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SOLUBILITY ENHANCEMENT OF POORLY SOLUBLE SAQUINAVIR BY USING SELF-EMULSIFYING DRUG DELIVERY SYSTEM

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ABSTRACT

A self-micro-emulsifying drug delivery system (SMEDDS) has been developed to enhance dissolution rate and oral bioavailability of saquinavir. The solubility of saquinavir was checked in different oils, surfactants and co-surfactants and ternary phase diagrams were constructed to evaluate the micro emulsion domain. The saquinavir SMEDDS was prepared using Capmul MCM (oil), Cremophor RL100 (surfactant) and labrafil (co-surfactant). The particle size was determined. Dissolution rate of saquinavir was measured by using 0.1 N HCL as diffusion media. Results of diffusion rate of saquinavir SMEDDS were compared with those of pure drug solution. Diffusion of saquinavir SMEDDS showed maximum drug release when compared to pure drug solution.

KEYWORDS: Saquinqvir, Self-micro-emulsifying Drug Delivery System.

INTRODUCTION

Solid dispersions can be defined as “dispersion of one or more active ingredient in an inert excipient or matrix” wherein the active ingredient exists in a finely crystalline, solubilized or amorphous state.

Lipid based drug delivery: It consists of delivering a drug dissolved in a mixture of one or more excipients which may be a mono, di and tri-glyceride, lipophilic and hydrophilic surfactants and a co-surfactant. When the drug is dissolved state throughout its transit in the GIT.[1]

Table. 1: lipid formulation classification system (LFCS) showing typical proportions of lipid formulations.

Excipient in formulation	Content of formulation (% w/w)				
	Type I	Type II	Type IIIA	Type IIIB	Type IV
Oils :triglyceride or mono and diglyceride	100	40-80	40-80	<20	-
Water insoluble surfactants (HLB<12)	-	20-60	-	-	0-20
Water soluble surfactants (HLB >12)	-	-	20-40	20-50	30-80
Hydrophilic co solvents (ex: PEG, propylene glycol)	-	-	0-40	20-50	0-50

OBJECTIVES OF STUDY

- To identify the drug Saquinavir as per the existing standard.
- Toformulatethe Self Emulsifying Drug Delivery System of Saquinavirwith differentOil & surfactantratio.
- To evaluate the Self Emulsifying Drug Delivery Systemparameters.

MATERIALS AND METHODS

Materials

Drug: Saquinavir mesylate, an HIV protease inhibitor which acts as an analogue of an HIV protease cleavage site. It is highly specific inhibitor of HIV-1 & HIV-2 proteases[12].

Excipients[4,5]

Capmul MCM C8
Cremophore EL
Labrasol
Labrafil M1944CS

Methodology[6]

Standard Graph of Saquinavir

Preparation of standard stock solution: Standard stock solution was prepared by dissolving accurately weighed 100 mg of saquinavir in solvent methanol and final volume made up to 100ml in volumetric flask(stock solution-I, 1000 mcg/ml). 10 ml of stock solution-I was diluted to 100 ml with methanol (stock solution –II, 100 mcg/ml). The absorbance of resulting solution was measured against respective blank solution in the UV region of 200-400 nm, which shows maximum absorbance at 239 nm.

Preparation of standard curve: Appropriate volume of aliquots from standard saquinavir stock solutions were transferred to a series of 10 ml volumetric flasks capacity. The volume was adjusted to the mark with methanol to obtain concentrations of 10,20,30,40,50 mcg/ml. absorbance spectra of each solution against methanol as a blank were measured at 239 nm and the absorbance values were shown in table. The obtained values are plotted against the concentration of saquinavir to get the calibration graph and were represented in figure.

Screening of Smedds Formulation

Solubility studies: The solubility of saquinavir in various oils, surfactant, and co-surfactant was determined as follows.

