column	Poroshell 120 EC-C18, (150 x 4.6 mm. 2.7µm)
2	Flow rate	0.8 ml/min.
3	Injection volume	20 µL
4	Run time	8 minutes
5	Detector wavelength	260 nm
6	Buffer	2.72 g potassium dihydrogen orthophosphate dissolved in 900 ml distilled water, volume made to 1000 ml with distilled water.
7	Mobile phase	A Buffer: acetonitrile: methanol 75:15:10 v/v B Buffer: acetonitrile: methanol 22:60:18 v/v
8	Diluent	Water: Acetonitrile 1:1 v/v

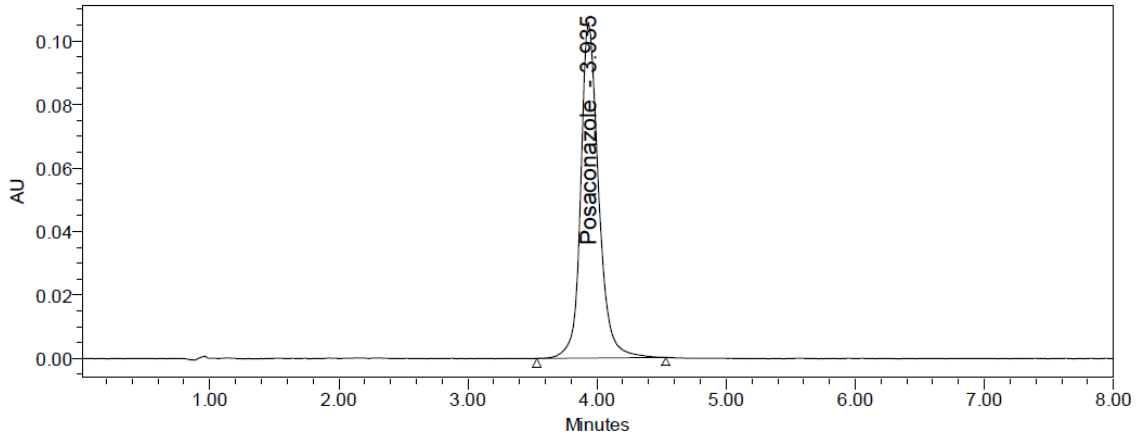


Figure 2.7 HPLC chromatogram of Posaconazole

System suitability and % assay for a test sample

System suitability were found was found to be satisfactory and supported that the equipment was working to generate reliable data. Results are depicted in Table 2.19, %RSD was 0.7% and tailing factor was 1.13 both being well within limits. % assay, Table 2.18, shows data within acceptance criteria.

Table 2.17 System suitability results for Posaconazole

S.No.	Parameter	Observation	Acceptance criteria
1	% RSD for five replicate injections of standard solution	0.7	<2.00
2	Tailing factor of Posaconazole peak	1.13	<2.00

Table 2.18 % assay of test solutions for Posaconazole

S.No.	Parameter	Assay % w/w	Acceptance criteria
1	Assay of test solution A	100.1	98-102%w/w
2	Assay of test solution B	99.7	
	Average	100.5	

Specificity

There was no interference of the excipients, Figure 2.8 and 2.9, within retention time of drug. This indicated that the developed method was specific for Posaconazole.

Solution stability

Test solution, standard solution and mobile phase were found to be stable for 48 hours when stored at room temperature. Acceptance criteria was that % assay of test, standard and test

solution in mobile phase should be 98-102% w/w and difference in % assay from initial should not be more than 1%. Table 2.19 and 2.20 represent the data.

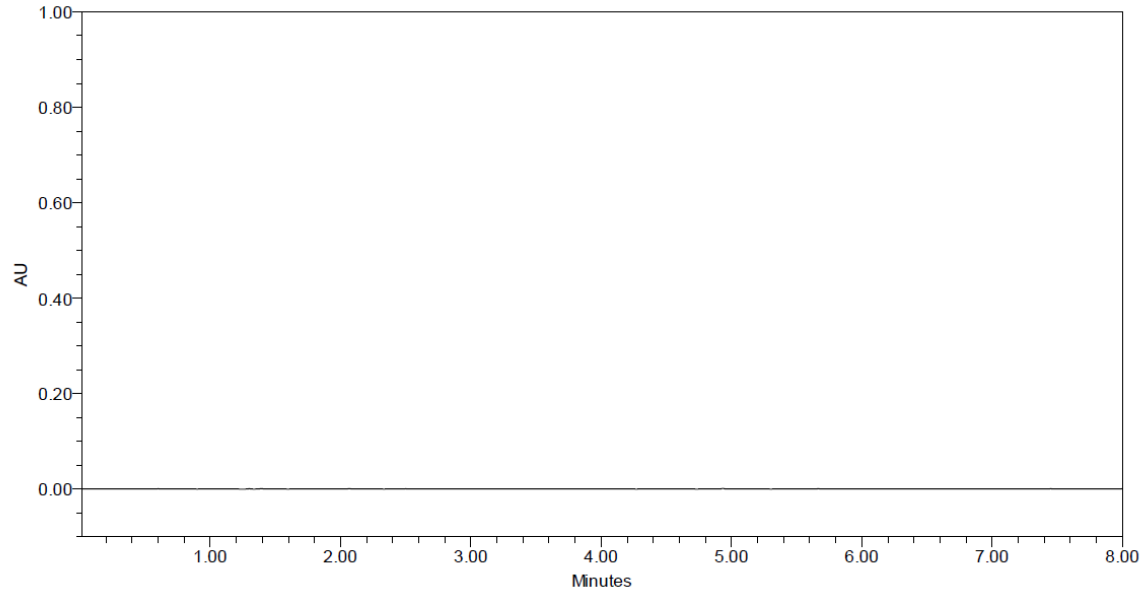


Figure 2.8 Chromatogram of blank for Posaconazole

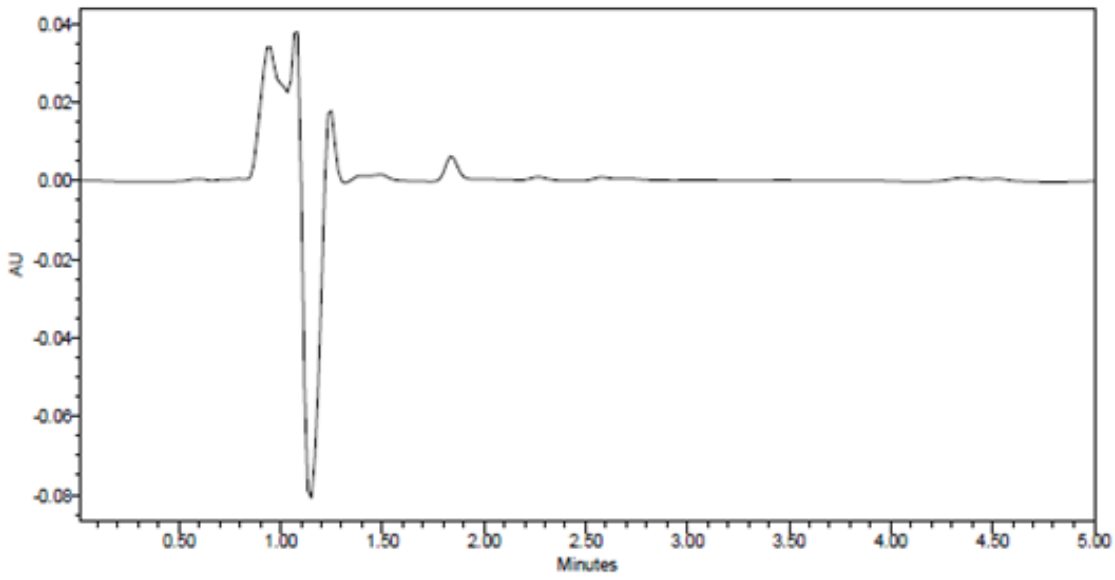


Figure 2.9 Chromatogram of placebo for Posaconazole

Table 2.19 Test solution stability study

S.No.	Parameter	Assay % w/w	Difference in % assay from initial
1	Initial	99.8	-
2	Day-1	99.7	0.1
3	Day-2	100.1	-0.3

Table 2.20 Standard solution stability study

S.No.	Parameter	Assay % w/w	Difference in % assay from initial
1	Initial	100.1	-
2	Day-1	99.9	0.2
3	Day-2	100.0	0.1

Linearity

A linear response for chosen concentration range with a regression coefficient (R^2) value of 0.9994 was observed. Variation of the tested concentrations was evaluated by determining the %RSD values.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOQ values were found to be 0.0003 mg/ml and LOD was found to be 0.0001 mg/ml

Accuracy and precision

Accuracy of the developed method was estimated by recovery method or standard addition method. Data was found to confirm to recoveries more than 98%. Precision was carried out by evaluating the variation of the intra-day and inter-day samples. RSD was determined and found to be below 2%.

Table 2.21: Accuracy for the HPLC method of Posaconazole (*n* = 6)

Nominal concentration %	Sample amount (µg/mL)	Standard added (µg/mL)	%Recovery ± SD
50 %	50	25	97.2 ± 0.352
100%	50	50	99.4 ± 0.218
150%	50	75	98.8 ± 0.346

Table 2.22: System precision for the HPLC method of Posaconazole

Concentration (µg/ml)	Intra-day precision		Inter-day precision	
	% assay Mean[a] ± SD (n=5)	%RSD	% assay Mean[b] ± SD (n=10)	%RSD
75	100.10 ± 1.136	1.13	100.2 ± 0.462	0.46
100	100.17 ± 0.998	1.00	99.87 ± 0.784	0.19
175	100.00 ± 0.999	1.00	100.1 ± 0.928	0.93

a Mean of 5 replicates in a day± standard deviation,

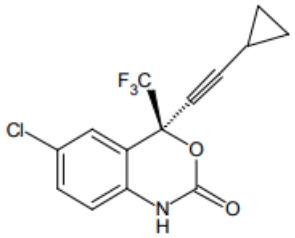
b Mean of 5 replicates per day for 2 days± standard deviation

2.3.6 Conclusion: From above data it is concluded that the developed method is suitable for use in formulation development of Posaconazole. This simple method is a phase appropriate analytical method validated for quantitative estimation of Posaconazole. The validation data is satisfactory for all tested method parameters. This method is specific, precise, accurate and linear. Hence, it can be used for routine testing in development stage.

2.4 Chemical analytical method development and validation for Efavirenz

Efavirenz is an antiretroviral drug that is indicated for treatment of AIDS. It is poorly water soluble. Physico-chemical properties of Efavirenz are provided in Table 2.23

Table 2.23 Key properties of Efavirenz

S. No.	Parameters	Details [8]
1.	Generic name	Efavirenz
2.	Molecular Structure	
3.	Molecular formula	$C_{14}H_{9F2}ClF_3NO_4$
4.	Molecular weight	315.68
5.	CAS number	154598-52-4
6.	Melting point	139-141ºC

All activities were similar to that performed with Itraconazole in above sections.

2.4.1 Materials

Efavirenz was obtained from MSN Laboratories Ltd. (Hyderabad, India). HPLC grade acetonitrile and methanol were purchased from Rankem (Mumbai, India), ammonium acetate was purchased from Merck (Mumbai, India)**.** Milli–Q water purification system (EMD Millipore, Billerica, USA) was used for obtaining high quality HPLC grade water.

2.4.2 Instruments

High performance liquid chromatography (HPLC) system consisted of Waters Alliance® separation module 2695, photo diode array detector 2996, UV detector 2487 (Waters Corporation, Milford, USA) with Empower 2 software. Electronic weighing balance (Sartorius, Germany), pH meter (Mettler-Toledo, Switzerland) and sonicator (Enertec, India) were utilised.

2.4.3 Chromatographic Conditions

Based on the drug supplier analytical methods for drug substance, these methods were developed. Efavirenz concentrations were determined using isocratic elution with mobile phase A composition of aqueous solution of buffer (800 mg ammonium acetate in 1000 ml water), acetonitrile and buffer 50:50 v/v filtered through pre-wetted Fluoropore^{TM} PTFE membrane filter 0.45 µm (Millipore, USA). Prior to analysis, HPLC system was allowed to stabilize for about 30 minutes at flow rate of 1.0 ml/min. Chromatographic conditions are detailed in table 2.28.

2.4.4 Solution preparations

Stock solution of concentration 1 mg/ml was prepared by dissolving 100 mg Efavirenz standard in diluent (Acetonitrile). 1 ml of this stock was further diluted with diluent to obtain standard solution of 100µg/ml. Separately, two test solutions were prepared. For each test solution 100 mg Efavirenz was dissolved in 100 ml of diluent and further 1 ml of this solution was diluted with diluent to 10 ml giving a concentration of 100 µg/ml. These solutions were labeled as test solution A and test solution B.

System suitability testing is performed to ensure that parts of the HPLC system are operating within acceptable range. As per regulatory requirements, it should be performed before and after an analytical method is executed. It ensures that the integrity of HPLC system and samples is same throughout the analytical procedure. Five replicates of standard solution (prepared as as described in section 2.1.4) were bracketed between blank solutions. Injection sequence is listed in Table 2.3. %RSD and tailing factor were recorded.

2.4.5 Results and discussion

After iterations, finalized chromatographic conditions are listed in Table 2.24. Elution time of 9.15 minutes was obtained with this method.

Table 2.24 Chromatographic conditions for Efavirenz

S.No.	Parameter	Details
1	Column	Hypersil BDS C18 (250x4.6 mm, 5um)
2	Flow rate	1.0 ml/min.
3	Injection volume	20 µL
4	Run time	16 minutes
5	Detector wavelength	252 nm
6	Buffer	800 mg ammonium acetate dissolved in 900 ml distilled water, volume made to 1000 ml with distilled water.
7	Mobile phase	A Buffer: acetonitrile 1:1 v/v
8	Diluent	Acetonitrile

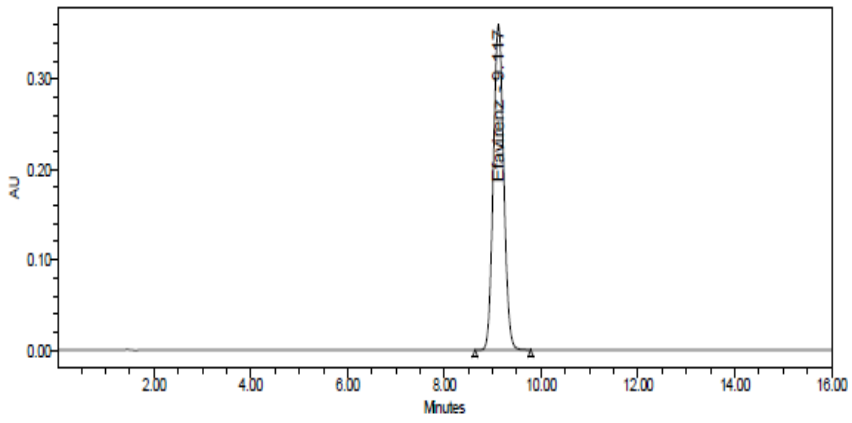


Figure 2.10 HPLC chromatogram of Efavirenz

System suitability and % assay for a test sample

System suitability were found was found to be satisfactory and supported that the equipment was working to generate reliable data. Results are depicted in Table 2.25. % assay calculated for two test solutions, was found to be satisfactory as shown in Table 2.26

Table 2.25 System suitability results for Efavirenz

S.No.	Parameter	Observation	Acceptance criteria
1	% RSD for five replicate injections of standard solution	0.15	<2.00
2	Tailing factor of Efavirenz peak	1.10	<2.00

Table 2.26 % assay of test solutions for Efavirenz

S.No.	Parameter	Assay % w/w	Acceptance criteria
1	Assay of test solution A	100.2	98-102%w/w
2	Assay of test solution B	99.9	
	Average	100.0	

Specificity

There was no interference of the excipients within retention time of drug. This indicated that the developed method was specific for Efavirenz. Refer Figure 2.11

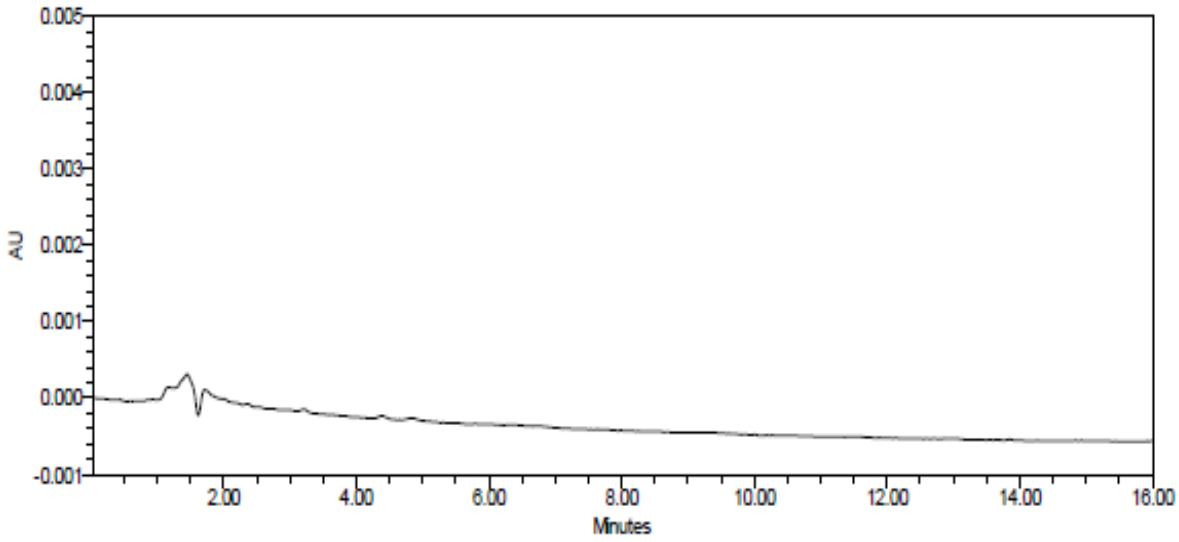


Figure 2.11 Chromatogram of placebo for Efavirenz

Solution stability

Test solution, standard solution and mobile phase were found to be stable for 48 hours when stored at room temperature. Acceptance criteria was that % assay of test, standard and test solution in mobile phase should be 98-102% w/w and difference in % assay from initial should not be more than 1%. Table 2.27 and 2.28 represent the data.

Table 2.27 Test solution stability study

S.No.	Parameter	Assay % w/w	Difference in % assay from initial
1	Initial	100.0	-
2	Day-1	99.9	0.1
3	Day-2	98.7	1.3

Table 2.28 Standard solution stability study

S.No.	Parameter	Assay % w/w	Difference in % assay from initial
1	Initial	100.1	-
2	Day-1	100.0	0.1
3	Day-2	100.0	0.1

Linearity

A linear response for chosen concentration range with a regression coefficient (R^2) value of 0.9998 was observed. Variation of the tested concentrations was evaluated by determining the %RSD values.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOQ values were found to be 0.0008 mg/ml and LOD was found to be 0.0002 mg/ml

Accuracy and precision

Accuracy of the developed method was estimated by recovery method or standard addition method. Data was found to confirm to recoveries more than 97%. Precision was carried out by evaluating the variation of the intra-day and inter-day samples. RSD was determined and found to be below 2%. Data represented in Table 2.29 and 2.30.

