Andrographolide is poorly soluble in water, it has low oral bioavailability. Objective of present study was to enhance solubility of andrographolide through solid dispersion technique and conversion it into tablet dosage form. Solubility of extract was found to be more from solid dispersion prepared by using Soluplus than that of prepared using PEG 6000. Compatibility study using IR, DSC and XRD showed that, ethanolic extract is compatible with soluplus and can be used for preparation of stable formulation. Pharmacokinetic study showed that, there was a significant increase in absorption of andrographolide from solid dispersion tablet. andrographolide. The relative bioavailability of andrographolide from solid dispersion tablet to that of pure andrographolide was found to be 177.59±14.6%. From tissue distribution study it can be concluded that, concentration of andrographolide in all tissues get increased in animals in which solid dispersion tablet was administered orally. It reveals that absorption of andrographolide is getting increased after conversion of extract into solid dispersion tablet. Accelerated stability study indicates that prepared solid dispersion was stable.

Dr. Sachin Annasaheb Nitave working as Principal at Dr. J. J. Magdum Trust's Anil Alias Pintu Magdum Memorial Pharmacy College, Dharangutti, Tal-Shirol, Dist-Kolhapur, Maharashtra, India. He secured the first rank in the M. Pharm examination at Shivaji University. He published 63 papers in different journals. He has undergone more than 50 training.

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Sachin Annasaheb Nitave
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Dr. Sachin A. Nitave

Abstract:

Andrographis paniculata Wall. ex Nees (Kalmegh) is one of the nineteen species of the genus *Andrographis*, which is indigenous to India. The herb is distributed in tropical Asian countries with hot and humid climate. Its major diterpenoid constituent is andrographolide. As andrographolide is poorly soluble in water, it has low oral bioavailability. Hence objective of present study was to enhance solubility of andrographolide through solid dispersion technique and conversion it into tablet dosage form. Solubility of extract was found to be more from solid dispersion prepared by using Soluplus than that of prepared using PEG 6000. Compatibility study using IR, DSC and XRD showed that, ethanolic extract is compatible with soluplus and can be used for preparation of stable formulation. Pharmacokinetic study showed that, there was a significant increase in absorption of andrographolide from solid dispersion tablet as compared to pure andrographolide. The relative bioavailability of andrographolide from solid dispersion tablet to that of pure andrographolide was found to be 177.59±14.6%. From tissue distribution study it can be concluded that, concentration of andrographolide in all tissues get increased in animals in which solid dispersion tablet was administered orally. It reveals that absorption of andrographolide is getting increased after conversion of extract into solid dispersion tablet. Accelerated stability study indicates that prepared solid dispersion tablet of *Andrographis paniculata* extract, F3 formulation was stable. The further research work intends to perform clinical trials for solid dispersion tablet of *Andrographis paniculata* extract as well as scale-up of developed solid dispersion tablet.

List of Abbreviations:

AP	:	*Andrographis paniculata*
APE	:	*Andrographis paniculata* extract
KEE	:	Kariyat ethanolic extract
K	:	Elimination rate constant
$AUC_{(0-t)}$	:	Area under curve up to measurable concentration
$AUC_{(0-\alpha)}$	:	Area under curve zero to infinity
C_{max}	:	Maximum drug concentration
DSC	:	Differential scanning calorimeter
LBD	:	Loose bulk density
TBD	:	Tapped bulk density
%F	:	Percent friability
F_{rel}	:	Relative bioavailability
FTIR	:	Fourier transform infrared spectroscopy
g	:	gram
GC-MS	:	Gas chromatography – Mass spectroscopy
$t_{1/2}$	:	Elimination half life
%	:	Percentage
HPLC	:	High Performance Liquid Chromatography
HPTLC	:	High performance thin layer chromatographic
Kg	:	Kilogram
PEG6000	:	Polyethylene glycol 6000
MCC	:	Micro crystalline cellulose
mL	:	Milliliter
NMR	:	Nuclear magnetic resonance
T_{max}	:	Time at which maximum C_{max} occurs

UV	:	Ultra violet
MRT	:	Mean residence time
XRD	:	X-ray Diffractometry
ng	:	Nano gram
µg	:	Microgram
TLC	:	Thin layer chromatography
PC	:	Paper chromatography
CC	:	Column chromatography
AAS	:	Atomic absorption spectroscopy
p.o.	:	Per oral
ppt	:	Precipitate

Table of Contents:

CHAPTER I: NEED AND OBJECTIVES

1.1 NEED OF WORK:

Drugs which are used presently for the management of pain and inflammatory conditions are either steroidal (corticosteroids) or non steroidal (aspirin). All of these drugs possess toxic effects at variable levels like renal failure, allergic reactions, nausea, vomiting, epigastric pain, gastro-intestinal bleeding, peptic ulceration, occasionally hearing loss and they can increase the risk of hemorrhage by affecting platelet function. Hence it is necessary to investigate analgesic and antipyretic effect of medicinal plant as it has less side effect.

The herbal drug technologies have facilitated the drug utilization of phytoconstituents and bioactives in a more precised manner. Interest in natural product research has been rekindled by discoveries of various novel natural molecules. But, therapeutic potential of natural molecules may often be limited by low solubility, bioavailability and instability associated with herbals. Exploration of solid dispersion technique provides various advantages including enhancement of solubility and bioavailability, protection from toxicity, enhancement of stability, sustained delivery, protection from physical and chemical degradation etc.

Literature survey reveals that, principle constituent of *Andrographis paniculata* i.e. andrographolide has poor aqueous solubility and hence poor oral bioavailability. Among the available approaches, the solid dispersion technique has often proved to be the most commonly used method in improving dissolution and bioavailability of the drugs because of its simplicity and economy in preparation and evaluation. Exploration of solid dispersion technique provides various advantages including enhancement of solubility and bioavailability, protection from toxicity, enhancement of stability, sustained delivery, protection from physical and chemical degradation etc. Hence it is necessary to increase aqueous solubility, dissolution and hence absorption and oral bioavailability of andrographolide through solid dispersion technique.

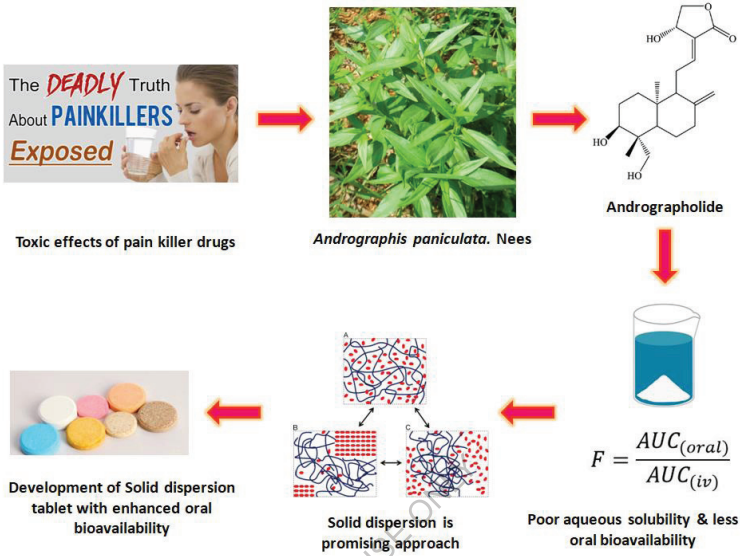

Fig. 1.1: Need of work

1.2 OBJECTIVES OF WORK:

I. Extraction and characterization of aerial part of *Andrographis paniculata.*

II. Analgesic activity of ethanolic extract of *Andrographis paniculata* by using following models:

- Hot plate method in mice.
- Acetic acid induced writhing test model in mice.

III. Antipyretic activity of ethanolic extract of *Andrographis paniculata* using

- Brewer's yeast induced Hyperpyrexia in rats.

IV. Screening of Prostaglandins inhibition in rats.

V. Formulation and Evaluation of Solid dispersion tablet of ethanolic extract of *Andrographis paniculata.*

VI. Pharmacokinetic and tissue distribution study of Solid dispersion tablet of ethanolic extract of *Andrographis paniculata*.

VII. Accelerated stability study of solid dispersion tablet.

CHAPTER II: MATERIALS AND METHODS

MATERIALS:

The plant materials, excipients, chemicals/ reagents, instruments and animals used for various experiments are enlisted in Table 2.1, 2.2 and 2.3.

