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FORMULATION AND EVALUATION OF LIQUID FILL FORMULATIONS FOR SOFT GELS OF ELVITEGRAVIR

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ABSTRACT
The present investigation includes the preparation and evaluation of liquid fill formulations for soft gels using an anti retro-viral drug Elvitegravir(EVG), in order to improve its dissolution properties and there by its bioavailability. Formulations were prepared using excipients like polyethylene glycol-400(PEG 400), propylene glycol (PG), Polyvinylpyrrolidone (PVPK-30), DMSO, SLS and ethyl alcohol. Prepared formulations were evaluated for appearance, pH, drug content uniformity, viscosity, stability, and *in-vitro* dissolution studies. The compatibility between the drug and excipients in formulations was confirmed by FT-IR spectra. The drug content of the liquid fill formulations was found to be in the range of 97.55 to 99.63 and the viscosity was in the range of 34-132cps for all the developed formulations. Formulation F5 prepared using ethyl alcohol(20.5% w/v),PEG-400(20% w/v), PG(15% w/v), DMSO(35% w/v) and 1% SLS w/w showed superior drug release when compared to other formulations (F1 , F2, F3 &F4), hence F5 was selected as optimized formulation and the complete release(100%) was observed at the end of 120seconds. Stability studies were conducted for all the formulations for a period of 6 months at room temperature(~30ºC/65%RH). From the studies, it can be concluded that EVG liquid fill formulations for soft gels were successfully prepared with *in vitro* dissolution properties superior when compard to EVG laboratory prepared formulation.EVG was earlier identified as a P-gp/MDR1 substrate. High concentrations of EVG present relatively briefly in the intestinal lumen during drug absorption can inhibit intestinal efflux transporters, such as P-gp.

KEYWORDS: Elvitegravir, Bioavailability, Viscosity, Soft gels, p-gp efflux.

INTRODUCTION
Acquired immunodeficiency syndrome (AIDS) is one of the greatest challenges to humankind. AIDS and HIV infection represent global health hazards, obvious targets for drug discovery and vaccination, and both are enormous social and economical. FDA-approved therapies target three steps of the HIV life cycle: reverse transcription, proteolytic maturation and fusion. Triple therapy, commonly referred to as highly active antiretroviral therapy (HAART), is now the standard for treatment. It consists of a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) in combination with two nucleoside reverse transcriptase inhibitors. HAART, however, is often not well-tolerated by the patients. It requires discipline, is expensive and leads to multidrug resistance. Therefore, additional therapeutic approaches are warranted. One such approach is to target the third viral enzyme, integrase, and recent studies from the Merck group have demonstrated the feasibility and efficacy of integrase inhibitors in animal models.

It is estimated that more than 40% of new chemical entities (NCEs) coming out of the current drug discovery process have poor biopharmaceutical properties, such as low aqueous solubility and/or permeability. These sub-optimal properties pose significant challenges for the oral absorption of the compounds and for the development of orally bioavailable dosage forms. Development of soft gelatin capsule (soft gel) dosage form is of growing interest for the oral delivery of poorly water soluble compounds (BCS class II or class IV).

The extensive research on bioavailability of various medication forms has reportedly demonstrated that the extent of absorption and bioavailability was much better in the form of soft gelatin capsules. This is mainly the consequence of one of the best properties-the exact suspension of therapeutic substance in the liquid medium. Owing to that, absorption of medicament starts at the moment of capsule disintegration in the elimentary tract and is more effective because of small size of suspended or dissolved particles of the medicament.

The soft gel dosage form offers several advantages over other oral dosage forms, such as delivering a liquid matrix designed to solubilize and improve the oral bioavailability of a poorly soluble compound as a unit dose solid dosage form, delivering low and ultra-low doses of a compound, delivering a low melting compound and minimizing potential generation of dust during manufacturing and thereby improving the safety of production personnel.