Table 2.29: Accuracy for the HPLC method of Efavirenz (n = 6)

Nominal concentration %	**Sample amount (µg/mL)**	**Standard added (µg/mL)**	**%Recovery ± SD**
50 %	50	25	97.8 ± 0.601
100%	50	50	98.7 ± 0.492
150%	50	75	99.6 ± 0.536

Table 2.30: System precision for the HPLC method of Efavirenz

Concentration (µg/ml)	**Intra-day precision**		**Inter-day precision**	
	% assay Mean[a] ± SD (n=5)	**%RSD**	**% assay Mean[b] ± SD (n=10)**	**%RSD**
20	99.5 ± 0.265	0.265	99.4 ± 0.431	0.431
40	99.8 ± 0.404	0.404	99.2 ± 0.263	0.263
60	99.6 ± 0.306	0.306	98.8 ± 0.291	0.291

a Mean of 5 replicates in a day± standard deviation, b Mean of 5 replicates per day for 2 days± standard deviation

2.4.6 Conclusion: From above data it is concluded that the developed method is suitable for use in formulation development of Efavirenz. This simple method is a phase appropriate analytical method validated for quantitative estimation of Efavirenz. The validation data is satisfactory for all tested method parameters. This method is specific, precise, accurate and linear. Hence, it can be used for routine testing in development stage

2.5 Bioanalytical method development for Efavirenz

This method was developed and qualified for fit-for-purpose use in preclinical studies.

2.5.1 Materials

Efavirenz was procured from MSN (Hyderabad, India), Celecoxib of 99.8% purity was provided by Dr. Reddy's Lab. Ltd. (Hyderabad, India). All solvents were procured from Merck (Mumbai, India). Double distilled water were passed through the 0.2 micron Millipore membrane and degassed by ultrasonic bath.

2.5.2 Methods

The LC-MS/MS analysis was performed on Applied Biosystems/MDS SCIEX API 4000 LC-MS/MS-Q trap with a liquid chromatography system consisting of Agilent 1200 infinity series (Agilent Technologies, Germany). Electrospray ionization (ESI) was used as a source of ionization. Analyst software (version 1.6.2) was used for automation control and data processing.

Chromatographic separation of efavirenz and internal standard (IS) was achieved using Aglient Eclipse XDB-C18, 4.6*100 mm, 3.5 µM column. The mobile phase consisted of pump A: 0.1% Formic acid in water and pump B: 0.1% Formic acid in acetonitrile in the ratio of 15:85 v/v.

The seal wash solution contained acetonitrile and water in the ratio of 50:50 v/v. All separations were performed in isocratic mode at a flow rate of 1.2 mL/min and the injection volume was kept at 5 mL. The effluent was ionized by negative ion mode by ESI and detected by mass spectrometry. The retention times of Efavirenz and IS were 1.21 and 1.11 min, respectively.

Mass spectrometric data was acquired in negative ion mode with the following ESI MS parameters: curtain gas flow rate, 20 psi; collision gas flow rate, medium; ion source gases GS1 40 and GS2 45 psi, respectively; ion spray voltage, -4500 V; dwell time for each transition, 200 ms; inter channel delay, 5 ms; transfer capillary temperature,500^0C. The curtain gas and ion source gases were nitrogen. The compound parameters, viz., declustering potential, entrance potential, collision energy and collision cell exit potential, for Efavirenz and IS were -90, -10, -46, -1 and -80, -10, -30 and -20eV, respectively. Data was recorded in multiple reaction monitoring (MRM) mode. Efavirenz and IS dissolved in dimethyl sulfoxide (1 mg/mL) were used for the MS/MS optimization (tuning). Detection of the ions was performed in MRM mode, with the transition of m/z (Q1/Q3) 313.99/69.00 for Efavirenz and m/z (Q1/Q3) 380.00/316.40 for IS. Quadrupole Q1 and Q3 were set on unit resolution. Data acquisition was performed with Analyst software (version 1.6.2).

2.5.3 Study

Efavirenz standard was weighed and prepared using dimethyl sulfoxide to yield primary standard stock solutions of a concentration of 1 mg/mL. Working stock solutions for calibration curve and the quality control (QC) working stock solutions were prepared from primary stock solution. Final concentrations of the calibration standards (CS1-CS11) were 5.08, 10.16, 25.40, 50.80, 101.59, 253.98, 507.96, 1015.92, 2031.84, 4063.68 and 5079.60 ng/mL, the concentrations of QC samples were 15.41 ng/mL (low quality control, LQC), 2589.60 ng/mL (medium quality control, MQC) and 3984.00 ng/mL (high quality control, HQC). The IS was

weighed and dissolved in dimethyl sulfoxide to make 1 mg/mL concentration. The working concentration of 100 ng/mL was prepared in acetonitrile.

Efavirenz extraction from rat plasma was carried out by protein precipitation method using acetonitrile as precipitating solvent. Calibration curve samples were prepared by spiking 5 µL of calibration curve spiking solutions to 45 µL of blank rat plasma followed by 500 µL of 0.1% formic acid in acetonitrile containing IS (100 ng/mL). The study samples (50 µL) were processed by adding 500 µL of 0.1% formic acid in acetonitrile containing internal standard. Samples were vortexed for 3 min and then centrifuged at 13,000 rpm for 5 min at 4°C. Clear supernatant (250 µL) was separated and transferred to labeled LC-MS vials.

Male Wistar rats (8–10 weeks of age) weighing 230–250 g were procured from Aurigene Discovery Technologies Limited animal house and were group housed in polypropylene cages for one week prior to the experiment. Animals had free access to normal laboratory diet (Envigo Teklad, 2014C, Madison, WI, USA) and potable water. The animal room was maintained at 23–25 °C and 40–70% humidity with a 12 h light/dark cycle with lights on at 7:00 a.m. All experimental protocols were approved by the Institutional Animal Ethical Committee.

Prior to the day of study, animals (n = 3) were fasted overnight (12 h) with free access to water. On the day of the study, Efavirenz AP1 (F1) , Efavirenz AP1(F2) +Polymer (Poloxamer 407) and Melt as Efavirenz API (F3) + Polymer (Product) was formulated as an oral suspension using 0.5% Tween 80 and 0.5% methyl cellulose . Efavirenz formulation was administered orally at 10 mg/kg dose, and blood samples (150 µL at each time point) were collected by retro-orbital puncture at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h post dose in K_2EDTA tubes placed on crushed ice. Plasma was harvested by centrifuging the blood samples at 13000 rpm for 5 min at 4°C, transferred to labeled tubes and stored below -20°C until analysis.

Data acquisition and peak integration were performed using Analyst software (version 1.6.2) and the peak area ratios of Efavirenz to the IS were plotted against Efavirenz nominal concentrations in plasma. Linear regression with a weighting factor of 1/x2 was used for the calibration curve. Efavirenz concentrations in rat plasma were quantified using the corresponding matrix-spiked calibration curves. The oral pharmacokinetic parameters of Efavirenz were calculated by noncompartmental analysis using Phoenix WinNonlin software (version 8.0, Pharsight Corporation, St Louis, MO, USA). Efavirenz plasma area under the plasma concentration–time curve (AUC) was calculated by linear trapezoidal method from time zero to the last detectable time point, post dose. Mean plasma concentration–time profile of Efavirenz after single oral administration at 10 mg/kg dose in male Wistar rats. Rat were fasted overnight (12 h) and fed 4 h post dose with free access to water.

Plasma matrices were obtained from different sources and assayed to evaluate the selectivity of the method and the detection of interference at the analyte and IS retention times. Efavirenz and IS were well separated from the rat plasma matrix components under the described chromatographic conditions at retention times of ~1.21 and ~1.11 min, respectively. No endogenous peak from rat plasma was found to interfere with the elution of either the drug or the IS. The LLOQ (5.08 ng/mL) could be measured with acceptable accuracy and precision for the analyte. These data indicate that the developed method is highly specific and selective for the analysis of Efavirenz in rat plasma samples.

2.5.4 Results

For specificity drug free plasma were analysed. No interference of the endogenous materials was observed. 89% recovery efficiency was observed for Efavirenz. Obtained calibration curve (n=4) were suitable with regression within limits. Accuracy and precision were found satisfactory, details in Table 2.31 and Table 2.32. Samples were stable for 48 hours at -70ºC.

Table 2.31 Back calculated concentrations and accuracy for Efavirenz in calibration standards and quality control samples

Sample name	Nominal concentration (ng/mL)	Back Calculated concentration (ng/mL)	% of Accuracy
CS1	5.08	5.34	105.11
CS2	10.16	9.48	93.28
CS3	25.4	23.85	93.90
CS4	50.8	48.79	96.04
CS5	101.59	100.36	98.79
CS6	253.98	239.56	94.32
CS7	507.96	509.29	100.26
CS8	1015.92	1056.32	103.98
CS9	2031.84	2135.15	105.08
CS10	4063.68	4066.26	100.06
CS11	5079.6	5546.34	109.19
LQC1	15.41	16.76	108.74
LQC2	15.41	18.12	117.62
MQC1	2589.6	2794.3	107.90
MQC2	2589.6	3073.11	118.67
HQC1	3984	4242.54	106.49
HQC2	3984	4692.33	117.78

Table 2.32 Mean oral pharmacokinetic parameters of Efavirenz following single oral administration at 10 mg/kg dose in male Wistar rats

Parameters	Units	F-1		F-2		F-3	
		Mean	SD	Mean	SD	Mean	SD
$t_{1/2,\beta}$	h	1.85	0.14	2.34	0.14	1.84	0.12
C_{max}	ng/ml	1327	160.07	1499	29.95	2037	271.20
T_{max}	h	0.50	0.00	0.50	0.00	0.33	0.14
AUC $_{(0\text{-last})}$	(h*ng/mL	4113	108.54	4483	224.55	6014	1175.75
AUC $_{(0\text{-inf})}$	(h*ng/mL	4368	120.26	4858	221.32	6299	1169.95
MRT	h	2.37	0.13	2.24	0.09	2.15	0.09

SD, Standard deviation; $AUC_{(0–t)}$, area under the plasma concentrations-time curve; $AUC_{(0\text{-inf})}$, area under the curve with extrapolation to infinity; $t_{1/2}$, elimination half-life; C_{max}, peak plasma concentration; T_{max}, time to reach peak plasma concentrations; MRT, mean residence time.

2.5.5 Conclusion

Plasma matrices were obtained from different sources and assayed to evaluate the selectivity of the method and the detection of interference at the analyte and IS retention times. Efavirenz and IS were well separated from the rat plasma matrix components under the described chromatographic conditions at retention times of ~1.21 and ~1.11 min, respectively. No endogenous peak from rat plasma was found to interfere with the elution of either the drug or the IS. The LLOQ (5.08 ng/mL) could be measured with acceptable accuracy and precision for the analyte. These data indicate that the developed method is highly specific and selective for the analysis of Efavirenz in rat plasma samples. Application of this method is illustrated in chapter 5.

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Chapter 3

Alternative solvents for sustainable manufacturing process

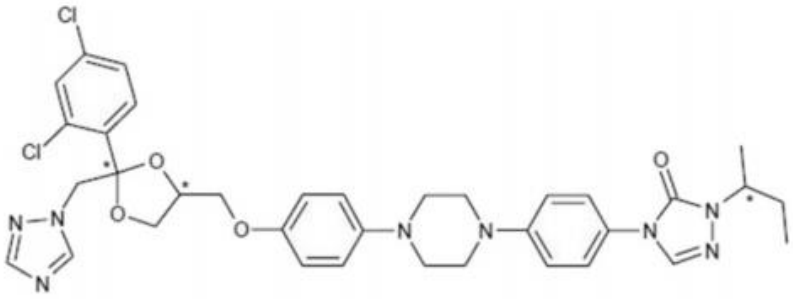
3.0 Introduction

Selection of drug delivery approach is a crucial decision for the clinical and commercial success of a drug, particularly for poorly water soluble drugs that require solubility and bioavailability enhancement. Recent reviews provide detailed description of such drug delivery/formulation approaches [1-5]. Selection of a solubility enhancement technique should be based upon the application for which enhanced solubility is required. The term 'solubility' is relevant at various stages of drug development with different objectives for a chemist, a preformulation scientist, a process engineer, a formulation scientist etc. For instance, a drug can be dissolved in organic solvent like dichloromethane for a process solubility enhancement (eventually evaporating this solvent to obtain a desired solid drug substance /drug product), however that solubility data is not useful for a formulation scientist trying to formulate an intravenous injection of the drug. As a result of these different requirements for information, a lot of solubility data is generated during drug development and drug product formulation. A holistic view of these data could provide new insights into addressing product and process solubility challenges.

This research explores one such approach, utilizing observations from drug salt screening to formulate amorphous solid dispersions (ASDs) of a poorly water soluble drug that is weakly ionic and not very amenable to formation of stable drug salts. Typical challenges associated with isolation of stable drug salts [6-9] and use of hazardous solvents [10] for industrial processes like spray drying that are popularly used for preparation of amorphous solid dispersions, are circumvented in this approach. *In vivo* study substantiates the feasibility of this approach.

Itraconazole (Figure 3.1) was taken as a model drug for the following reasons:

1. It is a poorly water soluble drug (about 1 ng/mL at neutral pH) and is classified as BCS class II [11, 12].
2. It is a very weak base (pKa 2 and 3.7) which is not ery amenable to salt formation and literature reports challenges in establishing a robust process for obtaining crystalline drug salt of Itraconazole [13].
3. Crystalline and amorphous forms of drug salt have been reported in salt screening with different solvents [14].
4. Present market formulation of Itraconazole (Sporanox® capsules, Janssen) is an amorphous solid dispersion that is prepared in a solvent mixture of dichloromethane and ethanol [15]. Dichloromethane is considered as a hazardous solvent and substitution with greener solvents is recommended [16-18].



Lipinski Properties Value

Freely Rotatable Bonds	:	11
H Acceptors	:	12
H Donors	:	0
H Donor/Acceptor Sum	:	12
LogP	:	4.995±0.826
Molecular Weight	:	705.63

Figure 3.1 Structure and properties of model drug Itraconazole

In this study salification was used for dissolving the drug in 'preferred solvents' [18] and mixed with suitable polymers to produce amorphous solid dispersions. Hypothesis for this research is derived from interesting prior research data on how salification works in different solvents [19-21] and how process solvent selection can be guided considering safety, environmental impact, occupational health hazard etc. [22].

3.1 Materials and methods

3.1.1 Materials

Itraconazole (ITZ) was supplied by Nosch labs (Hyderabad, India). Hypromellose (HPMC, Methocel™ E5) was procured from Dow Wolff Cellulosics Gmbh (Bomlitz, Germany) Hypromellose Acetate Succinate (HPMC-AS, AquaSolve™) was procured from Ashland Speciality Ingredients (Wilmington, USA) and Polyvinylpyrrolidone (PVP, Kollidon® 30) was procured from BASF (Ludwigshafen, Germany). All solvents and reagents were of analytical or high-performance liquid chromatographic grade procured from Rankem (Mumbai, India).

3.1.2 Methods

Salification requires two to three unit difference in the pKa of the acid and base [23]. Itraconazole ('Itraconazole base') has pKa 2 and 3.7 (13) requiring strong acids (pKa < 1.7) for salification. Preliminary study was performed with inorganic acid (hydrochloric acid, sulphuric acid and nitric acid) addition to drug slurry. Briefly the procedure involved screening 50 µL, 100 µL, 500 µL and 1000 µL of 0.1 N acids. Once acid selection was narrowed to 0.1 N HCl, 50 µL-1000 µL was tested in increments of 50 µL. 600µL was required for dissolving 100 mg of Itraconazole. Hydrochloric acid was selected from preliminary screening and a three stage experimental plan (Figure 3.2) was designed to assess feasibility of concept. It included:

a) Solvent screening

b) Polymer screening

c) Prototype screening

Glass forming ability of Itraconazole was confirmed with differential scaning calorimetry DSC Q1000 (TA instruments, New Castle, DE). Data was analysed with Universal Analysis 2000 thermal analysis software (TA instruments, New Castle, DE). Crystalline Itraconazole was heated to 10°C above its melting point of 166°C and rapidly cooled at 20°C/min. This sample was reheated at 2°C/min. till 170°C. Glass transition event confirmed the glass forming ability of Itraconazole. Separately, characteristic XRD peaks of crystalline Itraconazole were identified. For XRD study of amorphous Itraconazole, a melt-quenched sample was prepared by melting Itraconazole and quenching in liquid nitrogen.

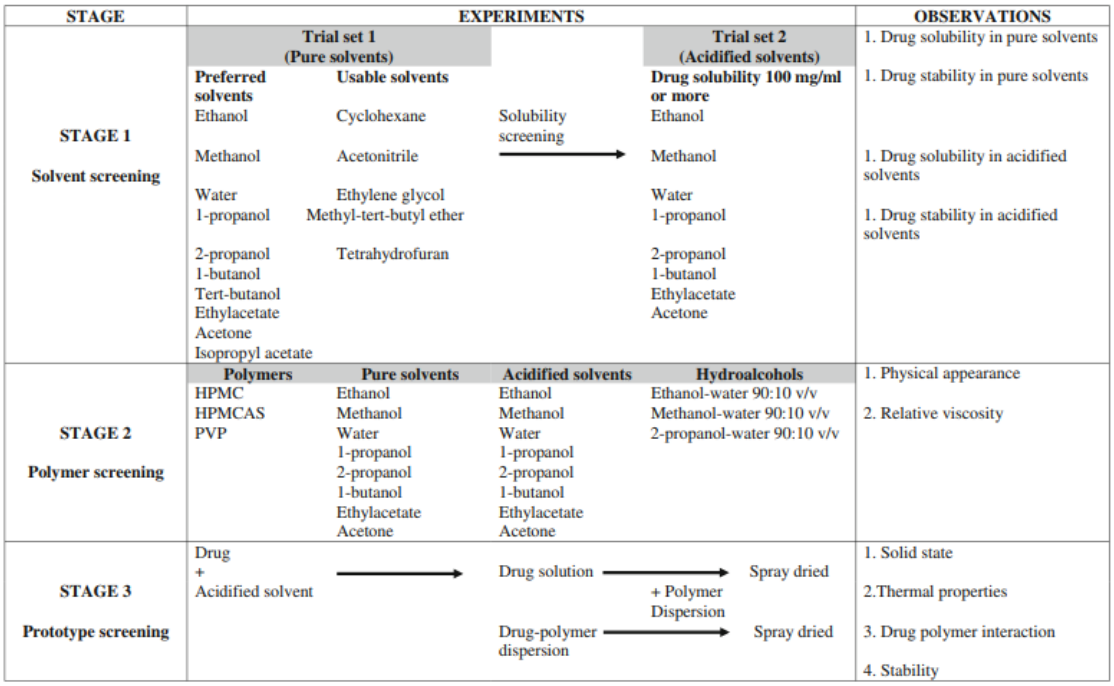


Figure 3.2 Scheme of experiments for feasibility testing of concept

3.1.3 Solvent screening

Solvent screening had two sets of trials. Trial set 1 had excess drug added to 1 mL of each solvent, enlisted in Figure 3.2 as preferred solvents and usable solvents, incubated for 24 hours and solubility estimated by HPLC. In trial set 2, 100 mg of drug (one dose equivalent) was suspended in 1 mL of each solvent (shortlisted from set 1) and sonicated for dispersing the drug. Incremental amounts of concentrated hydrochloric acid (50 µL - 1000 µL) was added to this suspension and intermittently sonicated to obtain a clear solution. Assuming a practical process time for ASD preparation by solvent evaporation/ antisolvent preparation to be not more than 24 hours, a 24 hour chemical stability study was performed for the above described drug solutions using HPLC where the mobile phase consisted of dibasic potassium phosphate and mixture of Acetonitrile and Tetrahydrofuran. 100 x 4.0 mm Hypersil ODS, 3 µm column was used at ambient conditions with a flow rate of 1.0 mL/min and UV detection at 225 nm. For ensuring integrity of parent molecule in salified drug stock solution, mass spectrophotometry was used. Mass number was measured in Waters mass spectrometer (Milford, USA) with ESI source, at intervals of 6 hours upto 24 hours. To understand proton affinity of the drug's functional moieties 1H NMR spectra were recorded at 400 MHz on a Varian Mercury Plus (California, USA).