Table 2.1: The drug, excipients, chemicals/ reagents used for various experiments

Material	Collected from or Gifted/Supplied by
0.9% Sodium chloride injection USP	Baxter
3,5-dinitrobenzoic acid	Pallav Chemicals, Mumbai
Acetic acid	Pallav Chemicals, Mumbai
Ammonia solution	Pallav Chemicals, Mumbai
Andrographis paniculata	Surrounding areas of Taluka Shirol, District Kolhapur, Maharashtra
Aspirin IP	Pallav Chemicals, Mumbai
Barfoed's reagent	Pallav Chemicals, Mumbai
Biuret reagent	Pallav Chemicals, Mumbai
Brewer's yeast	Sigma Chemicals, Mumbai
Castor oil	Sigma Chemicals, Mumbai
Chloroform	Pallav Chemicals, Mumbai
Conc. Hydrochloric acid	Pallav Chemicals, Mumbai
Conc. Nitric acid	Pallav Chemicals, Mumbai
Conc. Sulphuric acid	Pallav Chemicals, Mumbai
Copper sulphate	Pallav Chemicals, Mumbai
Dichloromethane	Pallav Chemicals, Mumbai
Disodium hydrogen phosphate	Sigma Chemicals, Mumbai

Ethanol	Pallav Chemicals, Mumbai
Fehling's A	Pallav Chemicals, Mumbai
Fehling's B	Pallav Chemicals, Mumbai
Ferric chloride	Pallav Chemicals, Mumbai
Gelatin	Pallav Chemicals, Mumbai
Glycerin	Pallav Chemicals, Mumbai
Heparin 100 I.U./ml	Wockhardt Ltd.
Iodine	Pallav Chemicals, Mumbai
Lead acetate	Pallav Chemicals, Mumbai
Magnesium acetate	Pallav Chemicals, Mumbai
Magnesium stearate	Pallav Chemicals, Mumbai
Magnesium turnings	Pallav Chemicals, Mumbai
MCC	Pallav Chemicals, Mumbai
Methanol	Pallav Chemicals, Mumbai
Millon's reagent	Pallav Chemicals, Mumbai
Ninhydrine solution	Pallav Chemicals, Mumbai
PEG-6000	Merck India Ltd. (Mumbai).
Phloroglucinol	Pallav Chemicals, Mumbai
Picric acid	Pallav Chemicals, Mumbai
Potassium bismuth iodide	Pallav Chemicals, Mumbai
Potassium hydroxide	Pallav Chemicals, Mumbai
Potassium iodide	Pallav Chemicals, Mumbai
Potassium mercuric iodide	Pallav Chemicals, Mumbai
Pure Andrographolide	Sigma-Aldrich
Resorcinol	Pallav Chemicals, Mumbai
Ruthenium red	Pallav Chemicals, Mumbai

Sodium bicarbonate	Pallav Chemicals, Mumbai
Sodium dihydrogen phosphate	Sigma Chemicals, Mumbai
Sodium hydroxide	Pallav Chemicals, Mumbai
Sodium nitroprusside	Pallav Chemicals, Mumbai
Sodium picrate	Pallav Chemicals, Mumbai
Soluplus	BASF Chemical, Mumbai
Sulfur powder	Pallav Chemicals, Mumbai
Talc	Pallav Chemicals, Mumbai
Tannic acid	Pallav Chemicals, Mumbai
Trichloroacetic acid	Pallav Chemicals, Mumbai
Water	Pallav Chemicals, Mumbai
Zinc dust	Pallav Chemicals, Mumbai
α-naphthol	Pallav Chemicals, Mumbai

Table 2.2: Instruments used for various experiments

Name of Equipment	Company Name/Model
HPLC	Shimadzu [(UV2075plus)/(JascoUV-1575)] ODS Hypersil C18 column (4.6 mm x 250 mm, 5µm)
FTIR spectrophotometer	Brucker Alpha – T, India
UV Spectrophotometer	Jasco V-530, Japan
GC-MS	TURBOMASS 2017
HPTLC	CAMAG Linomat instrument
Chromatographic columns	Ambassadar
Eddy's Hot Plate	Hicon
Rotary flash evaporator	Hahnvapor, HahnshinScifintic Korea

DSC	Mettler Toledo, DSC1
XRD	Philips PW-3710
	Bruker Avance 300, Switzerland
Cold centrifuge	Remi
Digital balance	Preci-Tech
Hot air Oven	Ambassadar
Muffle furnace	Microtech
Microwave Oven	LG
Micropipettes	Tarsons
Electric water baths	Inco
Rotary tablet press	Dolphin
Disintegration test Apparatus	Dolphin
Dissolution test apparatus	Dolphin
Hardness tester	Cadmatch
Roche Friabilator	Lab Hosp
Vernier caliper	Mitutoyo Corporation India
Motic microscope	Motic microscope – B1 series
Stability chamber	Aditi associates, Mumbai

Table 2.3: Animals used in experiment

Albino rats:	
Species / Common name	Wister rat
Weight	250-300 g
Gender	Male and female
Proposed source of animals	Animal House of S. E. T. College of Pharmacy, S. R. Nagar Dharwad
Albino mice:	

Species / Common name	Swiss
Weight	25 g
Gender	Male and female
Proposed source of animals	Animal House of S. E. T. College of Pharmacy, S. R. Nagar Dharwad

METHODS:

2.1 DEVELOPMENT OF SOLID DISPERSION TABLET

2.1.1 Preformulation study:

Preformulation testing is the first step in the rational development of dosage forms of a drug. It can be defined as an investigation of physical and chemical properties of drug substance, alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms, which can be mass-produced.

A thorough understanding of physicochemical properties may ultimately provide a rationale for formulation design or support the need for molecular modification or merely confirm that there are no significant barriers to the compounds development. The goals of the program therefore are:

- To establish the necessary physicochemical characteristics of a new drug substance
- To establish its compatibility with different excipients

Hence, a preformulation study on the procured sample of drug includes conducting physical tests and compatibility studies. (Lachman L., et al., 1990 and Howard Y. A., et al., 2000)

UV Spectroscopic study:

Molecules absorb energy and this energy can bring about translational, rotational or vibrational motion or ionization of the molecules depending upon the frequency of the electromagnetic radiation they receive. Excited molecules are unstable and quickly drop down to ground state again giving off the energy they have received as electromagnetic radiation. The wavelength and intensity of the electromagnetic radiation absorbed or emitted can be recorded to get a spectrum.

Spectral analysis yields qualitative and quantitative information about the matter under study. (Hamid H., 2007)

It is based on measurement of transmittance *T* or absorbance *A* of solution contained in transparent cells having a path length of *b* centimeters. Ordinarily, the concentration of an absorbing analyte is linearly related to absorbance as given by Beer's law:

$$\mathbf{A = -\log T = \log \frac{P0}{P} = \epsilon bc} \qquad \mathbf{(2.1)}$$

Where A: Absorbance, T: Transmittance, P0: Incident radiant power, P: Transmitted radiant power, ϵ: Molar absorptivity, b: Path length, c: Concentration of absorber. (Skoog D. A., et al., 2007)

Determination of λ_{max}:

The stock solution of andrographolide extracted from *Andrographis paniculata* was prepared by dissolving accurately 20 mg of *Andrographis paniculata* extract (equivalent to 50% Andrographolide) in methanol in a 100 mL volumetric flask to obtain a concentration of 100µg/mL. The UV spectrum was recorded on UV spectrophotometer (Jasco V-530, Japan) at 1 cm slit width.

Preparation of Standard Curve:

20 mg of *Andrographis paniculata* extract (equivalent to 50% Andrographolide) was dissolved in a small amount of methanol in 100 mL volumetric flask and the volume was made up to 100 mL using methanol to obtain a concentration of 100µg/ml. From this solution 10 mL was withdrawn and diluted to 100 mL with methanol. From this stock solution serial dilutions were made to obtain the solutions in concentration ranging from 2-10 µg/ml. The absorbance of the solution was measured at 224 nm.

2.1.2 Preparation of Solid dispersions of *Andrographis paniculata* extracts using solvent evaporation technique:

Solid dispersions of *Andrographis paniculata* extract and polymer such as soluplus and PEG-6000 were prepared by solvent evaporation method. The ratio of *Andrographis paniculata* extract (equivalent to 50 mg of andrographolide) to polymer was taken as 1:1, 1:2 and 1:3. Composition

of each ingredient is shown in Table 2.4. The *Andrographis paniculata* extract and polymer were separately dissolved in sufficient quantity of methanol. The clear solutions of extract and polymer were mixed and then the solvent was evaporated in water bath at 50°C till dryness. The dried sample of dispersion was kept in desiccator until further study. To this dried dispersion required quantity of micro crystalline cellulose (MCC) and talc were added as a diluent and lubricant respectively and then pulverized using a mortar and pestle. Mixture was passed through 50-mesh sieve (300µm) and used for evaluation of micromeritic properties. (Sharma A. et al., 2010 and Weerapol Y. et al., 2017)

Table 2.4: Composition of solid dispersion of *Andrographis paniculata* extract

Content	Formulation Code					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Andrographis paniculata extract (equivalent to 50 mg of andrographolide)	100	100	100	100	100	100
Soluplus	50	100	150	-	-	-
PEG 6000	-	-	-	50	100	150
Talc	2	2	2	2	2	2
Magnesium stearate	2	2	2	2	2	2
MCC	106	56	6	106	56	6

All ingredients were taken in mg per tablet.

2.1.3 Evaluation of solid dispersion:

Solid dispersion of *Andrographis paniculata* extract was evaluated for photo microscopic image and micromeritic properties such as angle of repose, bulk density, tapped density, Carr's index, hausners ratio, percent yield etc. (Aulton ME. 1988 and More HN. et al., 2010)

Photo microscopic image:

Photo microscopic image of solid dispersion was carried out by using motic microscope (Motic Microscope- B 1 Series) at 720 *576 resolutions.

Micromeritic properties of solid dispersion:

Angle of Repose:

The angle of repose of solid dispersion was determined by funnel method. Accurately weighed quantity of solid dispersion was taken in a funnel. Height of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap of dispersion. The dispersion was allowed to flow through funnel freely on to the surface. The diameter of the powder cone was measured and angle of repose was calculated by using the following equation.

$$\tan \theta = h/r \qquad (2.2)$$

Where θ = angle of repose, h = height of the cone and r = radius of the cone base

Bulk density:

Both loose bulk density (LBD) and tapped bulk density (TBD) were determined. A quantity of 2 g of solid dispersion of each formulation which was previously lightly shaken to break any agglomerates formed and introduced into a 10 ml measuring cylinder. After checking the initial volume was observed, the cylinder was allowed to fall under its own weight on to a hard surface from a height of 2.5 cm after every 2 seconds. The tapping was being continued until no further change in volume was noted. LBD and TBD were calculated by using the following formulas.

