Research Article

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Design and evaluation of microparticles containing didanosine for oral administration

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ABSTRACT

For the last so many decades of conventional dosage forms like tablets , capsules, pills, powders, parenteral preparations, emulsions, creams , ointments , solutions, suspensions and areosals are used in the treatment of acute and chronic diseases. However the objective of any drug delivery system is to provide drug in therapeutic amount to the proper site in the body to achieve immediately and then maintain the desired drug concentration. And hence only the aim of the current investigation is to design and evaluate the microparticles containing Didanosine. The short term stability study was performed as per ICH guidelines using selected Didanosine -loaded HPMC micro particles for a period of 3 months. The designed novel drug delivery system has provided a better drug bioavailability considering, high penetration property of the microparticles encapsulated agents through biological membrane and the stability. It has been found conducive to prepare the microparticles containing Didanosine.

Keywords: Micropaticles, kinetic models, Didanosine and biovaliability

INTRODUCTION

The gastrointestinal tract (GIT) comprises of a number of components (Fig. 1), their primary function being secretion, digestion and absorption. The mean length of the entire GIT is 450 cm. The major functional components of the GIT are stomach; small intestine (duodenum, jejunum and ileum) and large intestine (colon) which is grossy differ from each other in terms of anatomy, function, secretions and pH [1-5].

The stomach is an organ with a capacity for storage and mixing. The stomach lining is devoid of villi but consist of considerable number of gastric pits that contribute to the storage capacity of stomach. Under physiological conditions, the gastric absorption of most drug is insignificant as

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a result of its limited surface area (0.1-0.2 m^2) covered by a thick layer of mucus coating, lack of villi on the mucosal surface, and short residence time of most drugs in the stomach. Its acidic pH (1-3), due to secretion of HCl, favors absorption of acidic drugs if they are soluble in gastric fluid since they are unionized to the large extent in such a pH. The gastric pH aids dissolution of basic drugs due to salt formation and subsequent ionization which are therefore absorbed to a lesser extent from stomach because of the same reason [6-12].

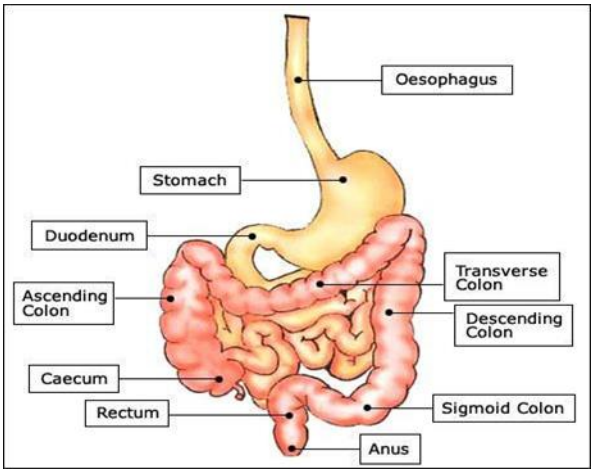

Figure 1: Anatomy of Gastro-intestinal tract

It is the major site for absorption of most drugs due to large surface area (200 m^2). The fold in the intestinal mucosa, called as the fold of Kerckring, result it three fold increases in the surface area. The surface of these folds possess finger like projection called as villi which increase the surface area 30 times. From the surface of villi protrude several microvilli resulting in 600 times increase in the surface area. All these above, combined with the great length of small intestine (approximately 285 cms), result in more than 200 square meters of surface which is several time that of stomach. The blood flow of the small intestine is 6 to 10 times that of stomach. Moreover, the pH range of 5 to 7.5 is most favorable for most drugs to remain unionized. The peristaltic movement of intestine is slow, transit time is long, and permeability is high. Thus, a contribution of the entire above factors make intestine the best site for absorption of most drugs [13-18].