EVG is an HIV integrase inhibitor that selectively inhibits the strand-transfer step of the integration process of viral DNA into host chromosomal DNA.EVG belongs to BCS class-II drug with a oral bioavailability of 20-30% and its absorption is dissolution rate limited. It is a potent anti-retro viral agent. The median terminal plasma half-life of Elvitegravir following administration of stribild is approximately 12.9 hours. After single dose administration of Ritonavir-boosted EVG, 94.8% of the dose was recovered in feces consistent with the hepatobiliary excretion of EVG; 6.7% of the administered dose was recovered in urine. Presently EVG is available only as film coated tablet dosage forms Vitekta (Elvitegravir) 150mg & Stribild (85mg/150mg).

Janice Soo Fern Lee *et al* carried work on Review of the safety, efficacy, and pharmacokinetics of Elvitegravir with an emphasis on resource-limited settings. Polina German *et al* investigated on pharmacokinetics and bioavailability of EVG. From the literature review, it is clearly evident that the most of the work were carried on the formulation of the immediate release and fim coated tablet dosage forms of EVG.Liquid fill formulations for soft gels avoid the step of disintegration. since they provide the solubilised drug instantly to the gastric environment.Hence,the present investigation was aimed at developing oral administrable soft gels (liquid fill) pharmaceutical formulations of EVG with improved rate of absorption.

MATERIALS AND METHODS

Materials

EVG was supplied by Hetero Ltd., Hyderabad, as a gift sample, PVPK-30 (Sisco Research Laboratories, Mumbai), PEG400 (Central Drug House, Mumbai), Propylene glycol (SD Fine Chemicals Mumbai).All the chemicals and reagents used in the study were of analytical grade.

Preparation of liquid fill formulations

Liquid fill formulations were prepared as per the formulae given in Table No.1 to a batch size of 1000mg. Initially PEG-400 and Propylene Glycol were taken into a small beaker and was mixed thoroughly. Accurate amount 85mg of EVG was weighed and transferred into the separate beaker containing DMSO and mixed thoroughly to dissolve the drug. In the formulations 85mg of EVG was taken because label claim of EVG available in market is 85mg.The drug solution was transferred into the PEG-400 and PG mixture and mixed thoroughly. Then PVP K-30 and ethyl alcohol were added to dissolve the EVG completely. The prepared formulation was sonicated for 3 minutes in order to remove any entrapped air. The weight of the liquid ingredients like Ethyl alcohol, propylene glycol, poly ethylene glycol-400 and DMSO was converted to volume from density values and taken accordingly.

The volume of the above ingredients was derived from the available values of density reported in standard literature (density of ethyl alcohol is 1gm/cm^3, propylene glycol is 1.038gm/cm^3, PEG-400 is 1.12gm/cm^3, DMSO is 1.100 g/cm^3). Empty soft gelatin capsules were incubated at 40ºC for 10 minutes with an objective of removing moisture taken up by the capsules during storage. Each oval shaped soft gelatin capsule of size 20 equivalent to 1.232 mL was taken for filling. Each capsule was filled by injection with 1.0 mL of each of the formulation. Each capsule should be filled up to 75 percent of its total volume. Using a glass syringe the liquid fill was injected into the capsule, which was then sealed by heat. The soft gelatin capsules filled with liquid fill formulations of EVG were then subjected to different tests to evaluate for various parameters.

Table No.1: Formulae of liquid fill formulations for soft gels of EVG.

Ingredients	F1(mg)	F2(mg)	F3(mg)	F4(mg)	F5(mg)	F5(using Basket)(mg)
EVG	85	85	85	85	85	85
PEG-400	200	200	200	200	200	200
PG	150	150	150	150	150	150
PVP K-30	_	_	_	50	_	_
ETHANOL	250	215	315	165	205	205
SLS	_	_	_	_	10	10
DMSO	315	350	250	350	350	350
TOTAL(mg)	1000	1000	1000	1000	1000	1000

Evaluation parameters for EVG Liquid Fill Formulations

Appearance

Appearance is one of the most important characteristic parameter of liquid fill formulations. All the formulations were evaluated for clarity and color change by visual observation against a black background.

pH

pH is one of the most important parameter involved in the liquid fill formulations. The developed liquid fill formulations were evaluated for pH by using Elico LI 120 pH meter and the estimations were carried out in triplicate.

