IN-VITRO DISSOLUTION AND IN-VIVO BIOAVAILABILITY OF A NOVEL SOLID DISPERSION OF LOSARTAN POTASSIUM AND HYDROCHLOROTHIAZIDE

A Dissertation submitted to

THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY
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In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY
IN
PHARMACEUTICS

Submitted by
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Under the Guidance of

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CONTENTS

1. INTRODUCTION

The enhancement of oral bioavailability of poorly water soluble drugs remains one of the most challenging aspects of drug development. Although salt formation, solubilization, and particle size reduction have commonly been used to increase dissolution rate and thereby oral absorption and bioavailability of such drugs,[1] there are practical limitations of these techniques.

For drugs whose GI absorption is rate limited by dissolution, reduction of the particle size generally increases the rate of absorption and/or total bioavailability. This commonly occurs for drugs with poor water solubility. For example, the therapeutic dose of griseofulvin was reduced to 50% by micronization [2], and it also produced a more constant and reliable blood level. The commercial dose of spironolactone was also decreased to half by just a slight reduction of particle size [3]. Such enhancement of drug absorption could further be increased several fold if a micronized product was used.[3,4]

Poorly water soluble drugs are allied with slow drug absorption leading to inadequate and variable bioavailability and G.I. mucosal toxicity of drugs [5]. Poorly water soluble drugs belong to BCS class II and Class IV [6] group of compounds. In the process of absorption of drug from oral route dissolution is the rate limiting step for poorly soluble drugs. In other words, in-vitro dissolution is the index of in-vivo absorption of poorly soluble drugs. Therefore it is necessary to enhance dissolution of these drugs to ensure maximum therapeutic utility of these drugs.

In 1961, Sekiguchi and Obi[7] developed a practical method whereby many of the limitations with the bioavailability enhancement of poorly water-soluble drugs just mentioned can be overcome. This method, which was later termed solid dispersion,[8] involved the formation of eutectic mixtures of drugs with water-soluble carriers by the melting of their physical mixtures. Sekiguchi and Obi[7] suggested that the drug was present in a eutectic mixture in a microcrystalline state. Later, Goldberg et al[9,10] demonstrated that all the drug in a solid dispersion might not necessarily be present in a microcrystalline state; a certain fraction of the drug might be molecularly dispersed in the matrix, thereby forming a solid

solution. In either case, once the solid dispersion was exposed to aqueous media and the carrier dissolved, the drug was released as very fine, colloidal particles. Because of greatly enhanced surface area obtained in this way, the dissolution rate and the bioavailability of poorly water-soluble drugs were expected to be high.

The term solid dispersion refers to the dispersion of one or more active ingredients in an inert carrier or matrix at solid state prepared by the melting (fusion), solvent, or melting-solvent method. The dispersion of a drug or drugs in a solid diluents or diluents by traditional mechanical mixing is not included in this category. The solid dispersions may also be called a solid-state dispersions, as first used by Mayersohn and gibaldi.[11]

Over the years, solid dispersion included physiologically inert carriers to improve dissolution and bioavailability of poorly soluble drugs. In the present study, a modified solid dispersion approach was followed, where in a poorly soluble drug was solid dispersed in another freely soluble drug both available in a fixed dose combination and further, this modified approach did not utilize physiologically inert carriers. Hydrochlorothiazide and Losartan potassium, a fixed dose combination was selected as a model for this novel approach. Hydrochlorothiazide and Losartan potassium is one of the fixed dose combinations that have been effectively employed in the management of hypertension[12]. Losartan potassium is an angiotensin receptor blocker (ARB), and controls hypertension through this mechanism. Hydrochlorothiazide is a thiazide diuretic and it indicated along with the suitable antihypertensive drugs in the management of hypertension. Hydrochlorothiazide inhibits Na+ and Cl- reabsorption in the early distal tubule and collecting system[14]. Previously, solid dispersions of Hydrochlorothiazide were prepared using physiological inert carriers and their improved dissolution and their bioavailability was reported.

Hydrochlorothiazide is poorly soluble whereas Losartan potassium is freely soluble in water. Owing to its poor solubility hydrochlorothiazide may pose dissolution rate limited absorption problem. An increase in dissolution rate of Hydrochlorothiazide effected by Losartan potassium in this novel approach will be beneficial for improving therapeutic efficacy of the combination[14].

2. REVIEW OF LITERATURE

Therapeutic effectiveness of a drug depends upon the bioavailability and ultimately upon the solubility of drug molecules. Solubility is one of the important parameters to achieve desired concentration of drug in systemic circulation for pharmacological response to be shown. Currently only 8% of new drug candidates have both high solubility and permeability.[15]The solubility of a solute is the maximum quantity of solute that can dissolve in a certain quantity of solvent or quantity of solution at a specified temperature. In other words the solubility can also be defined as the ability of one substance to form a solution with another substance. The substance to be dissolved is called as solute and the dissolving fluid in which the solute dissolve is called as solvent, which together form a solution. The process of dissolving solute into solvent is called as solution or hydration if the solvent is water.[16] The transfer of molecules or ions from a solid state into solution is known as dissolution.

Dissolution of drug is the rate-controlling step which determines the rate and degree of absorption. Drugs with slow dissolution rates generally show erratic and incomplete absorption leading to low bioavailability when administered orally. Since aqueous solubility and slow dissolution rate of BCS class II and class IV drugs is a major challenge in the drug development and delivery processes, improving aqueous solubility and slow dissolution of these Classes of drugs have been investigated extensively [17]. A review of new monograph (1992-1995) in European pharmacopoeia shows that more than 40% of the drug substances have aqueous solubility below 1mg/ml and the 32% have an aqueous solubility below 0.1mg/ml [18, 19].

The dissolution rate of a drug is directly proportional to its solubility as per Noyes-Whitney equation and therefore solubility of a drug substance is a major factor that determines its dissolution rate and hence its absorption and bioavailability eventually [20].

Noyes-Whitney equation illustrates how the dissolution rate of even very poorly soluble compounds might be improved to minimize the limitations to oral bioavailability:

$dc/dt = AD. (Cs - C) / h$

Where, dc/dt is the rate of dissolution, A is the surface area available for dissolution, D is the diffusion coefficient of the compound, Cs is the solubility of the compound in the dissolution medium, C is the concentration of drug in the medium at time t, h is the thickness of the diffusion boundary layer adjacent to the surface of the dissolving compound[21].

TECHNIQUES OF SOLUBILITY ENHANCEMENT

There are various techniques available to improve the solubility of poorly soluble drugs. Some of the approaches to improve the solubility are [22]

1) PHYSICAL MODIFICATIONS

a) Particle size reduction

- Micronization
- Nanosuspension
- Sonocrystalisation
- Supercritical fluid process

b) Modification of the crystal habit

- Polymorphs
- Pseudo polymorphs

c) Drug dispersion in carriers

- Eutectic mixtures
- Solid dispersions
- Solid solutions

d) Complexation

- Use of complexing agents

e) Solubilization by surfactants:

- Micro emulsions
- Self micro emulsifying drug delivery systems

2) CHEMICAL MODIFICATIONS

- Salt form
- Anhydrates
- Solvates

3) OTHER METHODS

- Cocrystalisation
- Co solvency
- Hydrotrophy
- Solvent deposition
- Selective adsorption on insoluble carrier
- Use of soluble prodrug
- Functional polymer technology
- Porous micro particle technology
- Nanotechnology approaches

SOLID DISPERSIONS

Solid dispersion was introduced in the early 1970s and refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug [23,24]. Chiou and Riegelman defined the term solid dispersion as "a dispersion involving the formation of eutectic mixtures of drugs with water soluble carriers by melting of their physical mixtures"[25], They classified solid dispersions into the following six representative types: Simple eutectic mixtures, solid solutions, glass solutions and glass suspensions, amorphous precipitations in a crystalline carrier, compound or complex formation, and combinations of the previous five types[26] while Corrigan (1985) suggested the definition as being a 'product formed by converting a fluid drug-carrier combination to the solid state'.[27]

CLASSIFICATION OF SOLID DISPERSIONS: solid dispersion can be classified as follows

1) First generation solid dispersions

The first description of solid dispersions was given from Sekiguchi and Obi in 1961 showed that formulation of eutectic mixtures improved the rate of drug release which in turn increases the bioavailability of poorly water soluble drugs. Later, Levy and Kaning developed solid dispersion systems, containing mannitol as carrier, by preparing solid solutions through molecular dispersions instead of using eutectic mixtures. They have the disadvantage of forming crystalline solid dispersions, which were more thermodynamically stable and did not release the drug as quickly as amorphous ones[28].

2) Second generation solid dispersions

It was noticed in the late sixties (Simonelli et al. 1969; Chiou and Riegelman, 1969), that Solid dispersion with drug in the crystalline state is not as effective as amorphous because they are thermodynamically stable (Simonelli et al. 1969; Vippagunta et al. 2006; Urbanetz, 2006). Therefore, second generations of solid dispersions were introduced having amorphous carriers instead of crystalline. Formerly, the drugs were molecularly dispersed in amorphous carriers which are usually polymers in random pattern (Vilhelmsen et al. 2005)[28].

3) Third generation solid dispersions

Third generation of solid dispersions appeared as the dissolution profile could be increased by using carriers having surface activity and self-emulsifying characteristics. These contain surfactant carriers or a mixture of amorphous polymers and a surfactant as carrier.

The third generation solid dispersions stabilize the solid dispersions, increase the bioavailability of the poorly soluble drugs and reduce recrystallization of drug[29]. Surfactants have been included to stabilize the formulations, thus avoiding drug recrystallization and potentiating their solubility[18].

SIGNIFICANT PROPERTIES OF SOLID DISPERSION:

There are certain parameters that are given below when successfully controlled, can produce improvements in bioavailability:

1. **Particle size reduction**

Solid dispersion represents the last state of the size reduction. It includes the principle of drug release by creating a mixture of poorly water soluble drug and highly soluble carriers, and after dissolution of carrier, the drug get molecularly dispersed in dissolution medium.

2. **Wettability**

Carriers having surface activity like cholic acid and bile salts, when used, can significantly increase the wettability properties of drug. Recently, in third generation solid dispersion surfactants have been included that is the emerging technique.

3. **Higher porosity**

Solid dispersions containing linear polymers produce larger and more porous particles than those containing reticular polymers and therefore, result in a higher dissolution rate.

4. **Amorphous state of drug particles**

Drug particles in amorphous state have higher solubility[30].

ADVANTAGES OF SOLID DISPERSION

1. Rapid dissolution rates that result in an increase in the rate and extent of the absorption of the drug, and a reduction in presystemic, both can lead to the need for lower doses of the drug.
2. Other advantages include transformation of the liquid form of the drug into a solid form (e.g., clofibrate and benzoyl benzoate can be incorporated into PEG 6000 to give a solid, avoidance of polymorphic changes and thereby bio-availability problems), as in the case of nabilone and PVP dispersion, and protection of certain drugs by PEGs (e.g., cardiac glycosides) against decomposition by saliva to allow buccal absorption[31].

DISADVANTAGES OF SOLID DISPERSIONS

Despite extensive expertise with solid dispersions, they are not broadly used in commercial products, mainly because there is the possibility that during processing (mechanical stress) or storage (temperature and humidity stress) the amorphous state may undergo crystallization [32-34]. The effect of moisture on the storage stability of amorphous pharmaceuticals is also a significant concern, because it may increase drug mobility and promote drug crystallization [20]. Moreover, most of the polymers used in solid dispersions can absorb moisture, which may result in phase separation, crystal growth or conversion from the amorphous to the crystalline state or from a metastable crystalline form to a more stable structure during storage. This may result in decreased solubility and dissolution rate [35]. Therefore, exploitation of the full potential of amorphous solids requires their stabilization in solid state, as well as during in vivo performance. Another drawback of solid dispersions is their poor scale-up for the purposes of manufacturing.

LIMITATIONS OF SOLID DISPERSION SYSTEMS

Problems limiting the commercial application of solid dispersion involve

a. Its method of preparation,
b. Reproducibility of its physicochemical properties,
c. Its formulation into dosage forms,
d. The scale up of manufacturing processes, and
e. The physical and chemical stability of drug and vehicle.

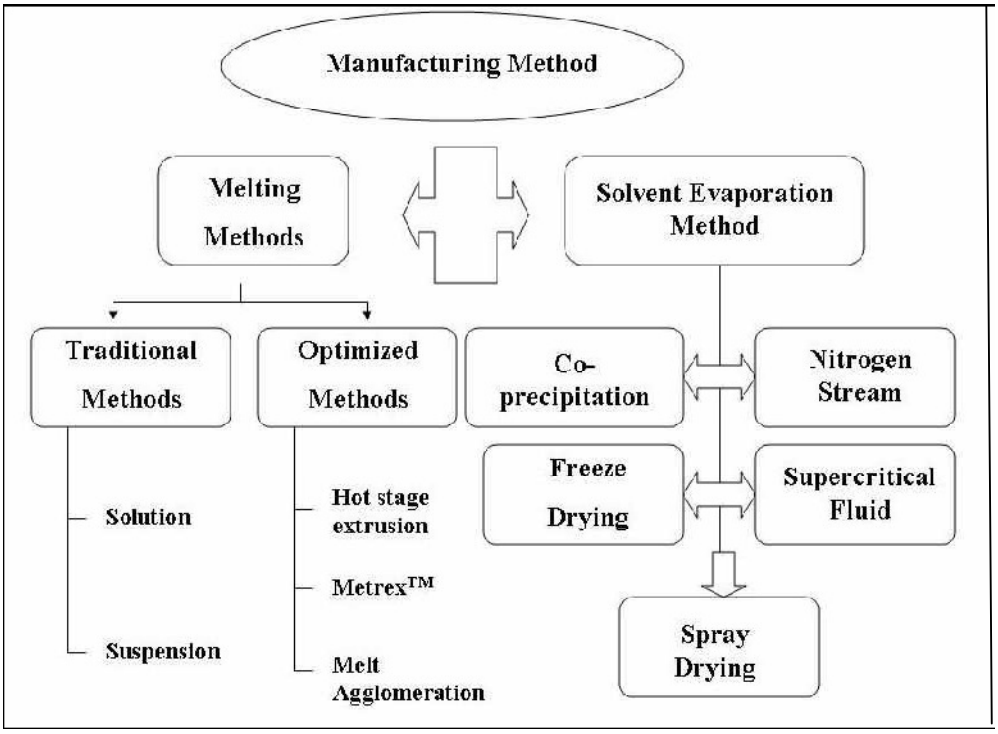
Table 1 **Classification of Carriers Enhancing Dissolution of Drugs** [17]

S.No	Category	Example of Carriers
1.	Polymers	Polyvinylpyrrolidone, Polyvinylpolypyrrolidone, Polyvinyl alcohol, Polyethylene glycols, Hydroxypropyl ethylcellulose, Hydroxypropyl cellulose, Poly (2-hydroxyethylmethacrylate), Methacrylic copolymers (Eudragit® S100 sodium salts and Eudragit® L100 sodium salts).
2.	Superdisintegrants	Sodium starch glycolate, Croscarmellose sodium, Cross-linked polyvinylpyrrolidone, Cross-linked alginic acid, Gellan gum, Xanthan gum, Calcium silicate.
3.	Cyclodextrins	β-Cyclodextrins, Hydroxypropyl-β-cyclodextrins.
4.	Carbohydrates	Lactose, Soluble starch, Sorbitol, Mannitol, β-(1-4)-2-amino-2-deoxy-D-glucose (Chitosan), Maltose, Galactose, Xylitol, Galactomannan, British gum,Amylodextrin.
5.	Surfactants	Poloxamers (Lutrol® F 127, Lutrol® F 68), Polyglycolized glyceride (Labrasol), Polyoxyethylene sorbitan monoesters (Tweens), Sorbitan esters (Spans), Polyoxyethylene stearates, Poly (beta-benzyl-L-aspartate) – b - poly (ethylene oxide), Poly (caprolactone) – b - poly (ethylene oxide).
6.	Hydrotropes	Urea, Nicotinamide, Sodium benzoate, Sodium salicylate, Sodium acetate, Sodium-o-hydroxy benzoate, Sodium-p-hydroxy benzoate, Sodium citrate.
7.	Polyglycolized glycerides	Gelucire 44/14, Gelucire 50/13, Gelucire 62/05.
8.	Acids	Citric acid, Succinic acid, Phosphoric acid.
9.	Dendrimers	Starburst® polyamidoamine (PAMAM).

Table 2 **Analytic method for characterization of solid forms**

S.No	Methods	Significance
1.	Thermal analysis a. Cooling Curve Method b. Thaw Melt Method c. Thermo microscopic Method d. Zone Melting Method e. DSC Studies f. DTA Studies	To study the morphology and degree of crystallinity. To find out the interaction between drug and carrier and formation of inclusion complex.
2.	X-ray Powder Diffraction Studies	To find out the crystalline or amorphous form of drug.
3.	FTIR, NMR, Raman spectra	To find out the complex formation between drug and carrier.
4.	Scanning Electron Microscopy	To find out the particle size and shape.
5.	Dissolution rate / diffusion rate studies	Rate and extent of dissolution.
6.	Thermodynamic study	Degree of crystallinity

Fig.1 **Methods of Preparation of Solid Dispersion** [36]



SOLVENT EVAPORATION METHOD

Though different methods have been followed for preparation of solid dispersion, the solvent evaporation method assumes significance in the present study and so a brief review of this method is presented. Commonly used method of preparing a solid dispersion is the dissolution of drug and carrier in a common organic solvent, followed by the removal of solvent by evaporation.[37-39]Because the drug used for solid dispersion is usually hydrophobic and the carrier is hydrophilic, it is often difficult to identify a common solvent to dissolve both components. Large volumes of solvents as well as heating may be necessary to enable complete dissolution of both components. Chiou and Riegelman[23] used 500 mL of ethanol to dissolve 0.5 g of griseofulvin and 4.5g of PEG 6000. Although in most other reported studies the volumes of solvents necessary to prepare solid dispersions were not specified, it is possible that they were similarly large. To minimize the volume of organic solvent necessary, Usui et al.[40] dissolved a basic drug in a hydro alcoholic mixture of 1 N HCl and methanol, with drug to cosolvent ratios ranging from 1:48 to 1:20, because as a

protonated species, the drug was more soluble in the acidic cosolvent system than in methanol alone. Some other investigators dissolved only the drug in the organic solvent, and the solutions were then added to the melted carriers. Vera et al.[41] dissolved 1 g of oxodipine per 150mL of ethanol before mixing the solution with melted PEG 6000. In the preparation of piroxicam-PEG 4000 solid dispersion, Fernandez et al.[42] dissolved the drug in chloroform and then mixed the solution with the melt of PEG 4000 at 70°C. Many different methods were used for the removal of organic solvents from solid dispersions. Simonelli et al.[38]evaporated ethanolic solvent on a steam bath and the residual solvent was then removed by applying reduced pressure. Chiou and Riegelman[23] dried an ethanolic solution of griseofulvin and PEG 6000 in an oil bath at 115 °C until there was no evolution of ethanol bubbles. The viscous mass was then allowed to solidify by cooling in a stream of cold air. Other investigators used such techniques as vacuum-drying,[42,43] spray-drying,[44-47] spraying on sugar beads using a fluidized bed-coating system,[48] lyophilization,[49] etc., for the removal of organic solvents from solid dispersions. None of the reports, however, addressed how much residual solvents were present in solid dispersions when different solvents, carriers, or drying techniques were used.

Solvent

Solvent to be included for the formulation of solid dispersion should have the following criteria:

1. Both drug and carrier must be dissolved.
2. Toxic solvents to be avoided due to the risk of residual levels after preparation E.g. chloroform and dichloromethane.
3. Ethanol can be used as alternative as it is less toxic.
4. Water based systems are preferred.
5. Surfactants are used to create carrier drug solutions but as they can reduce glass transition temperature, so care must be taken in to consideration. [39]

Class I Solvents (Solvents to be avoided)

Solvents included in this class are not to be taken in to use because of their deleterious environmental effects

Table. 3 **List of some Class I Solvents.**

Solvent	Concentration limit (ppm)	Effect
Benzene	2	Carcinogen.
Carbon tetrachloride	4	Toxic and environmental Hazards.
1,2-dichloroethane	5	Toxic.
1,1-dichloroethane	8	Toxic.
1,1,1-trichloroethane	1500	Environmental hazards.

Class II Solvents (Solvents to be limited)

These solvent should be limited used in pharmaceutical products because of their inherent toxicity.

Table 4 **Class II solvents in pharmaceutical products**

Solvent	PDE(mg/day)	Concentration limit(ppm)
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3880
1,2-dichloroethene	18.7	1870
Ethylene glycol	6.2	620
Methanol	30.0	3000
Pyridine	2.0	200
Toluene	8.9	890

PDE= Permitted Daily Exposure

Class III Solvents (Solvents with low toxic potential) [21]

Solvents included in this class may be regarded as less toxic and have the low risk to human health and as some are given in table 4.

Class IV Solvents (Solvents for which no adequate toxicological data was found)

Some solvents may also be of interest to manufacturers of excipients, drug substances, or drug products for example Petroleum ether, isopropyl ether. However, no adequate toxicological data on which to base a PDE was found[21].

Table 5 **Class III solvents which should be limited by GMP or other quality based requirements**[21]

Acetic acid	Heptane
Acetone	Isobutyl acetate
1-Butanol	Isopropyl acetate
2-Butanol	Methyl acetate
Butyl acetate	3-Methyl-1-Butanol
Dimethylsulfoxide	Pentane
Ethanol	1-Pentanol
Ethylacetate	1-Propanol
Ethyl ether	2-Propanol
Formic acid	Propyl acetate

PHARMACOLOGY OF HYPERTENSION

Hypertension is generally known as high blood pressure, i.e. evaluation of the arterial blood pressure above the normal range expected in a particular age group. Hypertension may be of unknown cause (essential hypertension or hyperpiesia). It may also result from kidney disease, including narrowing (stenosis) of the renal artery (renal hypertension), endocrine diseases (such as Cushing's diseases or pheaochromocytoma) or disease of the arteries (such as coarctation of aorta), when it is known as secondary hypertension or symptomatic hypertension.

$$BP = CO \times PVR \text{ (Cardiac Output} \times \text{Peripheral Vascular Resistance) and } MAP = CO \times TPR$$

$$MAP = \text{Mean Arterial Pressure} = (SBP - DBP) / 3 + DBP \quad CO = \text{Cardiac Output}$$
$$TPR = \text{Total Peripheral Resistance.}$$

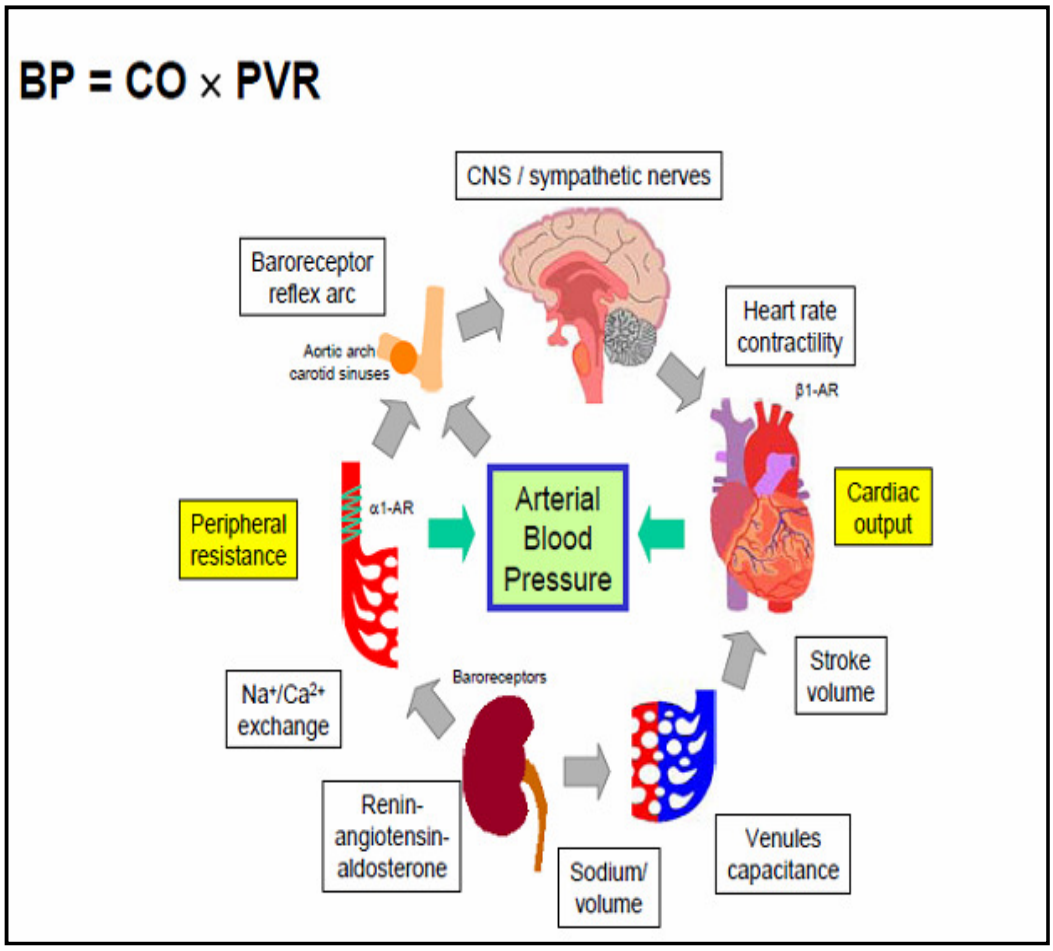


Fig.3: Schematic depiction of Pharmacology of Hypertension Processes

DRUGS AND MANAGEMENT OF HYPERTENSION

Classification and management of BP for adults

Category	Systolic		Diastolic	Lifestyle modification	Initial drug therapy
Normal	< 120	and	< 80	Encourage	Not needed
Pre-hypertension	120-139	or	80-89	Yes	No, or treat Compelling indications
Stage 1 hypertension	140-159	or	90-99	Yes	Diuretic, ACEI, ARB, β-blocker, CCB, Combination; + compelling indications
Stage 2 hypertension	≥ 160	or	≥ 100	Yes	Two-drug combo (diuretic and ACEI, or ARB or β-blocker or CCB; Also treat compelling indications

Based on 7th Report of the Joint National Committee on Detection, Evaluation, and Treatment of High BP (JNC 7)

Diuretic here means thiazide-type; ACEI, ACE inhibitor; ARB, angiotensin receptor blocker; β-blocker, β-adrenergic receptor blocker; CCB, calcium channel blocker

Table .6 **Classification and Management of BP for Adults.**

Categories of drugs

a. Diuretics
   i. Thiazides - Hydrochlorothiazide, chlorthalidone, Indapamide.,
   ii. High ceiling diuretics - Furosemide.
   iii. Potassium sparing - Spironolactone, triamterene, amiloride.

b. Angiotensin Converting Enzyme (ACE) inhibitors
   i. Captopril
   ii. Enalapril
   iii. Lisinopril
   iv. Perindopril
   v. Ramipril.

c. Angiotensin Receptor Blockers
    i. Losartan
    ii. Candesartan
    iii. Irbesartan.
d. Calcium Channel Blockers
    i. Verapamil
    ii. Diltiazem
    iii. Lacidipine
    iv. Felodipine
    v. Amlodipine
    vi. Nifedipine.
e. β Adrenergic Blockers
    i. Propranolol,
    ii. Metaprolol,
    iii. Atenolol.