Didanosine

IUPAC Name

9-[(2R,5S)-5-(hydroxymethyl)oxolan-2-yl]-6,9-dihydro-3H-purin-6-one

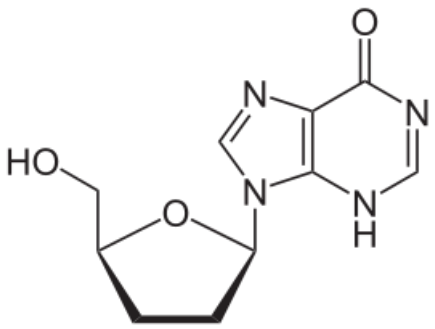

Figure 2: Structure of Didanosine

Formula	$C_{10}H_{12}N_4O_3$
Mol. mass	236.4 g·mol−1

Mechanism of action

Didanosine (ddI) is metabolized intracellularly by a series of cellular enzymes to its active moiety, dideoxyadenosine triphosphate (ddATP), which inhibits the HIV reverse transcriptase enzyme competitively by competing with natural dATP. It also acts as a chain terminator by its incorporation into viral DNA as the lack of a 3'-OH group in the incorporated nucleoside analogue prevents the formation of the 5' to 3' phosphodiester linkage essential for DNA chain elongation, and therefore, the viral DNA growth is terminated.

Absorption

Rapidly absorbed (bioavailability 30-40%) with peak plasma concentrations appearing within 0.5 and 1.5 hrs.

Route of elimination

Based on data from in vitro and animal studies, it is presumed that the metabolism of didanosine in man occurs by the same pathways responsible for the elimination of endogenous purines. Purines are eliminated by the kidneys.

Half life

30 minutes in plasma and more than 12 hours in intracellular environment.

MATERIALS AND METHODS

Didanosine is obtained as a gift sample from Aurobindo Pharma, Hyderabad, all other chemicals and reagents used in the research has been procured from Fine Chem, Mumbai.

Preparation of HCl pH 0.1

8.55 ml of Conc. HCl were taken and dissolved in 1000 ml of distilled water and then adjusted to pH 1.2 (0.1N) with Orthophosphoric acid.

Preparation of standard curve of Didanosine with HCl 1.2pH

100 mg of was accurately weighed and dissolved in a small portion of 0.1N HCl in a 100 ml volumetric flask then the volume was made up to 100 ml with 0.1N HCl. This was primary stock solution, contained 1000 µg/ml. From this primary stock solution 10 ml was pipette out and transferred in to a 100 ml volumetric flask and volume was made up to 100 ml with buffer pH 1.2 which contained the concentration of 100 µg/ml. From the second stock solution again 10 ml was pipette out and diluted up to 100 ml with buffer pH 1.2 to get concentration of 10µg/ml.

Preparation of microparticles

HPMC Microparticles were prepared according to the procedure first reported by Calvo et al. (1997b) based on the ionic gelation of HPMC with bicarbonate and alginate anions. Microparticles were prepared by using different drug to polymer ratio. Required quantity of drug was dissolved in 10 ml of mixture of methanol and dichloromethane (1:1). Polymer HPMC is separately dissolved in 2 % v/v acetic acid solution. Drug solution was dispersed into the polymer solution containing emulsifier Tween 80 added to the final solution with continuous stirring. The Emulsion was prepared with homogenous stirring. Then Emulsion was injected drop by drop into 20% w/v Calcium Chloride Solution. Because of using Calcium Chloride, HPMC polymer is not solubilised in the solution of Calcium Chloride which leads to formation of microparticles by precipitation [19-22].

Table 1: Formulation of different batches of Didanosine microparticles

Ingredients	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9	F-10	F-11	F-12
Didanosine	500	500	500	500	500	500	500	500	500	500	500	500
Na Alginate	100	200	300	400	100	200	300	400	100	200	300	400
HPMC K100	25	50	75	100	-	-	-	-	-	-	-	-
HPMC K15	-	-	-	-	25	50	75	100	-	-	-	-
HPMC K4M	-	-	-	-	-	-	-	-	25	50	75	400

Water	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	qs
Calcium chloridesolution	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%

In-vitro Release Studies

Drug release studies on the loaded HPMC microparticles were carried out using a USPXXI dissolution rate test apparatus for 30 h at a stirring speed of 100 rpm. An amount of microparticles equivalent to 150 mg of was placed in the dissolution medium Citric acid buffer pH 0.1 for 2 h. Then, after 2 h replaced phosphate buffer pH 7.4 maintained for 30 h at a temperature of 37± 0.5ºC. A 5 ml of sample aliquot of the dissolution medium was withdrawn at different time intervals and fresh dissolution medium was simultaneously used to replace the quantity withdrawn [24-26]. The samples were then filtered using a Whatmann No. 1 qualitative filter paper and assayed spectrophotometrically (Varian Carry 50 Bio, USA) at 254 nm to estimate the drug concentration. All experiments were performed intriplicate.