Both trial sets were studied for crystallization initiation by observing a drop of the solution (during solvent evaporation) under a polarized light microscope using Nikon Eclipse LV100POL Polarizing Microscope (Nikon, Japan) with objective of 10× and ocular magnification of 10×. Birefringence was considered as sign of crystallinity.

3.1.4 Polymer screening

Popular approach for polymer screening is use of theoretical computations like Hildebrand and Hansen solubility parameter and Flory-Huggins model [24], however, these methods are for binary systems (drug and polymer) and can have large deviations for complex systems

involving solvents. Therefore, polymer screening was performed with small scale experiments as per a three stage screening protocol as stated below:

1. Identifying a common solvent for the drug and polymer (based on observations of solvent screening for drug solution, acidified solvents were included in study)

2. Thermal analysis of solvent cast films, with different drug loadings, to ascertain drug-polymer miscibility.

3. Two point dissolution assay of drug-polymer dispersions to grade polymer performance in solubility enhancement and supersaturation.

For preparation of polymer dispersions in solvents, polymer powder was added to the solvent vortex, under continuous mechanical stirring. These dispersions were qualitatively characterised for appearance, homogeneity and relative viscosity. Pure solvents, acidified solvents and mixtures of alcohol and water were used in the study (Figure 3.2).

3.1.5 Prototype screening

With the selected solvent and polymer, drug solid dispersion was prepared using a spray dryer Labultima LU-222 (Mumbai, India). It was used in an open cycle configuration with inlet temperature 65°C, outlet temperature 45°C and atomising gas pressure 1.2 bar. Dried powders were characterized for physical, chemical and thermal properties. Samples were subjected to stress testing, in open pans, at 40°C/75% RH for four weeks.

3.1.6 Characterization of solid dispersions

3.1.6.1 Solid state of drug in solid dispersion

Solid dispersions were analysed with DSC Q1000 (TA instruments, DE, USA). Data was analysed with Universal Analysis 2000 thermal analysis software (TA instruments, Delware, USA). For these measurements, 3-5 mg powder sample was placed in standard aluminum pans,

equilibrated at 0°C and held isothermal for 5 min. Pierced lids were used to remove the volatile solvents in the sample. This was followed by heating at a ramp rate of 3°C/min from 0 to 180°C with a modulation temperature amplitude of 1°C and a modulation period of 40 s. All measurements were performed under nitrogen purge at a flow rate of 50 mL/min. Observations of DSC were further confirmed with X-ray diffraction study. At ambient temperature and humidity conditions, powder X-ray diffraction (PXRD) was performed with Bruker D8 Advance diffractometer (Bruker Corporation, Karlsruhe, Germany). A Cu K-a 1tube (wavelength 1.54056 Å) was the source, set at 40 KV and 40 mA. The divergence and scattering slits were set at 0.5° and the receiving slit was set at 0.15 mm. A θ–2θ continuous scan at 3°/min from 2.5 to 50° 2θ was used.

3.1.6.2 Chemical state of drug in solid dispersion

Raman spectroscopy was used as a chemical fingerprinting tool. Prototype solid dispersion was studied with InVia Reflex confocal microscope (Renishaw, UK). Data acquisition was done using an exposure time of 10 s for 10 accumulations. Five point mapping was performed. Raman spectra were collected using an extended 1200 groove per mm dispersive grating in a spectral window from 100 to 3500 cm−1. Comparative study was performed with crystalline drug and drug salt (without polymer). Data was further verified with FTIR studies. FTIR (Agilent Technologies Cary 660, USA) was used in conjunction with attenuated total reflection (ATR) accessories. This provides benefit of testing the samples without preparation with Nujol or KBr. Sample was placed on the internal reflection element (IRE) of the ATR sampling accessory (Pike Technologies, MIRacle ATR, USA) and infrared spectrum was captured with sample scans 16, background scans 64, resolution and aperture (cm-1) 4.

3.1.6.3 Stability of solid dispersion

Stability of prototype sample was assessed with a short term (4 weeks) stress testing, exposing the samples in open petri plates at 40°C/75% RH. Separately, moisture sorption study was performed using Dynamic Vapour Sorption (DVS) apparatus, DVS Advantage 1, (Surface Measurement Systems, UK). About 20 mg of sample was placed on the DVS pan at 25 °C, dried at 0% RH and then exposed to 90% RH with dm/dt stage type. Percentage of water sorbed or desorbed was recorded. Sample from DVS study was analysed with DSC and XRD to evaluate phase transformations.

3.1.7 *In vivo* study

Male Wistar rats were used for the bioavailability study. Study was approved by the Institutional Animal Ethics Committee of Aurigene (Hyderabad, India) and was in accordance with guidelines of the Committee for Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. Nine rats were divided into three groups with three rats in each group and kept under controlled laboratory conditions. There test preparations (Itraconazole, Itraconazole salt and solid dispersion) were prepared extemporaneously as suspensions and administered orally using a feeding tube 10mg/kg dose. Blood samples (approximately 300 μL) were collected, predose, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 hr, into a micro centrifugation tube containing Potassium EDTA as an anticoagulant. From the centrifuged samples, the supernatant plasma was separated and kept at −20°C until further analysis. Itraconazole detection was performed with LC-MS. The mobile phase consisted of mixture of formic acid in water and acetonitrile (25:75 v/v) and the flow rate was 1 mL/min. Acetonitrile was used as a precipitating agent for the plasma samples. The organic layer was analysed using HPLC. Plasma concentrations versus time profile and the pharmacokinetic parameters were estimated.

3.2 Results and discussions

Itraconazole had melting point of about 166°C. Glass transition temperature was observed at about 57°C (Figure 3.3a). Characteristic XRD peaks of crystalline Itraconazole were identified (Figure 3.3b). Melt quenched Itraconazole showed rapid re-crystallization therefore, ambient temperature XRD was not a useful supplementary technique to DSC for amorphous Itraconazole.

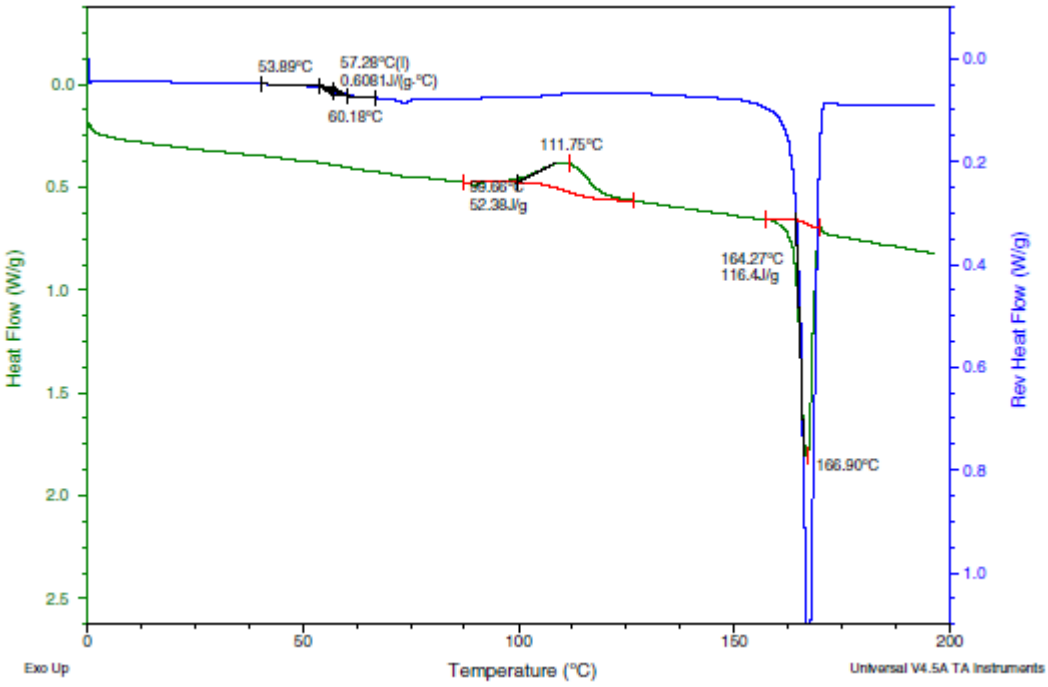


Figure 3.3a DSC thermogram of crystalline Itraconazole showing melting point and glass transition temperature of amorphous Itraconazole.

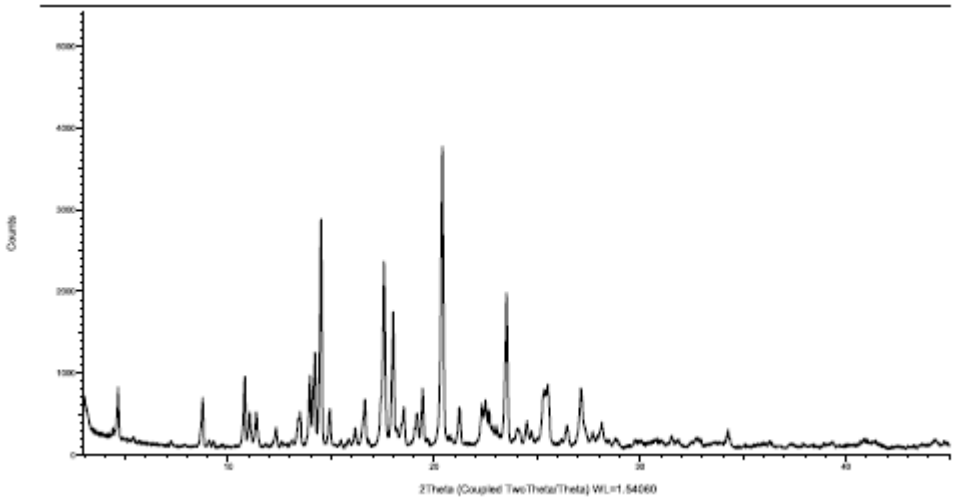


Figure 3.3b XRD of crystalline Itraconazole

Solvent screening protocol was designed with a defined selection criteria and a sequential screening process categorizing the solvents as per previously reported classification of preferred solvents and usable solvents (18). Selection criteria for this study were as below:

1) Minimum solvent requirement for dissolving one dose equivalent drug

2) On drying, these drug solutions should give either a stable amorphous drug or a slow crystalizing drug

3) Manufacturing process should be efficient and industrially feasible

Drug solubility was evaluated in preferred solvents and usable solvents (as per classification in reference 18). Pure solvents (without acid) and acidified solvents were used with both classes of solvents. Details provided in Figure 3.2.

Itraconazole was found to have solubility in various preferred solvents and acidification of these solvents increased the solubility further. When droplet of drug solution was observed for drying (under polarized light microscope), induction of crystallization (Figure 3.4) occurred at different rates. Solvents with high drug solubility had higher rates of crystallization and the same trend was seen in both pure solvents and acidified solvents. Methanol, Ethanol and

Isopropyl alcohol had relatively less drug solubility than Ethylacetate, however, when acidified they were able to readily dissolve one dose equivalent of drug in one mL. Crystal induction times graded the solvents in following order of Ethylacetate < ethanol < methanol < isopropyl alcohol, with rapid crystallization (least crystal induction time) in Ethylacetate and ethanol. Based on low chemical reactivity of methanol and acids [25] and its low boiling point [26], it was selected as solvent for preparing drug solution by salification. Stability of parent moiety was confirmed with HPLC analysis (Figure 3.5a) and monitoring mass number (Figure 3.5b). Protonation of the drug was studied with ^{1}H NMR (Figure 3.5c) and was found similar to previously reported literature [27] confirming salification.

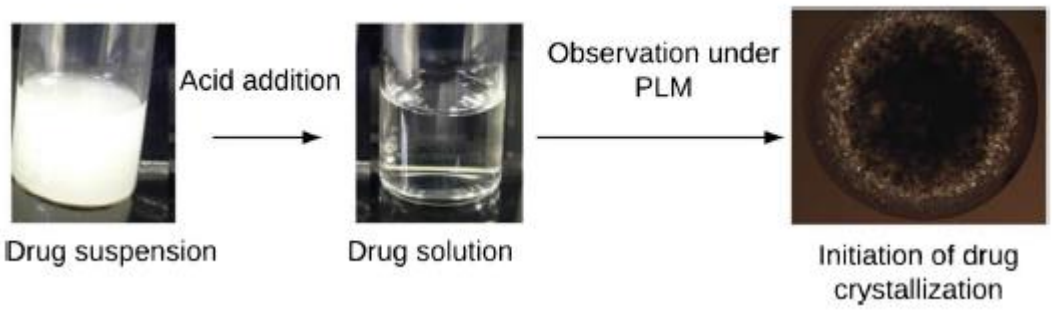


Figure 3.4 Crystallization initiation in drug solution droplet as observed under polarized microscope

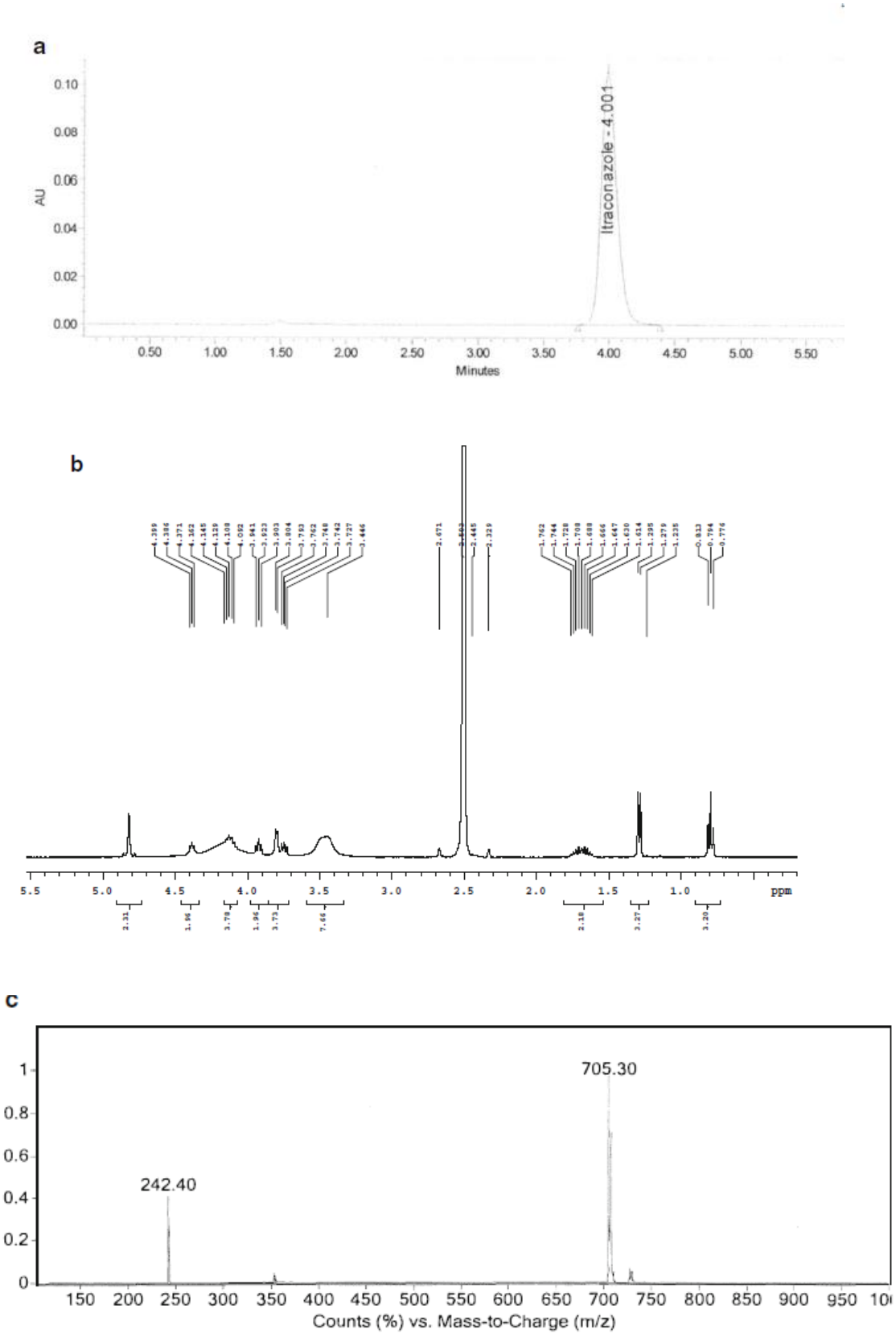


Figure 3.5 Drug solution stability estimated at 12 h by a HPLC, b mass spectroscopy and salification confirmed by c 1H NMR

To ensure molecular mixing of drug and polymer it is highly recommended to use a common solvent for them [28]. With methanol as solvent for drug solution, polymers were dissolved in methanol and methanol-water mixtures 90:10 w/w. Clear polymer dispersions were obtained with all polymers except HPMC in methanol, which was slightly hazy. Drug loading from 10-80% w/w were prepared by mixing drug stock solution and polymer dispersions. Cast films of these compositions were analysed with DSC (Figure 3.6) and dissolution studies (Figure 3.7). 60% w/w drug loaded HPMC provided highest drug concentrations in drug release studies in simulated intestinal fluids. At drug loading of 10% w/w PVP provided high drug concentrations but with increasing drug loads a decline in drug release was observed. Two solvent systems, methanol and methanol-water 90:10 v/v, were used for HPMC. Objective was to study the impact of small amount of water on the performance of solid dispersions Solid dispersions of HPMC with either methanol as the solvent or methanol water 90:10 v/v as solvent did not show difference in drug release. Based on these observations (Figure 3.7) it was evident that type of polymer and drug loading had impact on drug release. Solid dispersion with HPMC in methanol-water 90:10 v/v and 60% w/w drug loading was considered for prototype screening.