LBD = Weight of powder / Volume of packing (2.3)

TBD = Weight of powder / Tapped volume of packing (2.4)

Compressibility Index:

The compressibility of the granules was determined by Carr's Compressibility Index by using following formula.

Carr's compressibility index (%) = [(TBD – LBD) × 100] / TBD (2.5)

Hausners ratio:

A similar index like compressibility index has been defined by Hausners. Hausners ratio can be calculated by formula:

Hausners ratio = TBD/LBD (2.6)

Percent Yield:

The prepared solid dispersion was weighed and compared to the initial weight. The percentage yield of solid dispersion prepared by solvent evaporation method was determined by using the following formula:

$$\textbf{Percent yield} = \frac{\textit{Weight of preprated solid dispersion}}{\textit{Weight of extract+polymer}} \textbf{x 100} \qquad \textbf{(2.7)}$$

2.1.4 Determination of Saturation Solubility of *Andrographis paniculata* extract and Solid Dispersions:

Saturation solubility was determined by dispersing a known excess amount of *Andrographis paniculata* extract and solid dispersions into 10 ml of distilled water. The suspensions were stirred using mechanical shaker at 37 ± 0.5°C for 48 hrs. At the end of this samples were withdrawn and centrifuged at 10000 rpm for 10 min to separate un-dissolved extract. The supernatant was taken and diluted with methanol. This solution was filtered through 0.45µm whatman filter paper and solubility was quantified by using UV- Spectroscopy (Jasco V-530, Japan) at 224 nm. The results of triplicate measurements and their means were reported. (Changdeo JS. et al., 2011)

2.1.5 Preparation of Solid dispersion tablets:

To dried solid dispersion of each formulation, required quantity of micro crystalline cellulose (MCC) and talc were added as a diluent and lubricant respectively and then pulverized using mortar and pestle. Mixture was passed through 50-mesh sieve (300µm). Then required quantity of magnesium stearate was added as an anti-adherent and mixed well. The prepared dispersion was compressed into tablet using 8 mm punch on 8 station rotary tablet press. (Mamatha T. et al., 2017)

2.1.6 Evaluation of Tablets:

The solid dispersion tablets of *Andrographis paniculata* extract was evaluated for

different parameters like thickness and diameter, hardness, friability, weight variation test, drug content, disintegration test (Banker GS. 1990 and Indian Pharmacopoeia, 1996) and in-vitro drug release study. (Zhang D. et al., 2016)

Thickness and Diameter:

Thickness and diameter of tablet was determined using digital vernier caliper (Mitutoyo Corporation India). Five tablets from each batch were used and their average values were calculated.

Hardness:

For each formulation, the hardness of 6 tablets was determined using calibrated Monsanto hardness tester (Cadmach). The tablet was held along to its oblong axis in between the two jaws of the tester. At this point reading should be zero kg/cm^2. Then constant force was applied by rotating the knob until the tablet gets fractured. The value at this point was noted in kg/cm^2 which indicates hardness of tablets.

Friability:

For each formulation the friability was determined by using 20 tablets and Roche friabilator (Lab Hosp.). The tablets are subjected to combined effect of shock abrasion by utilizing a plastic chamber which revolves at a speed of 25 rpm and drops the tablets to a distance of 6 inch in each revolution. A sample of pre-weighed 20 tablets was placed in Roche friabilator which was then operated for 100 revolutions for 4 min. Then the tablets were de-dusted and reweighed. A loss of less than 1 % in weight is generally acceptable. Percent friability (% F) was calculated as follows:

$$\% \mathbf{F} = \frac{\textbf{Loss in weight}}{\textit{Initial weight}} \mathbf{x\ 100} \qquad \textbf{(2.8)}$$

Weight variation Test:

To study weight variation test 20 tablets of each formulation were weighed using an electronic balance (Citizen, CY-104) and the test was performed according to the official method.

Drug content:

Five tablets were weighed individually and these tablets were crushed in a mortar. Drug equivalent to 10 mg of powder was dissolved in a suitable quantity of methanol by using an ultrasonicator and filtered through Whatman filter paper. The drug content was determined at 224 nm using UV spectrophotometer (Jasco V-530, Japan) after suitable dilutions.

Disintegration test:

Disintegration test of solid dispersion tablet was carried out by using Disintegration test apparatus (Dolphin) at temperature 37ºC ± 2.0ºC. One tablet is placed into each tube & disc is

added to each tube. Suspend the assembly in the beaker containing water & operate the apparatus for the specified period of time.

In-vitro Drug Release Studies:

In-vitro drug release study for the prepared solid dispersion tablets was conducted using a six-station USP type II (paddle) apparatus at 37ºC ± 0.5ºC and 100rpm speed. The dissolution studies were carried out in two different dissolution media such as acidic buffer of pH 1.2 and phosphate buffer of pH 6.8. Samples were withdrawn at 5, 10, 15, 30, 45, 60, 90 and 120 min and replaced with fresh dissolution media. After filtration through Whatman filter paper, required quantity of methanol was added and concentration of andrographolide was determined UV-Spectrophotometrically at 224 nm.

2.1.7 Compatibility study:

Optimized formulation prepared by using soluplus containing solid dispersion of *Andrographis paniculata* extract was evaluated for compatibility study between andrographolide by using IR, DSC and XRD. (Chatwal GR. et al., 2002)

Infrared spectroscopy:

Fourier transform- infrared (FT-IR) spectra of *Andrographis paniculata* extract, soluplus and 1:1 physical mixture of extract and soluplus were obtained on FT-IR (Bruker Alpha-T, India). The spectra were scanned over the wave number range from 4000 – 600 cm^{-1}.

Differential Scanning Calorimetry:

Thermograms of *Andrographis paniculata* extract, soluplus and solid dispersion were obtained using a DSC instrument (Mettler Toledo, DSC1) equipped with an intracooler. Indium standard was used to calibrate the DSC temperature and enthalpy scale. The powder samples of granules was hermetically sealed in an aluminium pan and heated at constant rate 10ºC/min over a temperature range of 30ºC to 250ºC. Inert atmosphere was maintained by purging nitrogen at the flow rate of 100 ml/min.

X-ray diffraction:

The X-ray diffraction patterns of powders were determined using a Phillips PW- 3710 X-ray diffractometer. Sample of *Andrographis paniculata* extract, soluplus and solid dispersion were

irradiated with monochromatic Cu Kα radiation (1.542 A^0) and analysed at 2θ from 2^0 to 60^0 angles. The range and chart speed were $2x10^3$ cycle per second and 10 mm/2θ respectively.

2.2 PHARMACOKINETIC AND TISSUE DISTRIBUTION STUDY:

2.2.1 Pharmacokinetic study:

A simple, selective and rapid reverse phase high performance liquid chromatographic (RP-HPLC) method was selected for the analysis of *Andrographis paniculata* extract. (Sharma M. et al., 2012 and Kumaran KS. et al., 2003)

2.2.1.1 Instrumental specification:

A sample was resolved on a Shimadzu [(UV2075plus) / (Jasco UV-1575)] ODS Hypersil C18 column (4.6 mm x 250 mm, 5µm).

Mobile Phase:

65 volumes of methanol and 35 volumes of water were used.

Preparation of standard solution:

Standard stock solution was prepared by dissolving 10mg of pure andrographolide in 100mL of HPLC grade methanol to give a concentration of 100µg/mL. From the standard solutions the concentrations of 10, 20, 30, 40 and 50µg/mL were then prepared by suitable dilution with methanol. Finally the prepared standard solutions were filtered separately through 0.45µm filter paper and stored bellow 10°C.

Calibration curve range: 10, 20, 30, 40 and 50 µg/mL.

Preparation of plasma samples for calibration curve:

0.2 mL of rat plasma samples were vortexed (extracted) with methanol using 1mL v/v for 2 min and centrifuged up to 5min. After centrifugation supernatant layer up to 0.9 mL was separated and evaporated. Finally, dry residue was reconstituted with mobile phase.

2.2.1.2 Animals care and handling:

Healthy adult albino rats used for the study of different pharmacokinetic parameter. Rats will be divided into three groups which are control, standard and test formulation groups. Care of animals was taken and handled as per guidelines derived from Guide for the Care and Use of Laboratory Animals (ILAR, 1985).

Caging:

Animals were housed in cage of 7 inch and 17.78 cm height as per guidelines.

Feed and Bedding:

The animals were kept on standard supplemented diet and water for two weeks prior to experiment and maintained on it thereafter. Animals were kept in suspended-wire cages with noncontact bedding beneath the cages. Bedding was stored under controlled conditions to prevent contamination from vermin and chemicals, and must be kept dry.

Water:

Continuous access to fresh uncontaminated water was provided.

Sanitation:

Regular frequent sanitation of the animal's living space is necessary to minimize the impact of environmental contamination. Frequency of cleaning was maintained.

Environmental monitoring and maintenance:

Animals were monitored and maintained at controlled temperature, humidity, ventilation, and illumination.