Drug content uniformity

Drug content of developed Liquid Fill Formulations was estimated by weighing approximately 50 mg of fill formulation into a 5 mL of volumetric flask. To the flask few mL of methanol was added and mix thoroughly to dissolve the formulation and the volume was made upto the mark with remaining methanol. Samples were suitably diluted with 1% SLS+0.1 N Hcl and the samples were analysed for EVG content by measuring absorbance at 261 nm.The estimations were carried out in triplicate.

Rheological Studies

Viscosities of all the developed formulations were measured by using Brookfield DV-II +PRO viscometer**.** The formulations were taken in cup of Brookfield DV-II + PRO viscometer rotated with CP52 spindle. The angular velocity was fixed at 10-100 rpm. The viscosity measurements were made in triplicate using fresh samples each time at room temperature.

FTIR studies

Samples were analyzed using an ATR-FTIR spectrometer (Bruker, Germany). ATR spectra were measured over the wave number range of 4000–500 cm−1 at a resolution of 1.0 cm^{-1}. The powder or film sample is simply placed onto the ATR crystal and the sample spectrum is collected. The sample is then cleaned from the crystal surface and the accessory is ready to collect additional spectra. ATR analysis is less complicated, fast and a very small amount of the sample is needed than KBr pellets.

***In-vitro* drug release studies**

In vitro dissolution studies were conducted by using 900mL of 0.1N Hcl+1% SLS, as dissolution medium using USP XXI type I/II (paddle method) dissolution apparatus (DISSO 8000, LAB INDIA). A temperature of 37 ± 0.5ºC and a rotation speed of 50 rpm were maintained. Liquid formulations containing 85 mg of EVG were filled into empty soft gelatin capsules (size 20) and dissolution studies were performed. As the capsule tends to float in the dissolution medium, sinkers were used. A 5mL samples were withdrawn at predetermined time intervals over a period of 0, 15, 30, 45, 60, 90, 120, 240, 300, 360 seconds and then replaced with same volume of fresh dissolution medium. The filtered samples were suitably diluted and analyzed at 261nm using UV-Visible Elico SL150 spectrophotometer. Dissolution experiments were conducted in triplicate.

Stability studies

Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates. Liquid fill formulations (F1-F5) were observed for clarity, colour change, drug content, pH, viscosity and precipitation for a period of 6 months at room temperature (~30ºC/65% RH). The samples were withdrawn after 1, 3 and 6 months and evaluated for following parameters such as appearance, pH, drug content and *in vitro* drug release studies.

RESULTS AND DISCUSSIONS

Appearance

All liquid fill formulations of EVG were visually tested for clarity, color and precipitation of EVG. The results are given in Table No.2.The formulations were clear , homogeneous and free of precipitation. This may be due to the presence of DMSO which is a good solvent for Elvitegravir.

Table No.2: Evaluation parameters of EVG liquid fill formulations.

Formulations	Appearance	pH	Drug content (%) (Mean ± SD)	Viscosity (cps)
F1	Homogeneous, Clear, no color change	6.08	97.55 ± 0.55	34
F2	Homogeneous, Clear, no color change	6.0	98.58 ± 0.21	37
F3	Homogeneous, Clear, no color change	5.8	98.26 ± 0.105	65
F4	Homogeneous, Clear, no color change	6.2	99.03 ± 0.245	132
F5	Homogeneous, Clear, no color change	5.9	99.63 ± 0.140	85
F5(using Basket)	Homogeneous, Clear, no color change	5.9	99.63 ± 0.140	85

Ph

pH is another important parameter for liquid fill formulations. The two areas of critical importance are the effect of pH on solubility and stability. Liquid fill formulations should have a pH in the range of 2.5 to 7.5. At pH values below 2.5, gelatin is hydrolyzed causing leakage of the soft gel, whereas at pH values above 7.5, gelatin may be either hydrolyzed or tanned (i.e. crossslinked) resulting in decreased solubility of the gelatin shell. The pH of all the formulations was close to 6.0. The pH of the soft gelatin fill formulation without drug was found to be at 5.4 and therefore, all these batches of formulation pH are suitable for capsule filling. The results were given in Table No. 2 and shown in Fig.1.