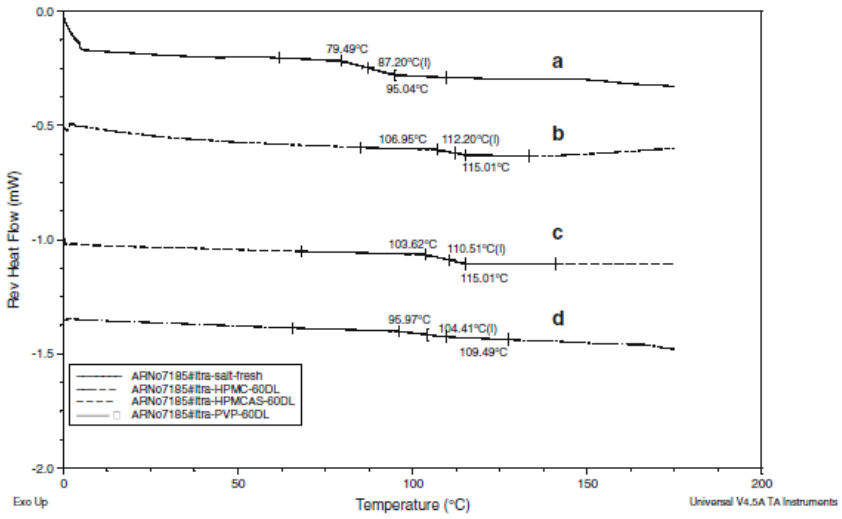


Figure 3.6 DSC thermograms of Itraconazole salt (a), HPMC solid dispersion (b), HPMCAS solid dispersion (c) and PVP solid dispersion (d). Drug loading at 60% w/w

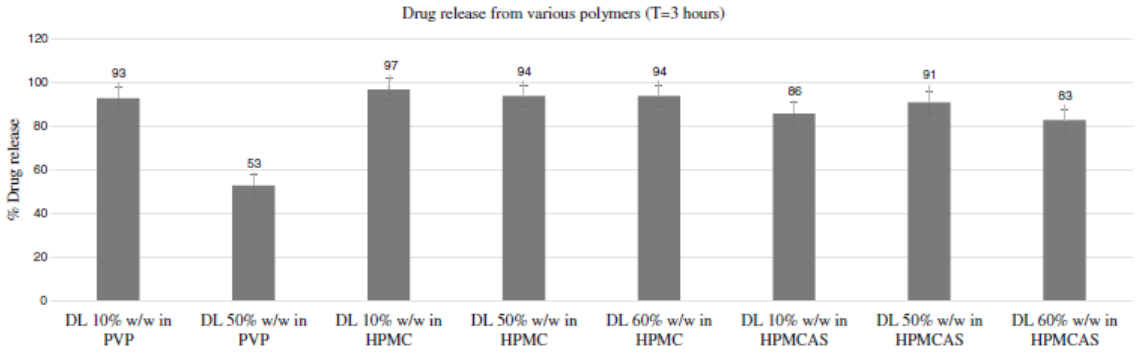


Figure 3.7 Dissolution drug release study of Itraconazole salt with different polymers (n = 3); error bars represent standard deviation

Spray dried solid dispersion of Itraconazole in HPMC showed a distinct Tg during thermal analysis indicating amorphous state of drug (Figure 3.8a). This was confirmed with XRD where characteristic peaks of crystalline drug were not found in the solid dispersion (Figure 3.9a). When the DVS treated solid dispersion sample was analysed with DSC (Figure 3.8b) and XRD (Figure 3.9b) no phase transformation (amorphous to crystalline) was observed, when compared to initial samples, indicating stability of the amorphous solid dispersions.

Raman spectra for Itraconazole and Itraconazole salt were compared with the spectrum of solid dispersion. Distinct peaks at 430, 1697 and 3123 cm^{-1}, corresponding to Itraconazole, were not observed in the solid dispersions. A comparison of Itraconazole with the Itraconazole salt indicated the absence of the Raman peak at 900 cm^{-1} (possibly shifted to 875 cm^{-1}). Presence of this peak was used as an indicator of the presence of Itraconazole salt form in the solid dispersions (Figure 3.10). These observations were further verified by the FTIR data (Figure 3.11) Characteristic peaks of Itraconazole were identified in regions of 1550-1660cm^{-1} (carboxylate group O-C-O), 1623 and 1560 cm^{-1} (N-H secondary stretching) and 1073cm^{-1} (C-N stretch). Presence of chlorine group in itraconazole salt was identified at 700-850cm^{-1}.

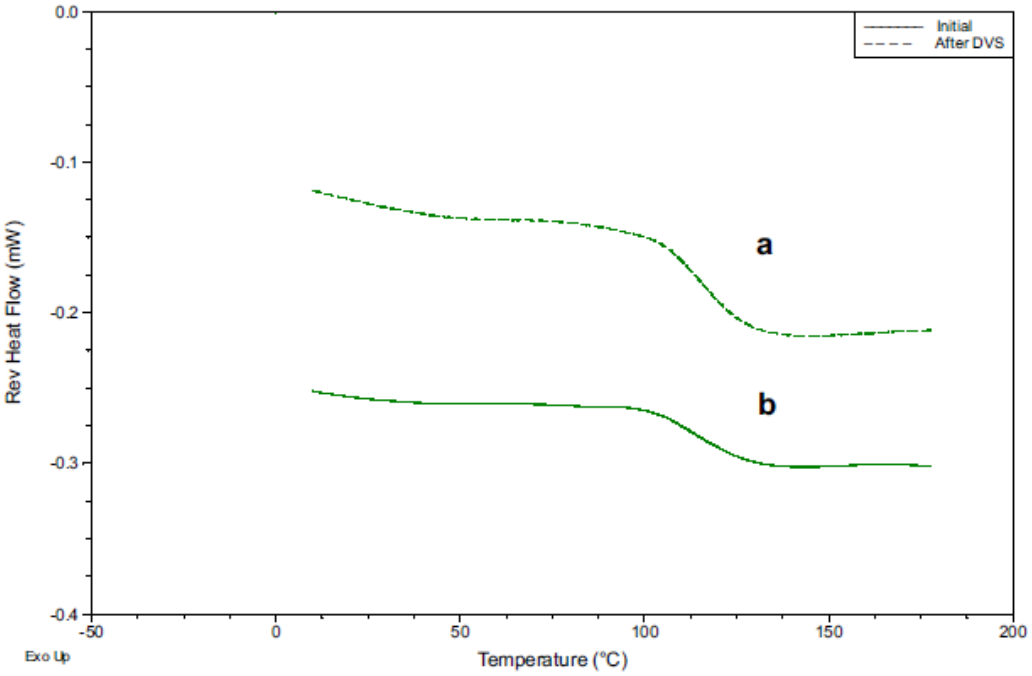


Figure 3.8 DSC thermogram of Itraconazole salt with HPMC solid dispersion before DVS study (a) and after DVS study (b)

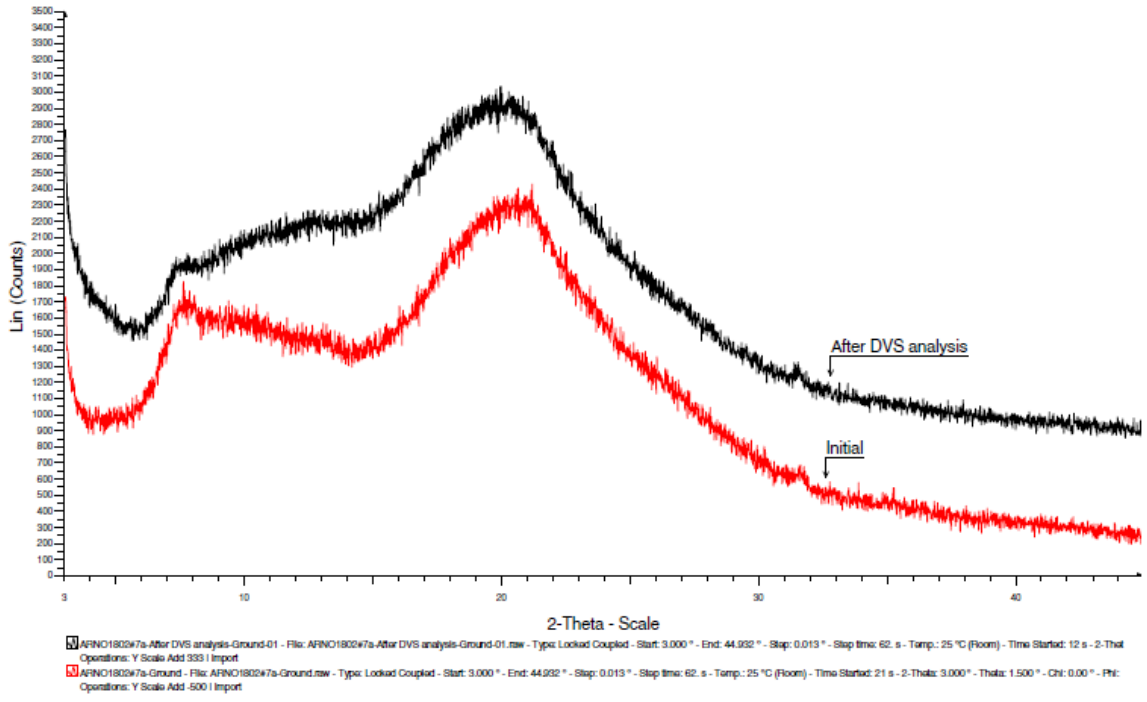


Figure 3.9 XRD of Itraconazole salt with HPMC solid dispersion (a) before DVS study and (b) after DVS study

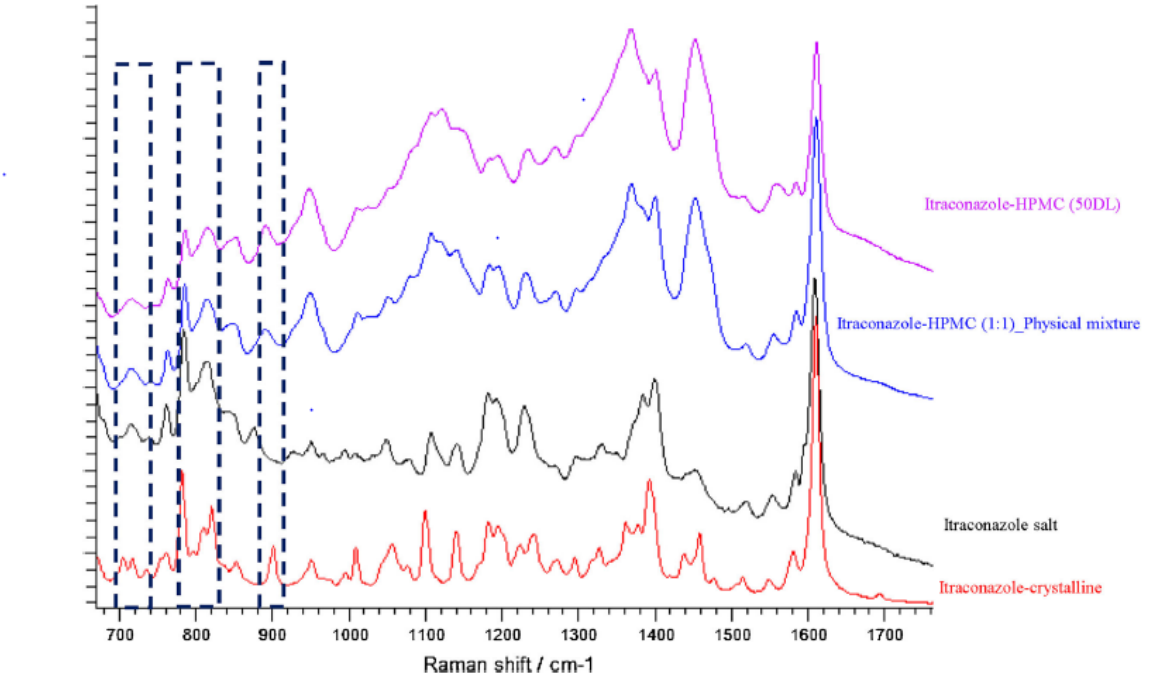


Figure 3.10 Raman spectrum of Itraconazole salt solid dispersion compared to crystalline Itraconazole and Itraconazole salt

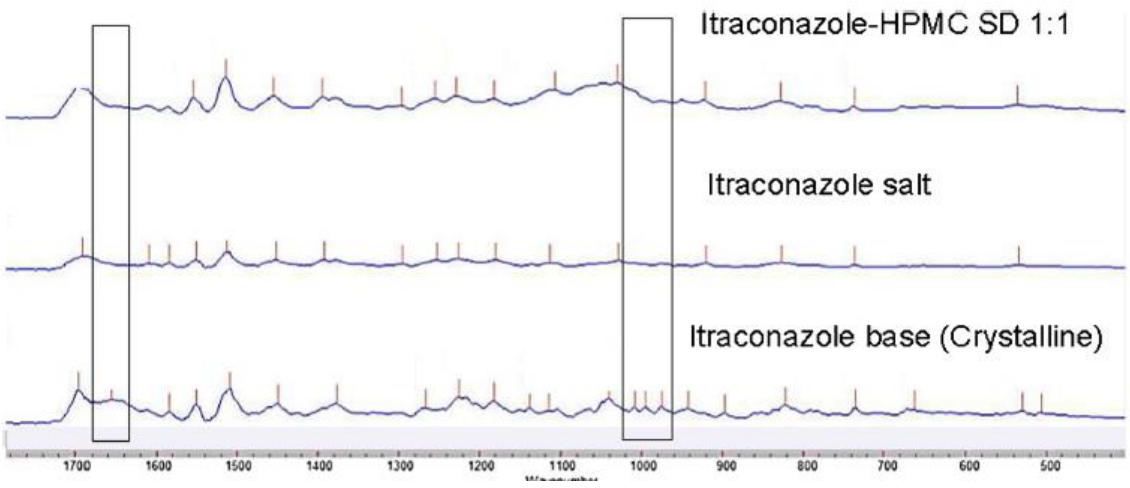


Figure 3.11 FTIR spectrum of Itraconazole salt solid dispersion compared to crystalline Itraconazole and Itraconazole salt

DSC and XRD study of stability samples indicated no phase transformation (amorphous to crystalline), compared to initial samples, indicating stability of the amorphous solid dispersions. In the DVS study, moisture sorption was found to be not more than 5.0% w/w.

When compared to naïve polymer it was <0.15% w/w. Karl Fischer titration was used to determine moisture content of samples. Initial moisture content for HPMC sample was 4.82% w/w and for solid dispersion it was 5.10%w/w. DVS samples were sufficient only for DSC and XRD study. DVS data served as indirect indicator of sample moisture content. For both samples no hysteresis was found between the isotherms of sorption and desorption, indicating that the samples were not significantly hygroscopic. Isotherm plots are presented in Figure 3.12 (a. HPMC polymer, b. solid dispersion).

In the DSC study of solid dispersion (sample before and after DVS study), shift in glass transition temperature was less than 3°C and in both samples Tg was well above the ambient storage conditions. It complied with rule of Tg-50°C for physical stability of amorphous solid dispersions. This assures that the solid dispersion has high probability of being stable.

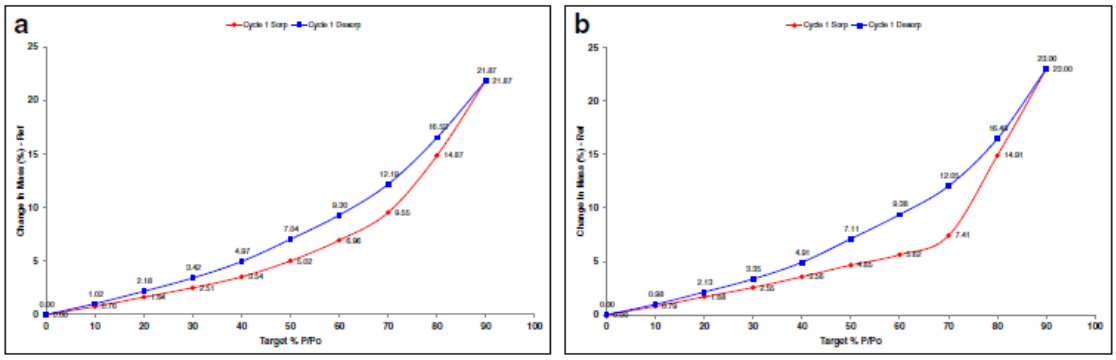


Figure 3.12 Isotherm plots of a HPMC polymer and b HPMC solid dispersion

Significant enhancement of the Cmax and AUC for Itraconazole salt and HPMC solid dispersion were observed compared to Itraconazole. HPMC solid dispersion formulation showed 6.7 folds and 45 folds higher exposure (AUC) compare to Itraconazole salt and Itraconazole, respectively. Pharmacokinetic parameters of tested samples are presented in table 1.

Table 3.1 Summary of Pharmacokinetic Parameters of Itraconazole, Itraconazole Salt and Itraconazole-HPMC Solid Dispersion, After Peroral Administration to Rats (n = 3)

PK parameters	Statistics	Formulations		
		Itraconazole-HPMC solid dispersion	Itraconozole salt	Itraconozole
C_{max} (ng/mL)	Mean	1006	195	40
	SD	263	66.7	15.1
	%CV	26.2	34.2	37.8
t_{max} (h)	Mean	8	5	7
	SD	0.0	2.31	1.15
	%CV	0.0	43.3	15.7
$AUC_{(0-24)}$ (ng/h/mL)	Mean	14,690	2199	326
	SD	3680	498	373
	%CV	25.1	22.6	114

SD standard deviation, C_{max} maximum concentration, T_{max} time to achieve maximum concentration, *AUC* area under the curve

In situ salification approach has its earliest references in the reports of drug solubilisation by pH adjustment, a common method of enhancing drug solubility of poorly water soluble drugs and obtaining solutions of drug for oral and parenteral applications. pH adjustment solubilizes drug by forming *in situ* salt/salification [29]. This work has differently applied pH adjustment principles and the two major differences are briefly described here:

1. Drug solution obtained by pH adjustment is used ‘as is’ and therefore requires careful selection of solvent (s), physical and chemical stability assessment of solution and inclusion of stabilizing additives (if required) to formulate product with sufficient shelf life. In this approach, drug solution is an intermediate stage of product manufacturing. A solid product is obtained by solvent evaporation and solvent is relevant only in trace amounts as residual solvent. Also, solution stability requirement is limited till solution is processed to obtain the solid powder.
2. Drug solutions prepared by pH adjustment approach have the risk of drug precipitation in body fluids. In presented approach, a precipitation inhibiting polymer was added to drug solution for ensuring that the drug is not precipitated and is obtained in a stable amorphous form that could provide maximum solubility of drug.

Salification reactions are governed by the intrinsic solubility and dissociation/ionization constants of reactants and reaction products. Literature reports variations in the solubility and dissociation constants of substances when measured in different solvents. For example Hydrochloric acid has pKa of -8 in water and 1.8 in DMSO (21). This variation could give a no-salt observation during drug salt screening in different solvents or limit the applicability of pH adjustment approach for dissolving a drug in solvent. Different solvent-acidifier combinations provide different solid forms of a drug (14). These observations indicate that desired drug form (amorphous, crystalline, salt, cocrystal etc.) can be engineered with a thorough understanding of the solute-solvent interactions and interplaying thermodynamic and kinetic factors. This also provides an opportunity to explore possibility of selecting green and sustainable solvents that could be suitably combined with acids/bases and drug to give the desired drug product. Hypothesis testing was done with model drug Itraconazole.