2.2.1.3 Pharmacokinetic study in rats:

Albino rats of either sex weighing between 250-300g were divided into 3 groups, each consisting of 6 animals. First group received ethanolic extract solid dispersion tablet (equivalent to 50 mg/Kg). Second group received a pure sample of Andrographolide (50 mg/ Kg) and third group was kept as control. The rats were restrained in a rat holder during blood sampling. The initial blood sample was taken by clipping the end of the rat's tail while the subsequent blood samples were collected by removing the clotted blood with cotton rinsed with 100 IU of heparin

solution. After each blood sampling, the wound was monitored for approximately 3 min to ensure that there was no excessive bleeding. Blood samples were immediately transferred to a heparinized micro centrifuge tube and centrifuged at 3000 rpm for 10 min at 5 ºC. The resulting plasma samples (0.2 mL supernatant) was transferred into 1.5 mL Eppendorf tubes and stored at –80 ºC until HPLC analysis. Blood samples were collected at time intervals of 0 min (predose), 0.5, 1, 2, 4, 8, 12, 16 and 24 hours after dosing. (Naidua SR. et al., 2009) The Institutional Animal Ethical Committee approved the protocol for this study (Ref. No. SETCP/IAEC/2017-2018/022).

2.2.1.4 Determination of various pharmacokinetic parameters:

The pharmacokinetic parameters such as a maximum concentration of andrographolide in plasma C_{max} and the time for the andrographolide to reach maximum concentration in plasma after administration t_{max} were computed directly from measured plasma concentration data. (Brahmankar DM. et al., 1995)

Area under the plasma andrographolide concentration-time curve up to measurable concentration $AUC_{(0\text{-}t)}$, area under the curve zero to infinity $AUC_{(0\text{-}\alpha)}$ and elimination rate constant (K) were calculated using MS-Excel based AUC calculation linear and log linear trapezoidal rule computer program. (Boomer Excel Calculation)

Elimination half-life ($t_{1/2}$) was calculated from following equation

$$t_{1/2} = 0.693/K \qquad (2.9)$$

To assess the degree of retardation of drug release, mean residence time (MRT) was calculated as follows.

$$MRT = 1/K \qquad (2.10)$$

Relative bioavailability (F_{rel}) of andrographolide from andrographolide extract tablet

to that of pure andrographolide was calculated using following formula. (Zhang Y. et al., 2012)

$$F_{rel} = \frac{AUC_{test} / Dose_{test}}{AUC_{reference} / AUC_{reference}} \times 100 \qquad (2.11)$$

All results were expressed as mean ± SD. The data from different groups were compared for statistical significance by students T test. The minimal level of significance was identified at $P<0.05$.

2.2.2 Tissue distribution study of ethanolic extract solid dispersion tablet of *Andrographis paniculata* in rats:

For *in-vivo* tissue distribution study albino rats of either sex weighing between 250-300g were used. Rats were acclimatized for at least one week before using them for experiments. Rats were divided into 2 groups (A and B) each containing 9 animals. Each group was further divided into 3 sub-groups (A1, A2, A3 and B1, B2, B3) for three different time points such as 1, 3 and 8h respectively. All animals of group A and B received ethanolic extract solid dispersion tablet (equivalent to 50 mg/Kg) and pure sample of Andrographolide (50 mg/Kg) respectively. Rats were then sacrificed by cervical dislocation at 1, 3 and 8 hours after dosing and heart, liver, kidney, lung, spleen and brain were collected. Tissue samples were washed with ice cold 0.9% w/v sterile physiological saline solution and immediately weighed and stored at −80°C until HPLC analysis. (Zhang X. et al., 2017)

2.3 ACCELERATED STABILITY STUDY

In any rationale design and evaluation of dosage forms for drugs, the stability of the active component must be major criteria in determining their acceptance or rejection. During the stability studies the product is exposed to normal conditions of temperature and humidity. However, the studies will take a longer time and hence it would be convenient to carry out the accelerated stability studies where the product is stored under extreme conditions of temperature.

In the present study, accelerated stability studies were carried out on F3 formulation. Tablets were stored at 40 ± 2°C/75 ± 5 % RH for duration of three months. After completion of three months sample was withdrawn and tested for thickness, hardness, disintegration time, drug content and *in-vitro* drug release. (Lakshmi PK. et al., (2013)

CHAPTER III: RESULTS AND DISCUSSION

3.1 Development of solid dispersion tablet

3.1.1 Preformulation study:

UV Spectroscopic study:

Determination of λ_{max}:

λ_{max} of ethanolic extract of *Andrographis paniculata* extract was found to be **224nm** in methanol which was shown in Fig. 3.1.

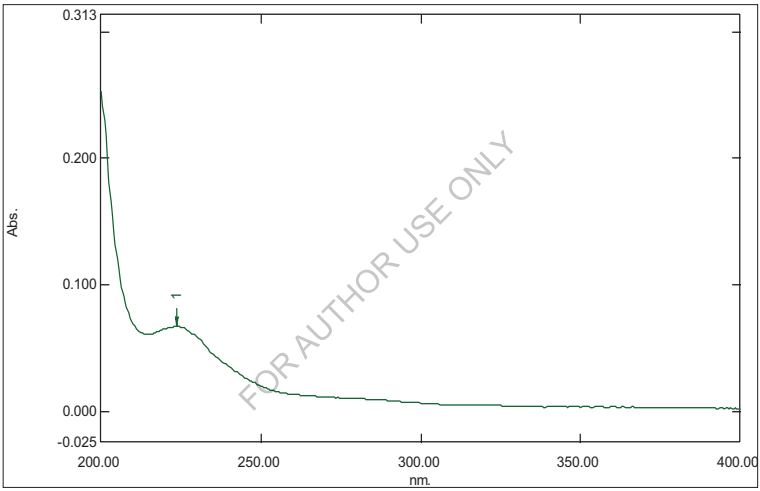

Fig. 3.1: λ_{max} of *Andrographis paniculata* extract

Preparation of Standard Curve:

Table 3.1: Reading of Calibration curve of *Andrographis paniculata* extract

Concentration (µg/mL)	Absorbance
0	0
2	0.037
4	0.086

6	0.115
8	0.17
10	0.217

The graph of absorption versus concentration for ethanolic extract of *Andrographis paniculata* was found to be linear in concentration range 2 to 10 µg/ml at 224 nm. The drug obeys Beer-Lambert's law in the range 2 to 10 µg/ml. It was shown in Fig. 3.2. The value of regression coefficient (R^2) is 0.994. Hence this calibration curve was found to be linear.

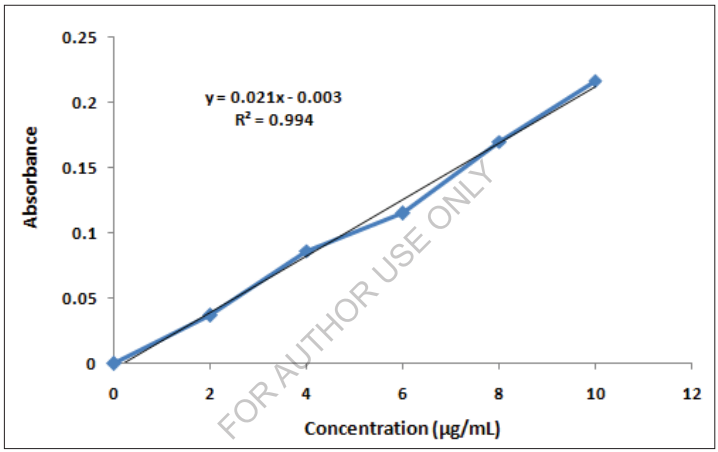

Fig. 3.2: Standard curve of *Andrographis paniculata* extract in methanol

3.1.2 Preparation of Solid dispersions of *Andrographis paniculata* extracts

Solid dispersion of *Andrographis paniculata* extract was prepared using solvent evaporation technique.

3.1.3 Evaluation of solid dispersion:

Photo microscopic image:

Photo microscopic image of *Andrographis paniculata* extract showed irregular crystals of extract. Whereas in the photo microscopic image of solid dispersion, crystals of extract was not

observed on the surface of solid dispersion. This may be due to complete dispersion of extract into carriers. It was shown in Fig. 3.3.

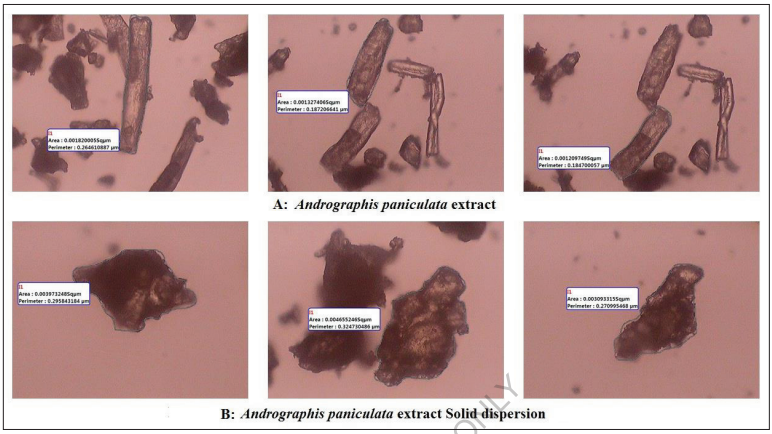

Fig. 3.3: Photo microscopic image

Micromeritic properties of solid dispersion:

All formulations of solid dispersions were found to be pale yellow, free flowing and odourless powder. Results of micromeritic properties of *Andrographis paniculata* extract like angle of repose, bulk and tapped density, compressibility index, Hausners ratio and percent yield are summarized in Table 3.2.