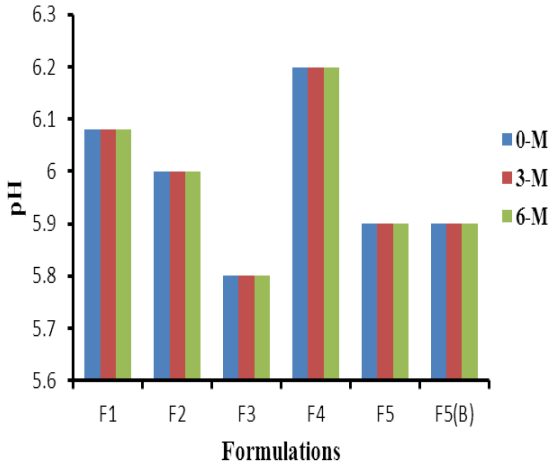

Fig.1: pH values of all the formulations (0-6M)

Drug Content Estimation

All the liquid fill formulations were subjected to drug content estimation within the labelled claim limits and it indicates that the amount of active ingredient in all these formulations were uniformly distributed and the values were satisfactory. The results are given in Table No.2 and shown in Fig.2.The percent drug content was found to be in the range of 97.39±0.11 to 99.63±0.140.

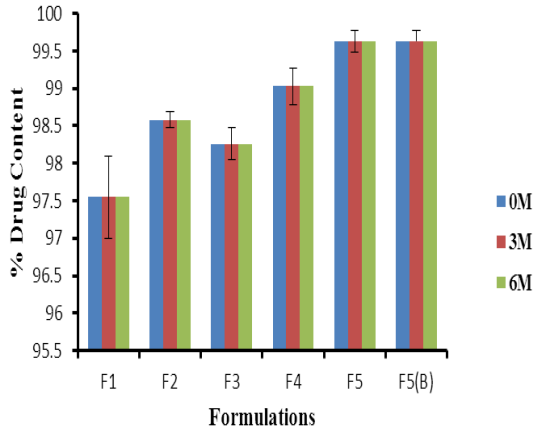

Fig.2: Drug content profiles of all the formulations (F1-F5) 0-6Months

Rheological Studies

Viscosity is one of the important parameters which provide vital information during the optimization of the liquid fill formulations for soft gels. In general, the viscosity of liquid fill formulations for soft gels is in the range of 0.222-3000 cps.

Rheological studies were carried out for all the liquid fill formulations by using Brookfield DV-II PRO viscometer. F1, F2, F3 and F5 had fluid like consistency, whereas F4 had thicker consistency due to the presence of PVPK-30 which is having high viscosity. The study showed that PVP K-30 solutions are Newtonian at high shear rates and non Newtonian fluid in the low shear rate region. The rheological data for the formulations (F1-F5) were given in Table No.2 and shown in Fig.3.