An aqueous solution of Itraconazole (10 mg/mL) is obtained by complexation with cyclodextrin and pH adjustment [30]. However, without cyclodextrin it has very low solubility even in highly acidified water [31]. In this study, when similar pH adjustment was attempted in organic solvents, substantial solubility improvement was observed. This formed the basis of presented study where Itraconazole was salified in-situ to obtain a solution and then suitably processed with polymers to obtain amorphous Itraconazole with benefits of maximum solubility (compared to crystalline form) in the form of amorphous solid dispersion. Previously reported salts [12], cocrystals [13] and non-salt acid mixtures [32] of Itraconazole have reported solubility enhancement, however, there are no published reports on their supersaturation and possible precipitation that may result from supersaturation. Theoretically, these products will dissolve to give supersaturated solutions at the drug absorption site and may have the risk of intraluminal precipitation that could adversely affect drug bioavailability. With the presented approach, an amorphous drug is obtained which will dissolve and can

precipitate/crystallize only if effective concentrations of polymer are not present. Polymers retard crystallization of amorphous drugs and sustain supersaturation [33].

Solvent selection, polymer selection, preparation and characterization of solid dispersions have been discussed in earlier sections. For ensuring industrial application of this research, solvent selection was the critical aspect. Other than the green chemistry and sustainability aspects, due consideration was required from perspective of the drying process efficiency (like solvent boiling point, heat of vaporization, solvent viscosity etc.) and achieving acceptable residual solvents in the final product. Methanol efficiently replaced dichloromethane as solvent for preparation of amorphous solid dispersion of Itraconazole.

Counterions of drug salts provide various physicochemical features to a product [34-36]. Unlike the ionic liquids [37] where regulatory understanding is still nascent, drug salts are better understood both *in vitro* and *in vivo*. This approach for salified amorphous solid dispersions requires further studies to understand effect of various product parameters process conditions and environmental factors.

3.3 Conclusion

Given the advantages of physical and chemical stability of crystalline materials, most drug salt screenings would continue to focus on generating crystalline drug salts. However, this study provides evidence that amorphous salts and ionic mixtures that are often not pursued during drug development, anticipating risks of instability and clinical surprises from their metastable state, may find a very different and useful application in drug product development. For weekly ionic drugs, drug salt preparation and isolation per se has limitations. However, when the same principles are applied *in situ* and are followed by subsequent processing stages (in this case formation of solid dispersion) it can provide a novel method for leveraging the solubility features of salts and amorphous state. This approach testifies the feasibility of alternative

processes for replacing hazardous organic solvents. In today's time it has become imperative that pharmaceutical industry inculcates principles of green solvents and sustainable processes in its research and manufacturing.

Encouraging *in vivo* performance of this product has reinforced the technical feasibility and potential industrial application of this approach.

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Chapter 4

Alternative solvent and manufacturing process for sustainable product development

4.0 Introduction

Most product manufacturing processes (irrespective of the industry that they belong to like paints, petroleum, steel etc.) have potential environmental impact, either the energy consumption for process, generated by-products or process waste etc. are of concern. Encouraging green and sustainable processes has become the need of the hour. While lot is being researched, very few are commercially implemented. Even the research is very fragmented among the different industries. One industry that needs to critically assess the pace of inculcating green processing as a product design philosophy is the pharmaceutical industry. Pharmaceutical industry's performance on the green metrics is of concern. Average waste-to-product ratio is 200 which means for every kilogram of active pharmaceutical ingredient (API) pharmaceutical factories generate 200 kilograms of waste [1]. This is in addition to the huge energy consumption in the form of electricity and fuels that is required to keep the manufacturing facilities working almost round-the clock [2].

Given the notion (perceived or real) that pharmaceutical industry is still not in the 'mandatory' zone to create ecolabels or standards for green products [3] and there are no clearly defined guidelines (neither from regulatory authorities nor from individual organizations) stating at what stage green principles should be implemented, we are far from reaching the objectives of 100% sustainable, environment friendly processes and zero landfills. In the last decade, pharmaceutical industry has started acknowledging the importance of green chemistry and sustainability. Sustainability is applicable to the overall life cycle including environment, people, economics etc. [4, 5]. while green chemistry is one of its core elements that is predominantly focused on design, production/manufacturing, energy efficiencies, throughputs etc [6]. Figure 4.1 depicts how elements of green chemistry, guided by the 12 principles, is one major approach for sustainable future.

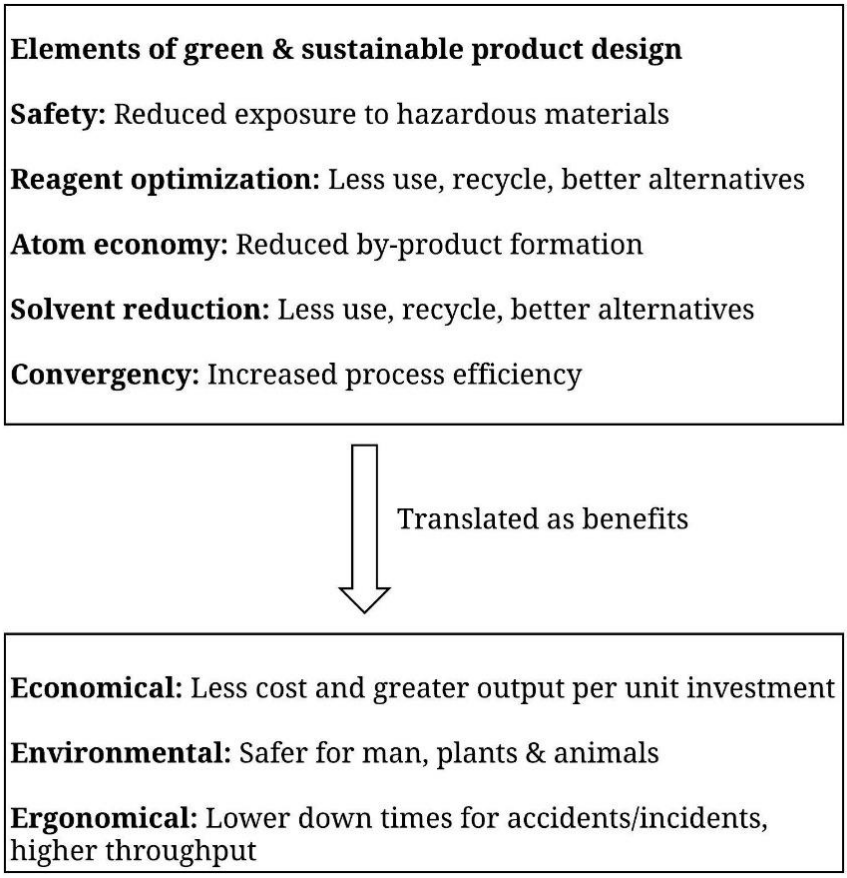


Figure 4.1 Elements of green chemistry impacting sustainability

While the term and principles have been more commonly associated with chemistry, they are equally applicable to other pharmaceutical processes including analytical methods (HPLC, derivatization reactions etc.) and drug product processes (solvent based tablet coatings, granulations, pellet coating, films, amorphous solid dispersions etc.). Green chemistry applications have won accolades for drug substance processes, worth mentioning are the EPA Presidential Green Chemistry awards for some of the major projects like the drug substance of Sitagliptin, Sertraline etc. and products of Taxol, Emend etc. [7]. Few nominations for sustainable processes of excipients like the one for Hydroxypropyl methylcellulose acetate succinate (HPMCAS) [8] are also encouraging. However, research for drug product manufacturing processes has been limited [9-12]. Field of work in these

researches has been particle engineering, predominantly nanoparticles. In terms of the energy consuming technologies or processes and the ones that utilize solvents [13] there are many other drug product manufacturing processes that require feasibility assessment to evaluate whether greener alternatives to the present processes are possible. Particularly while dealing with poorly water soluble drugs, most drug products require enabling technologies like nanoparticles [14], lipid drug delivery [15], amorphous solid dispersion [16] etc. which utilise solvents or thermal energy, in some cases both. One of our earlier research [17] provided evidence that processes can be designed to be benign.

This work attempts to evaluate if alternative solvents, in situ salification, and controlled precipitation could be used to replace hazardous solvents in preparation of amorphous solid dispersions (ASDs). Enabling technology of ASDs is widely utilized for enhancing solubility and bioavailability of poorly soluble drugs [18]. Manufacturing processes of ASDs utilise solvents, thermal energy or mechanical energy, and the former (solvents) dominates in most of the reported literature [19]. With over twenty commercial products [20] and about 50% of those utilizing solvents (Figure 4.2) it would be worthwhile to evaluate sustainable alternatives.

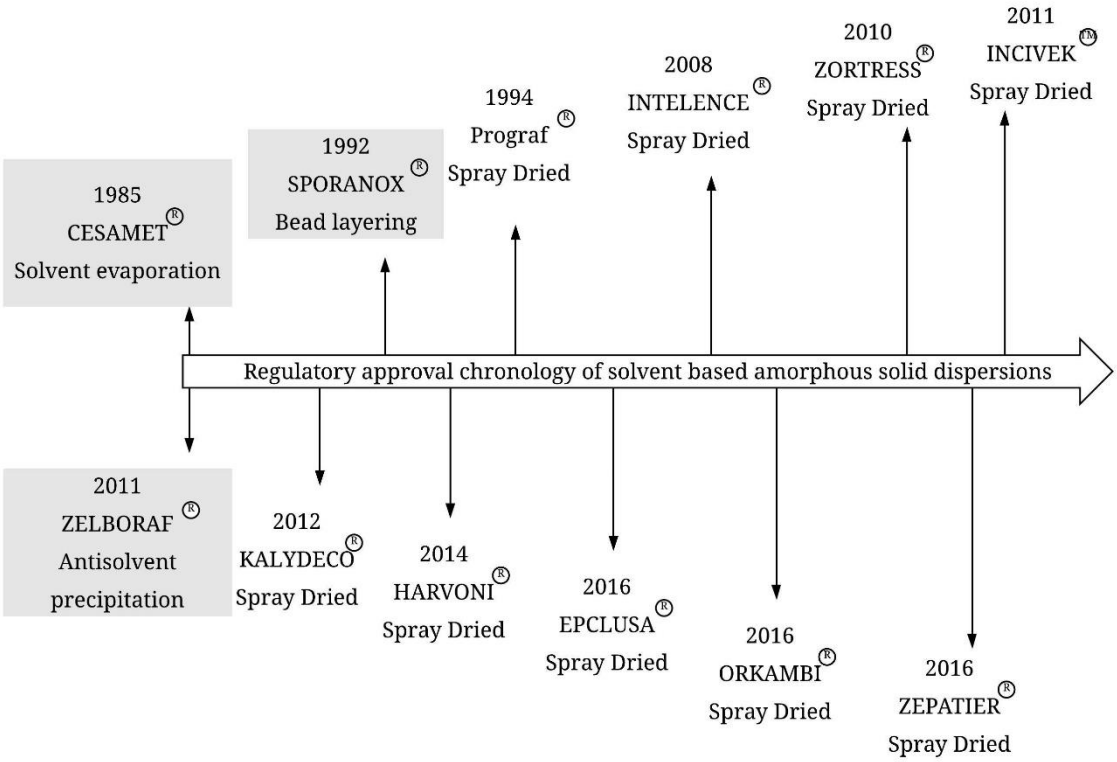


Figure 4.2 Regulatory approval chronology of solvent based amorphous solid dispersions. Spray drying leads over other solvent methods (grey scale items).

Antifungal drugs (Itraconazole and Posaconazole), antiretroviral drugs (Ritonavir, Telaprivir, Ledipasvir-Sofosbuvir etc.) and immunosuppressants (Tacrolimusand Everolimus) constitute the major therapeutic classes that utilise amorphous solid dispersion technology [21]. For the purpose of this research Posaconazole was selected as the model drug. This potent triazole antifungal agent is used in the prevention of invasive fungal infections due to aspergillosis and candida in high risk patients. It has low water solubility (0.027 mg/L) and cLogP (4.6) [22]. Its pharmacokinetic profiles indicate solubility limited drug absorption [23]. It is commercially available as solution for intravenous administration, delayed-release tablet and suspension for oral administration. Literature reports of amorphous Posaconazole utilize undesirable solvents like dichloromethane [24]. This solvent is categorized as problematic solvent in the solvent selection guides [25].

DCM is associated with both acute and chronic toxicity in humans, including respiratory toxicity, central nervous system toxicity, cardiovascular toxicity, carcinogenicity and genotoxicity. Additionally, DCM persists in the environment with a half-life of over 18 months in water [26]. Scheme of work for present research focuses on circumventing the need for such solvents and utilizing biodegradable polar solvents which could be recycled as media for dissolving drugs. Overview of work plan is presented in Figure 4.3. Differential scanning calorimetry (DSC), X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopy and Nuclear Magnetic Resonance (NMR) spectroscopy were utilised to characterise the product. High performance liquid chromatography and potentiometric titrations were utilized for chemical characterization. Considering requirement of a delayed release product, Hydroxypropyl methylcellulose acetate succinate (HPMCAS) was evaluated as the crystallization inhibiting polymer.

4.1. Materials and methods

Posaconazole was supplied by Dr. Reddy's Laboratories Ltd. (Hyderabad, India), Hypromellose Acetate Succinate (HPMC-AS, AquaSolve™) was procured from Ashland Speciality Ingredients (Wilmington, USA). All solvents and reagents were of analytical or high-performance liquid chromatographic grade procured from Merck (Mumbai, India) and Rankem (Mumbai, India) respectively.

4.1.1 Solvent screening

Preferred solvents were enlisted based on their safety and environmental impact [25] A common solvent for drug and polymer was evaluated. General properties of shortlisted solvents [27] are listed in Table 4.1 and Table 4.2.

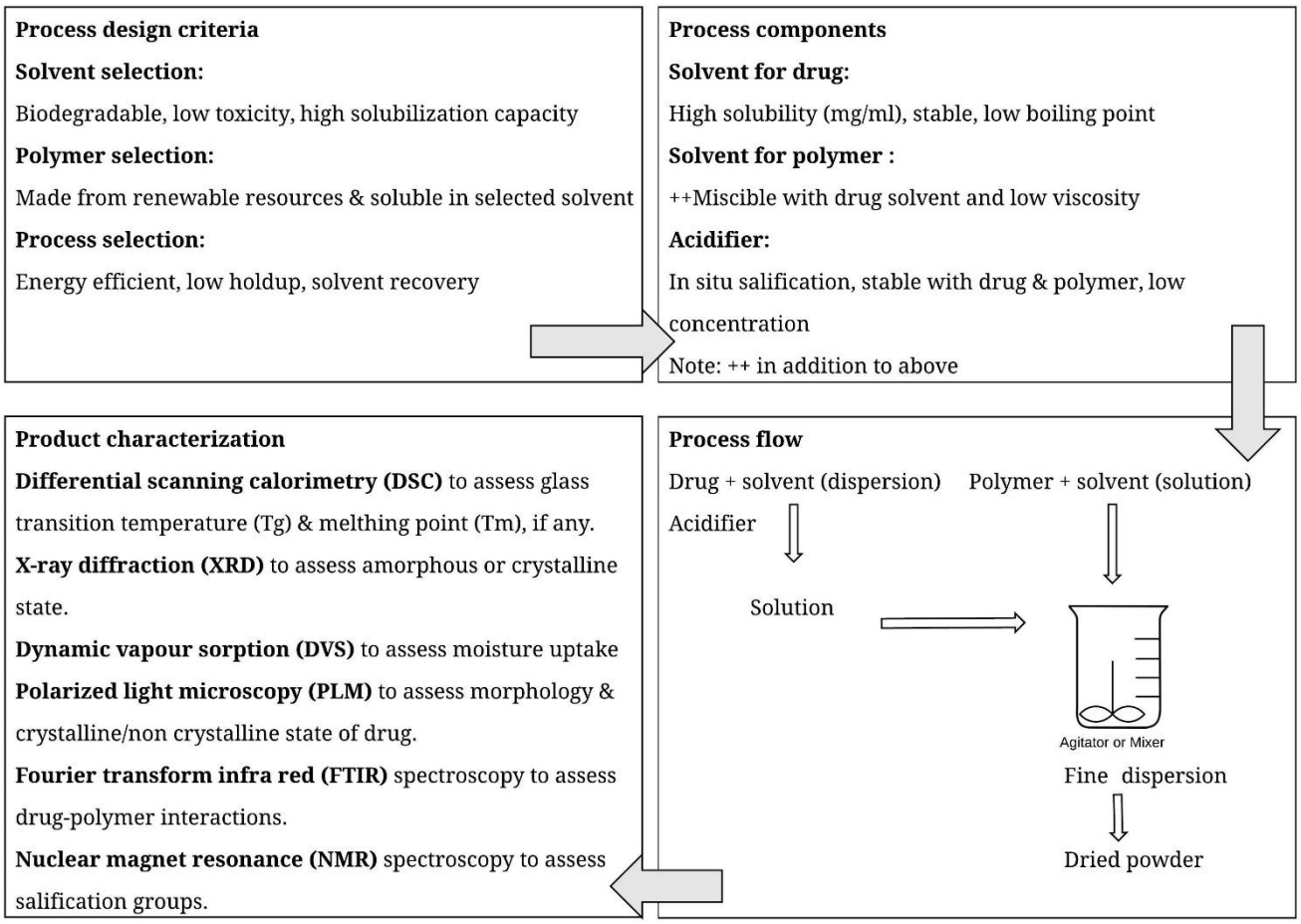


Figure 4.3 Work plan for preparation and evaluation of Posaconazole amorphous solid dispersion

Table 4.1 Solvents and their properties

Solvent	Boiling point	Dielectric constant
Ethanol	78	23.4
Methanol	65	33
Isopropyl alcohol	83	18.3
Ethyl acetate	77	6.02
Water	100	80

Table 4.2 Solvent miscibility chart

Solvent	Ethanol	Methanol	Isopropyl alcohol	Ethyl acetate	Water
Ethanol					
Methanol					
Isopropyl alcohol					
Ethyl acetate					
Water					
	Miscible			Immiscible	

4.1.2 Drug and polymer stability in solvents

Excess of drug and polymer were added to solvent and mixed well. After 6 hours, supernatant of drug samples were analysed with HPLC method and polymer samples were evaluated for visual clarity and solution pH. Separately, 100 mg of drug was added to 2 ml of solvent and dissolved with increments of 50-1000 µL of 1 N Hydrochloric acid (HCl). Clear solution was analysed for chemical stability till 24 hours.

4.1.3 Preparation of solid dispersion

Based on laboratory trials, a pilot scale batch was executed. Under mechanical stirring, 300 g drug was dispersed in 10 L solvent. 90 ml of concentrated HCl was added to it for obtaining a clear solution. This acidified drug solution was labelled as drug phase.

Separately, 2.7 kg HPMCAS was dissolved in 10 L solvent and labelled as polymer phase. With a peristaltic pump, drug phase was slowly added (20 g/min.) to the polymer phase and stirring was continued for 30 min. to obtain a uniform dispersion with total solid content of about 15% w/v. This dispersion was transferred into feed tank of agitated thin film evaporator (ATFD, Techno Force Ltd., India) and then dried in tray driers till constant weight (achieved after 1.5 hours). Dried powder was sieved through mesh 40 and characterized for moisture content, solid state, thermal and chemical properties. Same trials were repeated with different ratios of drug to polymer as 1:1 and 1:3.