Works done so far to improve solubility of Hydrochlorothiazide

1. Solid dispersions of hydrochlorothiazide in mannitol and in dihydroxypropyltheophylline were prepared by melting and solvent methods. (Abdel-Fattah et al., 1986)[50].
2. Binding of hydrochlorothiazide to milk improves its solubility. (Antimisiaris et al., 1989)[51].
3. Influence of Sodium Caseinate on the Dissolution Rate of Hydrochlorothiazide and Chlorothiazide. (Millar.F.c et al., 1991)[52].
4. Formulation and production of rapidly disintegrating tablets by lyophilisation using hydrochlorothiazide as a model drug. (Jean Paul Remon et al., 1997)[53].
5. Bioavailability of hydrochlorothiazide pellets made by extrution, Spheronisation, containing polyethelene glycol 400 as a dissolution enhancer. (Christvervaet et al., 1997)[54].
6. Valsartan and Hydrochlorothiazide alone and in Combination on Blood Pressure and Heart Rate in Conscious-Telemetered Spontaneously Hypertensive Rats (SHR) (Randy L.Webb et al., 1997)[55].

7. Solubility of chlorothiazide was improved in the presence of gelatin. (Antimisiaris et al., 2001)[56].
8. Direct moulding of isomalt co-extruded with either paracetamol or Hydrochlorothaizide has shown improved dissolution rate of Hydrochlorothiazide. (Ndindayino et al., 2001)[57].
9. Liquisolid formulations of hydrochlorothiazide tablets showed significantly greater extent absorption and bioavailability than the commercial tablets which was evaluated in beagle dogs (Khaled et al., 2001)[58].
10. Enhancing dissolution of hydrochlorothiazide using superdisintegrants (Sodium starch glycolate, Croscarmellose sodium) by direct compression. (Zhao et al., 2005)[59].
11. A novel drug-drug solid dispersion of hydrochlorothiazide-losartan potassium (N.N Rajendran., et al 2010)[60].
12. Micelles from PEO–PPO–PEO block copolymers as nanocontainers for solubilization of a poorly water soluble drug hydrochlorothiazide.(Yogesh Kadam et al.,2010)[61].
13. Pharmaceutical composition of Hydrochlorothiazide and β-cyclodextrin was prepared by spray-drying, freeze-drying and fluid bed drying to improve water solubility and bioavailability of hydrochlorothiazide. (Maria Arlete Silva Pires et al., 2011)[62].
14. Preparation of microparticles of hydrochlorothiazide by spray drying. (R.M. Martins et al., 2011)[63].
15. Solid dispersion of Hydrochlorothiazide – Captopril has improved bioavailability of hydrochlorothiazide by wet granulation method. (Padmapriya et al., 2011)[64].
16. Pellets made with co-processed MCC-Eudragit_ E incorporating the higher proportion of sorbitol (50%) show a very high dissolution rate of hydrochlorothiazide (HCT) and undergo rapid disintegration in the dissolution medium. (Ramón Martínez-Pacheco et al., 2011)[65].

BACKGROUND OF THE STUDY:

Literature survey indicates that over the years solid dispersion approach has been applied to poorly soluble drugs using physiologically inert carriers with a view to improve dissolution and bioavailability of these drugs. In recent past a novel drug-drug solid dispersion of Hydrochlorothiazide – Losartan potassium has been reported and improved dissolution of poorly soluble hydrochlorothiazide has also been documented. An improved pharmacodynamic activity of Hydrochlorothiazide, through this novel solid dispersion approach has also been established (unpublished data). Many approaches have been followed earlier to improve solubility (or) dissolution of Hydrochlorothiazide[50-65]. Pharmacokinetic profile of this novel solid dispersion of Hydrochlorothiazide - Losartan potassium will help understand its influence on improved dissolution and pharmacokinetic property and therefore, the present study was directed to investigate the pharmacokinetics of Hydrochlorothiazide as compared to physical mixture and corresponding commercial tablet dosage form.

3. AIM AND OBJECTIVE

AIM:

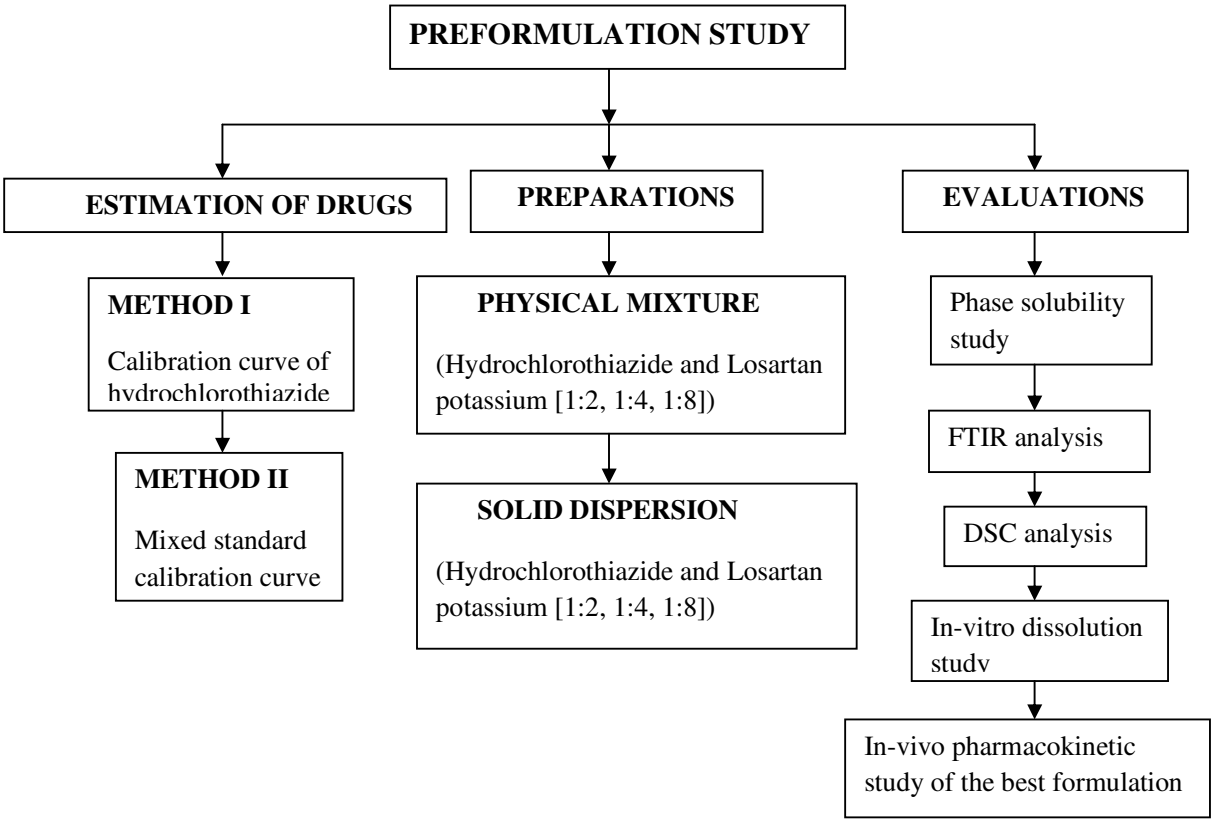
To investigate In-vitro dissolution and In-vivo bioavailability of a novel drug– drug solid dispersion of Hydrochlorothiazide – Losartan potassium.

OBJECTIVE

The following parameters were estimated

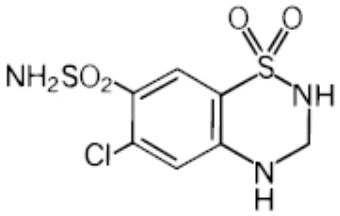
- ✓ Preparation of physical mixtures (1:2, 1:4, 1:8 ratios).
- ✓ Preparation of solid dispersions (1:2, 1:4, 1:8 ratios).
- ✓ Phase solubility study of Hydrochlorothiazide - Losartan potassium in deaerated water.
- ✓ FTIR analysis of pure drugs, physical mixtures and solid dispersions.
- ✓ DSC of pure drugs, physical mixtures and solid dispersions.
- ✓ In-vitro dissolution of pure drugs, physical mixtures and solid dispersions.
- ✓ In-vivo pharmacokinetic study of Hydrochlorothiazide from best In-vitro formulation.

4. PLAN OF WORK



5. DRUG PROFILES

HYDROCHLOROTHIAZIDE



$C_7H_8C_lN_3O_4S_2$ Molecular weight .297.73

Hydrochlorothiazide is 6 chloro,-3, 4-dihydro-2, 4-1, 2, 4-benzothiazide-7-sulfonamide1, 1 dioxide.

Category : Diuretic

Description : white, crystalline powder odorless.

Solubility : soluble in acetone, methanol, and dimethyl formamide, Sparingly soluble in ethanol, very slightly soluble in water, insoluble in ether and in chloroform.

Pharmacology

Hydrochlorothiazide is a thiazide type diuretic. This group of diuretic agent is actively transported by a probenecid-sensitive secretory mechanism in to the proximal tubule. They act on the luminal membrane of the cortical diluting segments of the distal convoluting tubule and inhibit specific Na^+ and Cl^- reabsorption. A 0.3% of diuretic action to be the result of carbonic anhydrase inhibition in the proximal tubule, resulting in decreased Na^+ and $HCO3^-$ reabsorption. The antihypertensive effect of diuretic is by modulating the activity of K^+ channels. ATP regulated K^+ channels in resistance arterioles may be activated by thiazide. This action leads to membrane hyper polarization, which opposes the smooth muscle Ca^{2+} entry and contraction and reduces peripheral vascular resistance.The other mechanism in reducing blood pressure is by contraction of extra cellular volume (ECV), reduced cardiac outputs and peripheral vascular resistance. Hydrochlorothiazide Also

increases $HCO3^-$, $PO4^-$,Mg^{2+} excretion (proximal tubule)and Na^+,Cl^-,$2K^+$ excretion(distal tubule). By contrast, calcium excretion is decreased with increase in plasma urate concentration through an enhanced reabsorption of urate in the proximal tubule and decreased excretion of urate by the tubular cells.

Pharmacokinetics

Following an oral dose little is absorbed from the stomach. Food and the volume of fluid do not affect absorption. Plasma protein binding of Hydrochlorothiazide is 40-60%. It is highly concentrated in erythrocytes as a result of binding to carbonic anhydrase. Peak plasma concentration is attained after 2-4 hrs of dosing. Volume of distribution is 0.83-301 lit/kg. Onset of diuresis occurs with in 2hrs of administration and lasts 6-12 hrs. Onset of antihypertensive effect is over 3-4 days. It is not metabolized in man and is excreted unchanged almost entirely 95% in the urine. It crosses the placental barrier and is excreted in breast milk.

Uses and administration

1. Edema
2. Control of hypertension
3. Management of diabetic insipidus.
4. Management of proximal renal tubular acidosis.
5. Idiopathic hypercalcaemia of calcium nephrolithiasis.

Contraindications

1. Anuria
2. Renal impairment
3. Diabetes mellitus
4. Hyperlipidema
5. Gout
6. Systemic lupus erythematous
7. Electrolyte imbalance.

Adverse reactions

1. Hyponatremia
2. Hypokalemia
3. Acute saline depletion
4. GIT-disturbances
5. Hearing loss
6. Allergic manifestations
7. Hyperuricaemia
8. Hyperglycemia
9. Hypercalcaemia
10. Hyperlipidemia
11. Magnesium depletion.

Hydrochlorothiazide available in combination with various antihypertensive agents namely captopril, enalapril, lisopril, timolol, sotalol, metaprolol tartrate, propranolol HCl.

Diuretics In Hypertension

The initial reduction in blood pressure during the first weeks of diuretic therapy is due to volume depletion. During this period, cardiac output falls, because of venous return. Total peripheral resistance is unchanged and plasma rennin activity increase. The long term antihypertensive effect of diuretic is the result of diminished vascular responsiveness to sympathetic nervous stimulation. Aoki and broadi (1969) demonstrated that antihypertensive effect of diuretics is mainly due to direct action on the resistance and capatacitance vessels. Diuretics produce antihypertensive effects by modulating the activity of K^+ channels. ATP regulated K^+ channels in resistance arterioles may be activated by thaizides. This action leads to membrane hyper polarization, which opposes smooth muscle Ca^{2+} entry and contraction and reduces peripheral vascular resistance.

Changes in extra cellular and intracellular electrolyte relationship resulting in diminished arteriolar tonicity reflected as reduction in TPR.

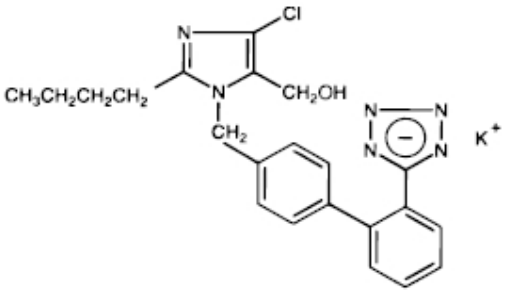
Green et al., (1961) demonstrated that a decrease in total circulation plasma volume due to thaizide diuretic resulted in diminished cardiac filling and reduced cardiac output.

Feisal (1961) indicated that decrease in BP was due to decreased response to endogenous vasopressor mechanism and by vasodilatation.

Table.7 **Marketed preparations of Hydrochlorothiazide with losartan potassium**

Zaart H	Cipla	Hy Hydrochlorothiazide 25 mg Los Losartan potassium 50mg
Losar H	Unichem	Hydrochlorothiazide 12.5 mg Losartan potassium 50mg
Covance	Ranboxy	Hydrochlorothiazide 25 mg Losartan potassium 50mg.

LOSARTAN POTASSIUM



$C_{22}H_{22}C_lKN_6O$ Molecular weight .461.01

2-butyl-4-chloro-1-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1*H*-imidazole-5-methanol monopotassium salt.

Category : Antihypertensive

Description : White to off-white free-flowing crystalline powder

Solubility : freely soluble in water, soluble in alcohols, and slightly Soluble in organic solvents, such as acetonitrile and methyl ethyl ketone.

Pharmacology

Angiotensin II [formed from angiotensin I in a reaction catalyzed by angiotensin converting enzyme (ACE, kininase II)], is a potent vasoconstrictor, the primary vasoactive hormone of the renin-angiotensin system and an important component in the pathophysiology of hypertension. It also stimulates aldosterone secretion by the adrenal cortex. Losartan and its principal active metabolite block the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor found in many tissues, (e.g., vascular smooth muscle, adrenal gland). There is also an AT2 receptor found in many tissues but it is not known to be associated with cardiovascular homeostasis. Both losartan and its principal active metabolite do not exhibit any partial agonist activity at the AT1 receptor and have much greater affinity (about 1000-fold) for the AT1 receptor than for the AT2 receptor. In vitro binding studies indicate that losartan is a reversible, competitive inhibitor of the AT1 receptor. The active metabolite is 10 to 40 times more potent by weight than losartan and appears to be a reversible, non-competitive inhibitor of the AT1 receptor.

Neither losartan nor its active metabolite inhibits ACE (kininase II, the enzyme that converts angiotensin I to Angiotensin II and degrades Bradykinin); nor do they bind to or block other hormone receptors or ion channels known to be important in cardiovascular regulation.

Pharmacokinetics

Losartan is an orally active agent that undergoes substantial first-pass metabolism by cytochrome P450 enzymes. It is converted, in part, to an active carboxylic acid metabolite that is responsible for most of the angiotensin II receptor

antagonism that follows losartan treatment. Losartan metabolites have been identified in human plasma and urine. In addition to the active carboxylic acid metabolite, several inactive metabolites are formed. Following oral and intravenous administration of 14C-labeled losartan potassium, circulating plasma radioactivity is primarily attributed to losartan and its active metabolite. In vitro studies indicate that cytochrome P450 2C9 and 3A4 are involved in the biotransformation of losartan to its metabolites. Minimal conversion of losartan to the active metabolite (less than 1% of the dose compared to 14% of the dose in normal subjects) was seen in about one percent of individuals studied.

The terminal half-life of losartan is about 2 hours and of the metabolite is about 6-9 hours.

The pharmacokinetics of losartan and its active metabolite are linear with oral losartan doses up to 200 mg and do not change over time. Neither losartan nor its metabolite accumulates in plasma upon repeated once-daily dosing.

Following oral administration, losartan is well absorbed (based on absorption of radio labeled losartan) and undergoes substantial first-pass metabolism; the systemic bioavailability of losartan is approximately 33%. About 14% of an orally-administered dose of losartan is converted to the active metabolite. Mean peak concentrations of losartan and its active metabolite are reached in 1 hour and in 3-4 hours, respectively. While maximum plasma concentrations of losartan and its active metabolite are approximately equal, the AUC of the metabolite is about 4 times as great as that of losartan. A meal slows absorption of losartan and decreases its Cmax but has only minor effects on losartan AUC or on the AUC of the metabolite (about 10% decreased).

The pharmacokinetics of losartan and its active metabolite were also determined after IV doses of each component separately in healthy volunteers. The volume of distribution of losartan and the active metabolite is about 34 liters and 12 liters, respectively. Total plasma clearance of losartan and the active metabolite is about 600 mL/min and 50 mL/min, respectively, with renal clearance of about

75 mL/min and 25 mL/min, respectively. After single doses of losartan administered orally, about 4% of the dose is excreted unchanged in the urine and about 6% is excreted in urine as active metabolite. Biliary excretion contributes to the elimination of losartan and its metabolites. Following oral 14C-labeled losartan, about 35% of radioactivity is recovered in the urine and about 60% in the feces. Following an intravenous dose of 14C-labeled losartan, about 45% of radioactivity is recovered in the urine and 50% in the feces.

Both losartan and its active metabolite are highly bound to plasma proteins, primarily albumin, with plasma free fractions of 1.3% and 0.2%, respectively. Plasma protein binding is constant over the concentration range achieved with recommended doses.

Indications and Usage

Treatment of hypertension; nephropathy in type II diabetic patients; reduce risk of stroke in patients with hypertension and left ventricular hypertrophy.

Dosage and Administration

Hypertension
Adults Initial dose

PO 50 mg/day; 25 mg/day if volume depleted or history of hepatic impairment.

Maintenance dose

PO 25 to 100 mg/day.

Children 6 yr of age and older Initial dose

PO 0.7 mg/kg (max, 50 mg) once daily.

Maintenance dose

PO 0.7 to 1.4 mg/kg/day (max, 100 mg).

Nephropathy in Type 2 Diabetes Adults Initial dose

PO 50 mg/day; the dose may be increased to 100 mg/day based on BP response.

Drug Interactions

Losartan, administered for 12 days, did not affect the pharmacokinetics or Pharmacodynamics of a single dose of Warfarin. Losartan did not affect the pharmacokinetics of oral or intravenous Digoxin. There is no Pharmacokinetic interaction between Losartan and Hydrochlorothiazide. Co-administration of Losartan and Cimetidine led to an increase of about 18% in AUC of losartan but did not affect the pharmacokinetics of its active metabolite. Co-administration of losartan and Phenobarbital led to a reduction of about 20% in the AUC of losartan and that of its active metabolite. A somewhat greater interaction (approximately 40% reduction in the AUC of active metabolite and approximately 30% reduction in the AUC of losartan) has been reported with Rifampin. Fluconazole, an inhibitor of cytochrome P450 2C9, decreased the AUC of the active metabolite by approximately 40%, but increased the AUC of losartan by approximately 70% following multiple doses. Conversion of losartan to its active metabolite after intravenous administration is not affected by Ketoconazole, an inhibitor of P450 3A4. The AUC of active metabolite following oral losartan was not affected by erythromycin, another inhibitor of P450 3A4, but the AUC of losartan was increased by 30%.

Contraindications

Losartan is contraindicated in patients who are hypersensitive to any component of this product.

Adverse reactions

- Fetal/Neonatal Morbidity and Mortality.
- Hypotension — Volume-Depleted Patients.
- Hypertensive Patients with Left Ventricular Hypertrophy.
- Nephropathy in Type 2 Diabetic Patients.

The following additional adverse reactions have been reported in post marketing experience:

- Digestive: Hepatitis (reported rarely).
- Hemic: Thrombocytopenia (reported rarely).
- Hypersensitivity: Angioedema.
- Metabolic and Nutrition: Hyperkalemia, Hyponatremia.
- Musculoskeletal: Rare cases of Rhabdomyolysis.
- Nervous system disorders: Dysgeusia.
- Skin: Erythroderma.
- Respiratory: Dry cough.

Table.8 **Commercial products**

Losartas		-50mg	- Tab
Covance		-50mg	- Tab
Zaart	-	50mg	- Tab

6. MATERIALS AND METHODS

Table.9

S.no	Drugs and chemicals	Manufactures/suppliers
1.	Hydrochlorothiazide USP	Medrich Pvt. Ltd.
2.	Losartan potassium USP	Medrich Pvt. Ltd.
3.	Methanol	Loba Chemie Pvt.Ltd.
4.	Tragacanth	Loba Chemie Pvt.Ltd.
5.	Alcohol	Loba Chemie Pvt.Ltd.

S.no	Equipments	Manufactures/suppliers
1.	UV – Spectrophotometer	Perkin Elmer
2.	Infra-Red Spectrophotometer	Perkin Elmer spectrum RX1 FT-IR
3.	Differential Scanning Calorimetry	Schimadzu, DSC 60.
4.	Weighing Balance	Schimadzu
5.	Dissolution Apparatus (USP)	Veego

Table.10

7. METHODOLOGY

7.1 ESTIMATION OF PURE DRUGS, PHYSICAL MIXTURES AND SOLID DISPERSIONS

Method I

Pure sample of Losartan and potassium were analyzed by Spectrophotometric method as described by M.Gandhimathi et.al. [66]

Preparation of Standard Solutions

Standard solution of 100mg each of Losartan potassium and Hydrochlorothiazide were made using methanol.