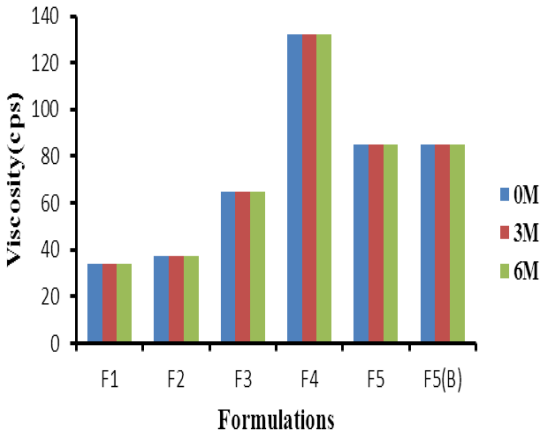

Fig.3: Viscosity of all the formulations (0-6 Months)

Drug-Excipient Compatibility Studies

The IR spectra of Elvitegravir pure drug and all other formulations were obtained by KBR pellet method by ATR-FTIR spectrometer (Bruker, Germany). The characteristic spectrums were observed for all the formulations within specified I.R ranges, indicates that there was no interaction between drug and excipients. The spectrums are shown in Fig.4,Fig.5.

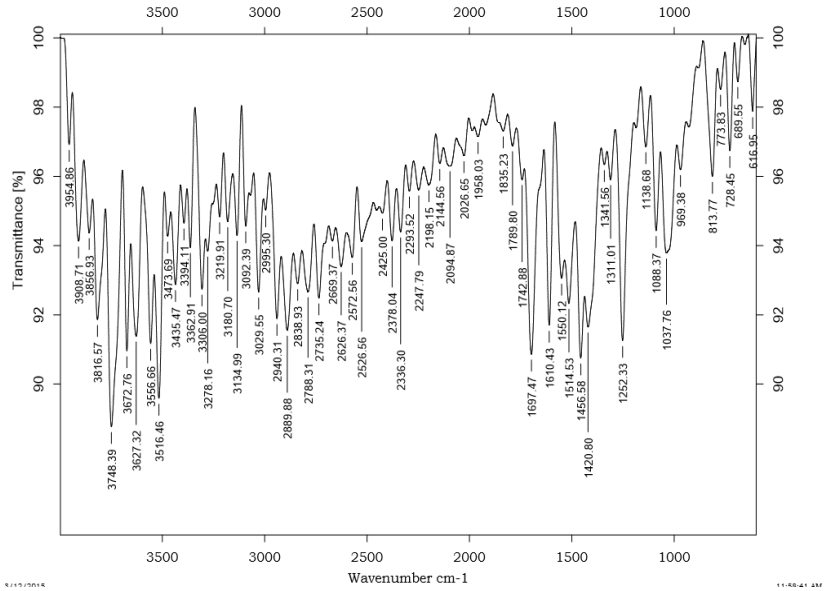

Fig.4: FTIR spectrum of pure EVG

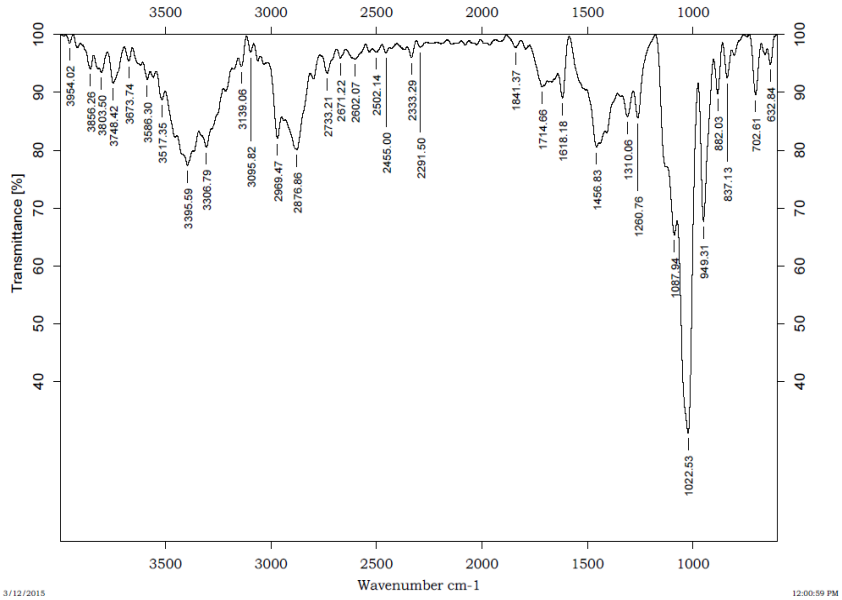

Fig.5: FTIR spectrum of F5

***In-vitro* drug release studies**

In vitro dissolution studies were carried out to evaluate the EVG release from liquid fill formulations & tablets. Dissolution studies were conducted in 900mL of 1%SLS+0.1 N HCl at 37 ± 0.5°c. Totally five different formulations of Elvitegravir Liquid filled capsules were prepared. The formula was given in Table No.1.Comparative dissolution profiles were shown in Fig.6 and Fig.7. *In vitro* release studies were performed to assess the dissolution parameters like drug percent released at 90 seconds (DP_{90}), and first order release kinetic data for EVG liquid fill formulations and DP_{90} data was shown in Fig.8.