The API is mixed with individual excipient. The binary mixture should contain an excess amount of drug. The mixture is replaced in a sonication bath for upto one hour to optimize the dispersion of API into the excipient. The mixture is left to pseudo-equilibrate for a periodof one week prior to analysis with UV spectroscopy. An aliquot for analysis taken following centrifugation which is filtered before analysis. Results of solubility study were given in table.[9]

Construction of pseudo ternary phase diagrams: Pseudo ternary phase diagrams were constructed in order to obtain the concentration range of components for the existing region of micro emulsions. The weight ratio of surfactant to co-surfactant (km) was varied as 7:3, 6:4, 5:5, 4:6 and 3:7. For each pseudo ternary phase diagram a specific surfactant/co-surfactant weight ratio, the oily mixtures of oil, surfactant and co-surfactant were prepared with the weight ratio of oil to the mixture of surfactant and co-surfactant at 30:70, 35:65, 40:60, 45:55, 50:50, 60:40, 65:35, 70:30 respectively. Water was added drop by drop to each oily mixture under proper magnetic stirring at 37◦ C until the mixture became clear at a certain point. The concentrations of the components were recorded in order to complete the pseudo ternary phase diagrams, and then the contents of oil, surfactant, co-surfactant and water at appropriate weight ratios were selected based on these results.[11]

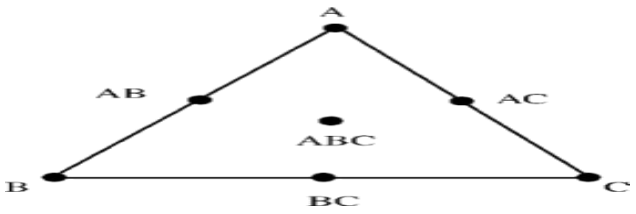
Preparation of SMEDDS formulations

Phase transformation was observed visually and phase boundary was decided. The phase diagram was constructed by using PCP disso software. Capmul MCM (oily phase), cremophore EL (surfactant) and labrafil (co-surfactant) was chosen for formulation from the experience of solubility studies conducted. The existence of micro emulsion region was

determined by using pseudo ternary phase diagram. SMEDDS wee diluted under agitation condition using water titration method. The mixture of oil and surfactant/co-surfactant at a certain weight ratios were diluted with water in drop wise manner. The ratio of surfactant and co-surfactant were prepared with specific manner that is 2:1, 1:1 and 1:2 (W/W). Each of these ratios was mixed with increase in percentage of oils that is 10%, 20%, up to 90% of oil to get phase diagram. Then each mixture was titrated with water and agitation was provided by magnetic stirrer. The formation of micro emulsion regions was monitored visually for turbidity-transparency-turbidity. These values of oil, surfactant, and co-surfactant were used to determine the boundaries of micro emulsion region. After the identification of micro emulsion region in the phase diagrams, the micro emulsion formulations were selected at desired component ratios.[8]

Effect of oily phase content on mean globule size: A series of SMEDDS were prepared with varying oil content to study effect of oil content on mean globule size. Ratio of surfactant to co surfactant was selected on the basis of phase diagrams and was maintained at 2:1. Briefly, oil, surfactant and co-surfactant were weighed into glass vials, mixed by stirring and heated (40-50◦C) to affect homogeneous mixture. Oil-surfactant mixture, 500 mg was dispersed in 500 ml of various aqueous phases' viz. double distilled water, buffer pH 1.2 and buffer pH6.8 with gentle stirring. Globule size was determined immediately after dilution.[8]