4.1.4 Characterization of intermediates and solid dispersion

Chemical properties were evaluated with 1H NMR and HPLC. Drug solution (drug dispersion dissolved with acid) was vacuum dried, dissolved in DMSO and analysed with 1H NMR at 400 MHz on a Varian Mercury Plus (California, USA). This was to understand proton affinity of the drug's functional moieties and possible sites of salification. Chemical stability of drug solution was analysed with HPLC as detailed in chapter 2.

Thermal properties of drug and solid dispersion were analysed with DSC Q1000 (TA instruments, New Castle, DE). 2-3 mg drug was used for studying melting point and glass transition temperatures. Samples were equilibrated and heated to temperature 10°C above the reported melting point of 170°C [27]. This sample was then rapidly cooled. Heat-cool-heat cycling was performed to assess the glass transition temperature. Data was analysed with Universal Analysis 2000 thermal analysis software (TA instruments, New Castle, DE). All measurements were performed under nitrogen purge at a flow rate of 50 mL/min.

Solid state properties confirming amorphous/crystalline state of drug was studied with X-ray diffraction study. Bruker D8 Advance diffractometer (Bruker Corporation, Karlsruhe, Germany) was utilised for the study. A θ–2θ continuous scan at 3°/min from 2.5 to 50° 2θ

was used. Chemical interaction studies between the drug, drug salt and polymer was studied with FTIR (Shimadzu FTIR-8400S spectrometer, Japan)

Stability of prototype samples was assessed for 12 months at standard stability study conditions of 25°C/60% RH, 30°C/65%RH and 40°C/75%RH. Samples were stored in 30 cc HDPE bottles. Samples were studied for birefringence with polarized light microscope (Nikon Eclipse LV100POL, Nikon, Japan).

4.2. Results and discussion

4.2.1 Solvent selection

Ethanol, methanol, isopropyl alcohol etc. were found suitable. Samples were observed for clarity and relative viscosity. For molecular level mixing of drug and polymer it was preferred to utilise a common solvent for drug and polymer. As the delayed release polymer would be insoluble in the acidic drug solution, careful selection of solvents was important to ensure maximum possible solubilisation for both components. Based on the solubility of polymer and drug stability in methanol it was selected as solvent for prototype solid dispersions. The 24 hour stability study data is depicted in Table 4.3.

Table 4.3 Solution stability of acidified Posaconazole solution

Time interval	%assay of test solution	Difference in % assay from initial
Initial	100.4	-
6 hours	100.3	0.1
12 hours	100.2	0.2
24 hours	100.2	0.2

For every 100 mg of drug in 2 ml of methanol, about 18µL 0.1N HCl was required for preparing a clear solution. HPMCAS was freely soluble in methanol.

4.2.2 Preparation and characterization of solid dispersion

Addition sequence of drug solution and polymer solution was found to be important. To ensure smooth dispersion, drug solution was added to HPMCAS solution. This created a fine suspension of drug distributed in polymer matrix. Continuous low speed stirring was required to prevent settling of particles till solvent evaporation.

Protonation of the drug as studied with 1H NMR (Figure 4.4) was found similar to previously reported literature confirming salification. Figure 4.5a depicts the DSC thermogram of Posaconazole. During standard run, two endothermic events where observed at 132.4°C and 176.4°C, later corresponding to the melting point and the former nearer to the reported values for the nematic phase [28] DSC of acidified Posaconazole, Figure 4.5b, showed a clear glass transition temperature (Tg) of over 120°C. This was higher than the Tg reported for glassy Posaconazole [28] at 58.9°C. These values conform the probability for generation of an amorphous form of Posaconazole, however to avert the risk of drug re-crystallization a stabilizing polymer was required. Tg for acidified Posaconazole-HPMC-AS as a polymer matrix was found to have a good Tg of 117°C. Prototypes with various drug loadings were prepared and were found to be amorphous as shown in Figure 4.5c. However, after 24 hours of preparation the 50% drug loaded samples started showing surface crystallization, refer Figure 4.6. Therefore, the 10% and 20% drug loaded samples were only taken forward to stability studies. It was found that both the drug loadings were stable for 12 months. Specific molecular interactions between Posaconazole and HPMCAS contributed to the stable amorphous solid dispersions. When HPMCAS was added to Posaconazole, it was observed that the peak at 1691 cm^{-1} and 1654 cm^{-1} disappeared with the addition of HPMC-AS, indicating interaction with HPMC-AS. Figure 4.7a and 4.7b shows that the later indicates interaction on the imine group of Posaconazole.

No changes were observed for the phenyl peaks at 1620 cm^{-1} indicating that it had no role in drug-polymer interactions.

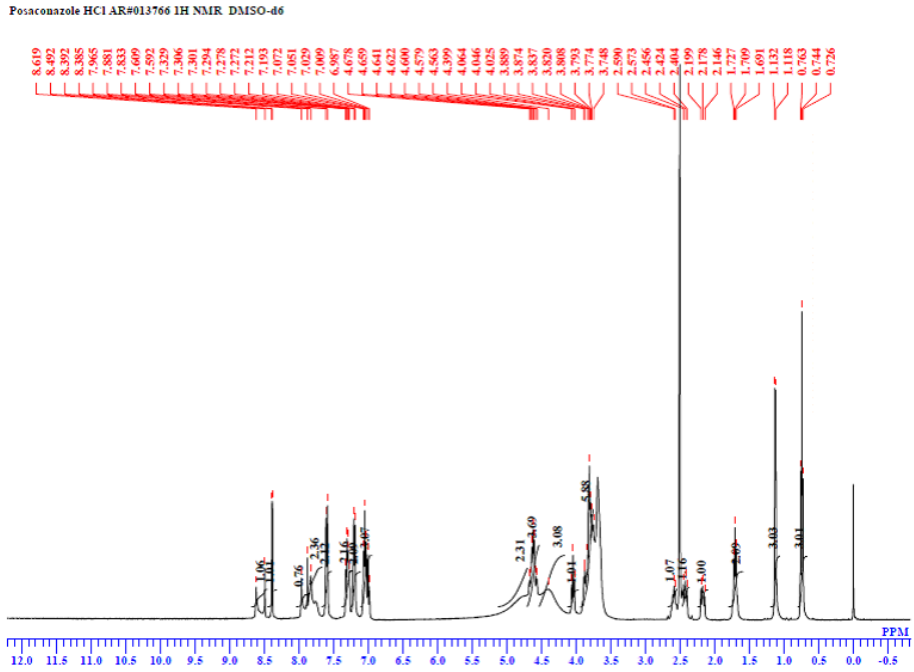


Figure 4.4 NMR study for acidified Posaconazole

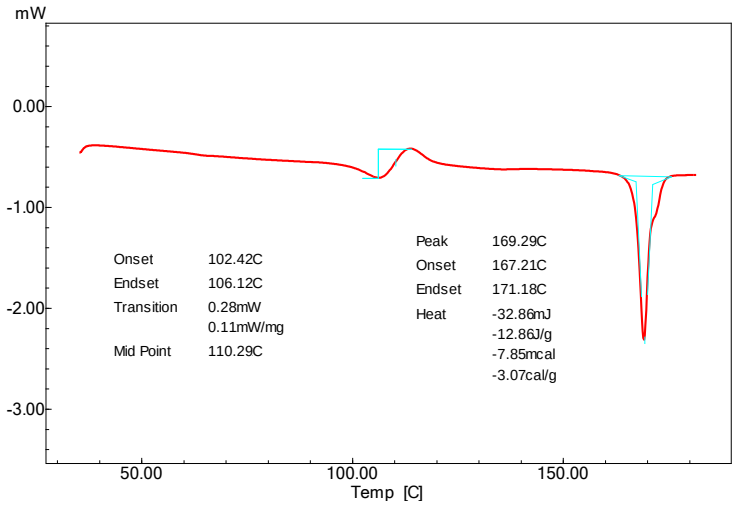


Figure 4.5a DSC thermogram of Posaconazole

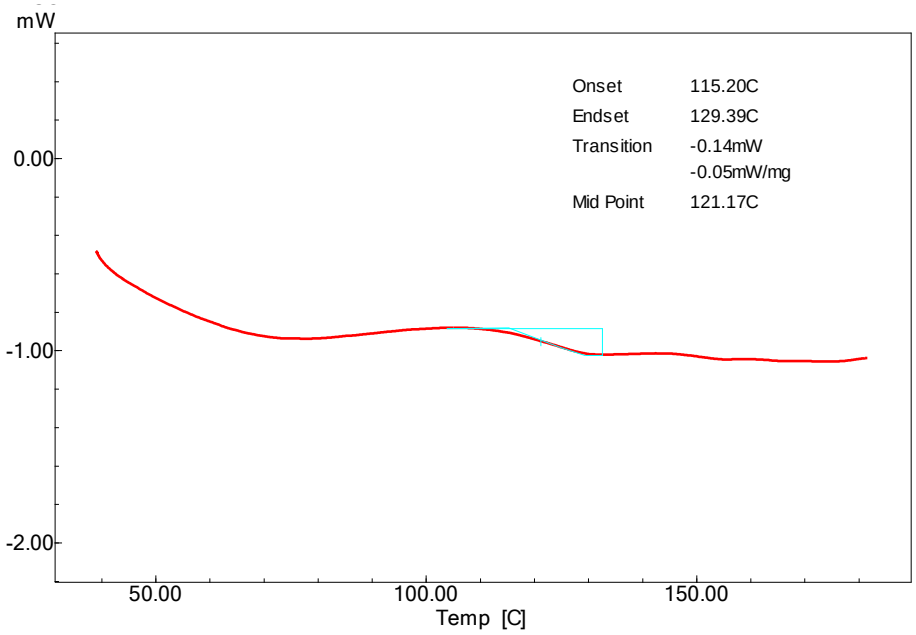


Figure 4.5b DSC thermogram of acidified Posaconazole

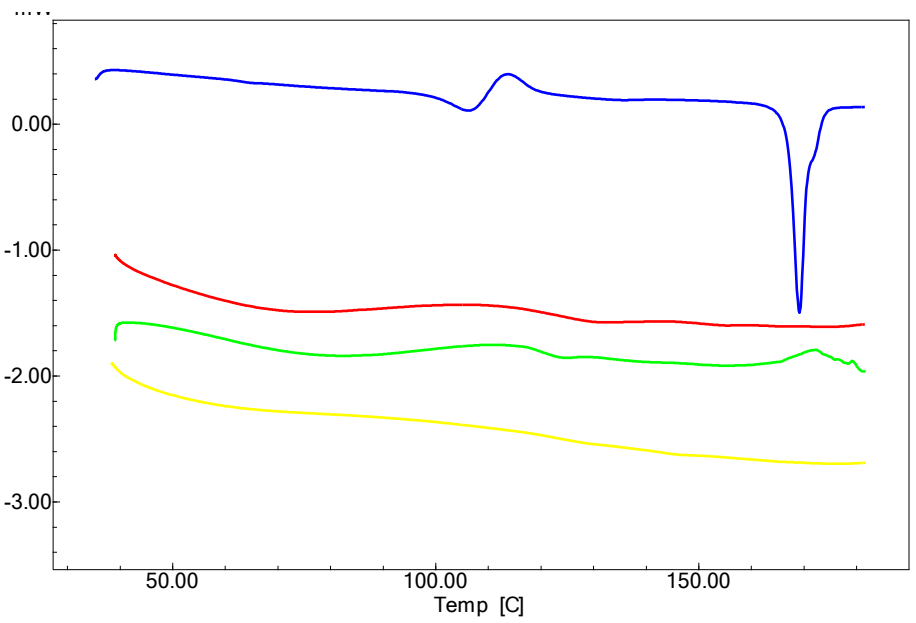
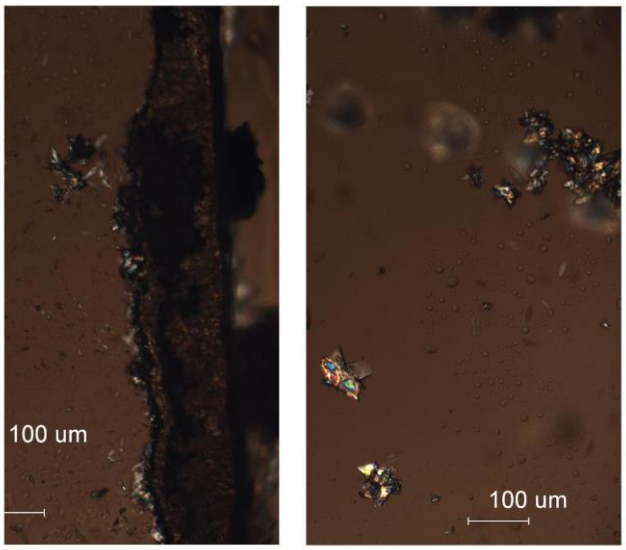


Figure 4.5c Overlay of DSC thermograms for crystalline Posaconazole (blue), acidified Posaconazole (red), acidified Posaconazole-HPMC-AS 20% w/w prototype (green) and acidified Posaconazole-HPMC-AS 10% w/w prototype (yellow)



a b

Figure 4.6 Polarised light microscopic images of drug crystallization in 50% w/w drug loaded samples, A Surface crystallization as observed at 10X magnification, B 50X magnified image of a section of A

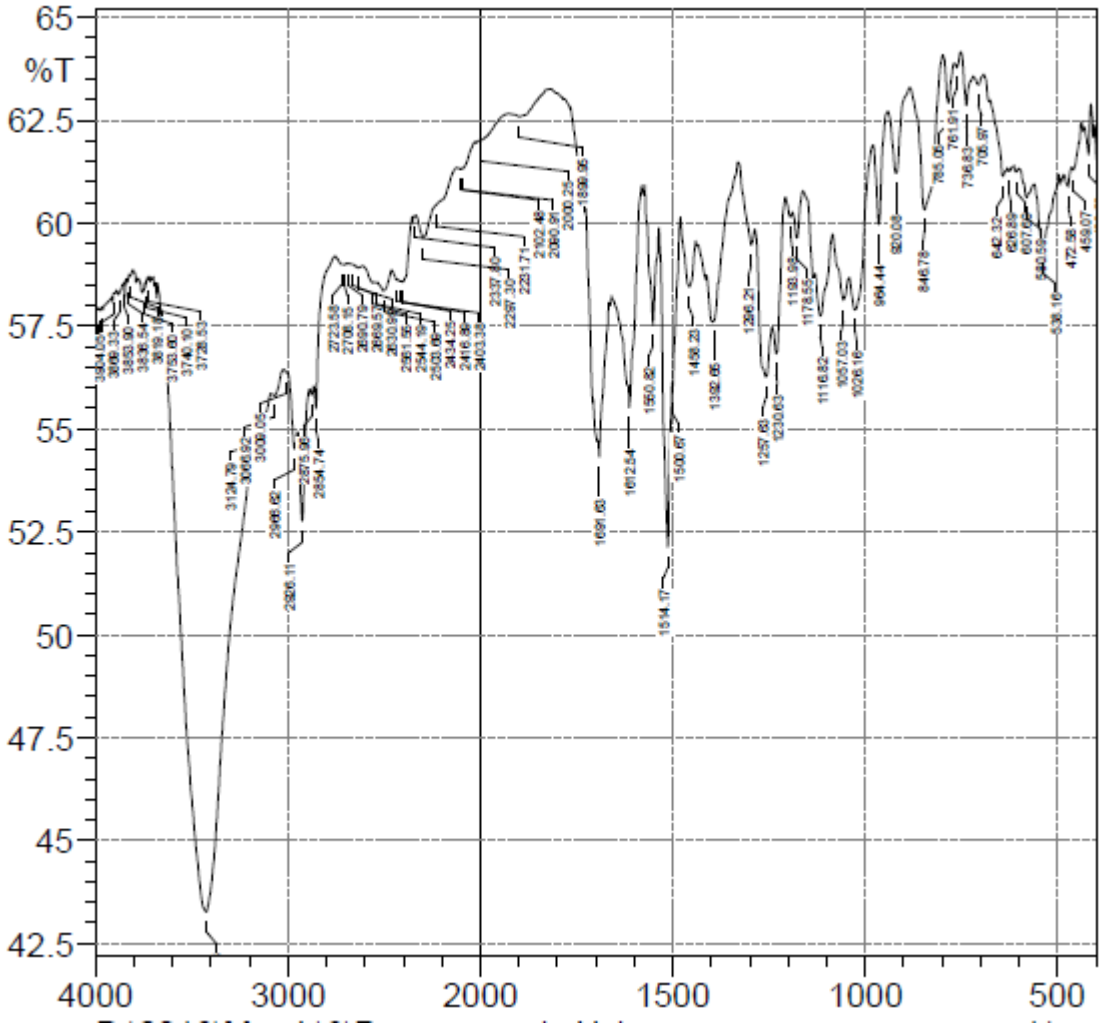


Figure 4.7a FTIR spectrum of acidified Posaconazole

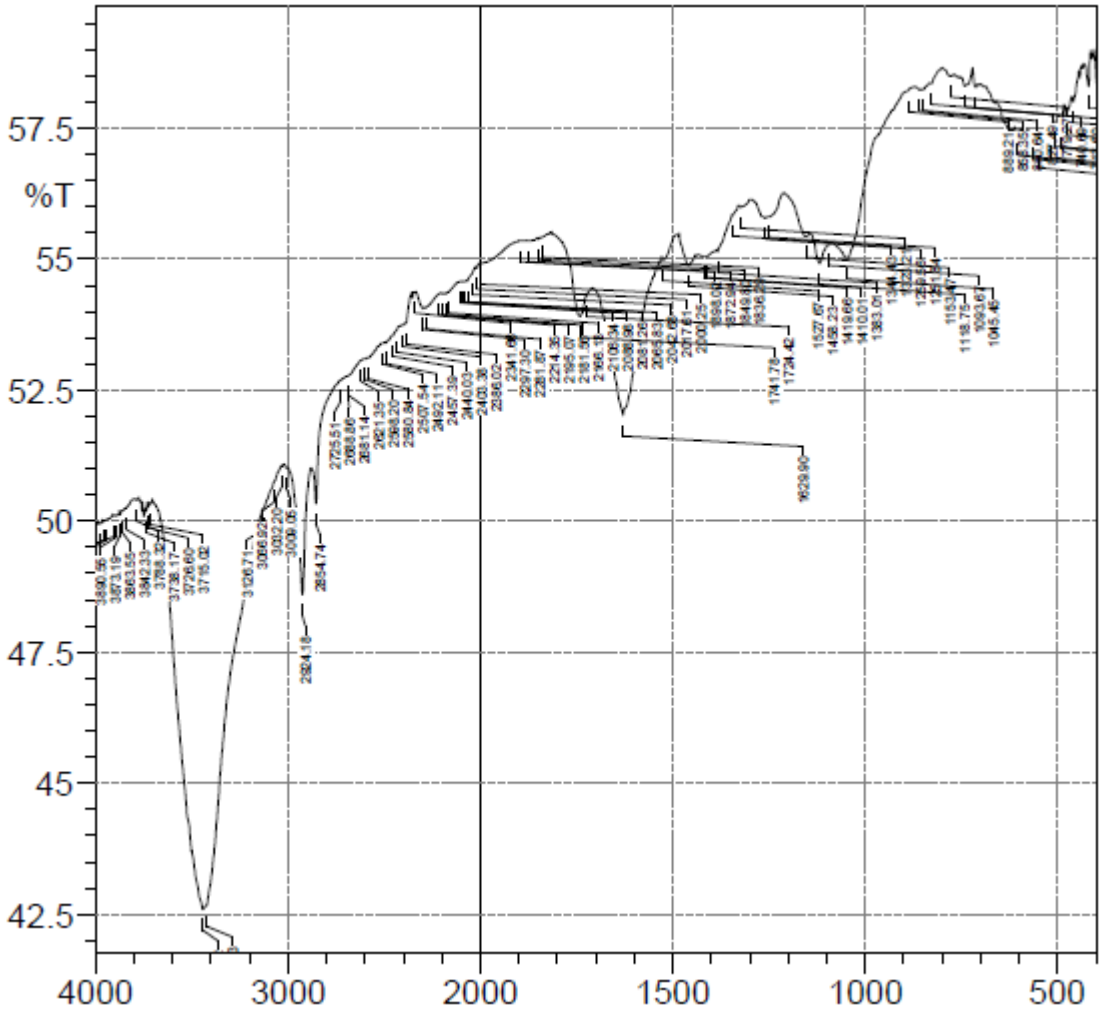


Figure 4.7b FTIR spectrum of acidified Posaconazole-HPMC-AS amorphous solid dispersion

4.3 Conclusion

Solvent selection is an important step in product development considering that it not only influences product manufacturing but also its stability. Empirical selection of process solvents and manufacturing processes could overstate limitations of a product development program and therefore, it is essential to evaluate these with regard to the drug, the drug product, target performance, environment and safety of people involved in its manufacturing. This research is an evidence that drug product manufacturing processes can be designed to be safer and efficient.