Mixed Standards

100mg of Losartan potassium and Hydrochlorothiazide standard solution were used in the mixed standards. The concentrations of the two components in the mixed standards are reported. All the mixed standard solutions were scanned in the range of 200-400 nm using the sample points 236 for Losartan potassium and 270nm for Hydrochlorothiazide. A standard curve was constructed by plotting the absorbance vs. concentration of the drug taken.

Method II

This method was adapted to pure Hydrochlorothiazide.100mg Hydrochlorothiazide was accurately weighed and dissolved in methanol in standard flask and diluted to 100ml with methanol. Further, dilutions were made to get 2, 4, 6, 8, 10µg/ml HCT and this solution was scanned at 270nm to obtain absorbance. Standard curve was constructed by plotting absorbance vs concentration of drug

7.2 PREPARATION OF PHYSICAL MIXTURE AND SOLID DISPERSION:

Preparation of physical mixture

Hydrochlorothiazide and Losartan potassium were accurately weighed at the ratio of 1:2, 1:4; 1:8 (12.5: 25mg, 12.5: 50mg, 12.5: 100mg) pulverized, and then

mixed thoroughly in a glass mortar with pestle until homogenous. The mixtures were passed through a 250µm sieve for further experiment.

Preparation of solid dispersion

Solid dispersion of Hydrochlorothiazide and Losartan potassium at three ratios of 1:2, 1:4, 1:8 (12.5:25mg, 12.5:50mg, 12.5:100mg) was prepared by solvent method. Hydrochlorothiazide and Losartan potassium were dissolved in methanol and mixed with magnetic stirring. Solvent was evaporated at reduced pressure a 40^{o}C in a rotatory evaporation apparatus. Subsequently solid dispersion was stored vaccum over silica gel for 12hrs at room temperature. After dried solid dispersion was passed through a 250µm sieve. Sample was stored in a desiccator and used for further investigation.

Table 11 **Formulation of physical mixtures and solid dispersions of Hydrochlorothiazide - Losartan potassium.**

S.No	Physical mixture (HTC:LSP)		Solid dispersion (HTC:LSP)	
1.	1:2	F1PM	1:2	F1SD
2.	1:4	F2PM	1:4	F2SD
3.	1:8	F3PM	1:8	F3SD

7.3Evaluation of formulations

The prepared formulations of solid dispersion and physical mixture were evaluated for the following

a. Physico-chemical characterization.
b. In vitro dissolution study.
c. In vivo pharmacokinetic study.

7.3.1 PHYSICO CHEMICAL CHARACTERIZATION:

Before formulation of drug substances into a dosage form, it is essential that drug polymer should be chemically and physically characterized. Preformulation studies give the information needed to define the nature of the drug substance and

provide a framework for the drug combination with pharmaceutical carrier in the fabrication of a dosage form.

Compatibility study:

One of the requirements for the selection of suitable excipients or carrier for pharmaceutical formulation is its compatibility. Therefore in the present work the compatibility study was carried out by using FTIR spectrophotometer to find out any possible chemical interaction of Hydrochlorothiazide and Losartan potassium in physical mixture and solid dispersion.

Compatibility between the drugs was determined by using Perkin Elmer spectrum RX1 FT-IR spectrometer model. The pellets were prepared at high compaction pressure by using KBr and the ratio of sample to KBr is 1:100.The pellets thus prepared were examined and the spectra of drug and other ingredients in the formulations were compared with that of the spectra of pure drugs.

7.3.2 Determination of phase solubility of Hydrochlorothiazide / Losartan potassium in Deaerated water.

Excessive amount of pure Hydrochlorothiazide (250mg) was added to 100mL of Deaerated Water containing varying concentrations of Losartan potassium (0025%, 0.005%, 0.01% w/v) in stoppered flasks. These suspensions were equilibrated by intermittent shaking for 72hrs maintained at 37±2^{o}C. These suspensions were filtered through a Whatman filter. The concentration of Hydrochlorothiazide was determined by method I described earlier and reported.

7.3.3 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) curve of Hydrochlorothiazide, Losartan potassium, physical mixtures and solid dispersions were measured with a DSC instrument (Mettler Tolero Model). The samples were accurately weighed and heated in closed aluminum crimped cells at a rate of 10^{o}C.min^{-1} between 30 and 300^{o}C temperature under a nitrogen gas flow of 40mL.min^{-1}during study.

7.3.4 Invitro Dissolution study

Dissolution profile of pure Hydrochlorothiazide, physical mixture and solid dispersion were evaluated according to the method described in USFDA. 900mL of the dissolution medium (Deaerated water) was placed in the vessel and temperature of medium was maintained at 37±0.5^{o}C. The sample was placed in the medium and the dissolution was performed at 100rpm. 10 ml samples were withdrawn at 10, 20, 30, 40……. .90 mins and equivalent amount of dissolution medium was added to maintain the sink condition. Samples were filtered (0.45 µm pore size) and analyzed by spectrophotometry at 270nm for HTC and 236nm for LSP. The experiments were carried out in triplicate and mean values and SD were recorded.

Kinetic analysis of in-vitro release rates of solid dispersion of Hydrochlorothiazide and Losartan potassium

The results of in-vitro release profile obtained for solid dispersion tablets were plotted in modes of data treatment as follows:

1. Zero – order kinetic model - cumulative % drug released versus time.

2. First – order kinetic model – log cumulative %drug remaining versus time.

a) Zero order kinetics

Zero order release would be predicted by following equation.

$$A_t = A_0 - K_0 t$$

Where,

A_t - Drug release at time 't'

A_0 - Initial drug concentration

K_0 - Zero-order constant (hr^{-1})

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero order kinetics, with a slope equal to K_0.

b) First order kinetics:

First order release would be predicted by the following equation:

$$\text{Log}C = \frac{\text{Log}\,C_0 - Kt}{2.303}$$

Where,

C = Amount of drug remained at time 't'

C = Initial amount of drug

K = First order rate constant (hr^{-1})

When the data plotted as Log cumulative percent drug remaining versus time yields a straight line, indicating that the release follow first order kinetics. The constant 'K' can be obtained by multiplying 2.303 with slope values.

7.3.5 In vivo Pharmacokinetic Study

The pharmacokinetic study was performed on physical mixture, solid dispersion and commercial product, all in 1:4 ratio of HTC and LSP.

Animals

New Zealand white rabbits weighing 1.5 to 2.5kg were obtained from the animal house of Swamy Vivekananda College of Pharmacy. The animals were fed with cabbage and water. They were maintained in standard laboratory conditions 21±2 ^{0}C and relative humidity of 55-60%. The animals were overnight fasted before the experiment. The study protocol was approved by the Institutional Animal Ethical Committee and the protocol number is SVCP/IAEC/M.Pharm/03/2011.

Drugs

Losartan potassium and Hydroclorothiazide pure gift samples were obtained from Medrich Company Pvt. Ltd**,** Banglore, Karnataka, India.

Chemicals

Tragacanth from Loba Cheme Pvt Ltd, Mumbai.

95% v/v alcohol

Requirements

- Cotton
- Surgical blade
- 26G needle
- Blood collecting tubes (EDTA tubes)
- Plasma sample collecting tubes (eppendrof's tube)

Sex: Both Male/Female

No. of animals: 9

Animal dose:

Hydrochlorothiazide: Losartan potassium: 0.892: 3.5 mg/kg p.o (1:4ratio)

Procedure for collection of blood:[67]

The animal was placed in a restrainer. Hair of the ear was shaved smoothly with blade without disturbing the blood vessels. Ear was cleaned with 95% v/v alcohol on the collection site and rapid rubbing on the ear to dilate blood vessels which is easy to collect the blood. 2G needle was used to collect the blood from marginal ear vein. After collecting blood, clean sterile cotton was kept on the collection site and finger pressure was applied to stop the bleeding. Blood samples were placed in K3 EDTA tubes and centrifuged at 4000RPM for 30 min. Plasma was separated by using micropipette and placed in epindroff tubes. Separated plasma samples were stored in refrigeration at -4ºc and analysed for HPLC.`

Experimental procedure: Rabbits were classified into 3 different groups each group consisting of 9 animals

Group I Physical mixture (Hydrochlorothiazide: Losartan potassium) (1:4) P.o.

Group II Solid dispersion (Hydrochlorothiazide: Losartan potassium) (1:4) P.o.

Group III Commercial product (Hydrochlorothiazide: Losartan potassium) (1:4) P.o.

Each group received Physical mixture or solid dispersion or commercial product suspended in distilled water using 1% tragacanth as a suspending agent. The suspension was administered P.o through an intragastric tube. Blood samples (1 ml) were collected in heparinized tubes from the marginal ear vein at 0, 0.5, 1, 1.5, 2, 4 and 8 hrs after drug administration and plasma was separated by using centrifugation at 4000 rpm and stored at -70°C. Samples were analysed by validated high performance liquid chromatography (HPLC).[68]

Bioanalytical work

HPLC chromatographic conditions

The HPLC system consisted of a Intersil C_{18} reversed phase analytical column (250mm × 4.6mm, 5µm) a shimadzu model SPD-6AV variable wavelength set as 230nm . The mobile phase consisted of water with 2.5% formic acid, methanol and acetonitrile {40:45:15,v/v/v(%)}. The separation was performed under isocratic conditions with a constant flow rate of 0.7mL/min the injection volume was 10µL.

Standard solutions and calibrators [68]

Stock standard solutions of Losartan potassium (800µg/mL) and Hydrochlorothiazide (400 µg/mL) were freshly prepared in acetonitrile. A series of working standard solution were diluted in acetonitrile to produce eight standard solutions. The calibration standards were prepared by spiking 100µL of each standard solution to a final volume of 10mL plasma.

Extraction of analytes from samples

To plasma aliquot (250µL) in an Eppendrof tube, a 0.5mL acetonitrile was added. The mixture was vortexted and centrifuged (4000rpm for 5min), 250µL aliquot of supernatant was transferred to a glass tube. The mixture was then evaporated to dryness. The residue was reconstituted with 1mL of water ; the mixture was then vortexted , and a 10µL aliquot of the solution was injected into HPLC system.

Pharmacokinetic parameters

The pharmacokinetic parameters were calculated for each rabbit of Group I, Group II, Group III by the semi logarithmic plot of plasma Losartan potassium and Hydrochlorothiazide concentration at various intervals. The following pharmacokinetic parameters were calculated:

1. Elimination rate constant (K_e): The elimination rate constant was determined using the formula

$$K_e = -2.303 \text{ x slope of extrapolated curve}$$

2. Elimination half life ($t_{1/2}$): $t_{1/2}$ was calculated using the formula

$$t_{1/2} = 0.693/K_e$$

3. Absorption rate constant (K_a): This was determined by the method of residuals. The log linear portion of the decline phase was back extrapolated for each curve. The plasma concentration along this extrapolated line was C_0. The observed plasma concentration C was subtracted from the corresponding extrapolated value at each time point. The semi logarithmic plot of residuals (C-C) against time yields a straight line.

$$K_a = -2.303 \text{ x slope of residual line}$$

4. Absorption half life**:** It was calculated using the formula

$$T_{1/2(a)} = 0.693/K_a$$

5. Apparent volume of distribution (V_d): It was calculated using the formula

$$V_d = \frac{K_a F X_0}{(K_a - K_e) \text{ y intercept}}$$

6. Time to C_{max} (t_{max}): t_{max} was calculated using the formula

$$t_{max} = \frac{\ln K_a - \ln K_e}{K_a - K_e}$$

7. Maximum plasma concentration (C_{max}): C_{max} was calculated using the formula

$$C_{max} = \text{Y intercept } (e^{-Ke.\ tmax} - e^{-Ka.\ tmax})$$

8. Area under curve (AUC_{0-12}): AUC_{0-12} was calculated using the formula

$$AUC = \frac{F X_0}{V_d.K_e}$$

9. $AUC_{0-\infty}$ was calculated using the formula

$$AUC_{0-\infty} = \frac{C_0}{K_e}$$

STATISTICAL ANALYSIS:

The values were expressed in mean ± SD and statistically analysed by one way ANOVA followed by Tukey-Kramer multiple comparison tests.

8. RESULTS

COMPATIBILITY STUDY

The FTIR spectra of pure drugs, physical mixture and solid dispersions of Hydrochlorothiazide and Losartan potassium are shown in Tables- 12,13 and Figures 3-10. The spectra exhibited presence of characteristic peaks of drugs in physical mixture and indicate that there was no chemical interaction between the drugs.

Table. 12 **FTIR spectra of hydrochlorothiazide in physical mixtures and solid dispersion**

Characteristics	**Pure HCT**	**F1 PM**	**F2 PM**	**F3 PM**	**F1 SD**	**F2 SD**	**F3 SD**
NH(Stretching)	3362.04-3173.01	3362.04-3174.94	3362.04-3178.79	3360.97-3182.65	3360.11	3358.18	3360.11
NH (bending)	1602.90-1518.03	1606.76-1514	1606.76-1506.46	1608.69-1506.46-	1599-1516	1600.97-1516.10	1600.97-1506.46
S=O(stretching)	1460.16	1460.16	1462.09	1462.09	1462.09	1462.09	1406.16
Aromatic(CH-Stretching)	2947.33	2955.04	2956.97	2956.97	2958.90	2956.97	2956.97
Aromatic(CH-Bending)	858.35-748.41	842.92-763.84	840.99-761.91	840.99-761.91	817.85-759.98	821.70-759.98	879.57-759.98
C-Cl (Stretching)	677.04-607.60	677.04-607.60	675.11-609.53	675.11-609.53	675-603	673.18-605.67	671.25-605.67

Table.13 **FTIR spectra of Losartan potassium in physical mixtures and solid dispersions**

Characteristics	**Pure LSP**	**F1PM**	**F2 PM**	**F3 PM**	**F1 SD**	**F2 SD**	**F3 SD**
NH(Stretching) OH(Stretching	3194.23	3174.94	3178.79	3182.65	3219	3215.44	3223.16
CH3-group CH bending	1425.44	1423.51	1425.44	1425.44	1425.44	1425.44	1423.51
Halogen compounds, C-Cl (Stretching)	761.91	763.84	761.91	761.91	759.98	759.98	759.98
OH primary alcohol, C-N(Stretching)	1259.56	1261.49	1259.56	1259.56	1255.71	1255.70	1255.70
Aromatic ring, C-C multiple bond (Stretching)	1575.89	1541.18	1575.89	1575.89	1562	1566.25	1575.97
Aromatic hydrocarbon chromospheres, CH(Stretching)	2956.97	2955.04	2956.97	2956.97	2958	2956.97	2956.97