Formulation optimization of Saquinavir loaded SMEDDS



Equilateral triangle representing simplex lattice design for three components

Simplex lattice experiment design was used to optimize the composition of SMEDDS (Subramanianet al., 2004). In this design, three factors were evaluated by changing concentrations of components simultaneously and keeping the total concentration constant. The simplex lattice design for a three-component system was represented by an equilateral triangle in two-dimensional space. Seven batches of SMEDDS carrying saquinavir were prepared, including three vertexes (A, B, C), three half-way points between vertices (AB, BC, AC), and one centre point (ABC). The concentrations of surfactant, co-surfactant and oil were selected as independent variables. The solubility of saquinavir in SMEDDS and the

mean particle size of micro-emulsification from SMEDDS were taken as responses. The responses for seven formulations were used to fit an equation for simplex lattice model (Mao et al., 2004; Patel et al., 2007), which can predict the properties of all possible formulations. Graphs of these properties in the form of contour plots were constructed by Matlab Software (Kompany-zareh and Khoshkam,2008; Casanova et al., 2007). With the aid of matlab software, the model equation was developed as the best representation of the relationship between the solubility or particle size and the measured characteristics[6].

Composition of SMEDDS of km=2.

Std order	Cremophore/labrafil(v/v)	Capmul (ml)
SMD1	425	75
SMD2	412	88
SMD3	400	100
SMD4	387	113
SMD5	375	125
SMD6	362	138
SMD7	350	150
SMD8	335	165
SMD9	325	175

Characterization of smedds containing saquinavir

Particle size analysis: Malvern zetasizer was used to measure the particle size and size distribution of different batches of formulation. It performs size measurements using dynamic light scattering. All the samples were diluted in phosphate buffer solution and was put to the particle size analyser. All the data presented are the mean values of three independent sample produced under identical production condition.[7]

Scanning electron microscopy: SEDDS was finely spread over a slab and dried under vacuum. The sample was shadowed in a cathodic evaporator with a gold layer (20nm thick). The surface morphology of the SEDDS was observed by SEM using a JSM-6400 scanning electron microscope.[7]

Stability study by visual assessment: It provides the information about the self-emulsifying property of the mixture and about the resulting dispersion. Shelf life as a function of time and storage temperature was evaluated by visual inspection of the SEDDS system at different time periods. Saquinavir SEDDS was diluted with purified distilled water and to check the temperature stability of samples, they were kept at two different temperature range (2-8◦C)

for 2 months and observed for any evidences of phase separation, flocculation or precipitation.[9]

Emulsification time: Selected oil and surfactant were mixed in 1:3 (w/w), heated at 40-50◦C and vortexed to form homogeneous mixture. Oil-surfactant mixture, 500 mg dispersed into 500 ml of double distilled water in a glass beaker with gentle stirring. Visual test was used to assess self-emulsification of surfactants in terms of dispersibility, ease of emulsification and final appearance using grading system. Various co-surfactants in 2:1 w/w ratios. Oily phase was added to this mixture in 1:3 (w/w), heated and vortexed gently to form homogeneous mixture.[8]

In-vitro drug dissolution studies: In-vitro drug dissolution studies were carried out using tablet USP II dissolution test apparatus. In the development of in-vitro dissolution tests were to show that, the solubility enhanced with SMEDDS and optimized formula is showing optimum drug release.[7]

$$\text{Percentage of drug release} = \frac{\text{C x D.Fx}\,900}{1000}\text{X}100$$

or

$$\frac{\text{Wt.of API}}{\text{Volume of diluent}}\text{x}\frac{1}{\text{D.F}}\text{x}\frac{900}{\text{dose}}\text{x}\frac{\text{D.F}}{1}\text{X}\frac{\%\text{Purity}}{100}\text{x}100 = \text{Percentage of drug release}$$

RESULTS AND DISCUSSIONS

Calibration curve of saquinavir: The calibration curve was plotted taking concentration between 10-50 mcg/ml, the maximum wavelength was found to be 239 nm from UV spectrum of saquinavir in methanol from the data obtained it can be said that the drug follows beer's law in this concentration range with correlation coefficient value 0.999.