Table 3.2: Micromeritic properties of solid dispersion of *Andrographis paniculata* extract

Formulation	Angle of repose* (^{0})	Bulk density* (g/mL)	Tapped density* (g/mL)	Compressibility index* (%)	Hausners ratio*	% yield
F1	18.34 ± 0.05	0.347±0.02	0.442±0.03	21.49 ± 0.04	1.27 ± 0.04	92.54
F2	16.87 ± 0.04	0.423±0.04	0.536±0.04	21.08 ± 0.03	1.26 ± 0.03	94.56
F3	14.85 ± 0.03	0.457±0.03	0.532±0.04	14.09 ± 0.02	1.16 ± 0.04	98.63
F4	22.52 ± 0.04	0.687±0.04	0.825±0.05	16.72 ± 0.02	1.20 ± 0.02	85.47
F5	23.65 ± 0.03	0.440±0.03	0.532±0.04	17.29 ± 0.04	1.20 ± 0.04	88.25
F6	25.32 ± 0.03	0.397±0.01	0.474±0.02	16.24 ± 0.04	1.19 ± 0.02	90.27

***** All values represent mean ± standard deviation (n=3)

Angle of repose of all solid dispersion formulations was found to be in between 14.85 ± 0.03 to 25.32 ± 0.03. Angle of repose of solid dispersion prepared by using soluplus as a carrier (F1, F2 and F3) was found to be less than 20. Hence showed excellent flow property than that of solid dispersion prepared by using PEG 6000 as a carrier (F4, F5 and F6) which showed angle of repose more than 20. Bulk density and tapped density of all solid dispersion formulations was found to be in the range of 0.347±0.02 g/mL to 0.687±0.04 g/mL and 0.442±0.03 g/mL to 0.825±0.05 g/mL respectively.

Compressibility index and Hausners ratio of all formulations were found to be in the range of 14.09 ± 0.02 % to 21.49 ± 0.04 % and 1.19 ± 0.02 to 1.27 ± 0.04 respectively. Compressibility index of solid dispersion prepared using soluplus and PEG 6000 was found to be approximate 21 and less. Hence showed fair to passable flowability. Among all formulations, compressibility index of solid dispersion of formulation F3 was found to be less than 15, hence it has excellent flowability. Hausners ratio of formulation F3 was found to be less than 1.25 which indicates that it has good flow property. Percent yield of all formulations of solid dispersion by solvent evaporation technique was found to be in the range of 85.47 to 98.63. Formulation F3 showed more percent yield that is 98.63 % than the other formulations.

Saturation Solubility of Andrographis paniculata extract and Solid Dispersion:

Result showed that, solubility of *Andrographis paniculata* extract in distilled water was found to be 2.98 ±0.32µg/ml. Solubility of extract from solid dispersion prepared by using soluplus shows maximum solubility than that of extract. Solubility of extract was found to be more from solid dispersion which was prepared by using soluplus than that of prepared using PEG 6000. Results also showed that as concentration of soluplus and PEG 6000 increases, solubility also increases. The study also showed that there is 5.77 fold increases in solubility of *Andrographis paniculata* extract when solid dispersion is prepared with 1:3 proportions of soluplus. It was shown in Table 3.3 and Fig. 3.4.

Table 3.3: Saturation solubility of *Andrographis paniculata* extract and solid dispersion formulations in water

Batch	Solubility (µg/mL)
Andrographis paniculata extract	2.98 ±0.32

F1	9.82 ±0.47
F2	13.24 ±0.74
F3	17.20 ±0.82
F4	4.48 ±0.88
F5	6.63 ±0.42
F6	7.25 ±0.56

All values represent mean ± standard deviation (n=3)

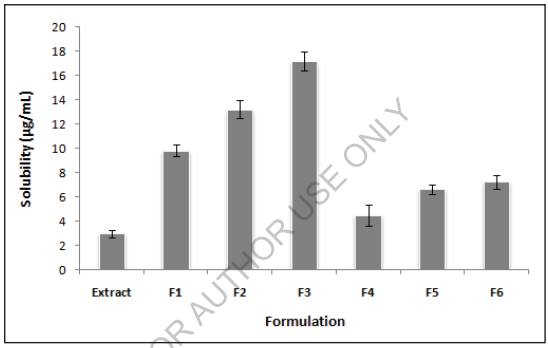

Fig. 3.4: Saturation solubility of *Andrographis paniculata* extract and solid dispersion formulations in water

3.1.4 Preparation of solid dispersion tablet:

Tablets of all formulations (F1 to F6) were prepared by direct compression technique.

Fig. 3.5: Solid dispersion tablet of *Andrographis paniculata* extract

3.1.5 Evaluation of solid dispersion tablet:

The prepared tablets were evaluated for different parameters such as thickness, hardness, weight variation, drug content, friability and disintegration time. All results are summarized in Table 3.4.

Table 3.4: Evaluation of solid dispersion tablet

Formulation code	Thickness (mm)	Diameter (mm)	Hardness (Kg/cm^2)	Friability (%)	Weight variation	Drug content (%)	Disintegration time (min)
F1	4.06±0.02	8.13±0.04	6.7±0.32	0.34±0.03	Passes	98.10±0.72	13±0.87
F2	4.08±0.04	8.13±0.02	6.7±0.42	0.42±0.02	Passes	98.77±0.30	11±1.52
F3	4.06±0.03	8.13±0.02	6.8±0.54	0.46±0.01	Passes	99.88±0.25	8±1.23
F4	4.05±0.03	8.13±0.03	6.4±0.22	0.82±0.03	Passes	95.25±1.05	14±0.46
F5	4.06±0.03	8.13±0.02	7.8±0.36	0.38±0.01	Passes	97.82±0.31	12±0.98
F6	4.08±0.02	8.13±0.04	8.2±0.28	0.31±0.02	Passes	98.20±1.59	10±1.41

All values represent mean ± standard deviation (n=3)

Examination of tablets from each batch showed flat circular shape with no cracks having pale yellow colour. The thickness of tablets ranged from 4.05±0.03 to 4.08±0.04 mm. All formulations showed uniform thickness. In weight variation test, the pharmacopoeia limit for percent of deviation for tablets of more than 250 mg is ± 5%. The average percent deviation of all tablets was found to be within the limit and hence all formulations passed the weight variation test. The drug content was found to be uniform among all formulations and ranged from 95.25±1.05 to 99.88±0.25 %. The hardness of tablets of all formulations were in the range of 6.4±0.22 to 8.2±0.28 Kg/cm^2. Disintegration time of all formulations was found to be less than 15 min. The friability of tablets of all formulations were in the range of 0.31±0.02 to 0.82±0.03% i.e. less than 1%.

***In-vitro* Drug Release Studies:**

The results showed that, drug released from solid dispersion tablet was found to be significantly higher as compared to plain *Andrographis paniculata* extract. It could be suggested that in the solid dispersion, molecular dispersion of drug in polymeric carriers may have led to particle size reduction and surface area enhancement, which results into improved dissolution rates. Furthermore no energy is required to break up the crystal lattice of a drug during dissolution process and improvement of drug solubility and wettability due to surrounding hydrophilic carriers. Thus this greater availability of dissolved extract from the solid dispersion tablet formulations may lead to higher absorption and higher oral bioavailability.

Results also showed that, rate of drug release are higher in acidic buffer at pH 1.2, which is shown in Table 3.5 and Fig. 3.6, as compared to that of in phosphate buffer at pH 6.8, shown in Table 3.6 and Fig. 3.7. Results also reveals that the solid dispersion prepared using soluplus gives higher dissolution rate as compared to that of solid dispersion prepared by using PEG 6000 as a carrier.

Table 3.5: *In-vitro* % drug release in acidic buffer at pH 1.2

Time (min)	% drug release						
	ANDG	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0	0
5	10.85±2.25	48.78±2.55	55.68±3.2	60.28±5.33	38.36±4.25	42.36±6.14	47.28±2.1
10	12.23±3.63	56.32±4.65	60.44±5.45	65.35±3.84	41.56±3.56	44.89±2.56	49.21±4.22
15	14.52±2.58	60.39±5.41	63.85±4.69	68.89±2.56	44.85±6.35	48.62±5.23	52.87±4.65
30	16.89±3.65	63.41±3.25	66.87±3.45	75.41±3.44	47.39±5.68	52.74±2.33	56.79±3.56
45	22.56±2.98	65.74±2.65	69.21±5.66	79.86±6.54	51.86±2.54	55.96±3.51	61.85±5.21
60	24.92±3.56	68.22±2.33	72.87±3.84	83.69±4.22	54.63±3.66	58.98±3.56	64.46±3.21
90	26.32±4.26	72.86±4.21	75.92±5.89	85.63±5.89	57.11±3.48	61.35±3.85	67.81±5.36
120	28.68±3.89	75.24±2.63	78.96±3.66	87.69±4.12	60.29±5.22	64.24±6.23	70.23±4.21

All values represent mean ± standard deviation (n=3), ANDG: *Andrographis paniculata* extract