Fig.3 **FTIR SPECTRA OF PURE HYDROCHLORTHIAZIDE**

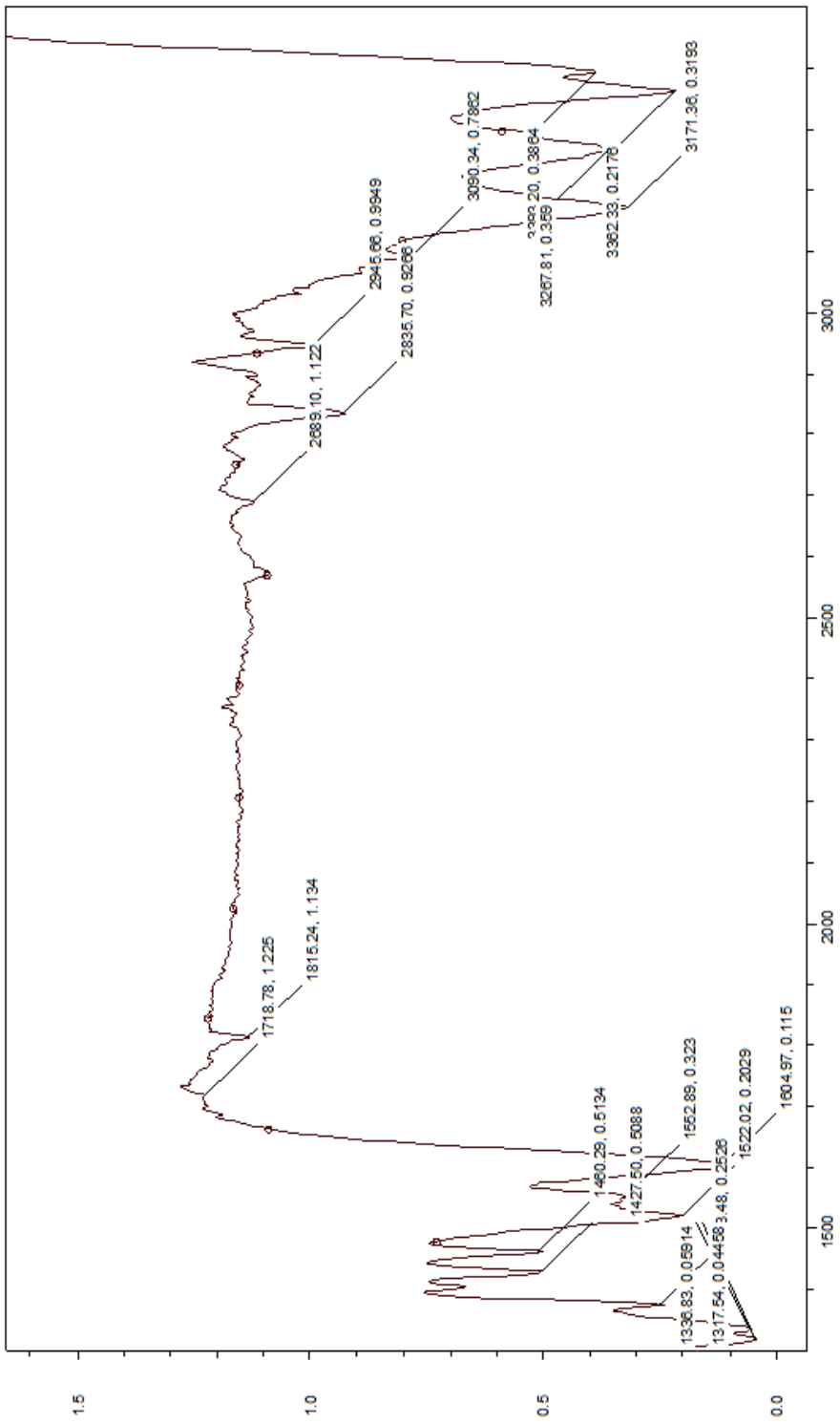


Fig.4 **FTIR SPECTRA OF PURE LOSARTAN POTASSIUM**

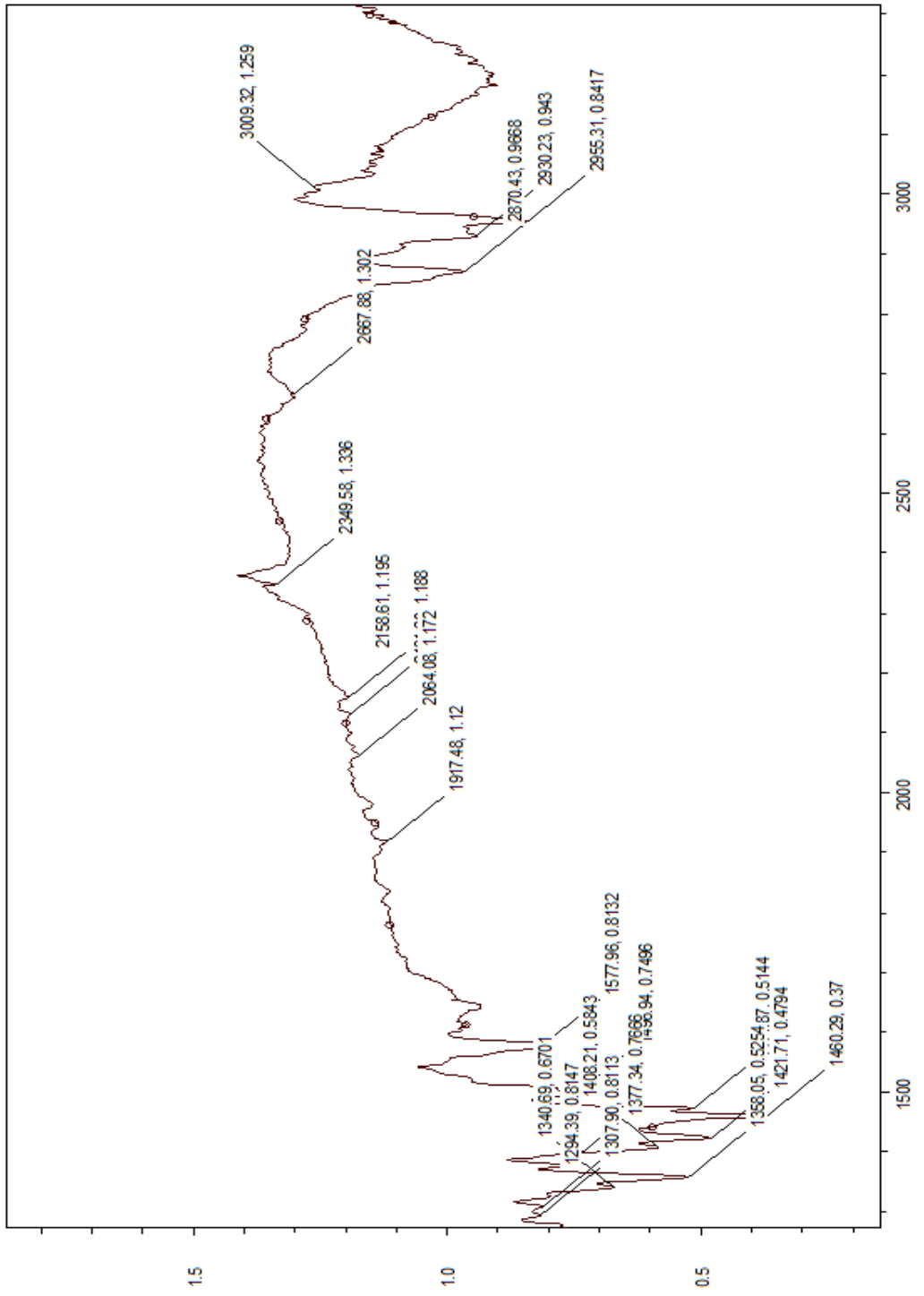


Fig.5 **FTIR SPECTRA OF HCT AND LSP (F1 S.D) (1:2)**

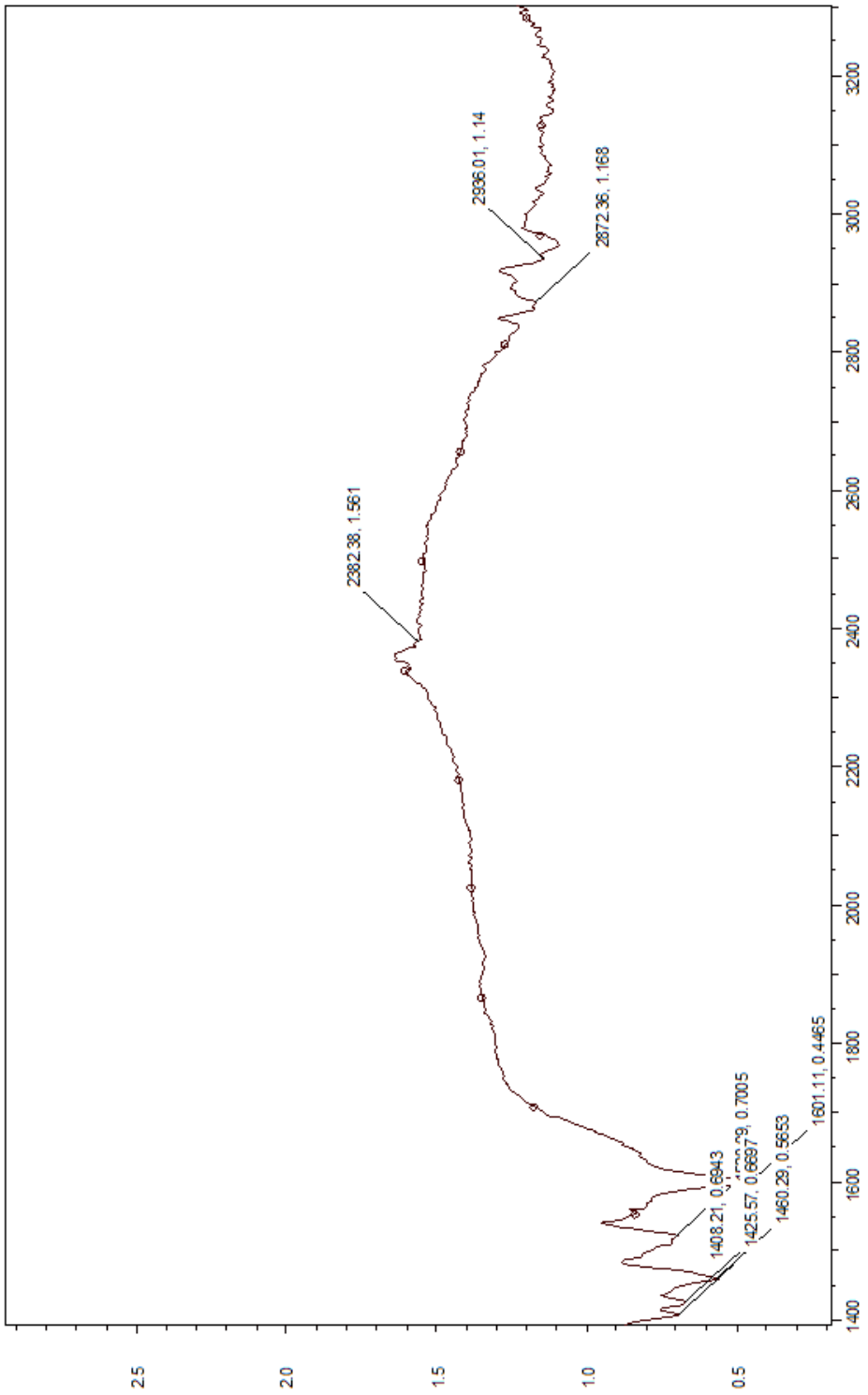


Fig.6 **FTIR SPECTRA OF HCT AND LSP (F2S.D) (1:4)**

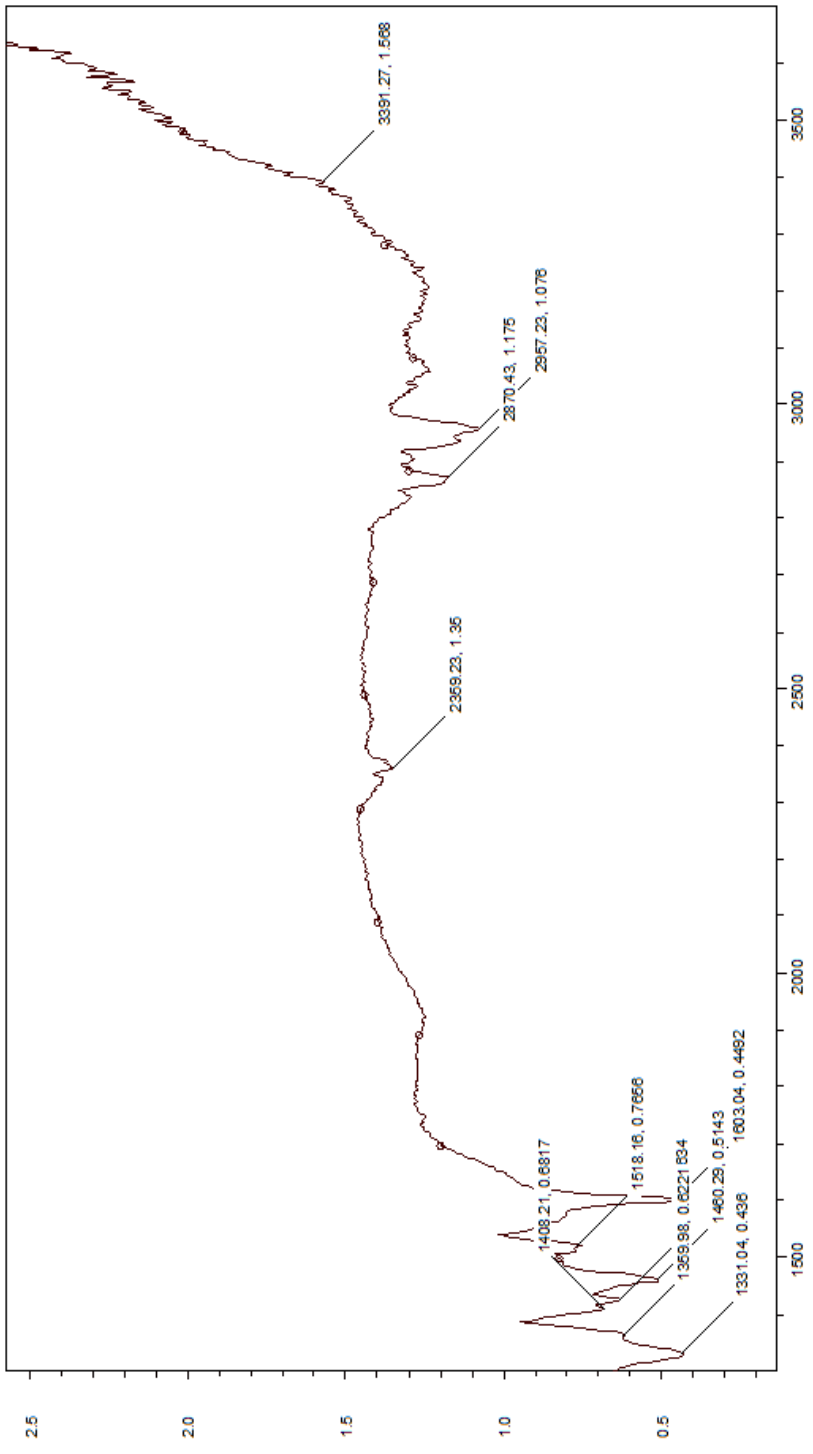


Fig.7 **FTIR SPECTRA OF HCT AND LSP (F3S.D) (1:8)**

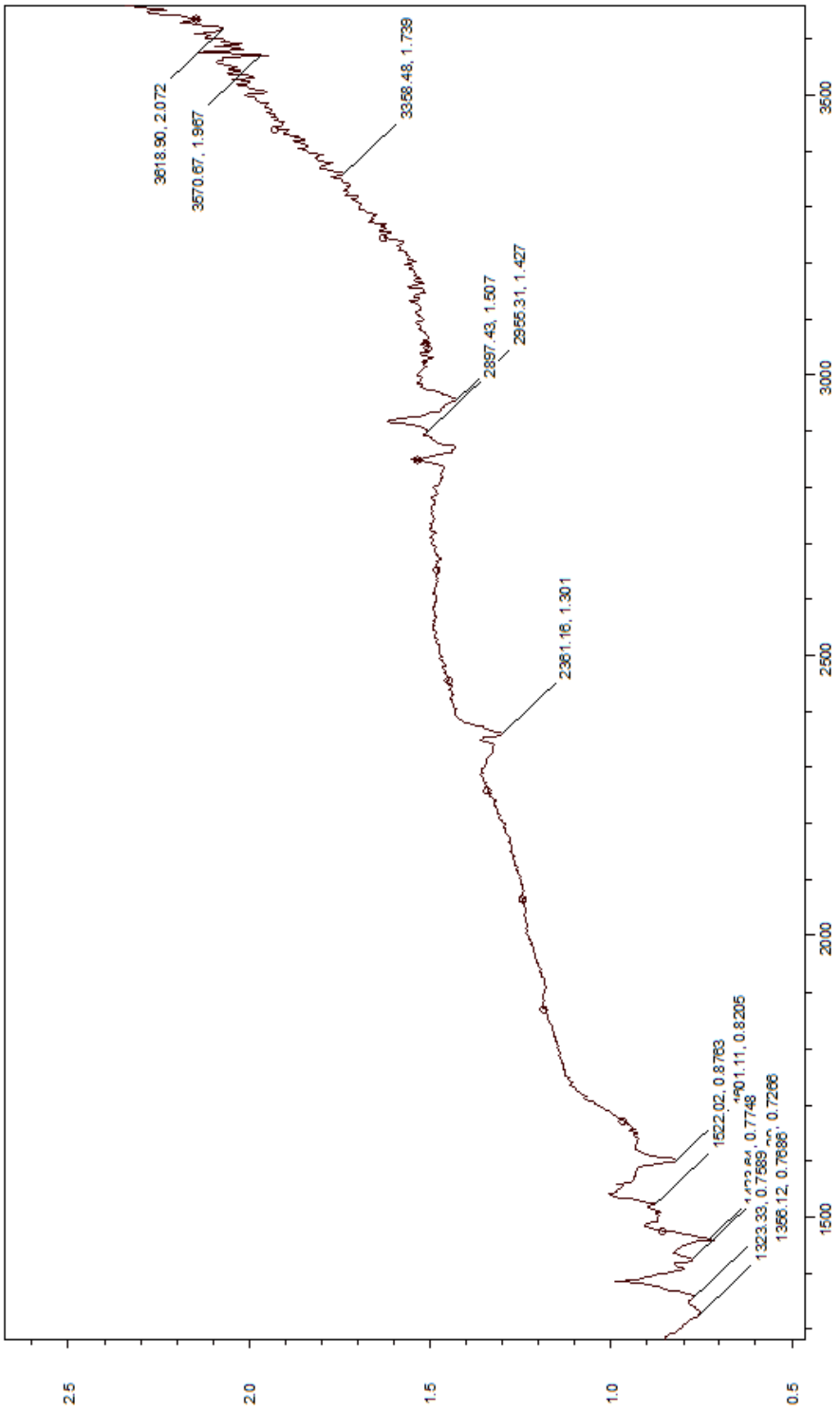


Fig.8 **FTIR SPECTRA OF HCT AND LSP (F1P.M) (1:2)**

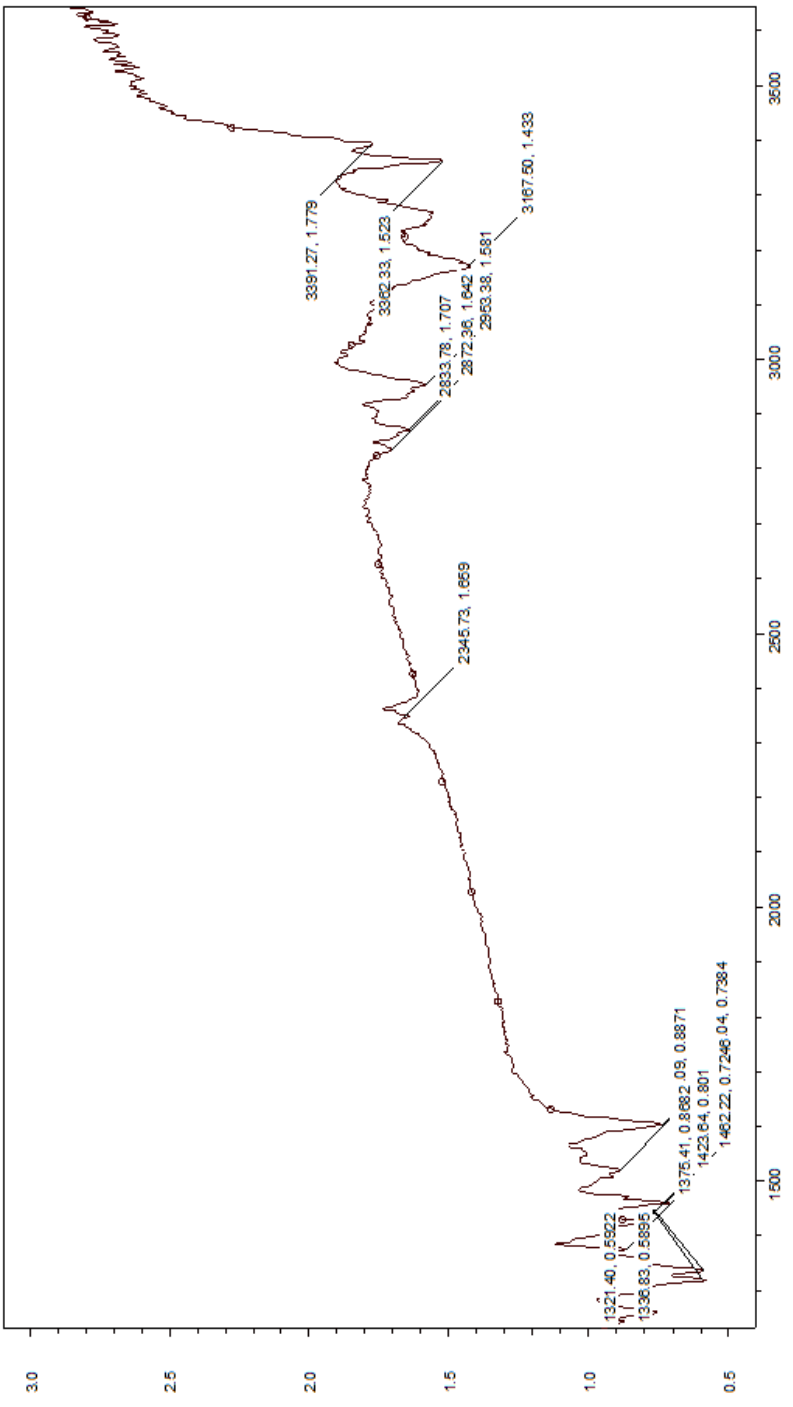


Fig.9 **FTIR SPECTRA OF HCT AND LSP (F2P.M) (1:4)**

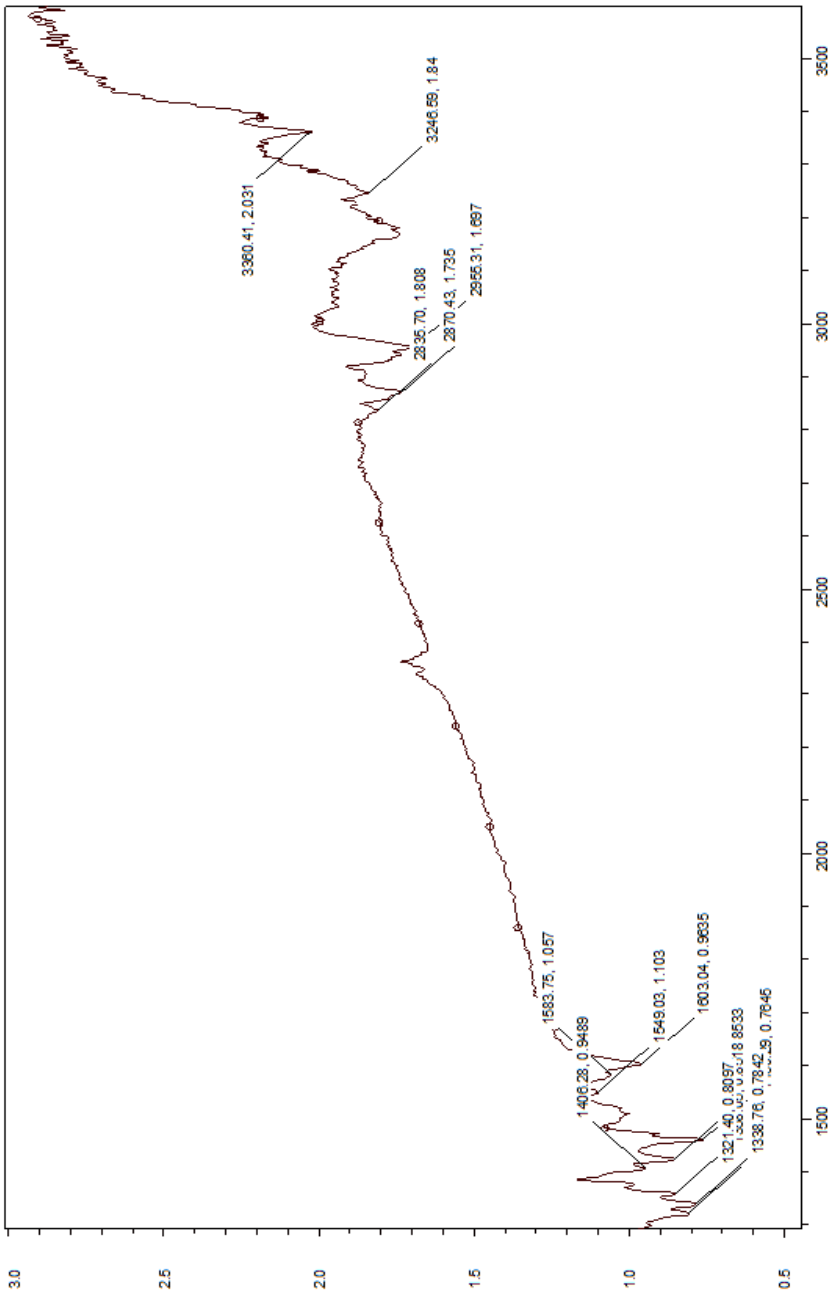
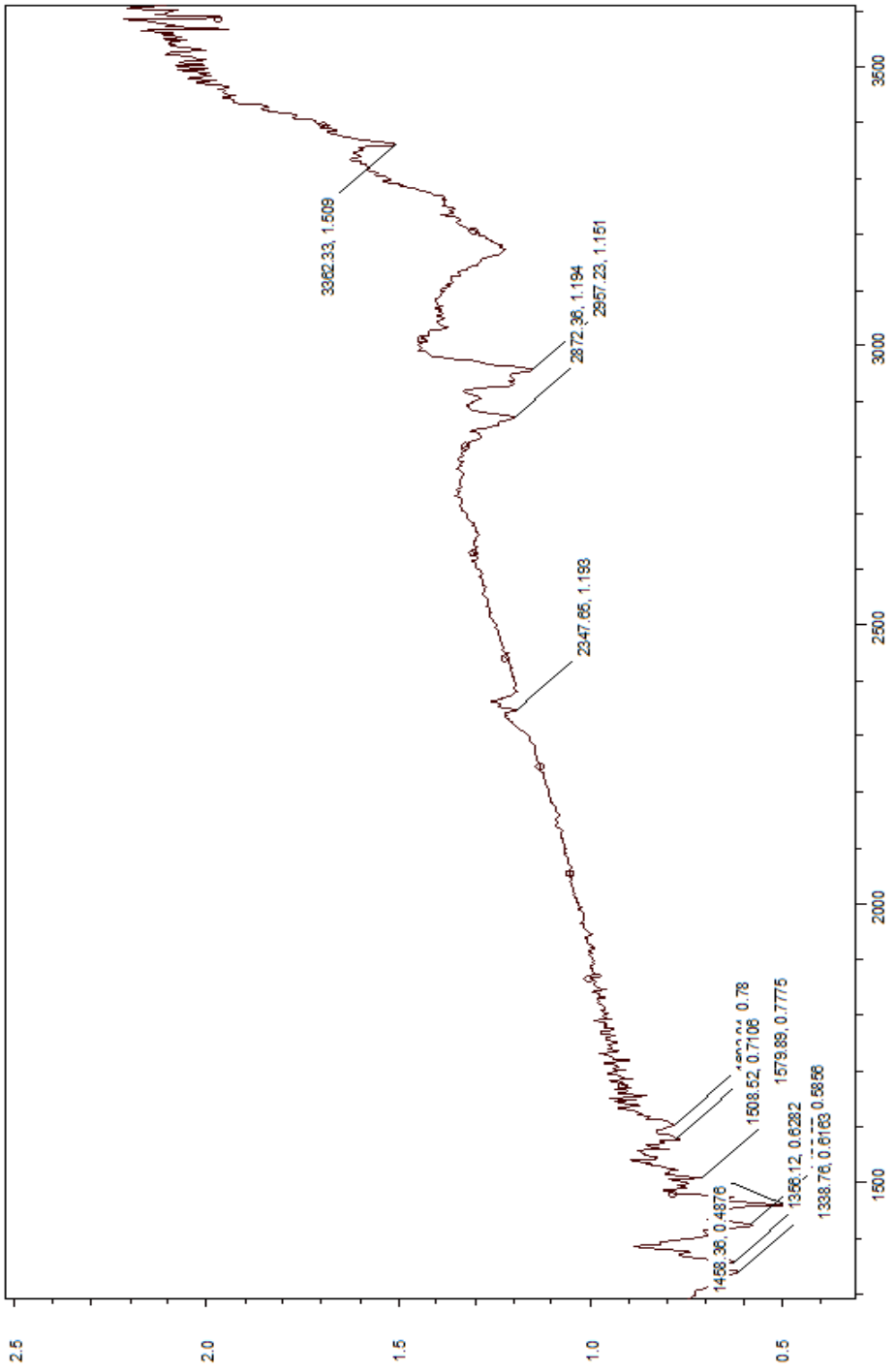


Fig.10 **FTIR SPECTRA OF HCT AND LSP (F3P.M) (1:8)**



DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The DSC thermo gram of Hydrochlorothiazide, Losartan Potassium, P.Ms and S.Ds are shown in Tables 10-15 and Figures 11-18. A sharp endothermic peak at 241.93°c and at 274.35°c was obtained for LSP and HCT respectively these peaks were corresponding to their melting point at their crystalline nature. In P.Ms the endothermic peak of HTC was shifted to lower melting temperature 226.53°c, 237.23°c, and 243.42°c for F1 P.M, F2 P.M and F3 P.M respectively and the peaks were widened. Where as in S.Ds the endothermic peak of HTC was shifted to further low melting temperature 211.41°c, 229.53°c, and 229.57°c for F1 S.D, F2 S.D, F3 S.D formulations respectively and the peaks of S.Ds were broader than those of P.M. LSP showed similar changes in both P.M and S.Ds in their thermogram. The endothermic peak of LSP was observed at 241.93°c was shifted to lower melting temperature 161.75°c, 212.78°c, 185.65°c for F1 P.M, F2 P.M and F3 P.M formulations for S.Ds respectively and shifted to further lower melting temperature 141.43°c, 187.60°c and 166.53°c for F1 S.D, F2 S.D, F3 S.D respectively and the endothermic peak in S.Ds were more broader than in P.Ms. The shifting and widening of endothermic peaks of HCT and LSP in both physical mixture and solid dispersions indicate change in solid state of the drug from crystalline to amorphous state. The endothermic peaks of HCT and LSP were broader in solid dispersions than observed in physical mixtures showing more amorphous state formulation in solid dispersions as compared to physical mixtures showing enhanced solubility of amorphous form of HCT.