Sl. No.	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.138
3	20	0.267
4	30	0.396
5	40	0.526
6	50	0.651

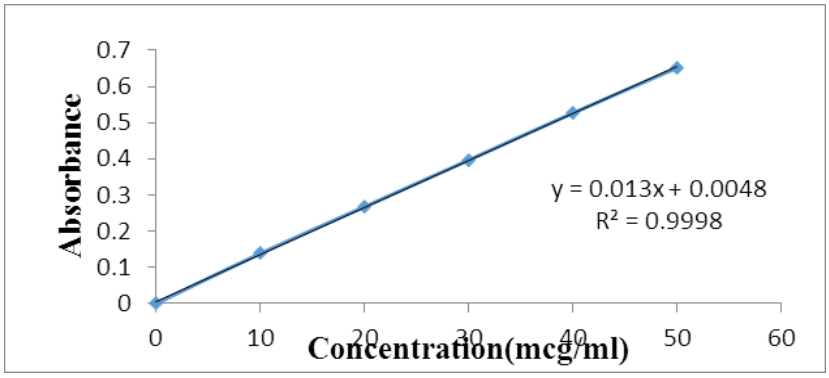


Figure: calibration curve of saquinavir.

Compatibility of saquinavir: The sample of saquinavir procured for study was identified by infrared spectrum as shown in figure.

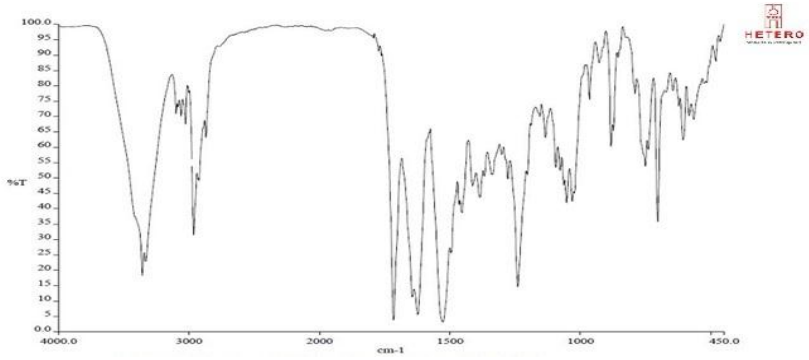


Figure: FTIR profile of Saquinavir + Cremophore EL + Labrafil + Capmul.

Solubility data of saquinavir: The self-emulsifying formulations consisted of oil, surfactants, co-surfactants and the drug should later be clear and monophasic liquid at ambient temperaturewhen introduced to aqueous phase, and hence it should have good solvent properties to allow presentation of the drug in solution. The solubility of saquinavir in various vehicles is presented in table.

Table: solubility studies of drug in different oils and solvents.

Oils	Solubility (mg/ml)	Surfactants	Solubility (mg/ml)	Buffers	Solubility (mg/ml)
Capmul Maisine Labrafac	110mg±4mg 95mg ± 3 mg 87mg ± 5mg	CremophoreEL Labrasol Captex Plurol olique Labrafil Transcutol p Tween 80 Capryol	50 mg±5 mg 45mg ± 3mg 27mg ± 2mg 18mg ± 4mg 30mg ± 2mg 22mg ± 2mg 47mg ± 3mg 45mg ± 4mg	0.1 N Hcl 0.2 pH 6.8 0.1 N NaOH	0.117±0.003 0.25 ± 0.002 0.107 ±0.002

Phase diagram study

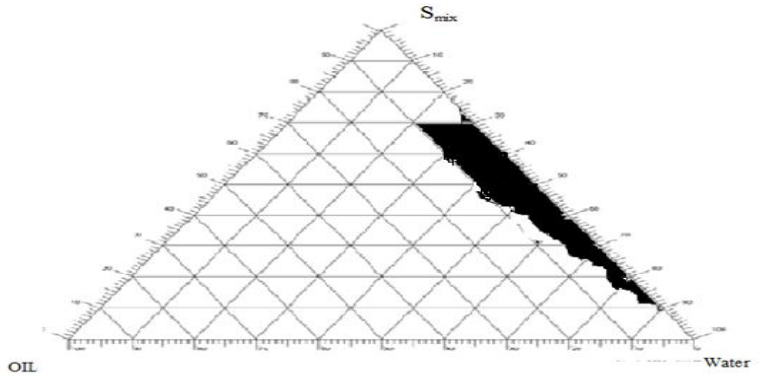


Figure. Phase diagram with Km (Surfactant/Co-Surfactant)=1.