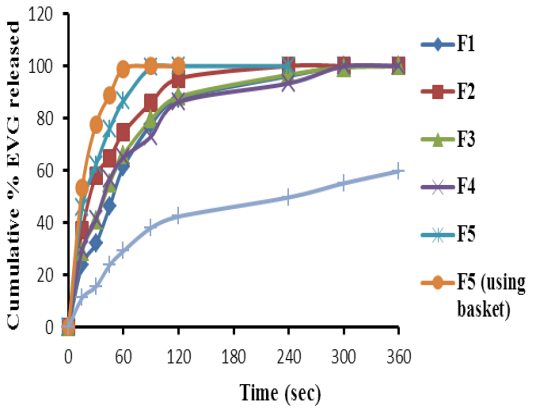

Fig.6: In-vitro dissolution profile of all EVG formulations, Laboratory formulation.

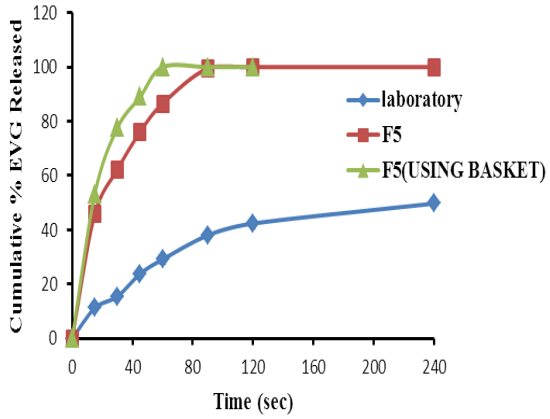

Fig.7: Comparative *in-vitro* dissolution profiles of F5, F5 (using Basket) and laboratory formulation

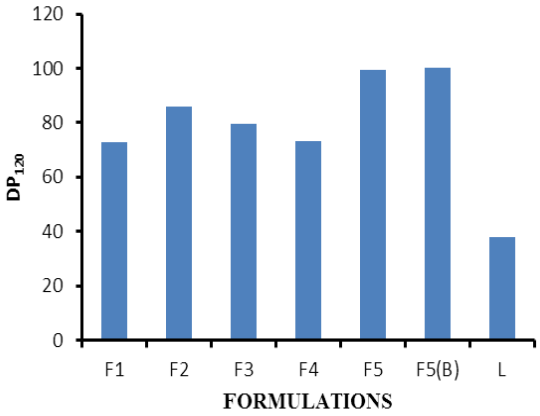

Fig.8: Comparative DP_{90} values of all the formulations (F1-F5), F5 (using basket) and laboratory formulation (L)

Formulations F1 and F2 prepared using Ethyl alcohol/PEG/PG/DMSO system showed superior drug release when compared to formulation F4 prepared using Ethyl alcohol /PEG/PG/DMSO/PVPK-30 system. This may be attributed to the higher solubility of EVG in DMSO when compared to ethyl alcohol.

Formulations F3 prepared using Ethyl alcohol/PEG/PG/DMSO system showed better drug release when compared to formulation F4 prepared using Ethyl alcohol/PEG/PG/DMSO/PVPK-30 system. This may be attributed due to the increase in the viscosity of the F4 formulation.