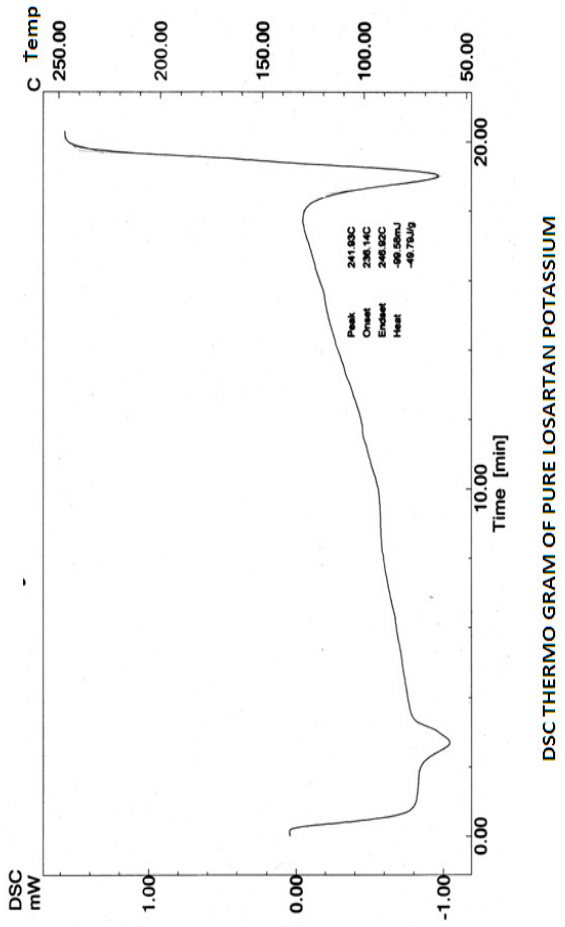

C Temp
250.00
200.00
150.00
100.00
50.00
DSC
mW
1.00
0.00
-1.00
0.00
10.00
20.00
Time [min]
Peak 241.93C
Onset 236.14C
Endset 245.92C
Heat -99.58mJ
-49.79J/g
DSC THERMO GRAM OF PURE LOSARTAN POTASSIUM

Fig.11

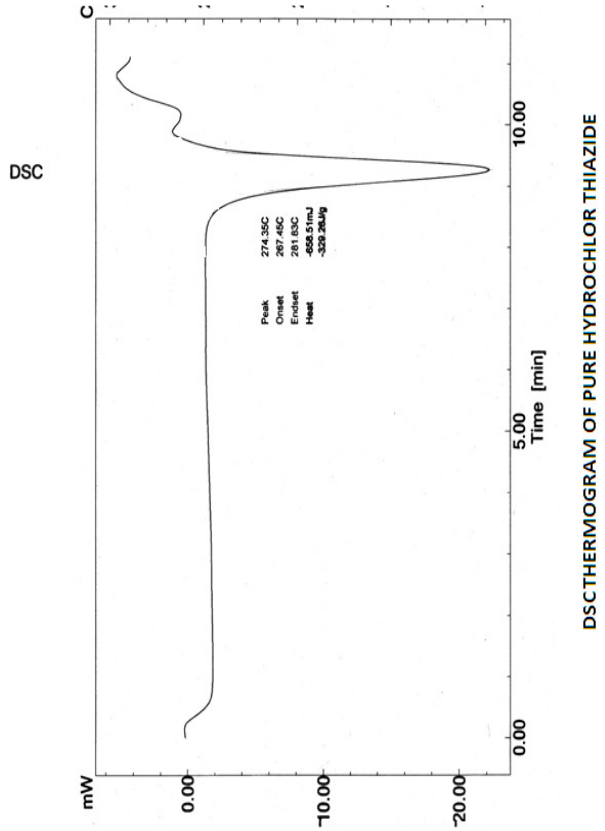
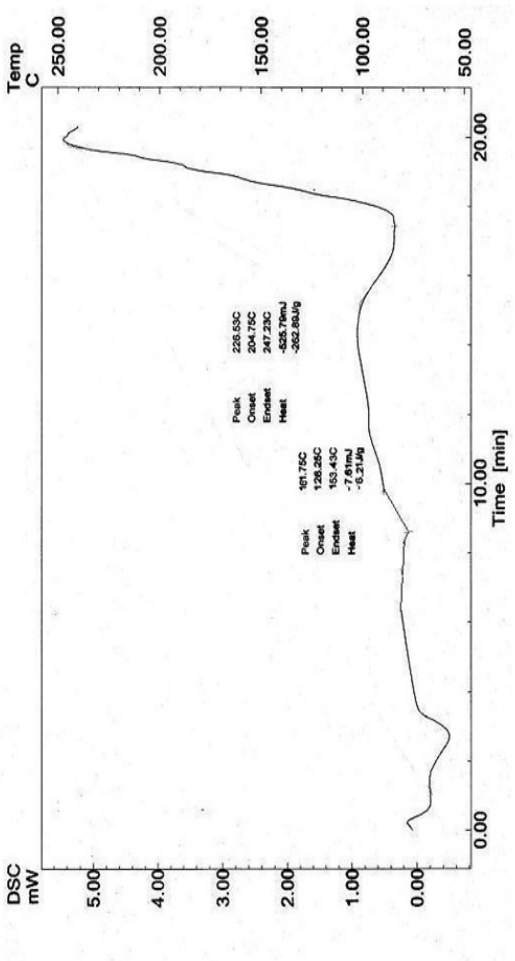
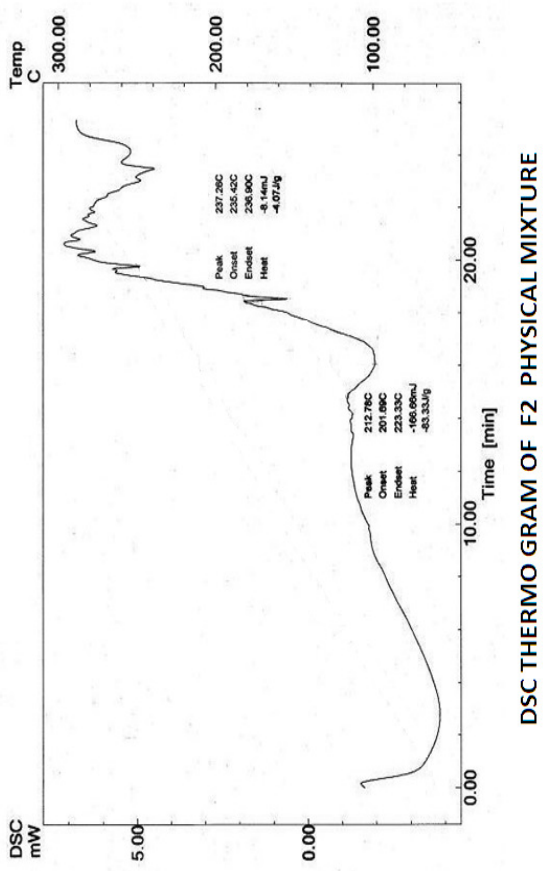

DSC
mW
0.00
-10.00
-20.00
0.00
5.00
10.00
Time [min]
Peak 274.35C
Onset 267.45C
Endset 281.63C
Heat -558.51mJ
-329.26J/g
DSCTHERMOGRAM OF PURE HYDROCHLOR THIAZIDE

Fig.12



DSC THERMOGRAM OF F1 PHYSICAL MIXTURE

Fig. 13



DSC THERMO GRAM OF F2 PHYSICAL MIXTURE

Fig.14

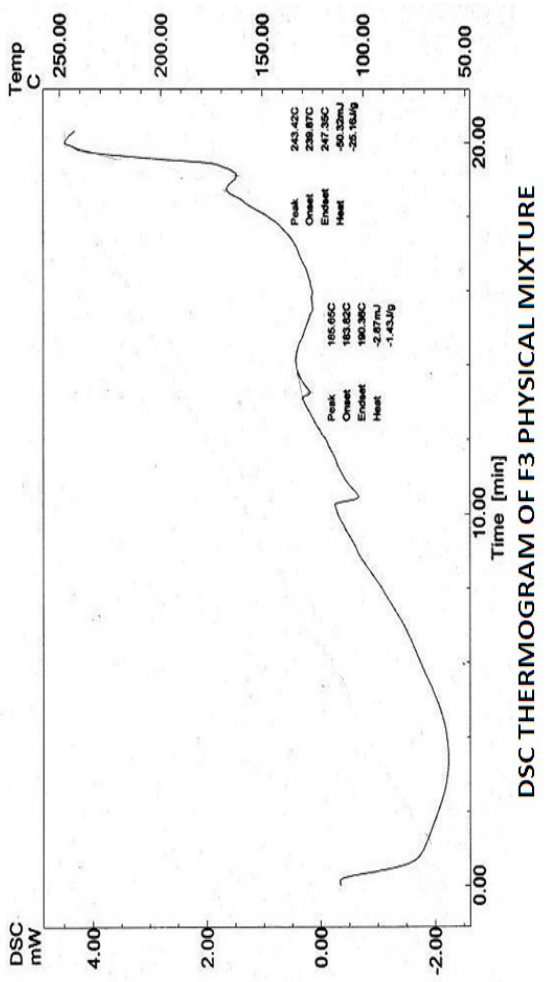

DSC THERMOGRAM OF F3 PHYSICAL MIXTURE
Temp
C
250.00
200.00
150.00
100.00
50.00
DSC
mW
4.00
2.00
0.00
-2.00
0.00
10.00
20.00
Time [min]
Peak 165.65C
Onset 163.62C
Endset 160.38C
Heat -2.87mJ
-1.43J/g
Peak 243.42C
Onset 239.87C
Endset 247.35C
Heat -50.32mJ
-25.16J/g

Fig.15

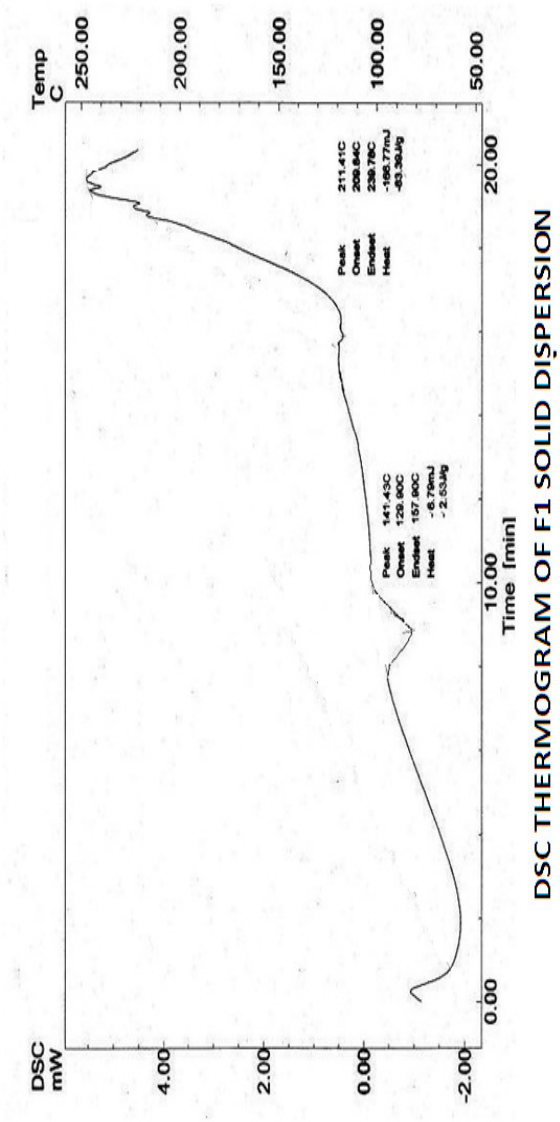
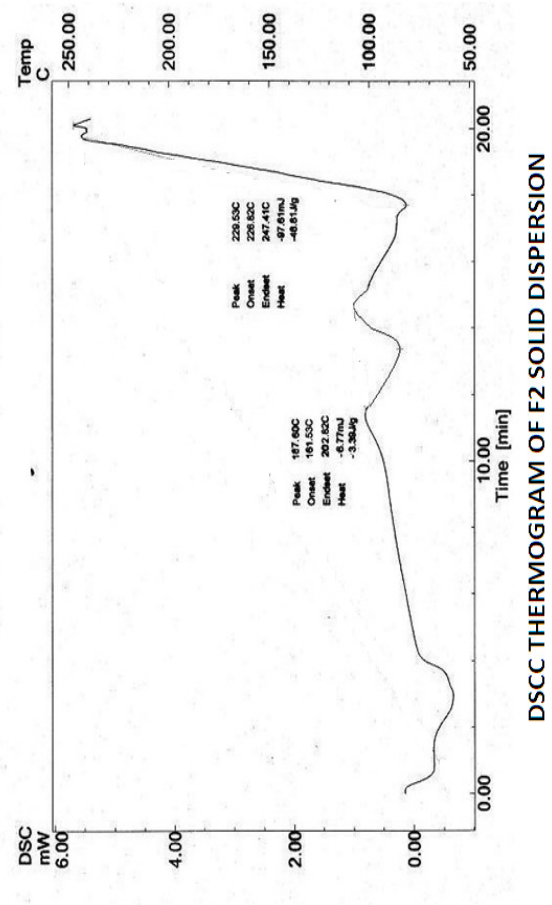

DSC THERMOGRAM OF F1 SOLID DISPERSION
Temp
C
250.00
200.00
150.00
100.00
50.00
DSC
mW
4.00
2.00
0.00
-2.00
0.00
10.00
20.00
Time [min]
Peak 141.43C
Onset 129.90C
Endset 157.90C
Heat -6.79mJ
-2.53J/g
Peak 211.41C
Onset 209.84C
Endset 239.78C
Heat -166.77mJ
-63.39J/g

Fig.16



DSCC THERMOGRAM OF F2 SOLID DISPERSION

Fig.17

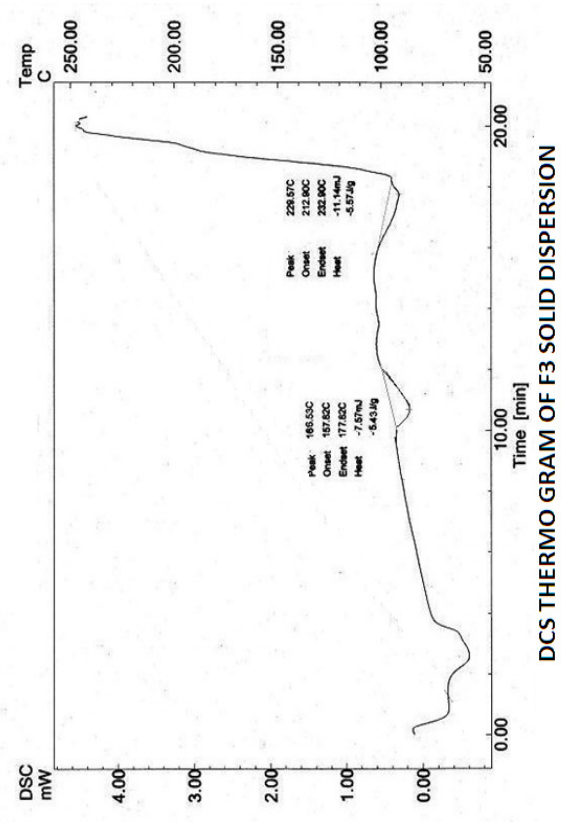

DSC
mW
Temp
C
Time [min]
Peak 229.57C
Onset 212.90C
Endset 232.90C
Heat -11.14mJ
-5.57J/g
Peak 166.53C
Onset 157.82C
Endset 177.82C
Heat -7.57mJ
-5.43J/g
DCS THERMO GRAM OF F3 SOLID DISPERSION

Fig.18

ESTIMATION OF DRUG CONTENT

Both methods I and II produced linearity in the graph obtained by plotting concentration versus absorbance. There was no interference in the analysis of drugs.

Table.14 **Calibration curve data for Hydrochlorothiazide-Losartan Potassium absorbance in combination**

s.no	Concentration 0f drug (µg/mL)	Absorbance of Hydrochlorothiazide at 270nm	Absorbance of Losartan potassium at 236nm
1	0	0.003	0.001
2	10	0.792	0.714
3	20	1.634	1.37
4	30	2.46	2.08
5	40	3.16	2.67
6	50	3.9	3.45

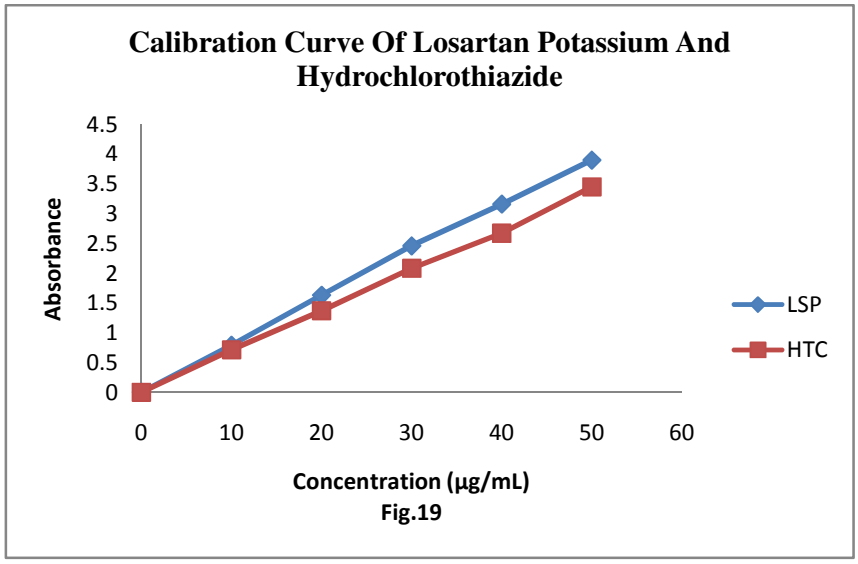


Fig.19

Table 15 **Calibration data of Hydrochlorothiazide**

S.NO	Concentration of Hydrochlorothiazide(µg/Ml)	Absorbance at 270nm
1	0	0
2	1	0.0615
3	2	0.135
4	3	0.1863
5	4	0.2475
6	5	0.3102

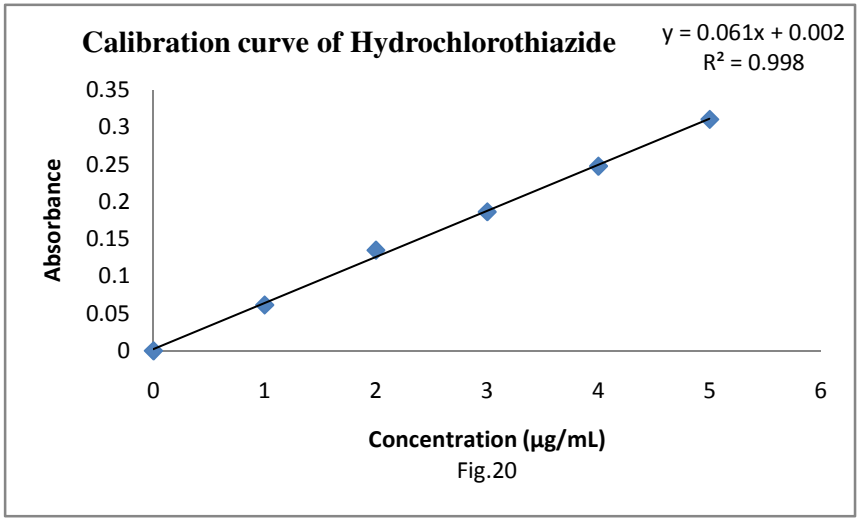


Fig.20

PHASE SOLUBILITY STUDY

Phase solubility study was carried out in order to ascertain the effect of LSP on the solubility characteristics of HTC. The results are presented in Table -14 and Figure -19. Solubility of HTC was increased as the concentration of LSP increased. The solubility of HTC was minimal in deaerated water and increased approximately eight fold at 0.01% w/v of LSP in deaerated water. These data indicates that LSP in deaerated water acted as a new vehicle and solubility of HTC was greatly enhanced, possibly due to the solvent effect of LSP.

Table:16

S.No	Concentration of Losartan Potassium (%w/v)	Solubility of Hydrochlorothiazide in Losartan potassium solution (mg/ml)
1	0	0.2123
2	0.0025	0.3610
3	0.0050	0.5270
4	0.010	1.6535

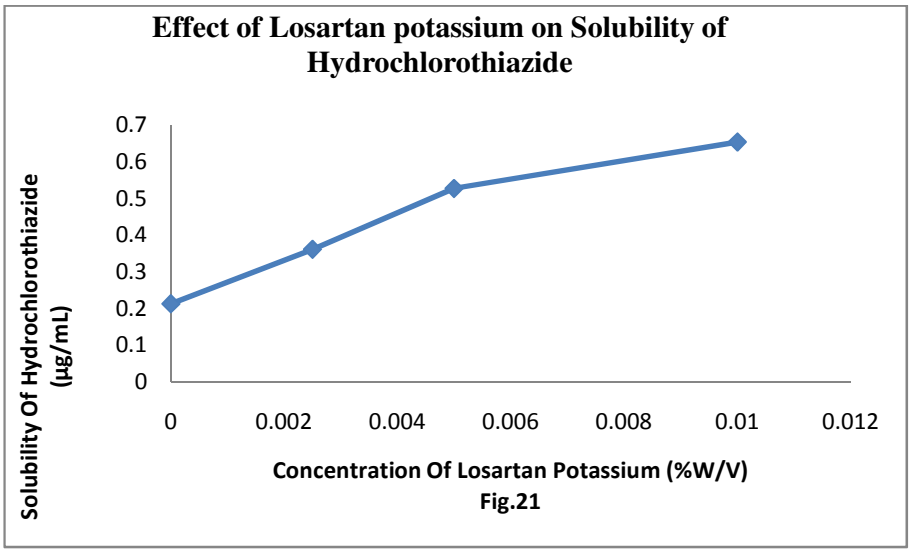


Fig.21

In-vitro dissolution studies

The data of the In vitro dissolution studies as cumulative % drug release Vs time performed on P.Ms, S.Ds and C.P on deaerated water are shown in Tables 17-26 and figures 22-45. There were differences in dissolution pattern of HTC between P.M 1, P.M 2 and P.M 3 formulations. The % drug dissolved at every time point intervals was statistically analyzed and it was observed at 10 min. The % drug dissolved from P.Ms was found to increase as the concentration of LSP is increased and all the P.M s showed about 90% release of HTC in 80min. When compared with pure HTC P.Ms showed faster dissolution while pure HTC dissolved 90% at 150 min the P.M dissolved 90% HTC in 80min. Thus showing faster dissolution of HTC from P.Ms as compared to pure HTC. Though there was a significant difference in dissolution pattern of HTC from P.Ms at different time intervals, no significant difference was observed in dissolution pattern all P.Ms at 80 min, at which fine point 90% dissolution of HTC was observed. Since LSP is freely soluble in dissolution media a uniform dissolution pattern of this drug was observed from P.Ms. S.Ds showed similar dissolution pattern as observed with P.Ms at different time point intervals and there were significant differences in percent (HTC) drug dissolved between different time point intervals. However S.Ds showed faster dissolution of HTC as compared to P.Ms. 90% dissolution of HTC was at shorter time (50min) from S.D3 formulation as compared to S.D1, S.D2 formulations which showed 90% dissolution of HTC at longer time (70min). These finding suggests that S.Ds showed faster dissolution rate of HTC as compared to P.M or pure drug. The enhanced dissolution of HTC from P.Ms is due to the solvent effect of LSP. The magnitude of dissolution was significantly higher from S.Ds as compared to P.Ms the mechanisms for improved dissolution of HTC from S.D is due to firstly, the solvent effect of LSP on the solubility of HTC; Secondly the change of physical state of HTC from crystallinity to amorphous state as seen in DSC thermogram and thirdly possible micronization of poorly soluble HTC, reduced particle size, larger surface area and so enhanced dissolution of the drug in the environment. The dissolution pattern of LSP whether from P.M or from S.D assumes no significance in the study as the drug is freely soluble.