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Chapter 5

Melt solubilisation: A lateral thinking approach for low temperature melting of drugs

5.0 Introduction

Drug development is a time and cost intensive process. From drug discovery to clinical studies and from there to market, multidisciplinary teams are involved in strategizing and implementing the drug program. Presumed time and cost considerations for drug development could be derailed if the drug poses low thermodynamic solubility at physiological pH and poses bioavailability challenge. Early identification and resolution of such issues is important [1]. Various pharmaceutical interventions address these challenges, particularly formulation strategies have been recognised as helpful for poorly soluble drugs [2]. For orally administered drugs, such enabling formulation strategies include salt formation [3], lipid based products [4], novel solid forms like cocrystals [5] etc.

One such interesting intervention is solid form modification of drug from crystalline to amorphous state. In recent years there has been a surge in the number of commercial products utilizing this approach [6]. Majority of these products are amorphous solid dispersions (ASDs) that utilize a polymer to stabilize the amorphous form and prevent or delay drug re-crystallization. Crystalline drugs are thermodynamically stable, low energy forms that have organised molecular structures as crystal lattice. The process of their dissolution involves disruption of the crystal lattice and dispersion of molecular units either by a solvent or thermal energy. Unlike this, amorphous drugs are metastable and do not have long range order of molecules. This high energy state generates higher apparent solubility and dissolution rate. Marketed ASDs are majorly manufactured by either spray drying or hot melt extrusion. Advantages and limitations of both these approaches are well documented [7]. Other than the glass forming ability and glass stability, practically there are two issues that restrict usage of ASDs:

1. Drugs that have low solubility in organic solvents, are high dose and have high melting points are not amenable to ASD manufacturing by spray drying or hot melt extrusion.
2. Drug to polymer ratios that are usually required for stabilization of ASDs are in the range of 1:1 to 1:5 w/w. This increases final product weight of high dose drugs.

Considering these observations, this research evaluated the feasibility of simplifying amorphization. Three components were considered as part of research strategy:

1. Retrospective assessment of amorphization: recapitulation of the process
2. Identification of intermediate phases: capturing of phase transition stages
3. Lateral thinking approach for design: starting from desired features

This micro scale evaluation was considered as prototype for hot melt extrusion (HME) process [8]. Pharmaceutical hot melt extrusion is a successfully adopted cross-industry technology that was popular in plastic and food industries. Numerous commercial products and plethora of research publications are evidence of its widespread acceptance by the pharmaceutical industry, drug regulatory authorities and research bodies in industry and academia [9]. Customizations have further enhanced the range of pharmaceutical applications that hot melt extrusion (HME) could be applicable [10-12]. Considered as a green technology, it offers advantages of continuous manufacturing, solvent-free process, limited unit operations, small footprint for equipment, easy scale-up and scope for implementation of process analytical techniques (PAT) [13,14]. However, its application is limited by the risk of thermal degradation of drug and polymers/additives [15-18]. HME process needs to be operated either in the miscibility regime (operation temperature would be higher than melting point of drug) or the solubilisation regime (operation temperature would be lower than melting point of drug). For high melting point drugs and/or those that are thermolabile, it is desirable to work in

solubilisation regime. However, the maximum drug loading that can happen in this regime is limited [19].

Lateral thinking was evaluated for challenging this limitation of solubilisation regime. Lateral thinking approach is a concept has been around in the pharmaceutical industry [20], its application has not been explicit but can be seen in cases like patents where a certain approach is required for creating patent non-infringing route or in new drug development where secondary indications are deduced from drug's side effects as was the case with Sildenafil which was developed as a drug for treatment of angina but is commercially successful as treatment for erectile dysfunction [21]. Lateral thinking is a problem solving approach that was introduced by Edward de Bono in 1967 [22]. It requires a problem statement to be assessed with unconventional solutions and create an unconstrained thought process that would seek solution alternatives, challenging dogmas and assumptions.

Efavirenz was selected as a model drug. This non-nucleoside reverse transcriptase inhibitor (NNRTI) is a preferred antiretroviral for first line therapy. Classified as BCS class II drug [23], it is an interesting candidate to evaluate solubility enhancing techniques because unlike most poorly soluble drugs that can be categorised as either 'brick dust' or 'grease ball', Efavirenz presents a borderline case for both with a high melting point (140°C) and high log P (4.34) values [24]. Prior studies with Efavirenz have evaluated applications of various techniques like nanocrystals [25], solid dispersions [26] etc.

In this study, Hot Stage Microscopy (HSM) was utilized for preliminary screening of additives. An additive that could lower the melting point of Efavirenz to an extent that the drug-additive mixture transforms to a liquid state with no solidification at ambient conditions was the selection criteria. PEG 6000, Poloxamer 188 and Poloxamer 407 were selected from the screening stage. Additive that could bring maximum melting point depression in minimum

concentration was evaluated further by FTIR, Raman spectroscopy, Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM) and X-ray diffraction (XRD).

5.1. Materials and methods

5.1.1 Materials

Crystalline Efavirenz was provided by Aurobindo Pharma Ltd. (Hyderabad, India). Poloxamer 188 and 407 were obtained from BASF Corporation (Mt.Olive, New Jersey), PEG 6000 was obtained from Merck (Mumbai, India) and Neusilin® UFL2 (magnesium aluminometasilicate) from Fuji Chemical Industry (New Jersey, USA). All other chemicals and reagents used were of analytical grade.

5.1.2 Methods

5.1.2.1 Melt stability of Efavirenz

Melting point of Efavirenz were determined with Differential Scanning Calorimetry (DSC), Q1000 (TA instruments, Delaware, USA). Data was analysed with Universal Analysis 2000 thermal analysis software (TA instruments, Delaware, USA). Efavirenz was heated to 10°C above its melting point of 140°C. Thermal gravimetric analysis (TGA) experiments were carried out on a Thermal Analyser (TA instruments, Delaware, USA) TGA Q500 system for temperature upto 250°C at a ramp rate of 10°C/min and modulation of ±0.5°C. Chemical stability was quantified with high performance liquid chromatography (HPLC) system (Waters Corporation, Milford, USA) consisting of a quaternary pump with gradient portion valve equipped with a 100 µL loop. The W2487 PDA detector was set to 252 nm. Equivolume mixture of acetonitrile (Merck, Mumbai, India) and ammonium acetate buffer (Sigma-Aldrich, Mumbai, India) was used as mobile phase. Elution at a constant flow rate of 1 mL/min was performed. 5-µL injection volume flowed through a Hypersil BDS C18 250*4.6, 5um column. Peaks were integrated using the Empower 2 software.

5.1.2.1 Glass forming ability of Efavirenz

Similar to above stated procedure Efavirenz was analysed with DSC. Efavirenz was heated to 10°C above its melting point of 140°C and rapidly cooled at 20°C/min to 0ºC. This sample was reheated at 2°C/min. till 150°C. Glass transition event was observed. This analysis was then repeated with two additional heating rates of 5°C/min and 10°C/min.

5.1.2.3 Selection of polymer

Binary physical mixtures of 50 mg Efavirenz and different ratios of PEG 6000, Poloxamer 188 and 407 were prepared. These mixtures were melted in micro crucibles. Samples were analysed with DSC to assess melting. Hot stage polarized light microscope was utilised to study the morphological changes accompanying melting. Based on melting point depression of Efavirenz and quantity of additive required for creating the depression, samples were graded to select one additive that was further studied.

5.1.2.4 Prototype formulation

Physical mixtures of drug-excipient (1:1 w/w) were prepared and studied for compatibility at 40ºC/75%RH for 4 weeks. For prototype formulation the drug excipient, drug-excipient ratio of 1:1 and 1:2 w/w were prepared by hot melt extrusion (Nano-16, Leistriz, Germany) at 75ºC. Unlike the conventional method of blending drug and polymer to make a physical mixture and then melting in hot melt extruder, here the polymer was melted first, drug was added and mixed. Then Neusilinº was added as adsorbent and mixed further, extruded and extrudates were milled to obtain lump-free powder.

5.1.2.5 Characterization of prototype formulation

Thermal analysis was performed with DSC with the parameters as stated in 3.1.2.3. XRD analysis was performed to assess the solid state of the drug. Morphology of samples with and

without Neusilin were studied with Scanning Electron Microscopy. Molecular interactions between the drug and polymer, polymer and adsorbent and drug and adsorbent with FTIR. Samples were analysed 'as is' with preparative techniques. In vivo assessments in male Wistar rats is detailed in chapter 2.

5.2. Results and discussion

From the DSC and TGA studies of Efavirenz melt, it was observed that the melting point and decomposition temperature of Efavirenz differ by over 20ºC (Figure 5.1) and therefore, the proposed process temperature is not likely to cause any drug degradation. This was confirmed by HPLC chemical analysis of Efavirenz melt. Assay of 100.3% (n=3) was observed. Glass transition event was observed at about 37ºC and this confirmed the glass forming ability of Efavirenz. Heating rates of 5°C/min and 10°C/min. showed the same glass transition temperature.

Binary physical mixtures showed varying melting points with different polymer concentrations. Larger depressions were seen with increasing concentrations of the polymer (Figure 5.2a, b and c). Hot stage polarized light microscope showed how the polymer melted first, spread over drug particles and eventually solubilized the drug particles (Figure 5.3), confirming the mechanism of melt solubilisation. Based on melting point depression of Efavirenz and quantity of additive required for creating the depression, Efavirenz-Poloxamer 407 1:2 w/w composition was selected for further studies.

Efavirenz was found to be compatible with Poloxamer 407. During hot melt extrusion the binary mixture of drug and poloxamer formed a tacky mass that was not appropriate for downstream processing. Addition of Neusilin® generated processable powders. Scanning Electron Microscopy showed the morphological difference wherein Neusilin mixed samples were adsorbed on the spherical particles of Neusilin and therefore, had better flow compared

to the binary melt (Figure 5.4). FTIR studies showed that weak hydrogen bond interactions were there between the drug and polymer. No chemical interactions was observed between with adsorbent (Figure 5.5).

In the Caco-2 study it was observed that membrane integrity was intact and there was relative difference between the drug, drug-polymer physical mixture and the prototype formulation but low recovery values (Table 5.1) indicate that this assessment should be considered as qualitative only and needed *in vivo* animal studies. Rat studies indicated relative differences between API and prototype formulation (Table 5.2). Considerable increase in systemic absorption (AUC (0-inf) was evidence of bioavailability enhancement with this approach.

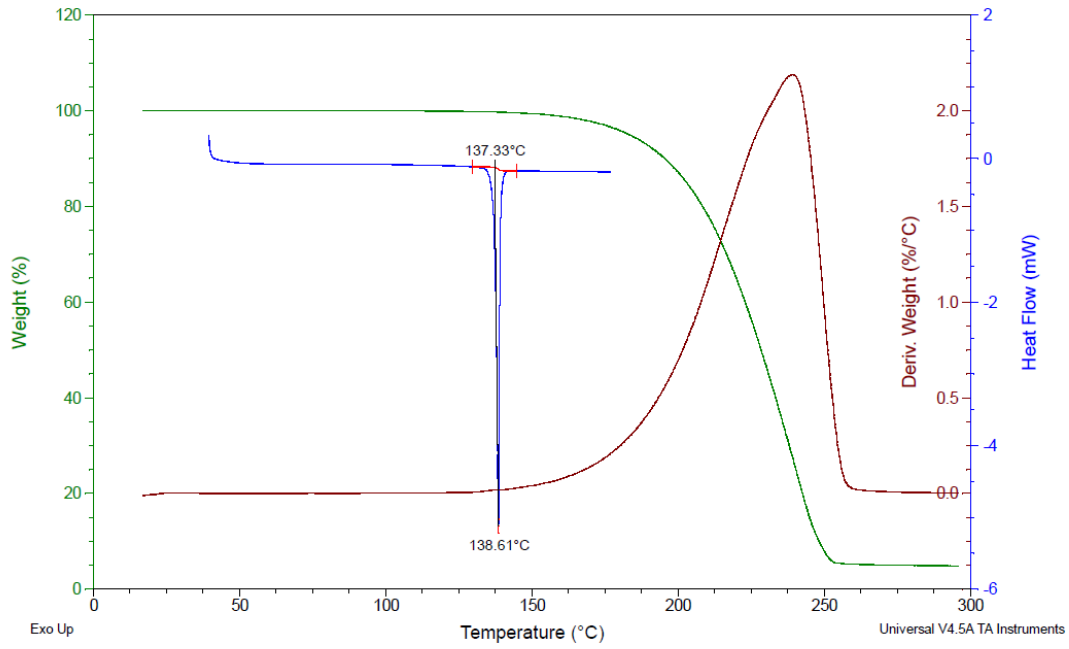
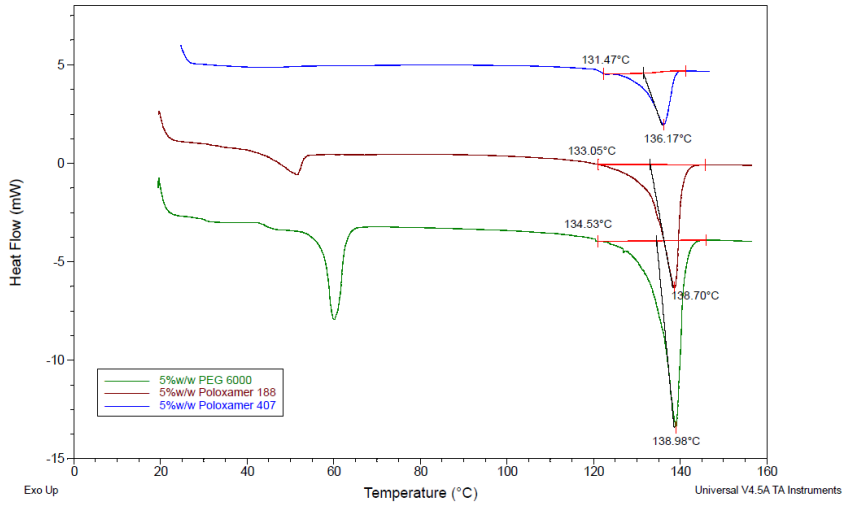
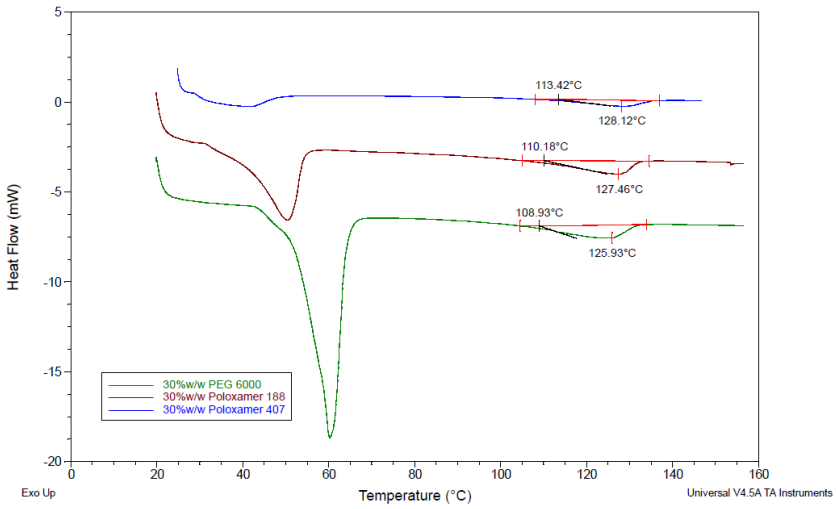


Figure 5.1 Overlay of DSC and TGA for Efavirenz drug



a



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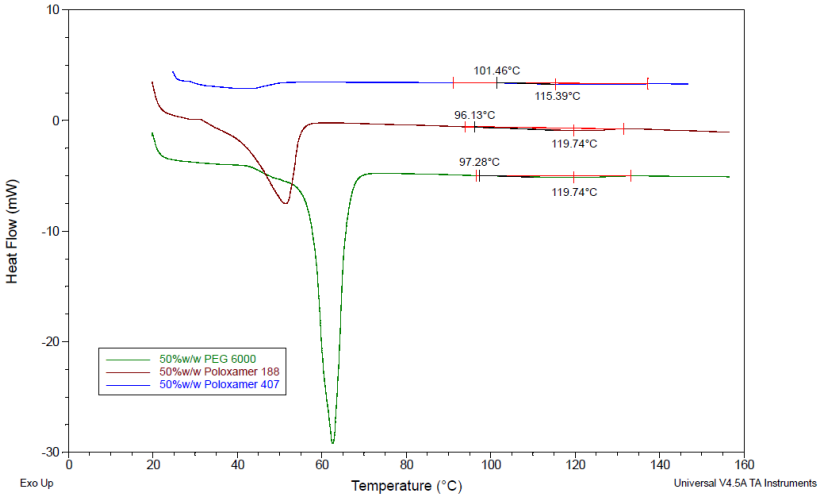
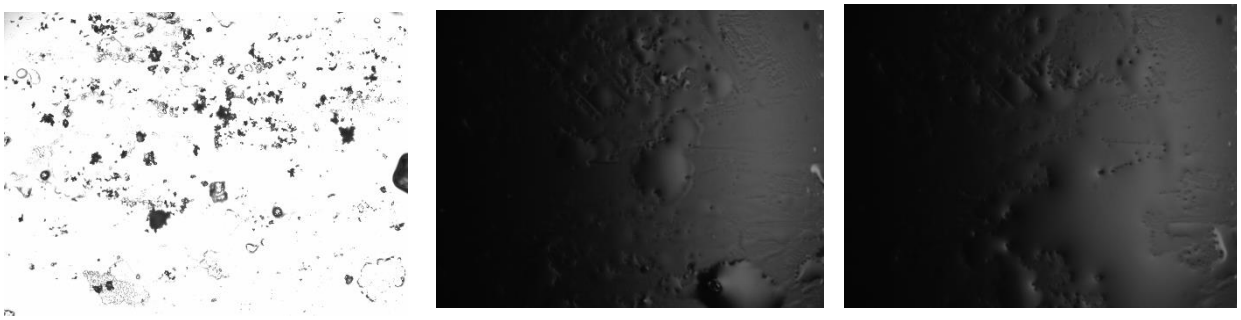