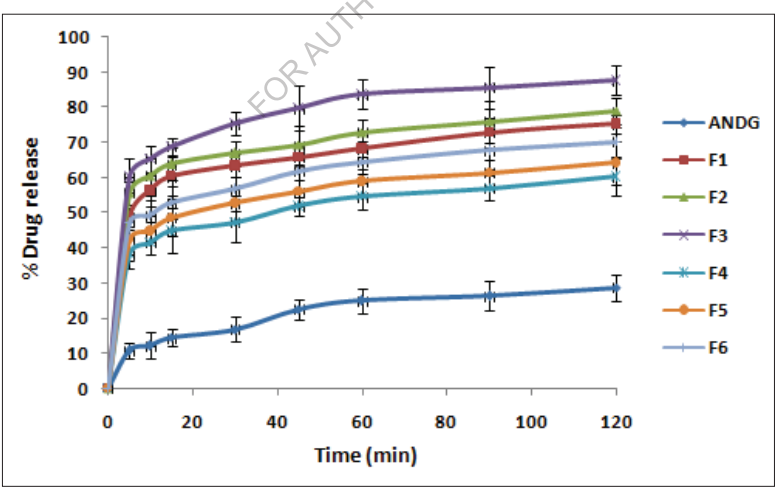

Fig. 3.6: *In-vitro* % drug release in acidic buffer at pH 1.2

Table 3.6: *In-vitro* % drug release in phosphate buffer at pH 6.8

Time (min)	% drug release						
	ANDG	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
0	0	0	0	0	0	0	0
5	6.28±3.21	32.82±3.47	38.74±4.22	44.39±4.84	26.38±3.89	29.78±5.78	39.79±3.57
10	8.41±2.86	35.29±5.86	41.85±3.59	48.78±4.36	30.17±3.41	34.27±4.96	42.88±5.79
15	11.26±4.84	40.75±4.18	45.29±4.85	54.81±5.25	33.89±2.36	38.96±5.32	50.98±6.32
30	13.74±5.96	45.32±3.87	49.67±3.97	59.32±4.34	38.92±5.23	41.89±3.69	54.86±5.33
45	14.98±4.71	49.87±5.32	55.47±5.12	63.71±3.87	41.79±4.97	46.84±2.84	58.71±6.24
60	16.47±6.01	54.98±6.02	59.63±3.95	68.38±3.63	46.78±3.65	51.39±3.86	61.55±4.33
90	18.29±5.72	60.28±4.11	65.87±4.56	73.92±4.26	51.02±4.89	55.74±5.74	65.48±5.74
120	20.59±4.98	64.78±3.86	70.52±5.01	78.23±4.11	54.21±5.71	58.79±5.86	69.41±5.29

All values represent mean ± standard deviation (n=3), ANDG: *Andrographis paniculata* extract

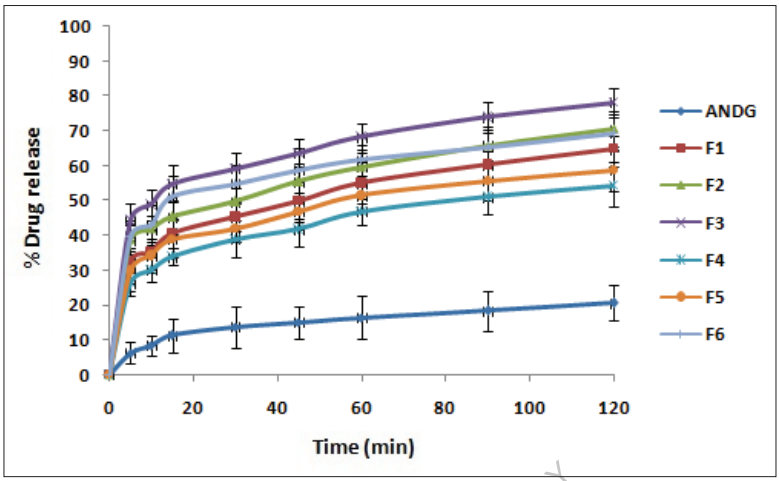

Fig. 3.7: *In-vitro* % drug release in phosphate buffer at pH 6.8

Selection of optimized formulation for further study:

From various batches of solid dispersion tablet of *Andrographis paniculata* extract formulation F3 was selected as optimized formulation by considering drug content, aqueous solubility, % yield and in-vitro % drug release.

3.1.6 Compatibility study:

Fourier transform-infrared (FT-IR) Spectroscopy:

FTIR study was done to assess whether any possible interaction between *Andrographis paniculata* extract and soluplus. Infrared spectrums of extract, soluplus and 1:1 physical mixture of extract and soluplus are shown in Fig. 3.8.

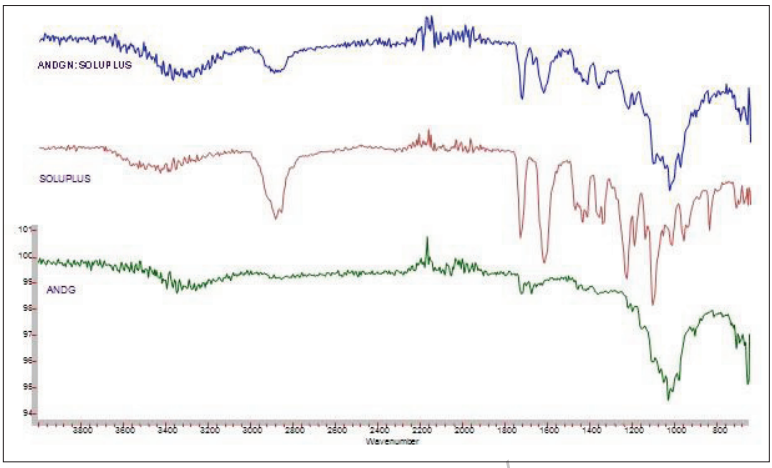

Fig. 3.8: FT-IR Spectrum of *Andrographis paniculata* extract, Soluplus and 1:1 Physical mixture of extract and Soluplus

Results of compatibility study using FTIR showed that the fundamental peak of *Andrographis paniculata* extract at 902.423 cm^{-1}, 1031.077 cm^{-1}, 1649.142 cm^{-1}, 2845.245 cm^{-1} and 3282.842 cm^{-1}attributable to various functional groups like primary and secondary amines, aliphatic amine, alkenes, alkanes, alcohols and phenols respectively are retained with slight shifting, which reveals the *Andrographis paniculata* extract is stable and can retain its functional ability with soluplus.

Differential Scanning Calorimetry (DSC):

Results of DSC Thermograms of *Andrographis paniculata* extract, soluplus and solid dispersion, showed peak at 69.07ºC, 61.05ºC and 72.76ºC which is shown in Fig. 3.9.

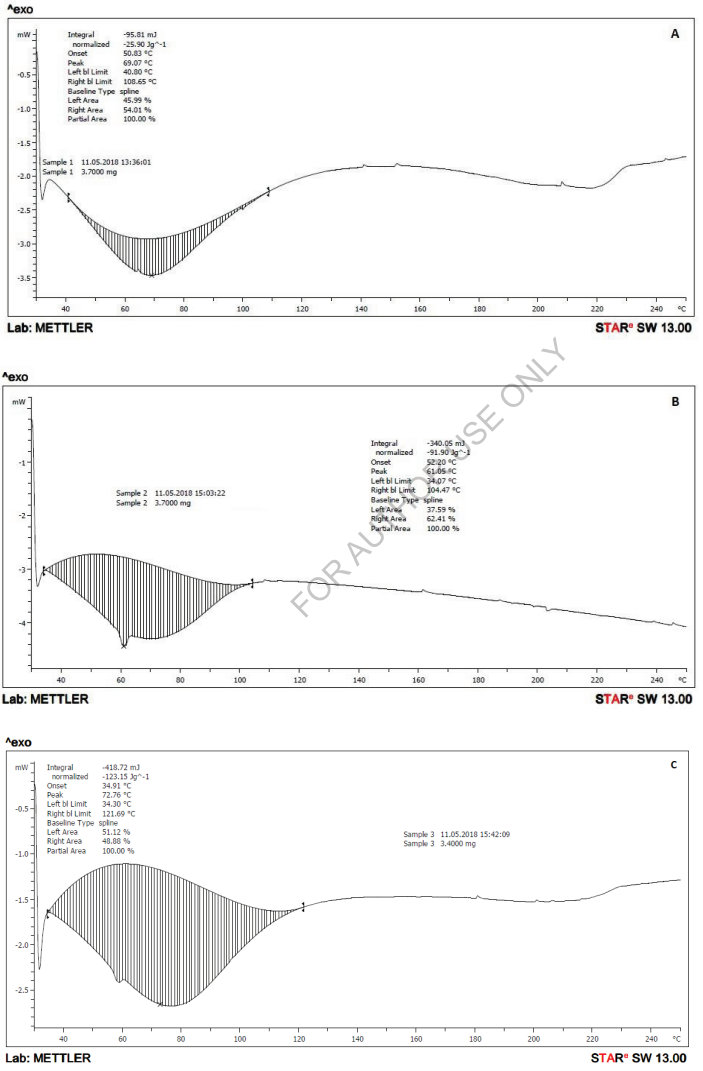

Fig. 3.9: DSC Thermograms of A: *Andrographis paniculata* extract, B: Soluplus and C: Solid dispersion

Peak of *Andrographis paniculata* extract is slightly shifted from 69.07°C to 72.76°C in DSC thermogram of solid dispersion. It may be due to solubilisation of extract in soluplus. The study conclude that, there is no or minor incompatibility between *Andrographis paniculata* extract and soluplus.

X-ray diffraction:

Characteristic peak of *Andrographis paniculata* extract was found in the diffractogram of solid dispersion, it means that there is no incompatibility between soluplus and ethanolic extract of *Andrographis paniculata*. The height of the characteristic peak intensity of *Andrographis paniculata* extract is remarkably reduced in case of diffractogram of solid dispersion. It is shown in Fig. 3.10. This indicates that *Andrographis paniculata* extract may have converted to a metastable amorphous form or may have dissolved in the matrix system.