Table 17 **In-Vitro Dissolution Profile of Pure Hydrochlorothiazide in deaerated water**

Time(min)	Trial 1	Trial 2	Trial 3	Mean cumulative %drug release
0	0	0	0	0
10	3.17	3.21	4.50	3.62 ± 0.756
20	9.15	8.97	9.12	9.08 ± 0.096
30	16.42	17.51	17.14	17.11 ± 0.6025
40	19.25	19.27	19.25	19.31 ± 0.092
50	24.19	25.12	24.11	24.47 ± 0.5615
60	34.45	33.21	34.36	34.00 ± 0.6914
70	45.15	44.17	44.84	44.72 ± 0.5009
80	53.7	54.73	54.30	54.24 ± 0.5173
90	59.9	60.1	60.2	60.06 ± 0.1528
100	65.2	66.24	66.1	65.84 ± 0.5644
110	67.42	68.43	67.2	67.68 ±0.655
120	72.81	72.92	73.32	73.01 ± 0.268
130	76.32	75.92	77.1	76.44 ±0.6001
140	85.12	84.89	85.3	85.103± 0.2055
150	92.13	92.15	93.16	92.48 ± 0.5890
160	96.62	95.92	96.53	96.3566 ± 0.3808
170	98.13	98.14	98.23	98.16 ± 0.055
180	98.39	99.2	99.62	99.07 ± 0.6252

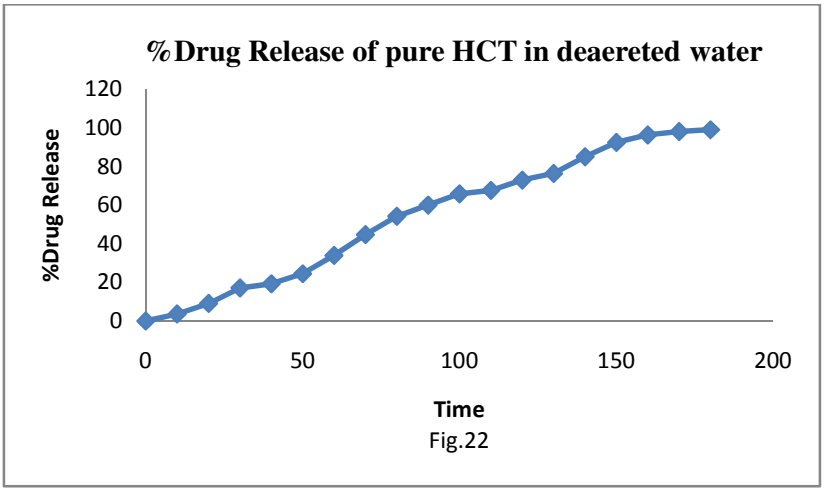


Fig.22

Table 18 **In-vitro Dissolution Profile for Physical Mixture F1 in deaerated water**

Hydrochlorothiazide					**Losartan Potassium**			
Time (min)	**Trial 1**	**Trial 2**	**Trial 3**	**Mean ± SD**	**Trial 1**	**Trial 2**	**Trial 3**	**Mean ± SD**
0	0	0	0	0	0	0	0	0
10	22.34	22.89	21.89	22.55±0.2972	80.71	78.91	80.68	80.01±1.031
20	41.91	41.70	41.15	41.586±0.3925	83.23	84.15	82.73	83.37±0.7203
30	62.28	63.19	60.99	62.1533±1.105	85.91	85.00	86.05	85.65±0.5701
40	70.64	69.43	70.43	70.16±0.6466	87.52	86.53	87.98	87.34±0.7401
50	77.93	75.32	77.83	77.026±1.479	90.23	91.13	88.37	89.91±1.408
60	81.54	80.48	81.63	81.216±0.639	91.03	92.24	92.48	91.916±0.77
70	88.59	88.62	87.48	88.23±0.6497	92.32	93.78	93.49	93.19±0.77
80	92.61	92.52	91.70	92.27±0.5014	95.18	94.79	95.39	95.12±0.3083
90	95.46	94.52	96.45	95.476±0.9651	96.47	95.68	97.66	96.60±1.9967

Release kinetics of HCT from F1 P.M in deaerated water

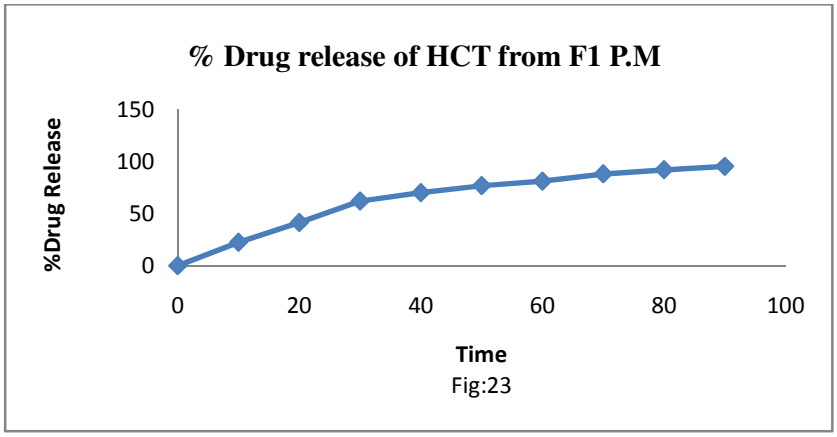


Fig:23

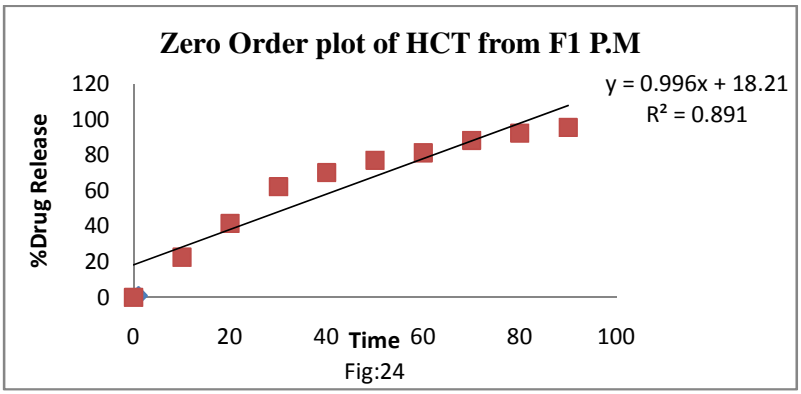


Fig:24

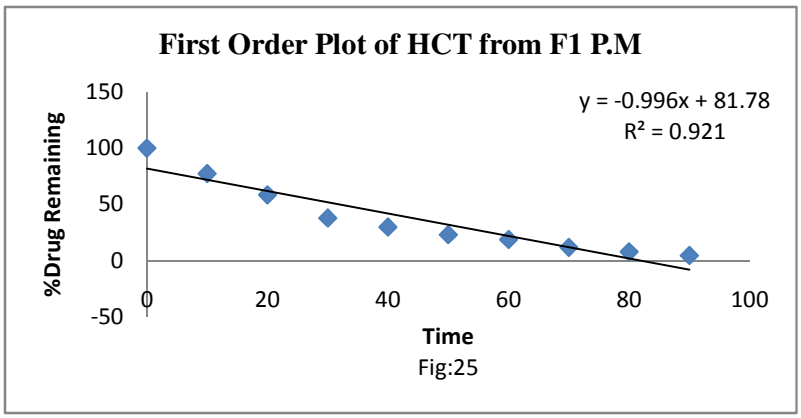


Fig:25

Table 19 **In-vitro Dissolution Profile for physical mixture F2 in deaerated water**

Hydrochlorothiazide					Losartan Potassium			
Time (mins)	**Trial 1**	**Trial 2**	**Trial 3**	**Mean ± SD**	**Trial 1**	**Trial 2**	**Trial 3**	**Mean ± SD**
0	0	0	0	0	0	0	0	0
10	19.88	19.18	20.23	19.76±0.5328	80.18	79.87	80.03	80.03±0.1527
20	37.01	36.30	37.35	36.89±0.5335	84.39	84.09	82.56	83.68±0.9819
30	65.30	64.61	65.65	65.19±0.5341	88.01	87.69	87.84	87.84±0.1530
40	71.31	70.61	72.36	71.42±0.8797	92.22	91.91	92.06	92.07±0.1532
50	78.71	78.01	79.07	78.60±0.5356	93.85	93.54	92.47	93.29±0.7229
60	84.38	83.68	85.08	84.38±0.7011	95.02	94.72	93.49	94.41±0.8101
70	88.66	87.96	89.37	88.66±0.7018	95.89	95.58	94.97	95.48±0.4695
80	93.65	92.94	94.01	93.53±0.5377	96.76	96.45	95.84	96.35±0.4700
90	95.15	94.44	97.24	95.61±1.4577	97.33	97.02	96.71	97.02±0.3093

Release kinetics of HCT from F1 P.M in deaerated water

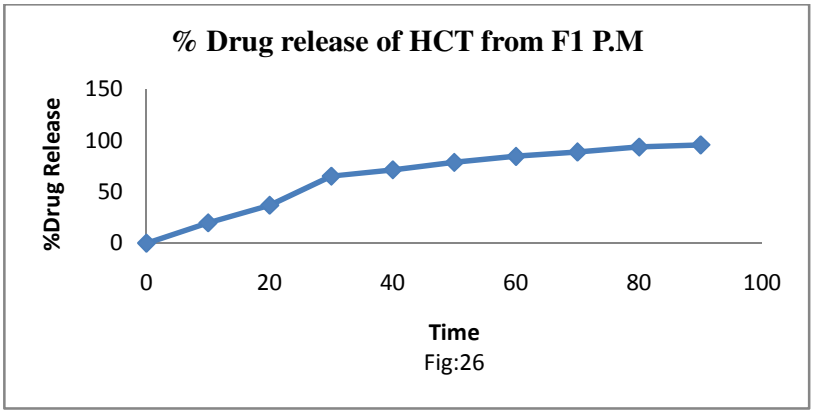


Fig:26

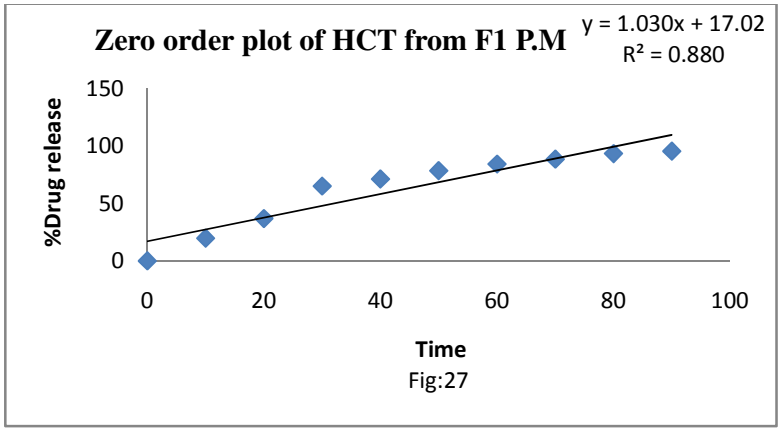


Fig:27

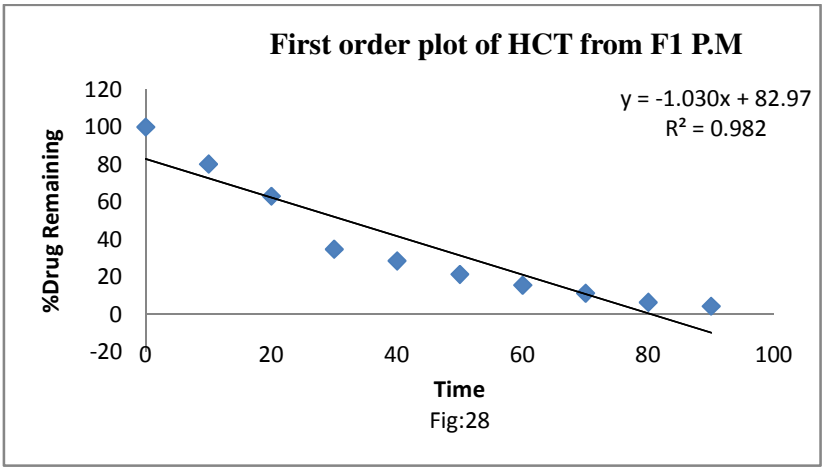


Fig:28

Table .20 **In-vitro dissolution profile for physical mixture F3 in deaerated water**

Hydrochlorothiazide					**Losartan Potassium**			
Time (min)	**Trial 1**	**Trial 2**	**Trial 3**	**Mean ± SD**	**Trial 1**	**Trial 2**	**Trial 3**	**Mean ± SD**
0	0	0	0	0	0	0	0	0
10	26.0	26.57	27.15	26.57±0.5778	80.33	84.17	84.34	82.95±2.2675
20	45.09	45.67	46.25	45.67±0.5784	88.77	89.11	89.27	89.05±0.2555
30	66.52	67.10	67.68	67.10±0.5790	91.38	92.05	93.72	92.38±1.2049
40	72.95	73.53	74.11	73.53±0.5797	94.99	95.32	96.16	95.49±0.6041
50	79.39	79.97	80.55	79.97±0.5803	96.09	96.43	96.77	96.43±0.3366
60	82.94	83.52	84.10	83.52±0.5810	97.03	97.20	97.71	97.31±0.3506
70	87.08	88.24	88.82	88.04±0.8863	97.48	98.15	98.32	97.98±0.4448
80	92.37	94.11	94.69	93.73±1.2074	97.92	98.59	98.59	98.37±0.3888
90	96.52	98.26	98.27	97.68±1.0063	98.69	99.03	98.87	98.86±0.1690

Release kinetics of HCT from F3 P.M in deaerated water

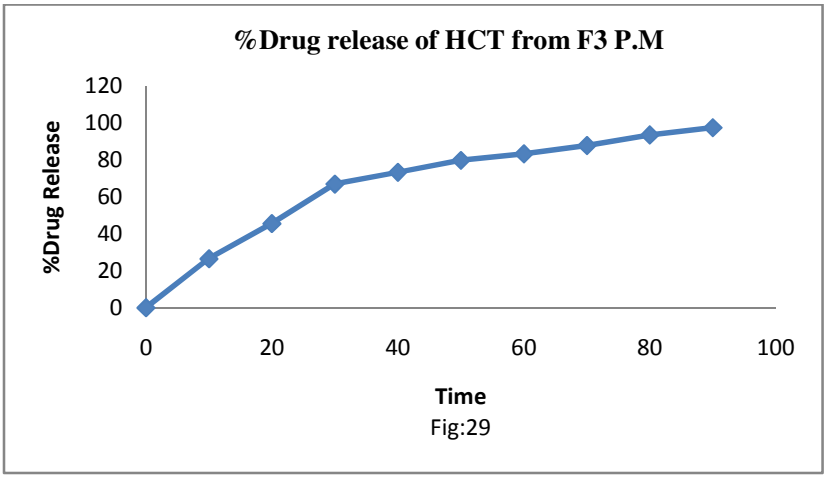


Fig:29

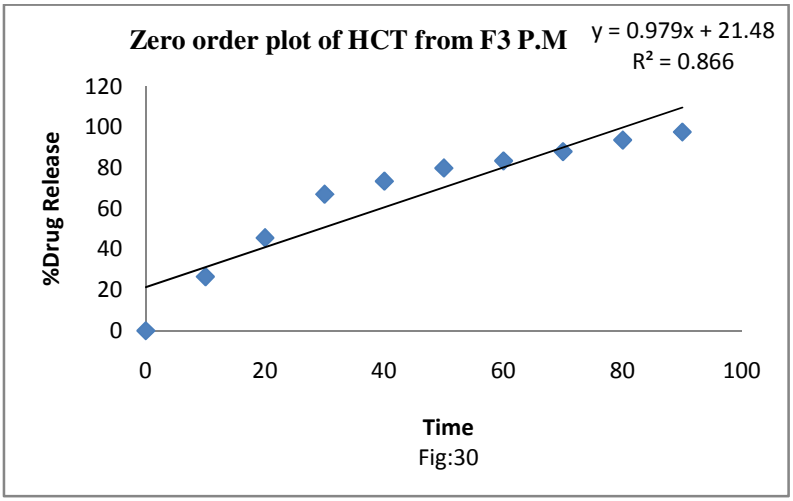


Fig:30

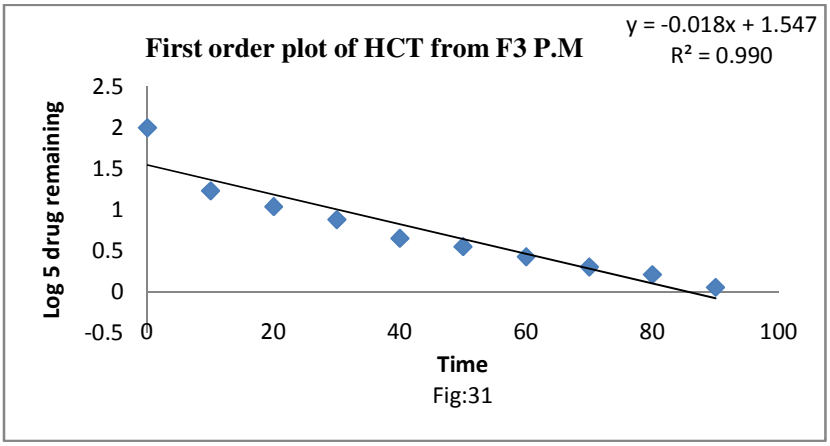


Fig:31

Table 21 **In-vitro dissolution profile for solid dispersion F1 in deaerated water**

Hydrochlorothiazide					**Losartan Potassium**			
Time (mins)	**Trial 1**	**Trial 2**	**Trial 3**	**Mean ± SD**	**Trial 1**	**Trial 2**	**Trial 3**	**Mean ± SD**
0	0	0	0	0	0	0	0	0
10	26.01	24.15	26.01	25.39±1.0774	81.80	77.71	79.76	79.75±2.0425
20	45.55	43.69	44.62	44.62±0.9300	83.94	79.84	81.89	81.89±2.0474
30	65.11	63.24	64.17	64.17±0.9317	88.94	88.52	88.73	88.73±0.2090
40	73.54	71.68	72.61	72.61±0.9320	90.67	90.26	90.46	90.46±0.2092
50	82.91	81.05	81.98	81.98±0.9331	93.64	93.22	93.43	93.43±0.2095
60	90.44	88.57	89.50	89.50±0.9341	94.56	94.14	94.35	94.35±0.2097
70	93.32	91.45	92.39	92.39±0.9352	95.69	95.27	95.48	95.48±0.2099
80	95.28	93.41	94.35	94.35±0.9362	96.61	96.19	96.40	96.40±0.2101
90	96.32	94.44	95.38	95.38±0.9372	97.34	96.94	97.29	97.19±0.2213

Release kinetics of HCT from F1 S.D in deaerated water

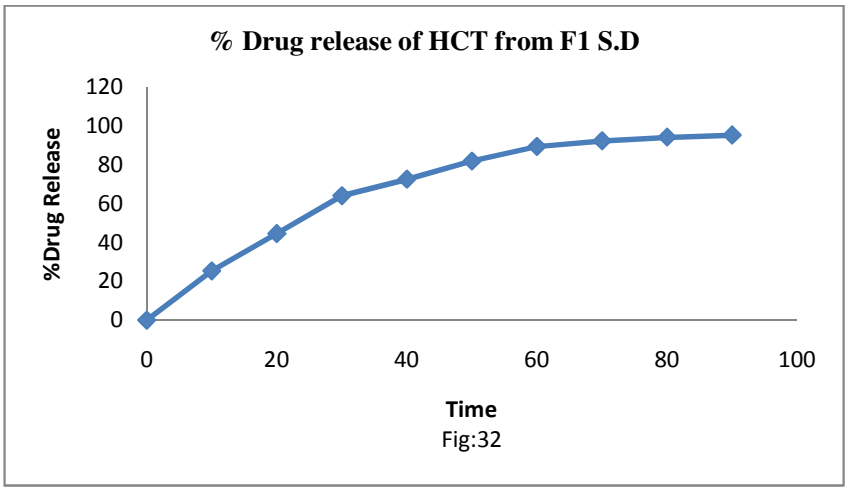


Fig:32

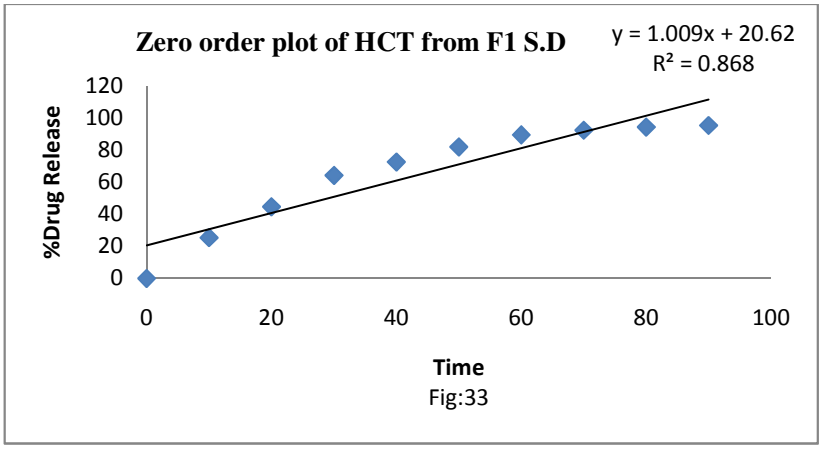


Fig:33

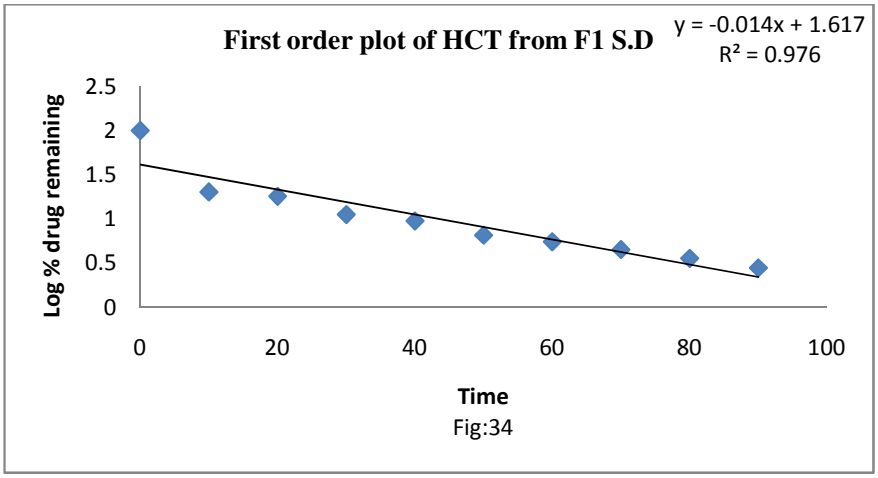


Fig:34

Table 22 **In-vitro dissolution profile for solid dispersion F2 in deaerated water**

Hydrochlorothiazide					**Losartan Potassium**			
Time (mins)	**Trial 1**	**Trial 2**	**Trial 3**	**Mean ± SD**	**Trial 1**	**Trial 2**	**Trial 3**	**Mean ± SD**
0	0	0	0	0	0	0	0	0
10	20.29	19.55	19.92	19.92±0.3687	83.31	83.00	83.15	83.15±0.1539
20	38.76	38.02	38.39	38.39±0.3694	84.78	84.48	84.63	84.63±0.1541
30	74.97	74.23	74.60	74.60±0.3698	89.96	89.65	89.81	89.81±0.1543
40	81.33	80.58	80.96	80.96±0.3702	91.91	91.60	91.75	91.75±0.1545
50	87.69	86.95	87.32	87.32±0.3706	94.01	93.70	93.86	93.86±0.1546
60	88.89	88.15	88.52	88.52±0.3710	96.89	96.58	96.73	96.73±0.1548
70	91.21	90.46	90.83	90.83±0.3714	97.31	96.99	97.15	97.15±0.1550
80	95.73	94.99	95.36	95.36±0.3718	97.72	97.41	97.56	97.56±0.1551
90	96.95	96.20	96.57	96.57±0.3722	98.60	98.29	98.44	98.44±0.1553

Release kinetics of HCT from F2 S.D in Deaerated water

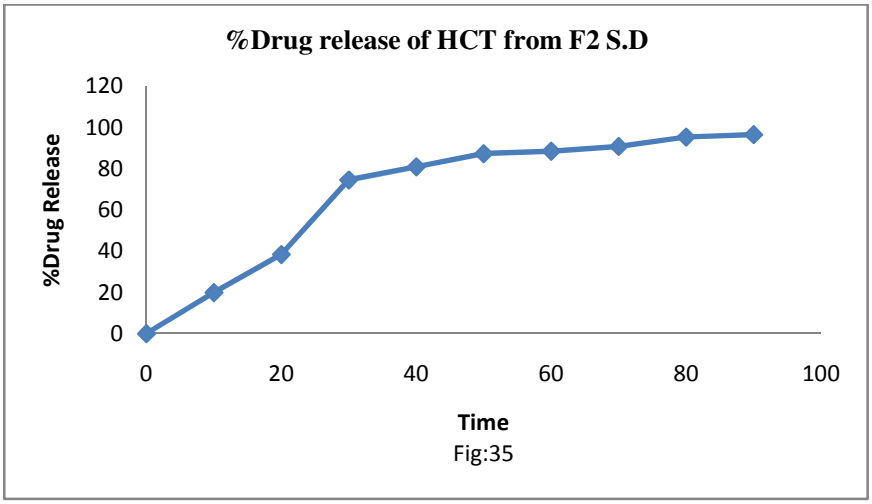


Fig:35

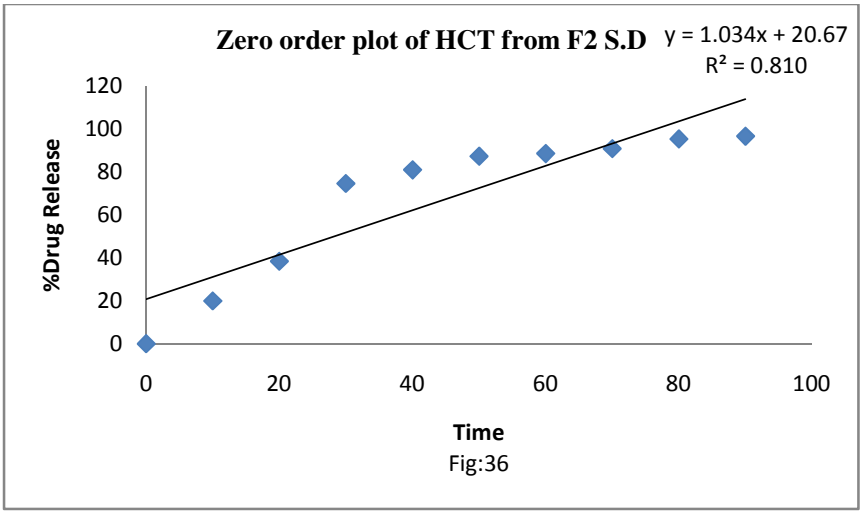


Fig:36

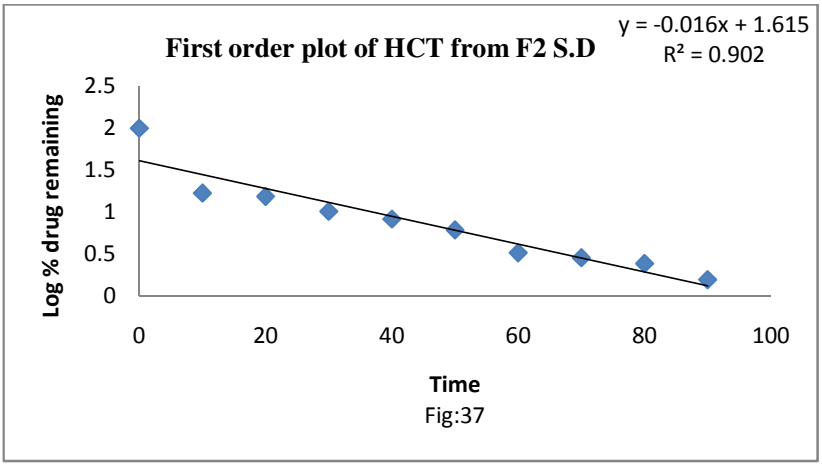


Fig:37

Table 23 **In-vitro dissolution profile for solid dispersion F3 in deaerated water**