Formulation F5 prepared using Ethyl alcohol/PEG/PG/DMSO/SLS showed superior drug release when compared to other formulations (F1, F2, F3&F4) .This may be attributed to the presence of 1% SLS (surfactant) in F5. Formulation prepared with SLS (F5), showed rapid drug release when compared to laboratory prepared tablet.Formulation F5 is having superior release properties and optimum viscosity. Hence F5 was selected as optimized formulation. This confirms that Ethyl alcohol/PEG/PG/DMSO/SLS system is better for EVG release than Ethyl alcohol/PEG/PG/DMSO and Ethyl alcohol /PEG/PG/DMSO /PVPK-30systems. Finally, the release kinetic was studied and showed that F5 better fits the first order release kinetics among all formulations.

First order release kinetics

To analyze the *in vitro* drug release data, various kinetic models were used to describe the release kinetics for both liquid filling and tablet formulations.

Both liquid fill and tablet formulations followed the First order release kinetics.

The first order kinetics data for liquid fill formulations was given in Table No.3 and shown in Fig.9.
The order of regression coefficient (R^2) values for all the formulations were in the order **F5≥F3>F4>L>F2≥F1.**

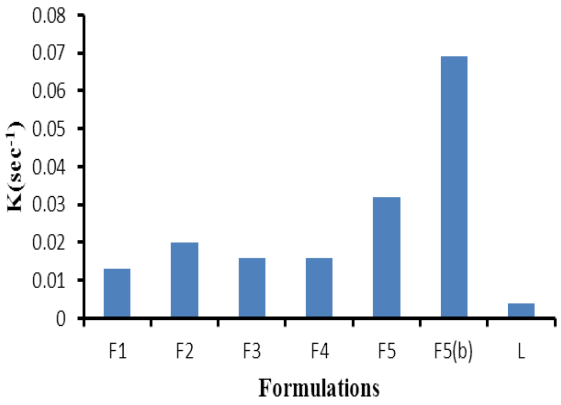

Fig.9: Comparative K (sec^{-1}) values

Stability studies

Liquid fill formulations were subjected to stability studies at room temperature for a period of 6 months. Stability studies were conducted for evaluation of various parameters such as drug content, viscosity, in-vitro drug release and pH. The results indicated that all the developed formulations (F1, F2, F3, F4, F5) have no significant changes were stable at room temperature without undergoing any degradation. [Fig.10].

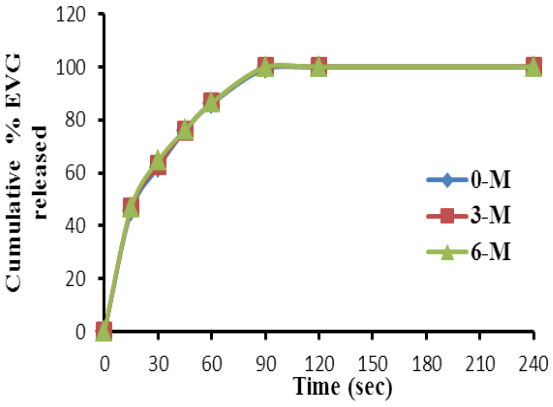

Fig.10: In-vitro dissolution profile of F5 (0-6M)

No change was observed in drug content, pH and viscosity for 6months at room temperature in liquid filling formulations. Formulations were stable with respect to color, clarity, homogeneity and precipitation for a period of 3 months and 6 months.After six months also same results were observed for all the developed formulations. All formulations showed good physicochemical properties.

CONCLUSION

EVG belongs to BCS class-II drug with a oral bioavailability of 20-30%. It is a potent anti-retro viral agent. There is a need for an anti-viral agent to show its action with in less time for better patient compliance. In the present investigation, the strategy of formulation into soft gelatin capsules is a novel approach of drug delivery.