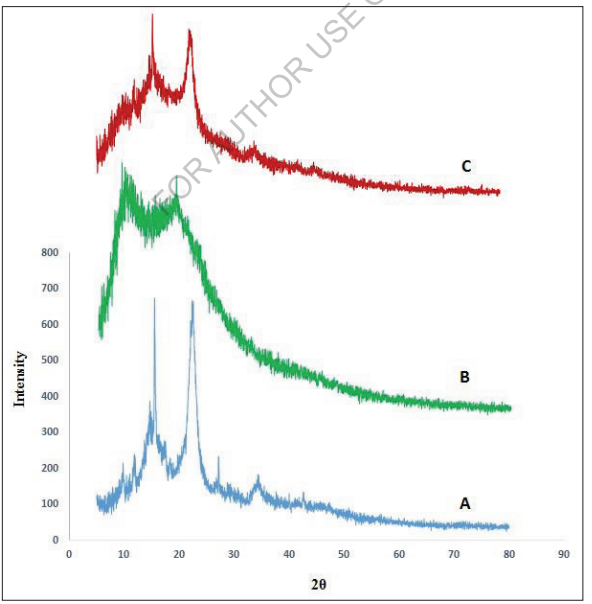

Fig. 3.10: XRD of A: *Andrographis paniculata* extract, B: Soluplus and C: Solid dispersion

3.2 PHARMACOKINETIC AND TISSUE DISTRIBUTION STUDY:

3.2.1 Determination of various pharmacokinetic parameters:

To determine various pharmacokinetic parameters of ethanolic extract solid dispersion tablet of *Andrographis paniculata* in rat a selective and rapid reverse phase high performance liquid chromatographic (RP-HPLC) method was used. Chromatogram of andrographolide is shown in Fig. 3.11. Peak overlay of different concentration of andrographolide is shown in Fig. 3.12.

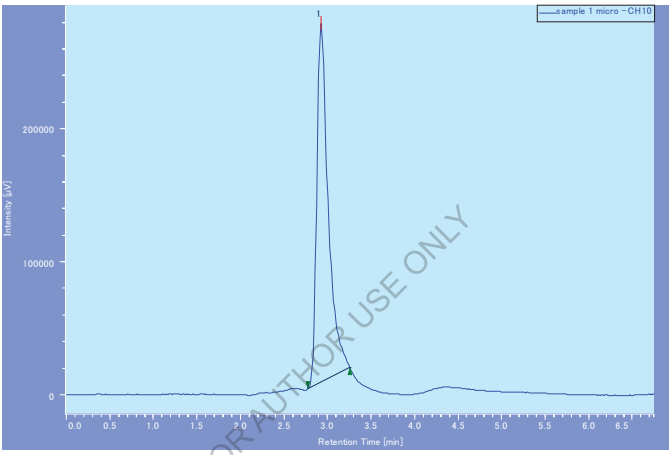

Fig. 3.11: Chromatogram of andrographolide

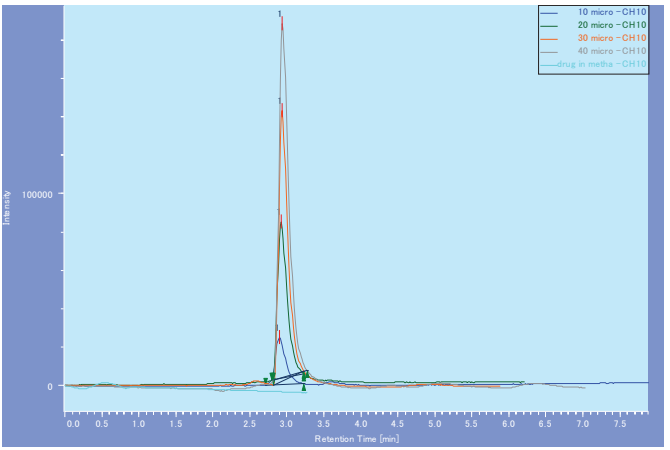

Fig. 3.12: Peak overlay of different concentration of andrographolide

Figure 3.13 shows calibration curve of andrographolide with linearity equation and R^2 value of 0.997. Hence this calibration curve was found to be linear.

Table 3.7: Reading for Calibration curve of andrographolide

Concentration (micron)	Area
10	237622
20	740930
30	1370681
40	2031842
50	2712694

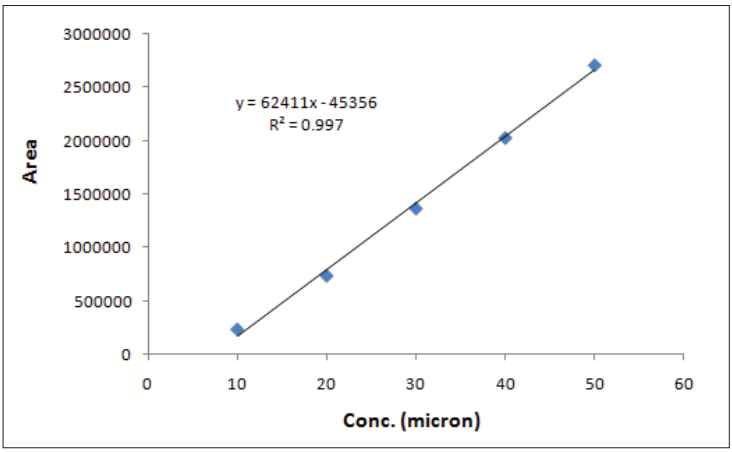

Fig. 3.13: Calibration curve of andrographolide

Plasma concentration of andrographolide from extract solid dispersion tablet and pure andrographolide versus time profile was determined after application of a single oral dose (50 mg/kg) of andrographolide in rats. The concentration versus time profile is shown in Table 3.8 and Fig. 3.14.

Table 3.8: Plasma concentration versus time profile of *Andrographis paniculata* ethanolic extract solid dispersion tablet and pure andrographolide

Time in hours	Plasma concentration (µg/mL)	
	Pure andrographolide	Solid dispersion tablet
0	0	0
0.5	24.42±4.20	32.82±3.54
1	27.24±3.33	35.22±3.52**
2	21.77±5.32	27.69±2.85
4	18.87±3.12	23.68±2.84
8	16.44±4.84	19.48±3.42
12	11.54±3.25	15.98±2.54
16	9.62±4.52	13.52±3.47
24	7.45±2.32	11.24±1.82

Table represents significant increase in plasma concentration in µg/mL values with compare to pure andrographolide. All values represent mean ± standard deviation (n=6), values are compared using students T test, P values of *P<0.05, **P<0.01 were considered statistically significant.

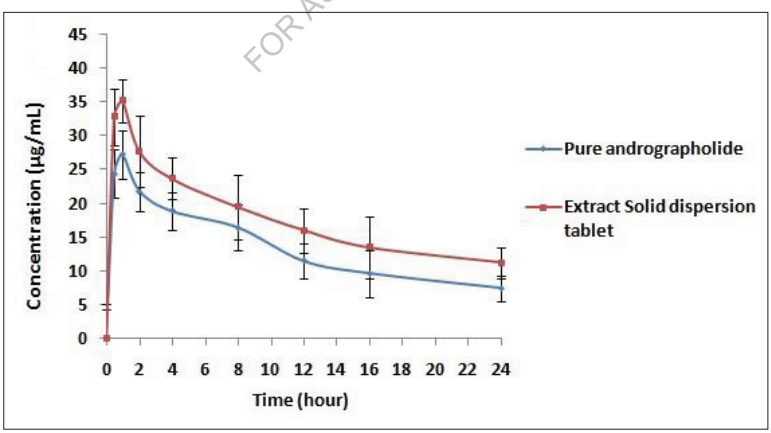

Fig. 3.14: Plasma concentration versus time profile of *Andrographis paniculata* ethanolic extract solid dispersion tablet and pure andrographolide

From Figure 5.32, maximum plasma drug concentration (C_{max}) of pure andrographolide and extract tablet was found to be 27.24±3.33µg/mL and 35.22±3.52µg/mL respectively. Similarly, time required to reach maximum concentration (t_{max}) was found to be 1hr for both. From trapezoidal rule, AUC_{0-t}, $AUC_{0-\alpha}$ and K was calculated. AUC_{0-24} was found 162.0±10.04µg.h/mL for pure andrographolide and 287.7±11.45µg.h/mL for extract tablet. Elimination rate constant was found to be 0.059±0.001h^{-1} for pure andrographolide and 0.047±0.002 h^{-1} for extract tablet. From the value of K, elimination half-life $t_{1/2}$ was calculated and it was found to be 14.74±0.02 h and 11.74±0.04 h for extract tablet and pure andrographolide respectively.

$AUC_{0-\alpha}$ i.e. area under the curve for zero to infinity was calculated and it was found 612.0±43.4µg.h/mL of extract tablet and 415.1±32.2µg.h/mL for pure andrographolide. Mean residence time (MRT) of extract tablet and pure andrographolide was found to be 21.27±3.1h and 16.94±4.3h respectively. All these pharmacokinetic parameters are summarized in Table 3.9. All results of pharmacokinetic parameters of andrographolide extract tablet were found to be better than that of pure andrographolide.

Table 3.9: Pharmacokinetic parameters of andrographolide extract tablet and pure andrographolide

Parameters	Extract tablet	Pure andrographolide
C_{max} (µg/mL)	35.22±3.54	27.24±3.23
T_{max} (h)	1	1
K (1/h)	0.047±0.002	0.059±0.001
Plasma half-life $T_{1/2}$ (h)	14.74±0.02	11.74±0.04
AUC_{0-t} (µg.h/mL)	287.7±11.45**	162.0±10.04
$AUC_{0-\alpha}$ (µg.h/mL)	612.0±43.4	415.1±32.2
MRT (h)	21.27±3.1	16.94±4.3
F_{rel} (%)	177.59±14.6	-

Table represents significant increase in values of different parameter compared to pure andrographolide. All values represent mean ± standard deviation (n=6), values are compared using students T test, P values of *P<0.05, **P<0.01 were considered statistically significant.