Hydrochlorothiazide					**Losartan Potassium**			
Time (mins)	**Trial 1**	**Trial 2**	**Trial 3**	**Mean ± SD**	**Trial 1**	**Trial 2**	**Trial 3**	**Mean ± SD**
0	0	0	0	0	0	0	0	0
10	35.17	33.96	35.78	34.97±0.9264	86.70	86.37	86.87	86.65±0.2522
20	60.04	58.82	60.64	59.84±0.9264	89.61	89.28	89.77	89.55±0.2525
30	73.38	72.17	73.99	73.18±0.9264	93.01	92.68	93.17	92.95±0.2528
40	82.48	81.26	83.08	82.28±0.9264	96.08	95.75	96.25	96.03±0.2531
50	90.97	89.76	91.57	90.77±0.9264	97.68	97.35	97.84	97.62±0.2534
60	94.01	92.79	94.61	93.80±0.9264	97.95	97.62	98.12	97.90±0.2536
70	96.43	95.21	97.03	96.22±0.9264	98.39	98.06	98.56	98.33±0.2539
80	97.59	95.82	97.64	97.02±1.0352	98.83	98.50	99.00	98.77±0.2542
90	98.25	97.03	98.85	98.04±0.9264	99.27	98.93	99.43	99.21±0.2545

Release kinetics of HCT from F3 S.D in deaerated water

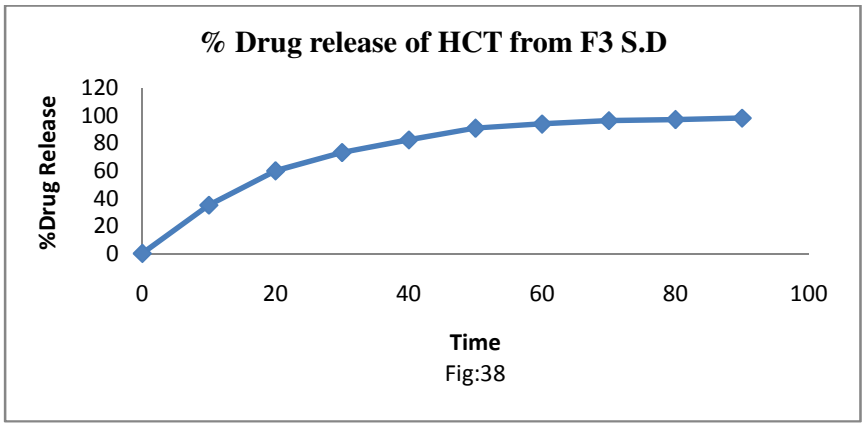


Fig:38

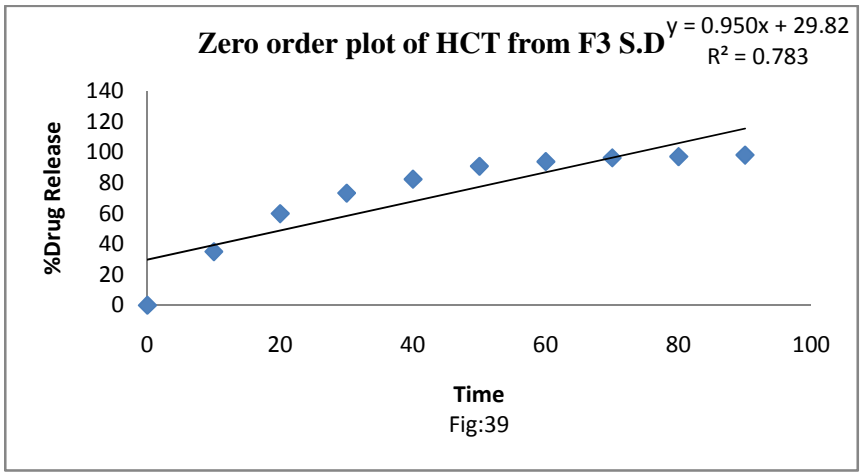


Fig:39

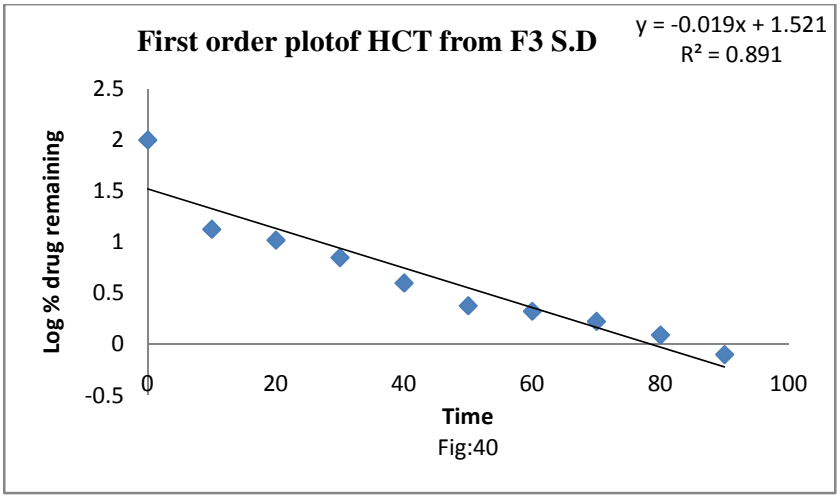


Fig:40

Table-24 **In-Vitro dissolution profile for F2 commercial product in deaerated water**

Hydrochlorothiazide					Losartan Potassium			
Time (mins)	**Trial 1**	**Trial 2**	**Trial 3**	**Mean ± SD**	**Trial 1**	**Trial 2**	**Trial 3**	**Mean ± SD**
0	0	0	0	0	0	0	0	0
10	21.72	23.32	21.73	22.25±0.92	75.75	73.75	75.23	74.91±1.03
20	31.73	30.54	32.73	31.66±1.09	82.49	80.95	83.49	82.31±1.28
30	45.19	44.82	44.28	44.76±0.45	88.97	86.22	88.97	88.05±1.58
40	54.89	63.25	62.19	60.11±4.55	90.12	90.47	93.91	91.5±2.09
50	72.97	71.96	72.86	72.59±0.55	94.41	91.10	94.44	93.31±1.92
60	74.79	76.00	73.69	74.82±1.15	95.73	94.71	92.74	94.39±1.52
70	80.48	80.77	81.02	80.75±0.27	96.24	96.33	95.68	96.08±0.35
80	83.88	83.25	82.81	83.31±0.53	98.12	98.89	98.56	98.52±0.38
90	85.81	87.29	85.81	86.30±0.55	99.02	99.66	100.15	99.61±0.56

Release kinetics of HCT from F2 C.P in deaerated water

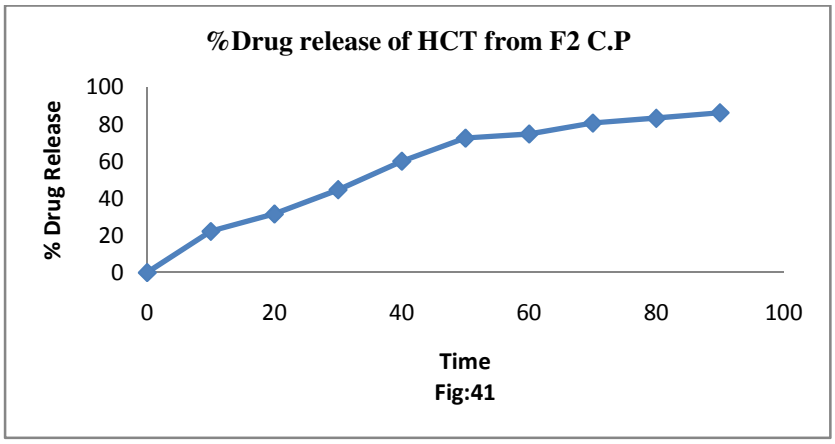


Fig:41

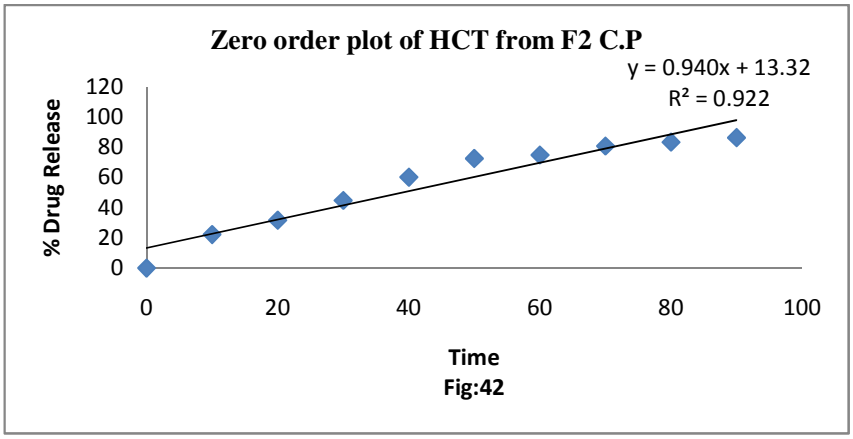


Fig:42

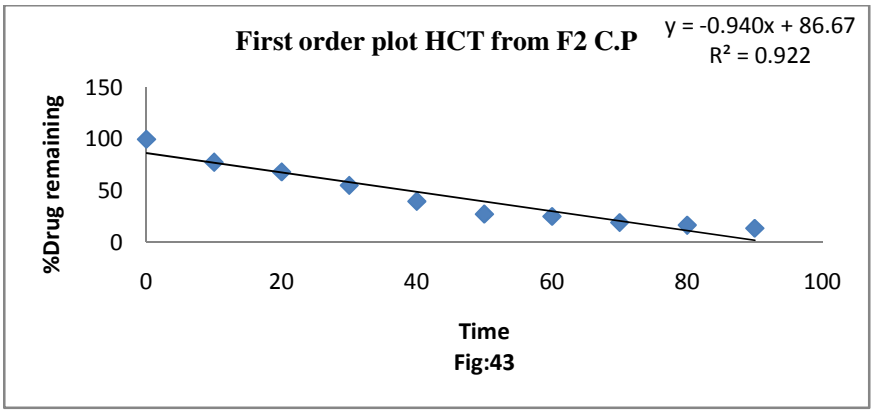


Fig:43

Table 25 **Release kinetics of Physical Mixtures**

Formulation code	HYDROCHLOROTHIAZIDE		
	F1 PM	F2 PM	F3 PM
Zero order "R^2"value	0.891	0.880	0.866
First order "R^2"value	0.921	0.982	0.990
Best fit model	First order	First order	First order

Table:26 **Release kinetics of Solid Dispersions**

Formulation code	HYDROCHLOROTHIAZIDE		
	F1 SD	F2 SD	F3 SD
Zero order "R^2"value	0.869	0.810	0.783
First order "R^2"value	0.976	0.902	0.891
Best fit model	First order	First Order	First order

COMPARATIVE DISSOLUTION OF PHYSICAL MIXTURES AND SOLID DISPERSIONS IN DEAERATED WATER

Table 27 **Physical mixtures**

Time (mins)	PM1		PM2		PM3	
	HCTZ	LSP	HCTZ	LSP	HCTZ	LSP
0	0	0	0	0	0	0
10	22.55	80.01	22.13	80.03	26.57	82.95055
20	41.589	83.37	41.22	83.68	45.67	89.05647
30	62.1533	85.65	61.94	87.84	67.10	92.38427
40	70.16	87.34	70.19	92.07	73.53	95.49296
50	77.026	89.91	76.80	93.29	79.97	96.43381
60	81.216	91.916	81.20	94.41	83.52	97.31992
70	88.23	93.19	87.71	95.48	88.04	97.9842
80	92.27	95.12	91.97	96.35	93.73	98.37075
90	95.476	96.60	94.54	97.02	97.68	98.86895

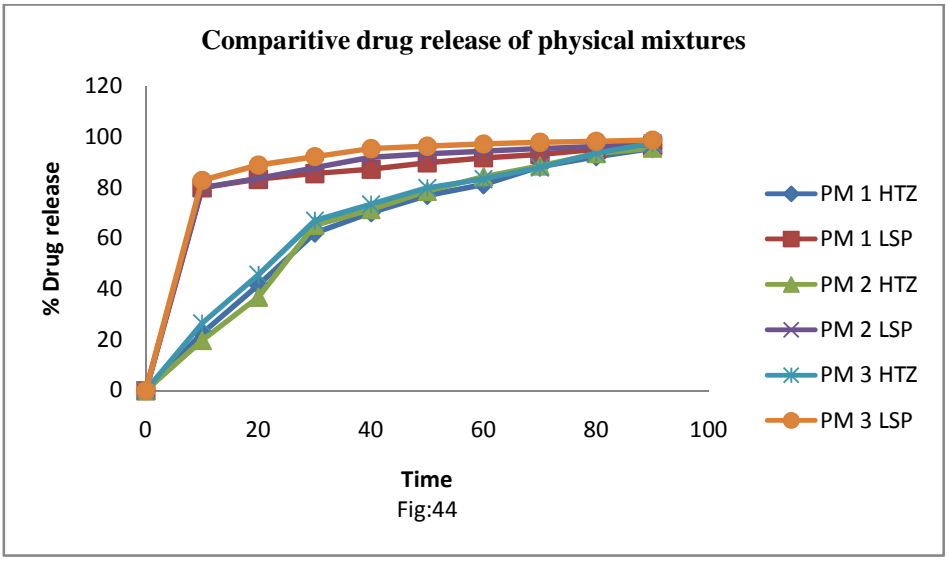


Fig:44

Table 28 **comparative dissolution profile of solid dispersions**

Time (mins)	SD1		SD2		SD3	
	HCTZ	LSP	HCTZ	LSP	HCTZ	LSP
0	0	0	0	0	0	0
10	19.92618	79.75968	25.39329	83.15735	34.97422	86.65235
20	38.39881	81.89524	44.62096	84.6357	59.8403	89.55629
30	74.60408	88.73518	64.17998	89.81148	73.18306	92.95883
40	80.96001	90.46971	72.61241	91.759	82.2804	96.03473
50	87.32291	93.43316	81.98315	93.86258	90.77125	97.62751
60	88.52669	94.35462	89.50617	96.73835	93.80371	97.9007
70	90.83872	95.4815	92.39234	97.15328	96.22965	98.33922
80	95.36725	96.40501	94.35257	97.56855	97.02034	98.77811
90	96.57964	97.19419	95.38585	98.44616	98.04913	99.21736

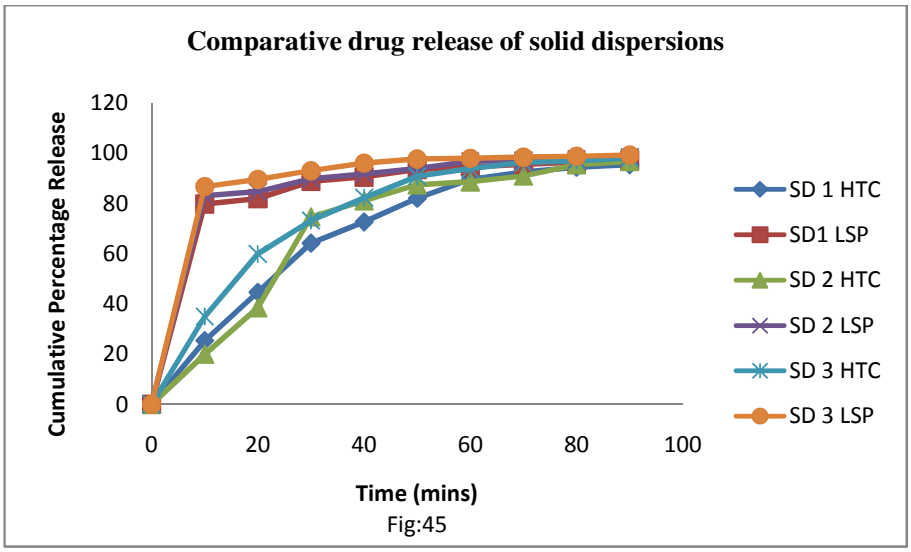


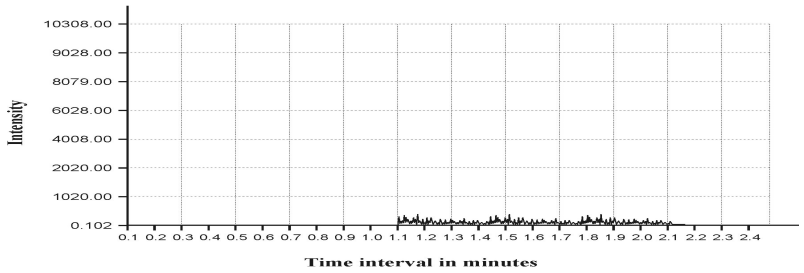
Fig:45

IN-VIVO PHARMACOKINETIC STUDY

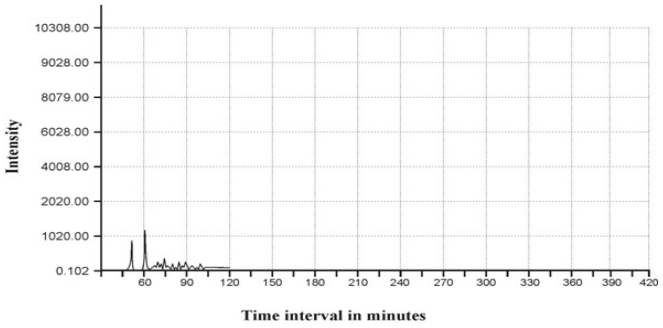
Pharmacokinetics of C.P, P.M and S.D are presented in Tables 26-29 and Figures. The Ka, K_E, C_{max}, V_d, AUC_{0-8} of HCT were significantly increased ($p< 0.05$) from solid dispersion as compared to P.M or C.P and these increases are as result of enhanced dissolution and absorption of HCT brought about by change of crystallinity to amorphous form and reduced particle size of HCT in solid dispersion. The pharmacokinetics of commercial product did not show significant difference with physical mixtures. The values of T_{max} and $t_{1/2}$ of HCT in solid dispersion decreased and this decrease was significant as compared to P.M or C.P ($p<0.05$). As Ka of HCT from solid dispersion was significantly increased, the T_{max} and $t_{1/2}$ values was decreased. These findings clearly suggest that the pharmacokinetics of HCT from solid dispersion was better than that of P.M and C.P.

Hplc Chromatographs of C.P, P.M and S.D

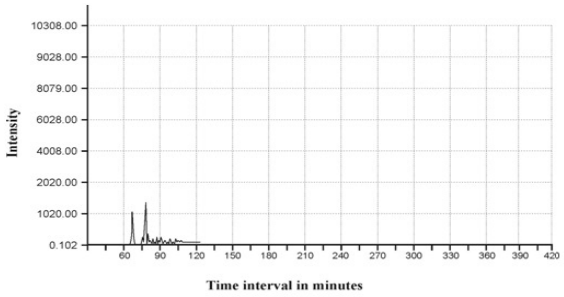
Commercial Product



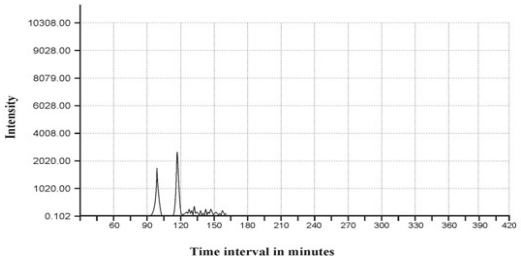
PLASMA SAMPLE AT 0 MIN.



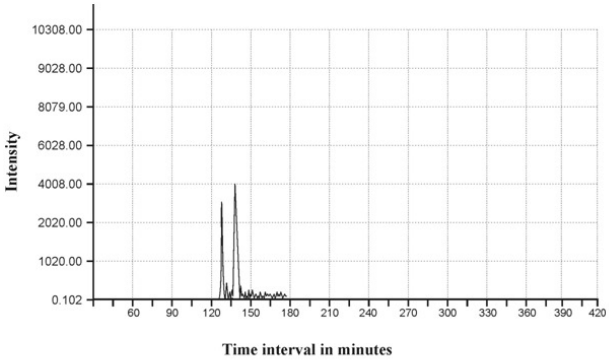
COMMERCIAL PRODUCT 1 : 4 (30 MIN)



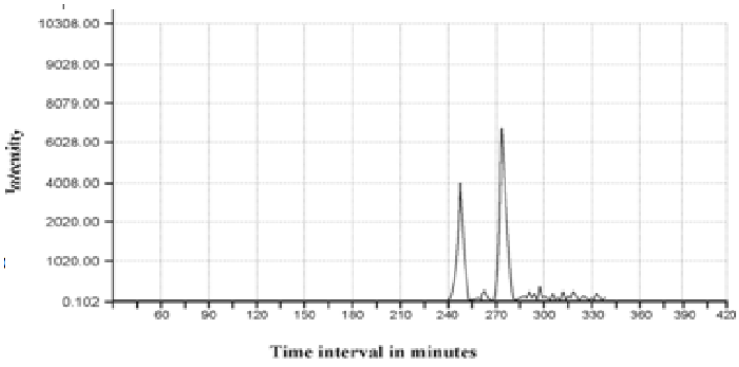
COMMERCIAL PRODUCT 1 : 4 (60 MIN)



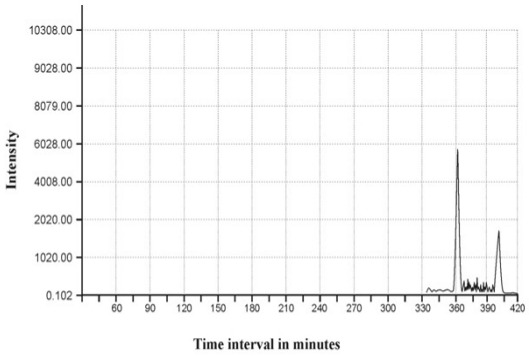
COMMERCIAL PRODUCT 1 : 4 (90 MIN)



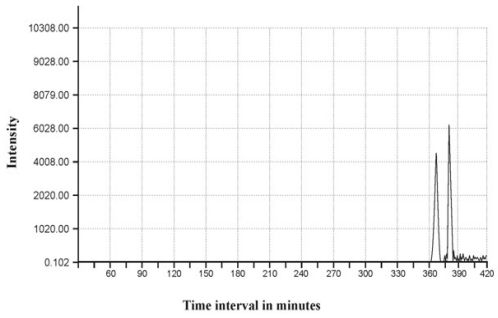
COMMERCIAL PRODUCT 1 : 4 (120 MIN)



COMMERCIAL PRODUCT 1 : 4 (240 MIN)

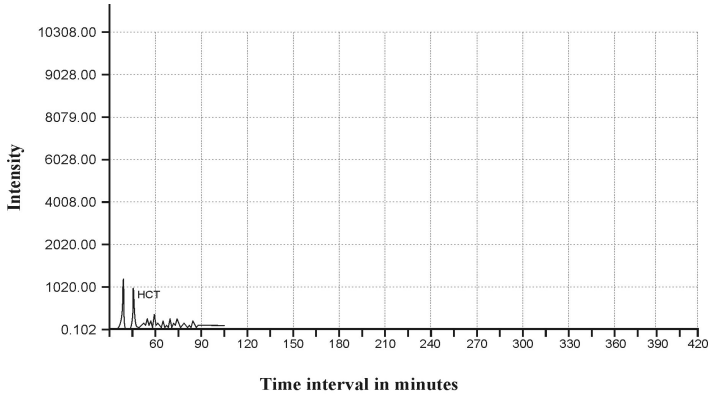


COMMERCIAL PRODUCT AT 420 MIN.