RESULTS AND DISCUSSION

Standard graph of Didanosine in 0.1 N HCl (lamda max 254nm)

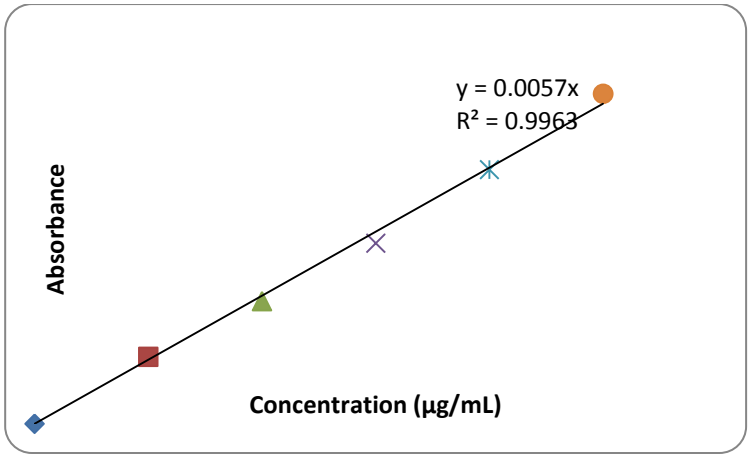

Figure 2: Standard graph of Didanosine 7.4 Buffer (lamda max 254nm)

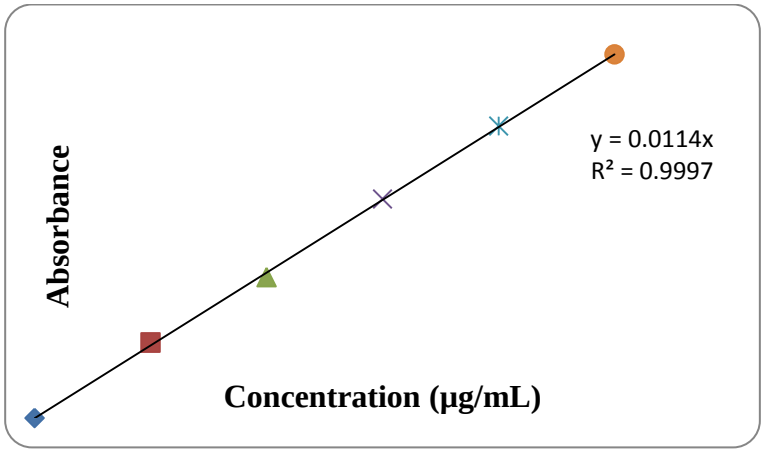

Figure 3: Standard graph of Didanosine 1.0 Buffer (lamda max 254nm)