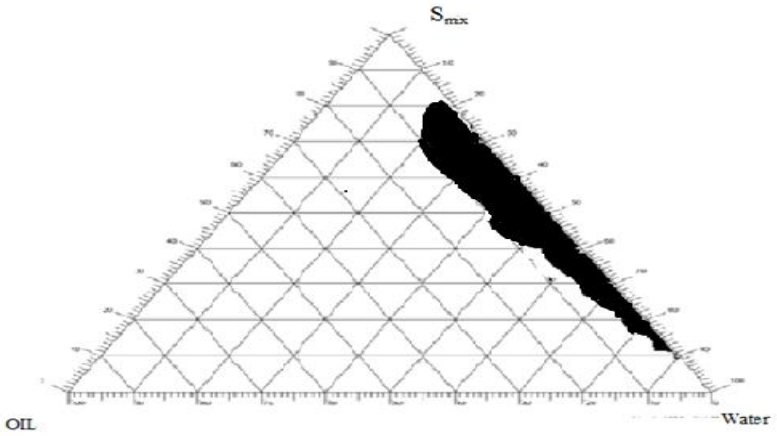


Figure 6.2: Phase diagram with Km (Surfactant/Co-Surfactant) =2.

Phase diagrams were constructed in the presence of Saquinavir, to find the optimum concentrations of oil, surfactant and co-surfactant. SEDDS form fine oil-water emulsions with only gentle agitation, upon its introduction into aqueous media. Since the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous. Surfactants form a layer around the emulsion droplets and reduce the interfacial energy as well as providing a mechanical barrier to coalescence. The visual test measures the apparent spontaneity of emulsion formation. The series of SEDDS were prepared and their self-emulsifying properties were observed visually. Pseudo-ternary phase diagrams were constructed to identify the self-emulsifying regions and optimized concentration of oil, surfactant and co-surfactant was used for formulation**.**

Formulation chart of SMEDDS

Std order	Cremophore/ capmul	Labrafil	Globule size (µm)	Visibility	Emulsification time
SMD1	425	75	75	Clear	>1 min
SMD2	412	88	70	Clear	>1 min
SMD3	400	100	78	Clear	>1 min
SMD4	387	113	80	Clear	>1 min
SMD5	375	125	81	Clear	>1 min
SMD6	362	138	85	Clear	>1 min
SMD7	**350**	**150**	**92**	**Clear**	**>1 min**
SMD8	335	165	110	Turbid	<1 min
SMD9	325	175	121	Turbid	<1 min

Every formulation contains 100 mg of drug, saquinavir. The ratio of surfactant and co-surfactant ratio is different in every formulation (1:1, 1:2 and 2:1) respectively. In each and every formulation there is an increase in 2.5% of oil ratio.

Optimized formulation

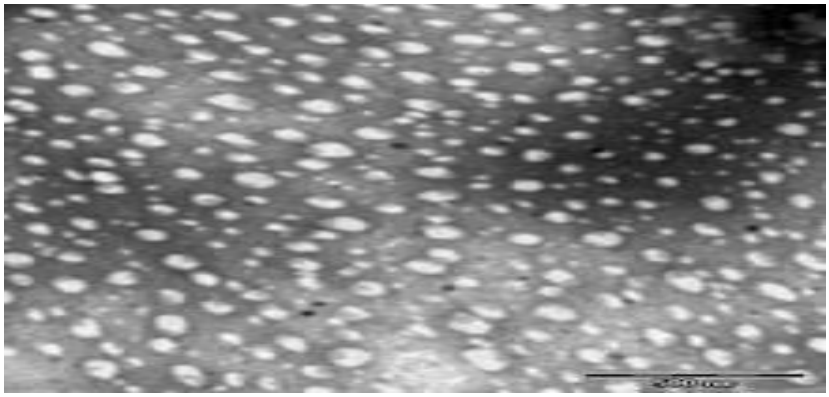
Optimized formula was decided by considering drug uptake that was SMD7. Though formulations from SMD1 to SMD6 have good globule size and emulsification time, precipitation of drug observed in those formulation.