Results of a pharmacokinetic study of ethanolic extract tablet and pure andrographolide showed that there was 1.29 fold improvement of C_{max} of ethanolic extract solid dispersion tablet as compared to that of pure andrographolide without a change in T_{max}. It reveals that there was a significant increase in absorption of andrographolide from solid dispersion tablet as compared to pure andrographolide. The difference was very significant ($p < 0.01$) as compared to pure andrographolide.

It was also observed that AUC_{0-t} of ethanolic extract solid dispersion tablet was 1.77 fold higher than that of pure andrographolide and difference was very significant ($p < 0.01$) as compared to AUC_{0-t} of pure andrographolide. The relative bioavailability of andrographolide from solid dispersion tablet to that of pure andrographolide was found to be 177.43±14.6%.

3.2.2 Tissue distribution study:

The distribution of pure andrographolide and solid dispersion tablet of *Andrographis paniculata* in the various tissues, namely heart, liver, kidney, brain, lung and spleen were determined. Results of tissue distribution of pure andrographolide are shown in Table 3.10 and Fig. 3.15 while that of ethanolic extract solid dispersion tablet of *Andrographis paniculata* in Table 3.11 and Fig. 3.16.

Table 3.10: Tissue distribution study of pure Andrographolide in rats

Organ	Concentration (ng/g) of andrographolide at time		
	1hr	3hr	8hr
Heart	49.58±5.65	29.45±7.2	4.98±1.2
Liver	90.25±9.68	68.36±8.45	7.1±2.41
Kidney	101.36±10.87	81.27±9.74	10.12±2.89
Brain	19.32±4.85	6.92±2.45	0.0
Lung	48.33±8.67	41.87±8.37	5.12±1.44
Spleen	61.85±9.72	50.18±6.76	14.98±3.57

All values represent mean ± standard deviation (n=3)

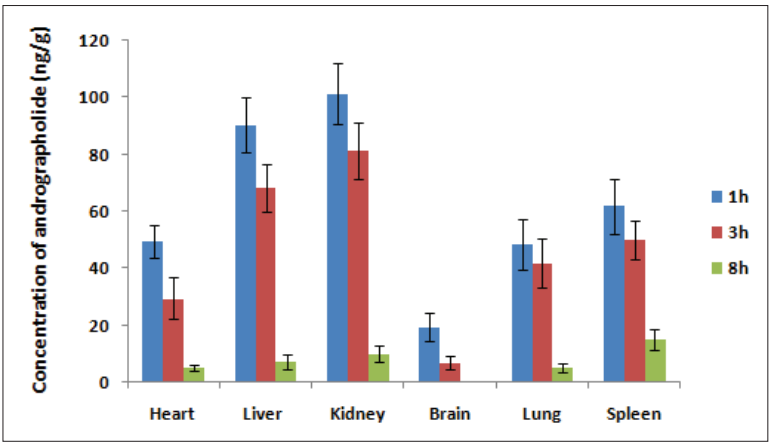

Fig. 3.15: Tissue distribution study of pure Andrographolide in rats

Table 3.11: Tissue distribution study of ethanolic extract solid dispersion tablet of *Andrographis paniculata* in rats

Organ	Concentration (ng/g) of andrographolide at time		
	1hr	3hr	8hr
Heart	51.23±6.28	30.12±6.74	5.1±1.12
Liver	121.3±8.75	79.56±9.52	14.97±3.43
Kidney	158.9±13.54	101.45±11.45	25.1±3.47
Brain	21.87±5.12	8.14±2.68	0.0
Lung	59.28±9.47	44.96±9.44	6.96±2.13
Spleen	81.45±8.39	64.83±7.28	20.15±4.17

All values represent mean ± standard deviation (n=3)

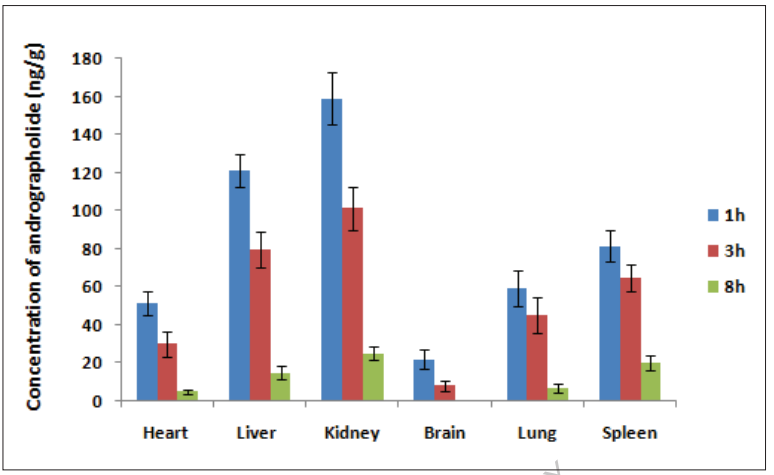

Fig. 3.16: Tissue distribution study of ethanolic extract solid dispersion tablet of *Andrographis paniculata* in rats

The maximum concentration was observed at 1 h in all tissues upon oral administration of andrographolide and solid dispersion tablet of *Andrographis paniculata* extract to rats at a dose 50 mg/kg. Highest concentration of andrographolide was found to be in kidney that is 101.36±10.87ng/g for pure andrographolide and 158.9±13.54ng/g for solid dispersion tablet. Concentration of andrographolide in all tissue goes on decreasing after 3 and 8 hours. Results also showed that, concentration of andrographolide in all tissues get increased in the animals in which solid dispersion tablet was administered orally. It reveals that absorption of andrographolide is getting increased after conversion of extract in to solid dispersion. Study also showed that, presence of andrographolide in brain after 1 and 3 h, this indicates that andrographolide can cross the blood brain barrier.

3.3 ACCELERATED STABILITY STUDY:

There was no considerable change in thickness, hardness, drug content and disintegration time of F3 formulation before and after accelerated stability study. It was shown in Table 3.12. Also there was no significant difference was found between *in-vitro* drug release in acidic buffer at pH

1.2 of F3 formulation before and after stability. It was shown in Table 3.13 and Fig. 3.17. Hence solid dispersion tablet prepared was found to be stable.

Table 3.12: Evaluation of F3 formulation before and after stability study

	Thickness (mm)	Hardness (Kg/cm^2)	Drug content (%)	Disintegration time (min)
Before stability study	4.06±0.03	6.8±0.54	99.88±0.25	8±1.23
After stability study	4.04±0.02	6.6±0.51	99.47±0.59	8±1.10

All values represent mean ± standard deviation (n=3)

Table 3.13: *In-vitro* Drug Release Studies of F3 formulation before and after stability study in acidic buffer at pH 1.2

Time(min)	% Drug release	
	Before stability study	After stability study
0	0	0
5	60.28±5.33	58.47±3.57
10	65.35±3.84	64.87±4.67
15	68.89±2.56	70.28±3.74
30	75.41±3.44	76.26±4.62
45	79.86±6.54	80.63±3.46
60	83.69±4.22	82.71±2.91
90	85.63±5.89	84.39±3.81
120	87.69±4.12	86.41±3.45

All values represent mean ± standard deviation (n=3)

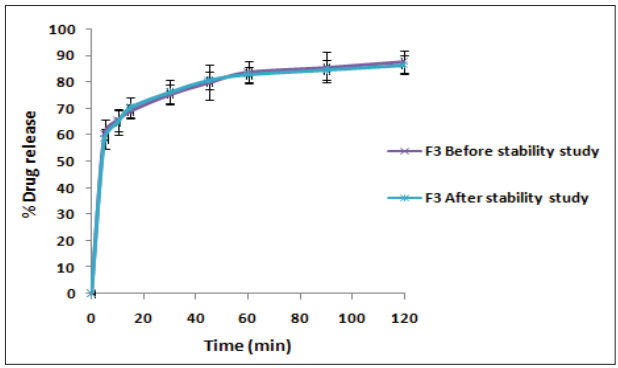

Fig. 3.17: *In-vitro* Drug Release Study of F3 formulation before and after stability study

CHAPTER IV: SUMMARY AND CONCLUSION

- Ethanolic extract was found to have poor aqueous solubility, hence need to enhance solubility.
- Solubility of extract was found to be more from solid dispersion prepared by using Soluplus than that of prepared using PEG 6000.
- From various batches of solid dispersion tablet of *Andrographis paniculata* extract formulation F3 was selected as optimized formulation by considering drug content, aqueous solubility, percent yield and percent drug release.
- Rate of drug release was found to be higher in acidic buffer at pH 1.2 as compared to that of in phosphate buffer at pH 6.8.
- Compatibility study using IR, DSC and XRD showed that, ethanolic extract is compatible with soluplus and can be used for preparation of stable formulation.
- Pharmacokinetic study showed that, there was a significant increase in absorption of andrographolide from solid dispersion tablet as compared to pure andrographolide.
- The relative bioavailability of andrographolide from solid dispersion tablet to that of pure andrographolide was found to be 177.59±14.6%.
- From tissue distribution study it can be concluded that, concentration of andrographolide in all tissues get increased in animals in which solid dispersion tablet was administered orally.
- It reveals that absorption of andrographolide is getting increased after conversion of extract into solid dispersion tablet. Accelerated stability study indicates that prepared solid dispersion tablet of *Andrographis paniculata* extract, F3 formulation was stable. Accelerated stability study indicates that prepared solid dispersion tablet of *Andrographis paniculata* extract, F3 formulation was stable.
- The further research work intends to perform clinical trials for solid dispersion tablet of *Andrographis paniculata* extract as well as scale-up of developed solid dispersion tablet.

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