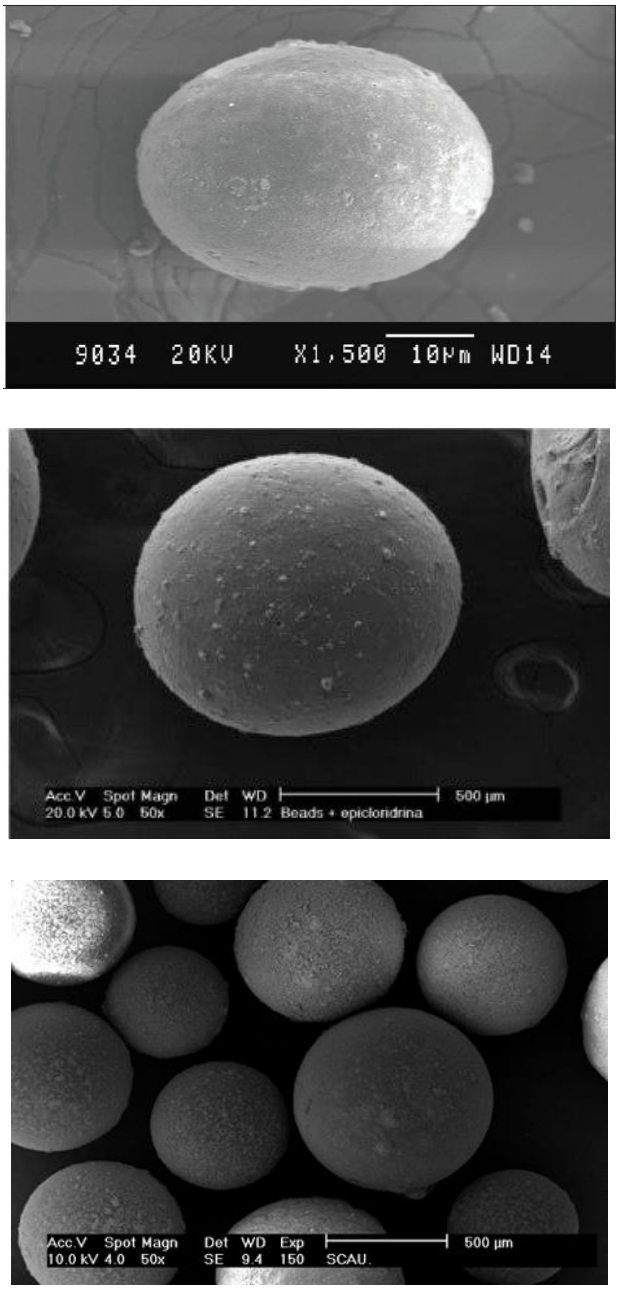

Figure 4, 5 & 6: SEM images and particle size of optimised formulation

In vitro Drug Release

Release studies were carried out by using two different release media. HCL pH 0.1 and Phosphate buffer at pH 7.4 were used in order to evaluate the influence of the pH inside gastric and intestine on Didanosine release from HPMC microparticles. In Figure, Didanosine release profiles from Didanosine-loaded HPMCmicroparticles at pH 0.1 and 7.4 buffer solutions respectively, are shown. As can be seen from the figures, an initial burst effect was observed from all

HPMC microparticles (between 13 and 22% of loaded Didanosine). After this initial burst, all studied microspheres released Didanosine at a lower rate. Didanosine release from the was pH dependent (faster release at pH 0.1 than at pH 7, 4). This is attributed to the higher solubility of the polymer at lower pH. In fact, as proposed earlier, HPMC microparticles can also provide pH responsive release profile by swelling in acidic

environment of the gastric fluid. When comparing the release profiles from cross-linked (with SS/SC) and with & without adding calcium chloride. By addition of the cations like calcium chloride, the drug release was diminished hence it was more controlled. we see that at pH 7.4 the release of Didanosine is substantially decreased in the cross-linked particles. It has been proposed before that addition in HPMC particles can be used as a method to modulate release kinetics of drugs, as demonstrated for theophylline. However, the difference between the release kinetics of Didanosine from the two types of HPMC particles is more or less diminished (or is a lot smaller) at pH 3.0, possibly due to the rapid swelling and increased solubility of this polymer a low pH, which results in a very fast release of particle- loaded Didanosine from all HPMC microparticles during the first 4-5 hours of incubation.

In vitro Drug release

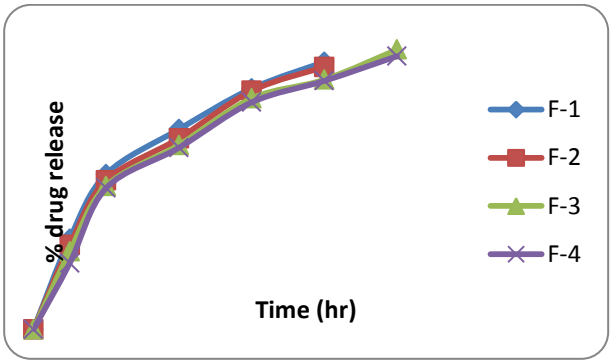

Figure 7: Invitro drug release profile

Table 2: Drug release profile-I

Time (hr)	% Drug release			
	F-5	F-6	F-7	F-8
0	0	0	0	0
1	14.6	12.8	11.45	8.34
2	28.32	27.53	25.3	20.6
4	48.4	46.9	44.8	41.9
6	64.2	61.8	58.5	56.36
8	82.6	79.6	78.2	74.7
10	94.6	91.3	90.3	89.4
12	-	-	-	97.4