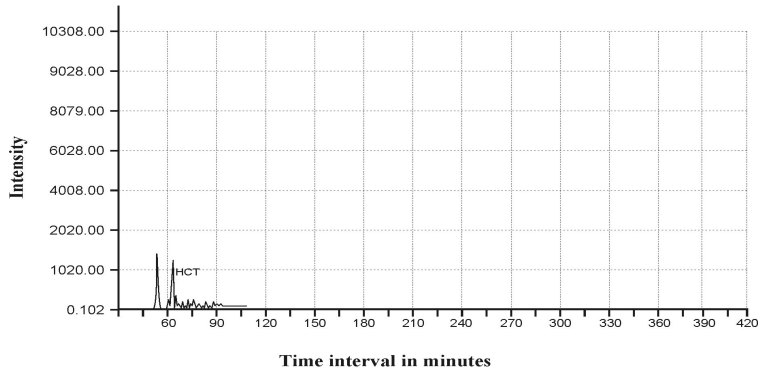


COMMERCIAL PRODUCT 1 : 4 (360 MIN)

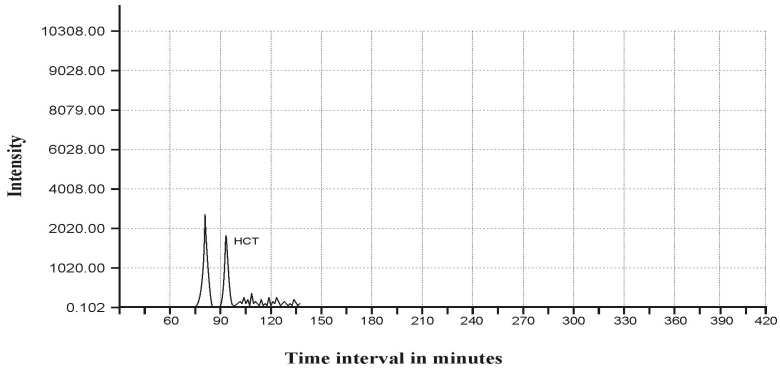
Physical Mixture



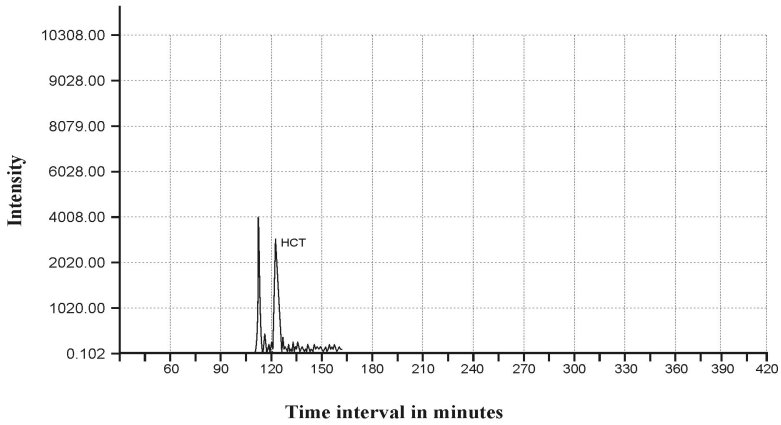
PHYSICAL MIXTURE 1 : 4 (30 MIN)



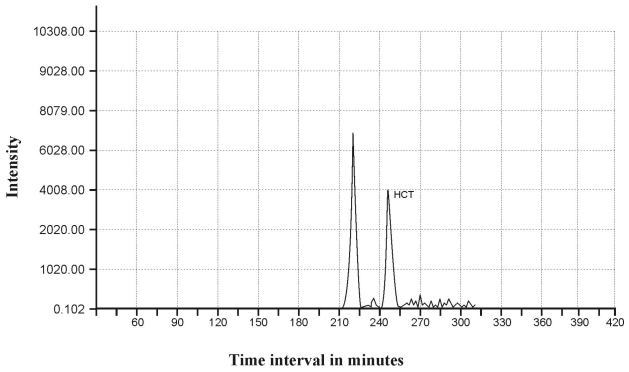
PHYSICAL MIXTURE 1 : 4 (60 MIN)



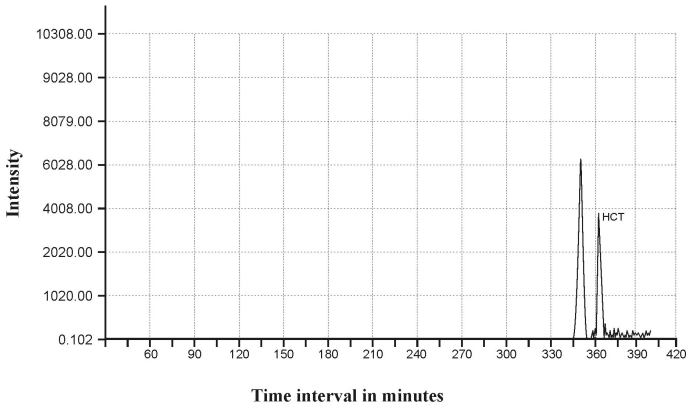
PHYSICAL MIXTURE 1 : 4 (90 MIN)



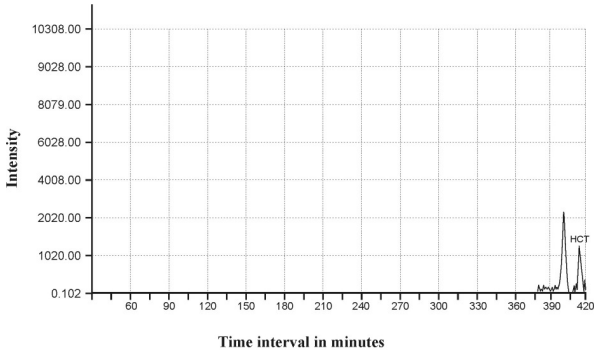
PHYSICAL MIXTURE 1 : 4 (120 MIN)



PHYSICAL MIXTURE 1 : 4 (240 MIN)

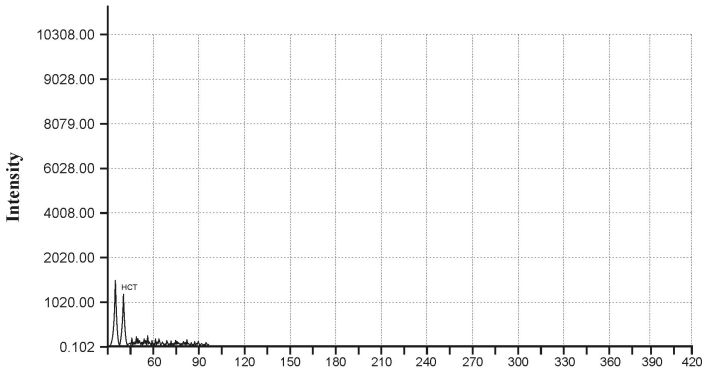


PHYSICAL MIXTURE 1 : 4 (360 MIN)

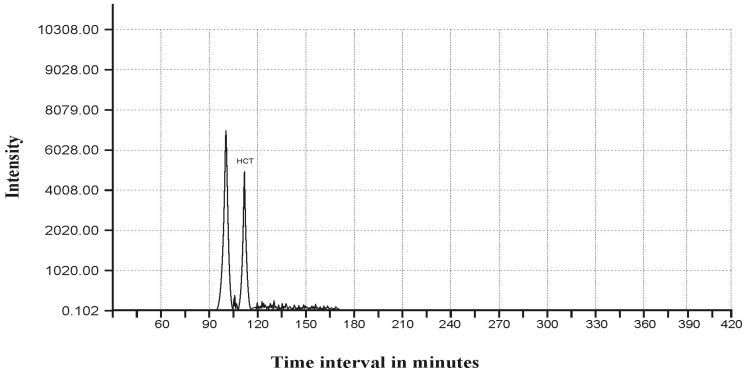


PHYSICAL MIXTURE 1 : 4 (420 MIN)

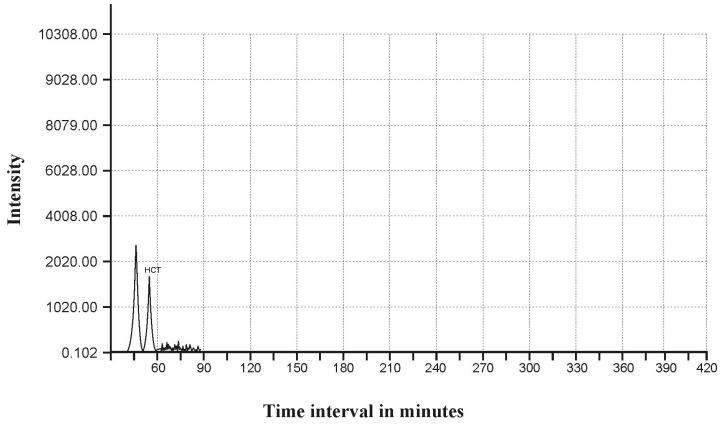
Solid Dispersion



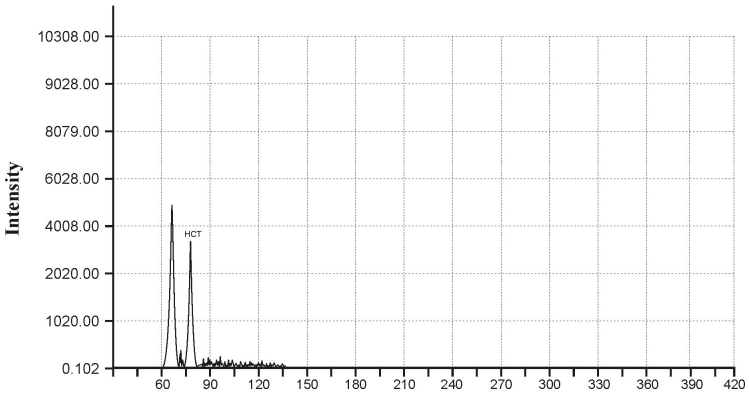
SOLID DISPERSION 1 : 4 (30 MIN)



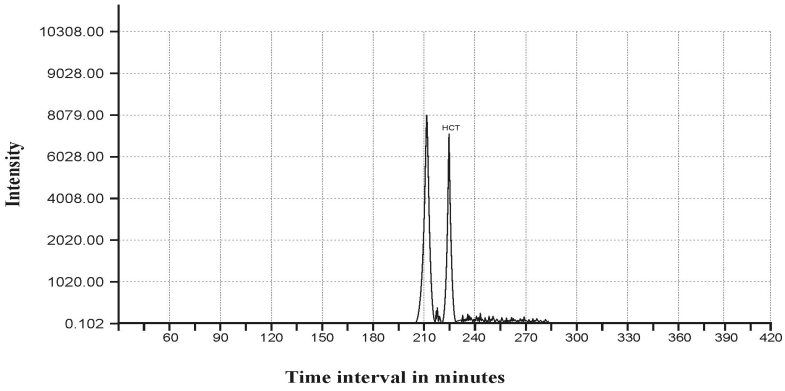
SOLID DISPERSION 1 : 4 (120 MIN)



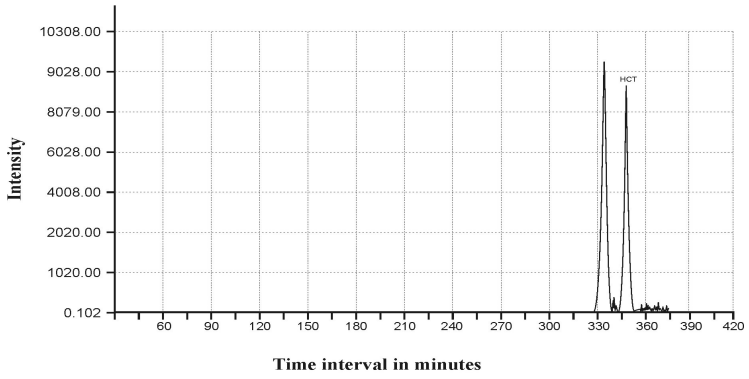
SOLID DISPERSION 1 : 4 (60 MIN)



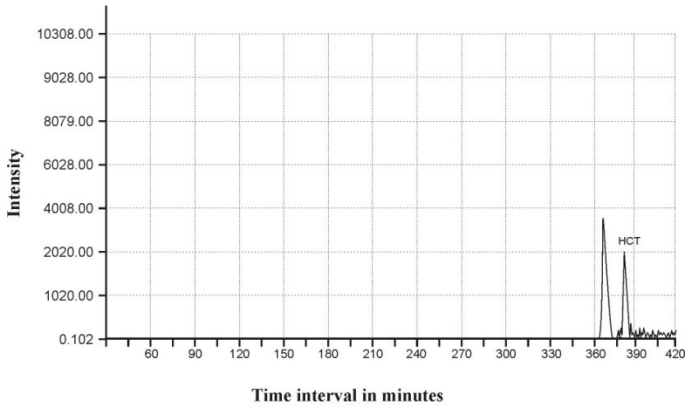
SOLID DISPERSION 1 : 4 (90 MIN)



SOLID DISPERSION 1 : 4 (240 MIN)



SOLID DISPERSION 1 : 4 (360 MIN)



SOLID DISPERSION 1 : 4 (420 MIN)

Table.29 **COMMERCIAL PRODUCT**

S.NO	Parameter	Animals (n=3)			Mean± S.D	S.E.M
		1	2	3		
1	**K_e (hr^{-1})**	0.14	0.19	0.2	0.17± 0.032	0.018
2	**K_a (hr^{-1})**	0.5	0.3	0.8	0.53 ± 0.25	0.14
3	**$t_{1/2}$**	3.01	3.46	3.3	3.35± 0.2	0.13
4	**V_d**	2.69	2.36	2.48	2.51± 0.16	0.096
5	**C_{max}**	1.11	1.09	1.13	1.11± 0.02	0.011
6	**T_{max}**	6.2	6.89	6.32	6.47± 0.36	0.212
7	**AUC_{0-8}**	2.7	2.63	2.82	2.71± 0.09	0.55

Table.30 **PHYSICAL MIXTURE**

S.NO	Parameter	Animals (n=3)			Mean± S.D	S.E.M
		1	2	3		
1	**K_e (hr^{-1})**	0.14	0.13	0.15	0.14± 0.010	0.005
2	**K_a (hr^{-1})**	0.32	0.36	0.34	0.34± 0.020	0.011
3	**$t_{1/2}$**	3.56	3.2	3.5	3.42± 0.19	0.11
4	**V_d**	1.99	2.0	2.2	2.063± 0.11	0.068
5	**C_{max}**	0.12	0.11	0.22	0.15± 0.06	0.0351
6	**T_{max}**	7.69	7.83	7.62	7.71± 0.106	0.061
7	**AUC_{0-8}**	2.79	2.2	2.4	2.46± 0.30	0.173

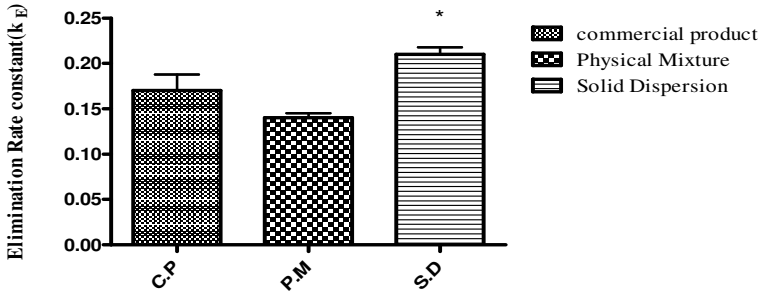
Table.31 **SOLID DISPERSION**

S.NO	Parameter	Animals (n=3)			Mean± S.D	S.E.M
		1	2	3		
1	K_e (hr^{-1})	0.23	0.2	0.21	0.21± 0.015	0.008
2	K_a (hr^{-1})	1.84	1.82	1.81	1.82± 0.015	0.008
3	$t_{1/2}$	2.1	2.3	2.6	2.33± 0.25	0.145
4	V_d	4.80	4.49	4.62	4.63± 0.155	0.089
5	C_{max}	1.4	1.2	1.30	1.3± 0.1	0.057
6	T_{max}	5.2	5.6	5.3	5.36± 0.20	0.120
7	AUC_{0-8}	4.4	4.2	3.18	3.92± 0.65	0.37

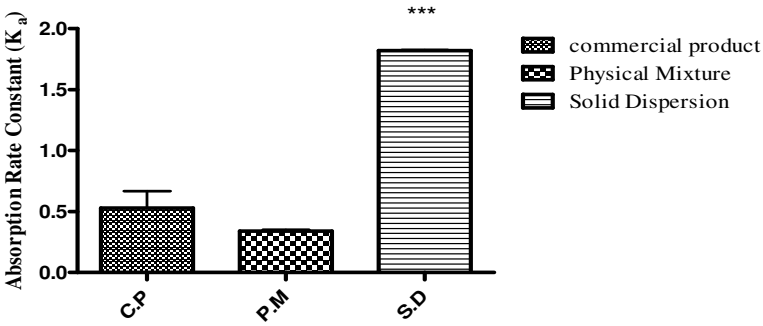
Table.32 **COMPARITIVE STUDY OF PHARMACOKINETIC PARAMETERS**

S.NO	Parameter	Mean ± S.D			P VALUE of S.D
		C.P	P.M	S.D	
1	K_e (hr^{-1})	0.17± 0.032	0.14± 0.010	0.21± 0.015	P<0.05
2	K_a (hr^{-1})	0.53 ± 0.25	0.34± 0.020	1.82± 0.015	P<0.001
3	$t_{1/2}$	3.35± 0.2	3.42± 0.19	2.33± 0.25	P<0.01
4	V_d	2.51± 0.16	2.063± 0.11	4.63± 0.155	P<0.001
5	C_{max}	1.11± 0.02	0.15± 0.06	1.3± 0.1	P<0.001
6	T_{max}	6.47± 0.36	7.71± 0.106	5.36± 0.20	P<0.01
7	AUC_{0-8}	2.71± 0.09	2.46± 0.30	3.92± 0.65	P<0.05

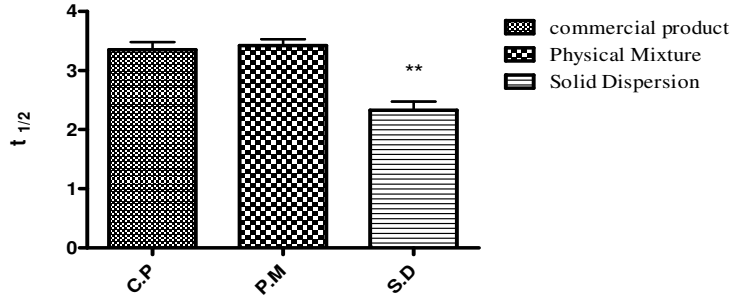
K_e (hr^{-1}) of Commercial product, Physical mixture and Solid Dispersion.



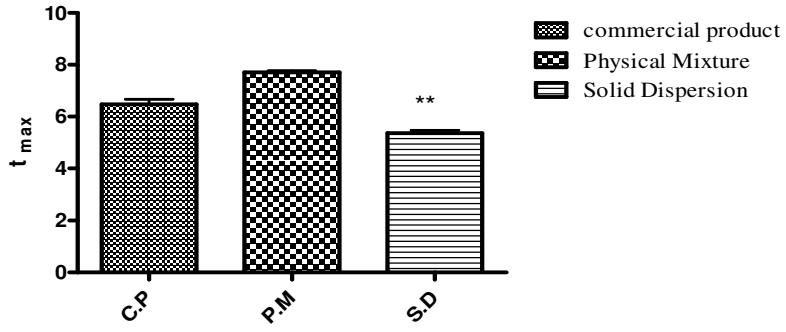
K_a (hr^{-1}) of Commercial product, Physical Mixture and Solid Dispersion



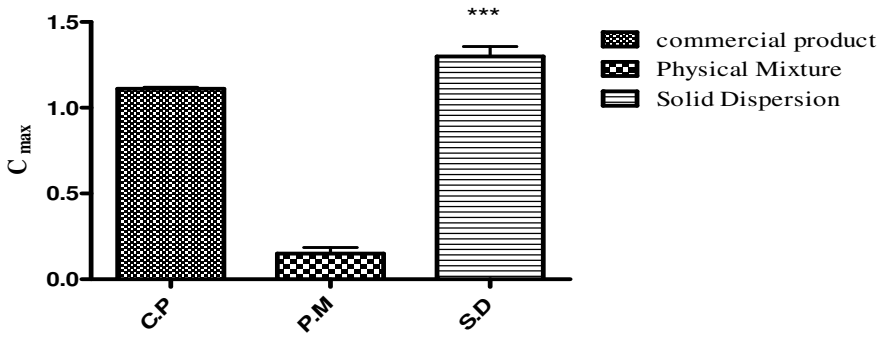
$T_{1/2\ of}$ Commercial Product, Physical Mixture and Solid Dispersion.



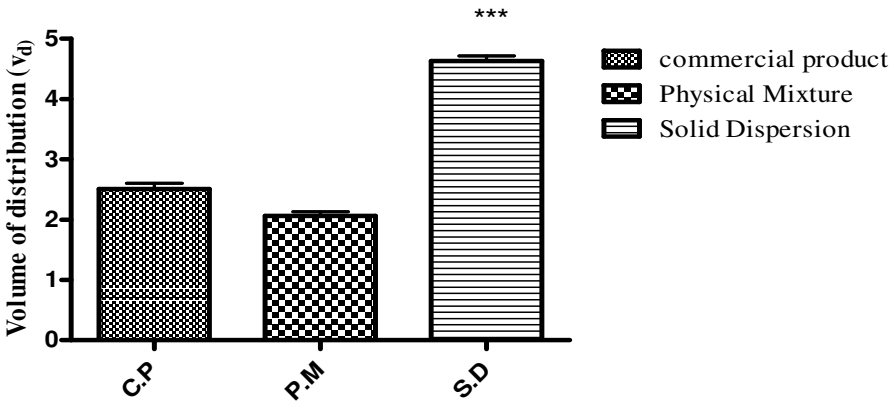
T_{max} of Commercial Product, Physical Mixture and Solid Dispersion



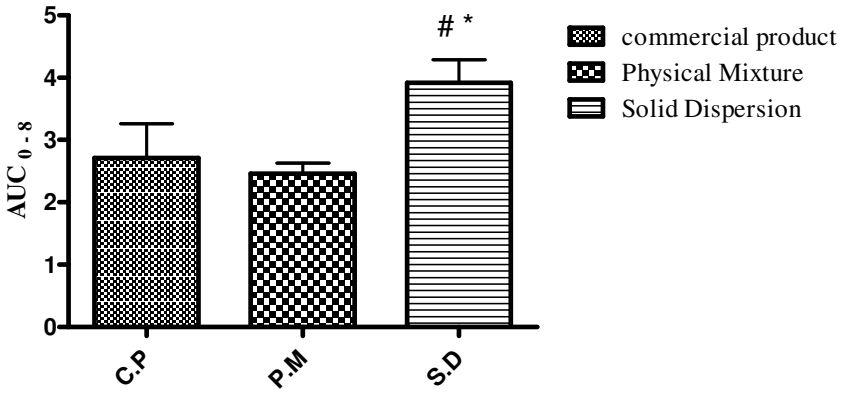
C_{max} of Commercial Product, Physical Mixture and solid Dispersion



V_d of Commercial Product, Physical Mixture and Solid Dispersion



AUC_{0-8} of Commercial Product, Physical Mixture and Solid Dispersion



9. DISCUSSION

The results of the present study demonstrate that a novel drug-drug solid dispersion approach can improve dissolution and pharmacokinetic characteristics of the poorly soluble drug that was presented with the soluble drug in fixed dose formulation. This novel approach will obviate the need for inclusion of physiological water soluble inert carriers in solid dispersion and so cost effective. Besides, this approach stabilizes the formulation from the effect of moisture that is normally encountered in solid dispersions prepared with physiological inert carriers.

In the present study HTC-LSP, the FDC used in the treatment of hypertension was selected as a model for this novel drug–drug solid dispersion approach and its physiochemical, *in vitro* release and pharmacokinetic characteristics were investigated. HTC though rapidly absorbed from the GIT following oral administration, its poor solubility may pose dissolution rate limited absorption problem. The *in vitro* release of solid dispersion has shown enhanced dissolution of HCT as compared to physical mixture or commercial product.

The HCT which was present in crystalline form in physical mixture was changed to amorphous form in the solid dispersion as evident from DSC thermogram. The amorphous form of HCT was more soluble than its crystalline form and so an improved dissolution of HCT was observed from solid dispersion. Additionally LSP which is freely soluble has increased the solubility of HCT due to its solvent effect as shown in phase solubility study.

To substantiate the effect of these changes in solid dispersion on the pharmacodynamic of HCT, a study was carried out in rats in our laboratory and it has observed that the improved solubility and dissolution of HCT in solid dispersion showed positive correlation with improved diuretic effect of HCT (unpublished data). These findings tempted to investigate further the pharmacokinetics of the solid dispersion in comparision with P.M and C.P. The solid dispersion has shown an improved pharmacokinetics of HCT which was significantly higher than that of P.M and C.P.

10. SUMMARY AND CONCLUSION

- The present study shows improved dissolution and pharmacokinetics of HCT from a modified novel drug-drug solid dispersion.
- Dissolution and pharmacokinetics of HCT was better from HCT-LSP solid dispersion as compared to physical mixture and commercial product.
- This novel solid dispersion is stable as no physiological inert carriers that are affected by moisture are used.
- Cost effective and economical as this approach is free of the economical burden of physiological inert carriers.
- Further studies in humans are recommended to address the therapeutic advantages of the novel solid dispersion in clinical practice.

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