SMD8 and SMD9 were showing turbidity.

Table: Composition of optimized SMEDDS7.

Ingredient	Quantity (mg per capsule)
Saquinavir	100
Cremophor EL	233.4
Capmul MCM C8	116.6
Labrafil	150
Total	600

Scanning Electron Microscopy



Dissolution studies

Table: Intrinsic dissolution of API.

Time (mints)	Absorbance		%Drug release
	Test Abs	Std Abs	
0	0	0.651	0
10	0.000	0.651	0
20	0.009	0.651	1.37%
30	0.020	0.651	3.05%
45	0.038	0.651	5.80%
60	0.048	0.651	7.33%
90	0.064	0.651	9.78%
120	0.09	0.651	13.75%
180	0.134	0.651	20.43%

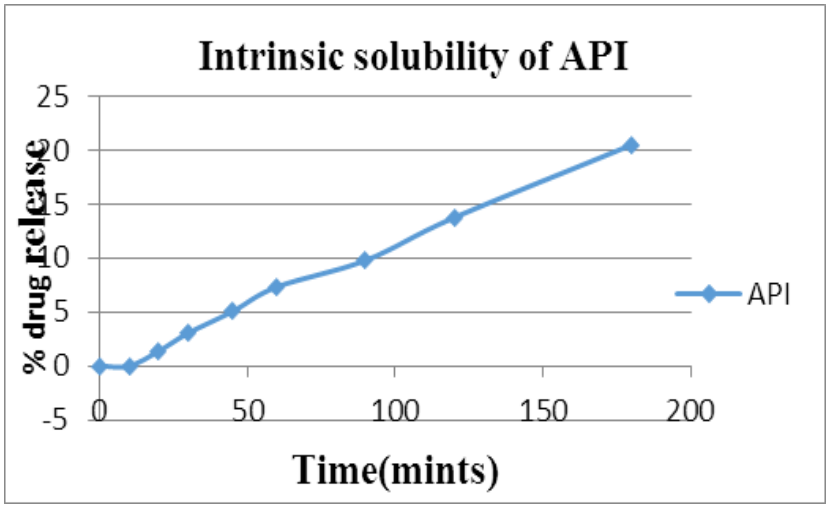


Figure. Dissolution profile of API.

Table: Dissolution data of SMD7.

Time (mints)	Absorbance		%Drug release
	Test Abs	Std Abs	
0	0	0	0
10	0.341	0.651	52.08%
20	0.570	0.651	87.06%
30	0.609	0.651	93.151%
45	0.644	0.651	98.55%
60	0.644	0.651	98.57%
90	0.644	0.651	98.57%
120	0.644	0.651	98.57%
180	0.644	0.651	98.57%