EVG soft gelatin capsules were formulated and evaluated which shows better results when compared to marketed tablet formulations.The present formulations avoids the step of disintegration which inturn increases the bioavailability thereby increasing the rate of absorption.

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REFERENCES

1. Rampurna Prasad G.Soft gelatin capsules. *J Pharma Sci*., 2010; 99: 4107-4148.
2. Cornaire G, Woodley J, Hermann P, Cloarec A, Arellano C, Houin G. Impact of excipients on the absorption of P-glycoprotein substrates in vitro and in vivo. *Int J Pharma.*, 2004; 278: 119-131.
3. Raghu Ram Jampala, V. Kiran Kumar, Appala Raju Nemala. Development and Application of Liquid Chromatographic method for Simultaneous Determination of Elvitegravir, Tenofovir Disoproxil Fumarate, Emtricitabine, and Cobicistat in Fixed Dosage Form. *Pharmaceutical Methods*, 2014; 5(1).
4. Swati S.Pardhi, Kiran B.Aher, Girija B. Bhavar, S.R. Chaudhari. Development and Validation of UV Spectrophotometric method for the estimation of Elvitegravir (EVG) in Bulk and Pharmaceutical Formulations. *Int J Pharma Sci Res*., 2015; 6(1).
5. Krishan K Pandey. Critical appraisal of Elvitegravir in the treatment of HIV-1/AIDS. *HIV/AIDS – Research and Palliative Care*., 2014; 6: 81–90.
6. Polina German, David Warren, MD, Steve West, MSPH, James Hui, and Brian P.Kearney. .Pharmacokinetics and Bioavailability of an Integrase and Novel Pharmacoenhancer-Containing Single-Tablet Fixed-Dose Combination Regimen for the Treatment of HIV. *J Acquir Immune Defic Syndr*.,1 November, 2010; 55(3).
7. Paul E Sax, Edwin DeJesus, Anthony Mills, Andrew Zolopa. Co-formulated Elvitegravir, Cobicistat, emtricitabine, and tenofovir versus co-formulated efavirenz, emtricitabine, and tenofovir for initial treatment of HIV-1 infection: a randomized, double-blind, phase 3 trial, analysis of results after 48 weeks. *Lancet.*, 2012; 379: 2439–48.
8. Pardhi Swati S, Aher Kiran B, Bhavar Girija B. Recent Research on Analytical Methods of Analysis of Raltegravir and Elvitegravir. *Int J Pharma Sci Research*., 2015; 6(2).
9. Janice Soo Fern Lee, Alexandra Calmy, Isabelle Andrieux-Meyer, Nathan Ford. Review of the safety, efficacy, and pharmacokinetics of Elvitegravir with an emphasis on resource-limited settings.*HIV/AIDS - Research and Palliative Care*., 2012; 45–15.
10. Custodio JM, Liu Y, Graham H: Bioequivalence of two pediatric formulations vs adult tablet formulation of Elvitegravir: Conference on Retroviruses and Opportunistic Infections. Boston MA, 2014; 3-6.
11. Jyothirmayee Devineni, Vijaya Ratna, Formulation and evaluation of soft gelatin capsules of nelfinavir mesylate, *Eur J Biomed Pharm Sciences*, 2016; 3(2): 398-407.
12. Venkat vardhan reddy, Jyothirmayee Devineni, Development of immediate release liquid fill formulations for soft gels of sumatriptan succinate, *Int J Res Pharm Sciences*, 2014; 5(4): 262-269.
13. Jyothirmayee Devineni, Tummala Harika, Venkat vardhan reddy, Development of immediate release liquid fill formulations for soft gels of paracetamol, *Int J Pharm Dev Tech*, 2015; 5(1): 75-82.
14. Jyothi Sanaboina, K.M. Maheswari, Seetha Sunkara, Sravanthi Deekonda, Buchi.N.Nalluri, Preparation and evaluation of Valsartan liquid fill formulations for soft gels, *J Pharm*, 2013; 1-8.