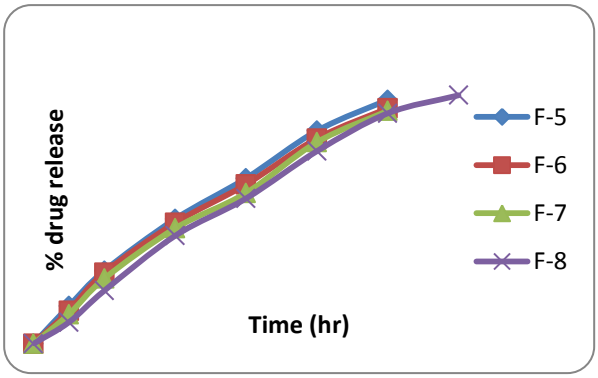

Figure 8: *In vitro* drug release profile-I

Table 3: Drug release profile-II

Time (hr)	% Drug release			
	F-9	F-10	F-11	F-12
0	0	0	0	0
1	20.2	18.61	15.3	12.16
2	42.68	36.9	32.72	27.8
4	58.4	52.8	48.3	42.6
6	66.46	60.72	54.34	50.46
8	72.8	64.7	62.2	61.6
10	78.4	72.98	70.46	68.2
12	84.8	81.6	82.8	79.42