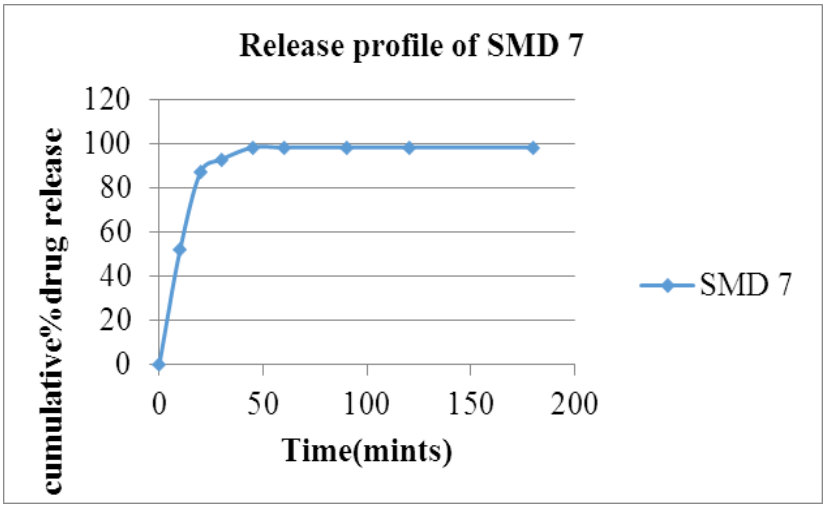


Figure: Drug release profile of SMD7.

More than **80%** of the drug was released before **20 mins** from the optimized formulation, which was greater than Marketed product.

Stability studies

Chemical and physical stability of SMD7 SMEDDS was assessed at 40±2 °C/75±5% RH as per ICH Guidelines.SMEDDS equivalent to 100 mg Saquinavir was filled in size '2' hard gelatin capsules, packed in aluminum strips and stored for 45 days.Samples were analyzed on 45th day for *in vitro* dissolution profile.

In-vitro drug release data of SMD7 after 45 days

From the stability studies, it was found that after 45 days, there was 1.78% decrease in % drug release.

Sl. No	Time (mints)	% Drug release	
		Initial	After studies
1	10	52.08%	51.99%
2	20	87.06%	86.81%
3	30	93.15%	92.07%
4	45	98.55%	97.80%
5	60	98.57%	96.77%

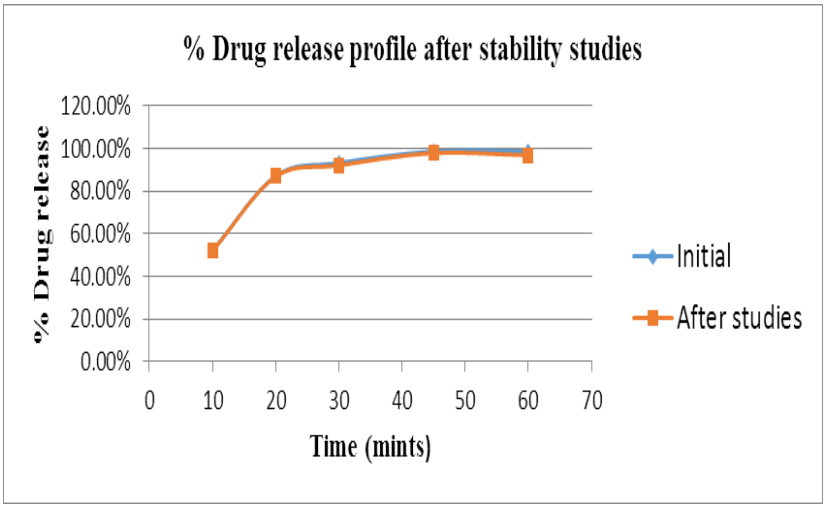


Figure. Release profile after 45 days.

From the stability studies, it was found that after 45 days, there was 1.78% decrease in % drug release.

CONCLUSION

From the studies the following conclusions can be drawn

The oral delivery of hydrophobic drugs can be made possible by SMEDDs, which have been shown to substantially improve oral bioavailability with future development of this technology.

Saquinavir, the Protease inhibitor can be used to develop the SMEDDS. The SMEDDS formulations prepared met the standard evaluation parameters with a slight deviation within the prescribed limits.The short term stability studies carried out were confirmative of the drug stability in the SMEDDS during the present study.The SMEDDS showed good release profile as drug delivery systems. Results of this study indicated that in vitro drug release of Formulation SMD7 was maximum up to **98.57 %** release at the time of 45 **min** from the formulation.

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