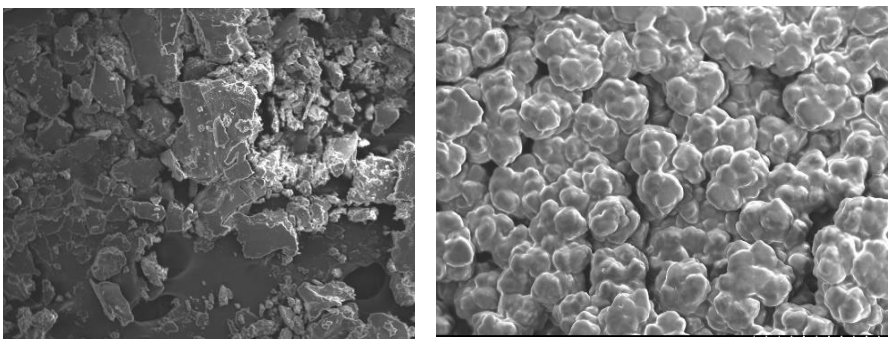
c

Figure 5.2 DSC thermograms of surfactant concentration dependent melting point depression in Efavirenz a) 5% w/w surfactants PEG 6000, Poloxamer 188 and Poloxamer 407 b) 30% w/w surfactants PEG 6000, Poloxamer 188 and Poloxamer 407 c) 50% w/w surfactants PEG 6000, Poloxamer 188 and Poloxamer 407



Initial At 30 seconds At 60 seconds

Figure 5.3 Hot stage microscopy study of melt solubilisation of Efavirenz and Poloxamer 407 at 70°C



a **b**

Figure 5.4 Scanning electron microscopy of Efavirenz-Poloxamer 407-Neusilin® mixture, a) Efaviren-Poloxamer 407 melt, b) Efaviren-Poloxamer 407-Neusilin® melt

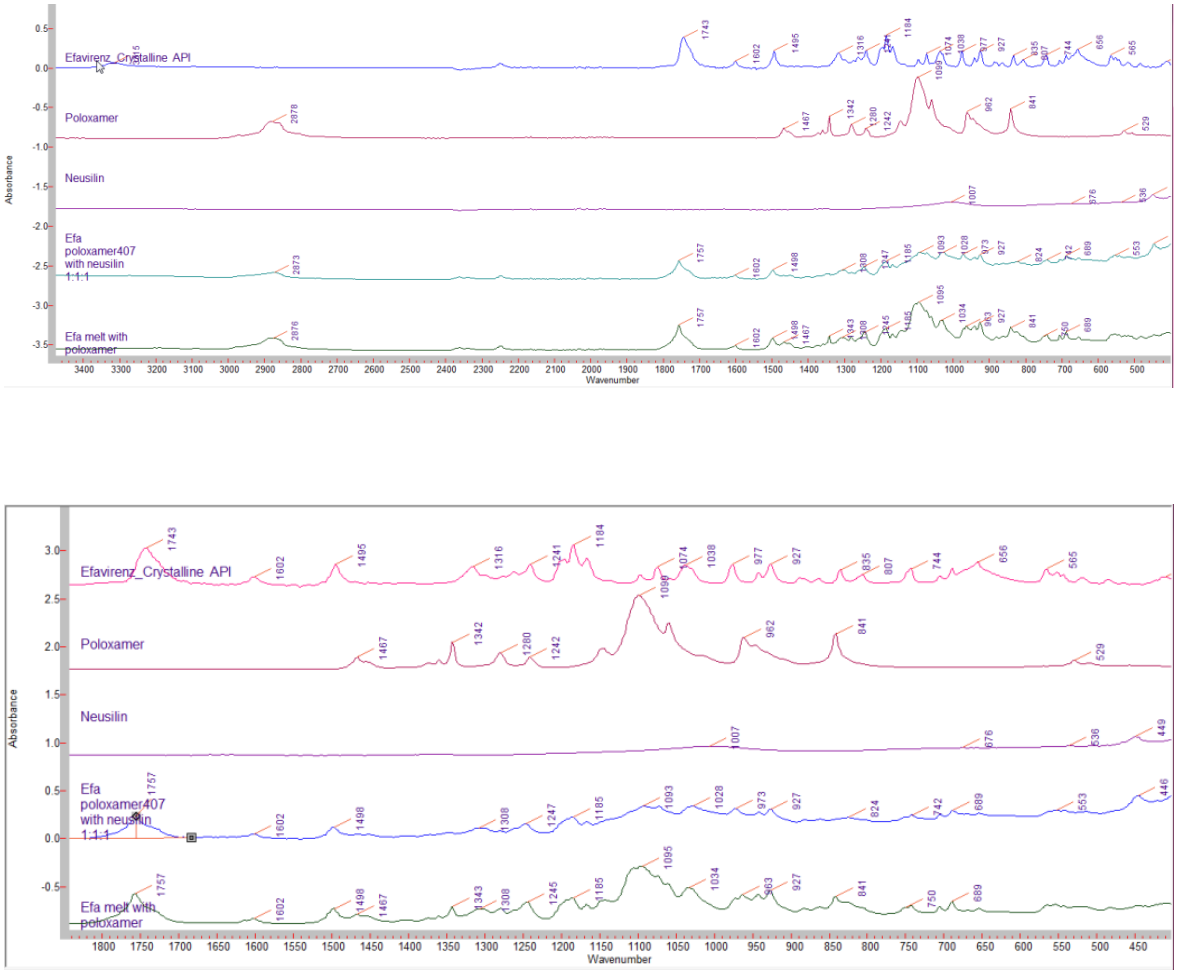


Figure 5.5 FTIR data for Efavirenz, Poloxamer 407, Neusilin and their mixtures

Table 5.1 Mean oral pharmacokinetic parameters of Efavirenz following single oral administration at 10 mg/kg dose in male Wistar rats

Parameters	Units	F-1		F-2		F-3	
		Mean	SD	Mean	SD	Mean	SD
$t_{1/2,\beta}$	h	1.85	0.14	2.34	0.14	1.84	0.12
C_{max}	ng/ml	1327	160.07	1499	29.95	2037	271.20
T_{max}	h	0.50	0.00	0.50	0.00	0.33	0.14
AUC (0-last)	(h*ng/mL	4113	108.54	4483	224.55	6014	1175.75
AUC (0-inf)	(h*ng/mL	4368	120.26	4858	221.32	6299	1169.95
MRT	h	2.37	0.13	2.24	0.09	2.15	0.09

SD, Standard deviation; $AUC_{(0-t)}$, area under the plasma concentrations-time curve; $AUC_{(0-inf)}$, area under the curve with extrapolation to infinity; $t_{1/2}$, elimination half-life; C_{max}, peak plasma concentration; T_{max}, time to reach peak plasma concentrations; MRT, mean residence time.

Conclusion

Efavirenz is a high dose poorly soluble drug that is a borderline case for ‘brick dust’ and ‘grease ball’ category of drugs making its solubility and bioavailability even more challenging. A miniature scale screening design was evaluated to assess low temperature melting. Exploiting the concept of melting point depression, attempt was to find polymers that could substantially depress melting point of Efavirenz and allow hot melt processing atleast 20°C below its melting point. Through screening studies it was observed that Poloxamer 407 was a suitable polymer for this purpose. Drug-excipient compatibility was established and the phase transitions during melting of Efavirenz and Poloxamer 407 mixtures were studied with hot stage microscopy. DSC and XRD characterization confirmed amorphous nature of the mixture. However, this melt was not processable into dosage forms because of its sticky nature. An amorphous adsorbent Neusilin® was utilised for this purpose. It was found that while this composition provided a free flowing powder, drug dissolution was negatively impacted with incomplete drug release. Considering it to be a constraint with in-vitro testing, Caco-2 studies were conducted. Additional objective of this study was to estimate effect of Poloxamer on cell membrane integrity because it is an amphiphilic surfactant that is known to form micellar dispersions. While the study indicated better permeation of the prototype formulation, compared to naïve Efavirenz and physical mixture of Efavirenz, Poloxamer and Neusilin® incomplete recovery was indicative of the limited application of this tool for this product. Therefore, animal studies were conducted in Wistar rats and it was observed that bioavailability was increased for the prototype formulation. Physico-chemical characterizations with DSC, XRD, FTIR, HSM etc. along with the animal study data indicates that low temperature melt solubilisation could be a very useful approach for solvent free, continuous manufacturing of amorphous solid dispersions.

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Chapter 6

Conclusion

6.0 Conclusion

Implementing change to a well-established process is a challenge, more so for industries like the pharmaceutical industry which is accustomed to certain ways of work that have evolved from many years of learnings. Sustainable product development is one such change. Bringing a change to the approach of people involved in key functions like research and development, manufacturing processes, analytical processes, supply chain etc. is difficult because of various reasons, major ones being familiarity and comfort with existing approaches, presumed higher cost, timelines or regulatory hurdles and business pressure to quicker deliveries of projects giving insufficient time for exploring alternative approaches to a process. While there are considerable attempts and few successes in application of sustainability principles to drug substance/active pharmaceutical ingredient (API) case studies, there is significant dearth of similar explorations in drug product development & manufacturing cases.

This research is an attempt to assess feasibility of sustainable alternatives to solvent based processes and energy intensive melt manufacturing processes that are utilised for drug product manufacturing. Amorphous solid dispersion technology was selected as an area of study where impact of such proposition could be quantified in descriptive or/and numerative terms. Extensive literature review supported in defining the two cases where such sustainable process interventions could provide environmental benefits and economic advantages. To ensure scope for future applications, model drug selection was based upon drug categories that pose solubility & bioavailability challenges like triazole antifungals Itraconazole and Posaconazole. Antiretroviral drugs are recognised as posing extreme case of bioavailability issues and therefore, Efavirenz was studied as a model drug in that category.

Three approaches were studied; one for solvent substitution for spray drying process, second for process substitution of spray drying with agitated thin film dryer and third for low temperature melt solubilisation of drug eliminating melting at high temperatures. In all these approaches objective was to evaluate target performance parameters: amorphization of drug and improved bioavailability. A systematics experimental plan was designed for each approach, majorly covering preformulation studies, formulation activities and analytical characterizations.

In Itraconazole amorphous solid dispersion, innovator product utilised dichloromethane which is known to be a hazardous solvent. While drug salts were attempted in prior literatures, when isolated they were found to have physico-chemical stability issues. In this approach, in-situ salification in polar protic solvent methanol provided an interesting circumvention to salt isolation while exploiting its solubility advantages. Amorphous state of drug was generated by bottom-up approach utilizing methanol as a solvent, HPMC as stabilizing polymer and spray drying as the manufacturing process. A free flowing powder of amorphous solid dispersion was obtained which showed improved bioavailability in animal studies. NMR studies showed that Itraconazole was salified (was not a physical mixture with acid). Amorphous state characterizations with DSC, XRD and PLM, drug polymer interactions with FTIR and Raman spectroscopy were performed and found satisfactory, validating the hypothesis that such approach is feasible.

Above stated salification approach was applied to Posaconazole. Spray dryer was replaced with an agitated thin film dryer (ATFD) which is an energy efficient, continuous manufacturing, low footprint processor. Utilizing DSC the miscibility of drug-polymer mixtures was estimated and HPMCAS was selected as a suitable polymer. Similar to Itraconazole, salification was performed with hydrochloric acid. Along with the polymer, an amorphous solid dispersion was obtained that was characterised by techniques as mentioned

above. ATFD provides large surface area for evaporation of solvent, drug-polymer solution transformed from solution to slurry to paste and then a thin film that is scrapped within the machine to provide fine free flowing powder. With its indirect heating principle it was found to avoid high temperature exposure of product. Unlike spray dryer where high solute concentration of feed creates nozzle block thereby reducing process efficiency per unit of solvent, ATFD was able to handle solid content of 20% w/w. With features of solvent recovery and continuous manufacturing, ATFD is a sustainable alternative to conventional spray drying. Extensive characterization showed that an amorphous solid dispersion was obtained by this process.

Another popular approach for large scale manufacturing of amorphous solid dispersions is hot melt extrusion. It is a solvent-free continuous manufacturing process that is underutilised mainly because of the thermal degradation risk for drugs & excipients. Efavirenz that is a high dose poorly soluble drug that is a borderline case for 'brick dust' and 'grease ball' category of drugs making its solubility and bioavailability even more challenging. A miniature scale screening design was evaluated to assess low temperature melting. Exploiting the concept of melting point depression, attempt was to find polymers that could substantially depress melting point of Efavirenz and allow hot melt processing atleast 20°C below its melting point. Through screening studies it was observed that Poloxamer 407 was a suitable polymer for this purpose. Drug-excipient compatibility was established and the phase transitions during melting of Efavirenz and Poloxamer 407 mixtures were studied with hot stage microscopy. DSC and XRD characterization confirmed amorphous nature of the mixture. However, this melt was not processable into dosage forms because of its sticky nature. An amorphous adsorbent Neusilin® was utilised for this purpose. It was found that while this composition provided a free flowing powder, drug dissolution was negatively impacted with incomplete drug release. Considering it to be a constraint with in-vitro testing,

Caco-2 studies were conducted. Additional objective of this study was to estimate effect of Poloxamer on cell membrane integrity because it is an amphiphilic surfactant that is known to form micellar dispersions. While the study indicated better permeation of the prototype formulation, compared to naïve Efavirenz and physical mixture of Efavirenz, Poloxamer and Neusilin® incomplete recovery was indicative of the limited application of this tool for this product. Therefore, animal studies were conducted in Wistar rats and it was observed that bioavailability was increased for the prototype formulation. Physico-chemical characterizations with DSC, XRD, FTIR, HSM etc. along with the animal study data indicates that low temperature melt solubilisation could be a very useful approach for solvent free, continuous manufacturing of amorphous solid dispersions.

Every drug that transitions from discovery to market undergoes extensive regulatory scrutiny and incurs huge drug development costs. When concepts like sustainability are to be implemented for developed drugs there is a serious challenge because it is difficult to predict how it could impact drug solid state, chemical stability, yield etc. This impact could be positive or negative, but in either case the risk (real or presumed) is a deterrent for pharmaceutical companies. The amount of effort required to re-submit drug approval/change applications to regulatory authorities involve time and cost which inhibits most companies from investing in sustainable approaches. For new drugs (new chemical entities) sustainability should be part of drug design and pharmaceutical companies should support, educate and emphasis benefits of sustainable product development. Green chemistry, solvent substitution, solvent free methods, reduced waste generation, zero emissions to land, water and air etc. are few of the approaches that are gaining grounds in pharmaceutical industry. American Chemical Society (ACS) roundtable, Dow Jones Sustainability Indices (DJSI) etc. are engaging more industries to adopt sustainability principles. Governments, regulatory authorities and companies need to incentivise sustainable innovations. Continued

focus at research and development stage, proper training to staff, appropriate metrics for evaluation of sustainability and holistic approach including all aspects of drug development, drug substance and drug product, would help percolate the culture of benign by design helping environment, employees and economic growth.

This research is one step towards exploring sustainable drug product development.

6.1 Limitations

Major limitations of present research are enlisted below:

1. Single solvent systems were evaluated in this research. For many drugs this approach would need modification including binary & tertiary solvent mixtures. Impact of such mixtures on the drug product performance and sustainability metrics needs assessment.
2. For salification approach, it is essential that drug should be ionisable and forms chemically stable salt. Amorphization along with reactive salts could negatively impact chemical stability of some drugs.
3. Melt solubilization approach could be limited by the dose of drug. Inducing melting point depression for high dose drugs could be challenging because it would require large quantities of polymer, increasing product weight and cost.

6.2 Future scope and directions

Present research is oriented to generate proof-of-concept data. Therefore, needs further detailed evaluation to conclude industrial application, both in terms of range of drugs that could benefit from these approaches and cost-benefit assessment. Major recommendations for future research are as below:

1. Applying these approaches to diverse drug categories so that the range of application is established.
2. Studying the *in vivo* behaviour of these prototypes in human clinical studies and establishing mechanisms of drug delivery from amorphous solid dispersions.
3. Creating a simple algorithm to identify which of these approaches could be utilised for what category of drugs and what could be the probability of success for that drug.

List of publications (from thesis)

Published

Journal

1. Nair R, Lamare I, Tiwari NK, Ravi PR, Pillai R. In Situ Salification in Polar Solvents: a Paradigm for Enabling Drug Delivery of Weakly Ionic Drugs as Amorphous Solid Dispersion. AAPS PharmSciTech. 2018; 19(1):326–37**.**
2. Nair R, The science of solubility and the success of amorphous solid dispersions. ONdrugDelivery. 2018; July: 26-30

Patent

1. Solid dispersions of in situ salts of triazole drug, IN 201741007573

Under communication

1. Nair R, Ravi PR, Pillai R. A step towards sustainable drug products: Case of amorphous solid dispersions. Sustainability in chemistry and pharmacy (manuscript submitted)
2. Nair R, Lamare I, Tiwari NK, Ravi PR, Pillai R. Low temperature melt solubilisation: A lateral thinking approach in hot melt technology, Drug development and industrial pharmacy. (manuscript submitted)

List of conferences

1. Select bioavailability enhancement strategies for early, clinical phase drugs: challenges of time, cost and drug, availability, 7th Drug Formulation, Solubility and Bioavailability (March 26–28, Boston)
2. Salification in polar protic solvents: A paradigm for enabling drug delivery of weakly ionic drugs, CRS 2016 (July 17-20, Seattle, Washington)
3. A Green Process for Preparation of Amorphous Solid Dispersion, AAPS 2013, (November 10-14, San Antonio)
4. Melt Solubilization and Controlled Cooling Enabled In Situ Micronization, CRS 2016 (July 22-24, New York)

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Dr. Raviraj Pillai is Head R&D, Abbott, India. He has over 27 years of industrial experience He completed his PhD from University of Illinois and since then has been author to various international research articles and holder of many patents. He is the supervisor to this research work. He has product development experience in Wyeth, Dr. Reddy's and Perrigo

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Rashmi Nair is Senior scientist, Dr. Reddy's Laboratories Ltd. She completed her postgraduation in Master of Pharmacy, 2006 and since then has been in R&D product development. She is credited with five research articles, three reviews and three patents. She has presented various posters and talks at international conferences.