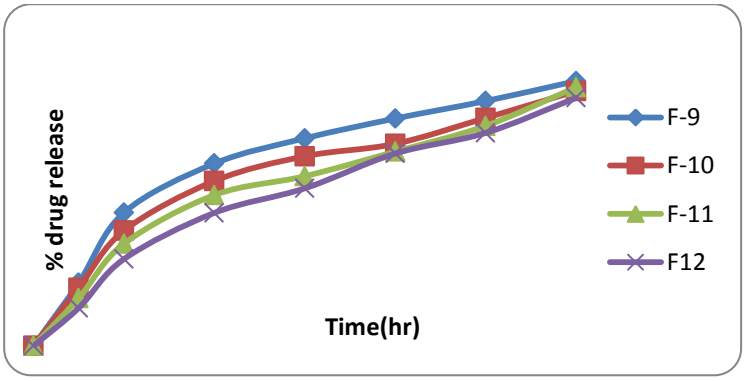

Figure 9: *In vitro* drug release profile-II

Optimized formula Graphs

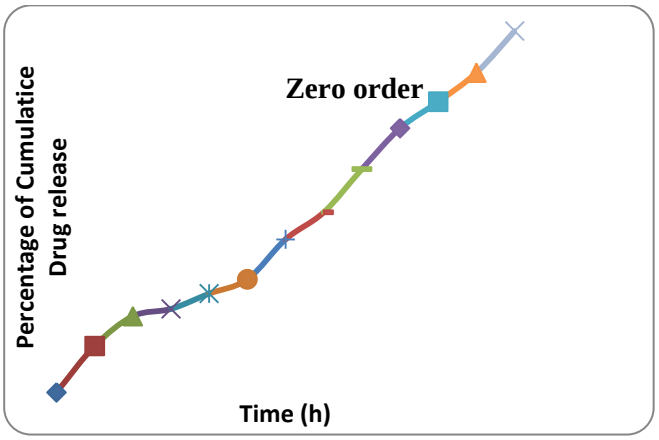

Figure 10: Zero Order Release

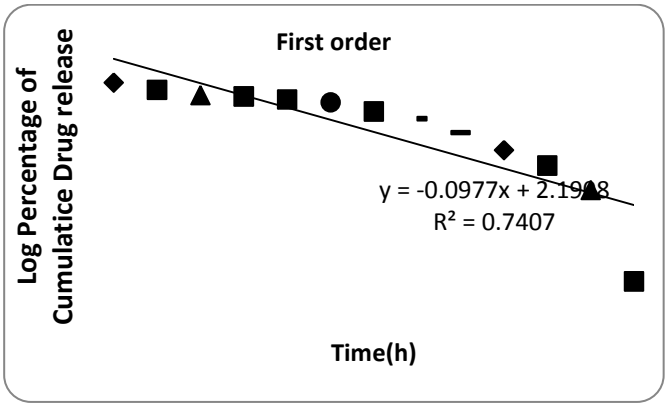

Figure 11: First Order Release

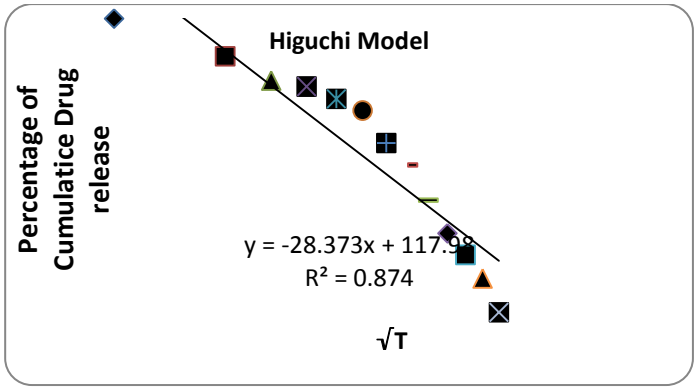

Figure 12: Higuchi Model

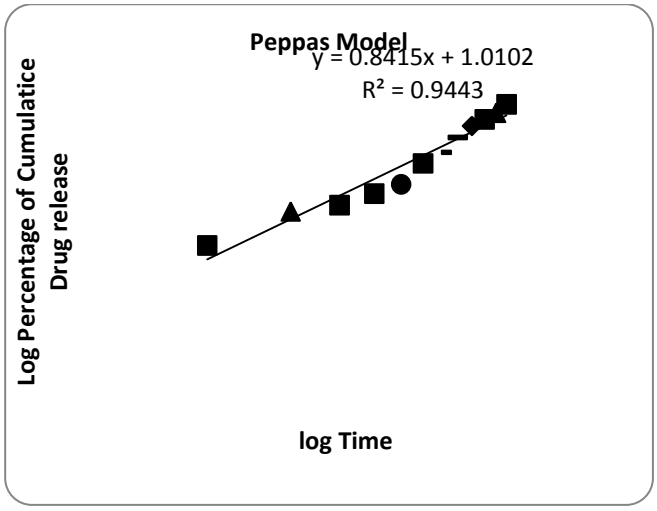

Figure 13: Peppas Model

Kinetic modeling

The various kinetic models were applied to *in vitro* release data for prediction of the drug release kinetic mechanism. The release constants were calculated from the slope of appropriate plots, and the regression coefficient (r^2) was determined. It was found that the *in vitro* drug release of microparticles was best explained by

First order kinetics as the plots shows highest linearity. The correlation coefficient (r^2) was 0.9877 for f8 formulation as shown in Table .For formulation correlation coefficient (r^2) is found to be 0.9566, indicating that the drug release was nearly dependent of concentration, followed by Higuchi's (r^2 = 0.9161).

In the current study, drug release kinetic according to korsmeyer-peppa's model is also followed. The values of release rate exponent (n), calculated as per the equation proposed by peppa's, and all the slope values range.9945 revealed the fact that the drug release follows a super case II transport.

Table 4: Kinetic models for drug release

Code	EE	Zero	Higuchi	First
F-1	69.11	0.9859	0.8269	0.9811
F-2	71.55	0.9865	0.8568	0.9919
F-3	72.67	0.9844	0.9045	0.9813
F-4	84.45	0.9835	0.9283	0.9816
F-5	67.91	0.9872	0.7941	0.9816
F-6	71.6	0.9858	0.7919	0.9879
F-7	86.64	0.9829	0.9161	0.9891
F-8	90.18	0.9877	0.9161	0.9887
F-9	56.56	0.9923	0.8612	0.9812
F-10	59.81	0.9918	0.8913	0.9823
F-11	74.62	0.9928	0.8913	0.9823
F-12	76.5	0.9898	0.9121	0.9719

Short term Stability Study

Stability study is the important part of the study for any pharmaceutical formulation. There are procedures given for the stability study in ICH guidelines.

Table 5: Short term stability study data of optimized

Duration (month)	Parameter studied	Formulation code
0	Drug content	82.11
	% Drug release	62.16
1	Drug content	80.54
	% Drug release	61.54
2	Drug content	79.83
	% Drug release	60.59
3	Drug content	79.05
	% Drug release	59.74

The short term stability study was performed as per ICH guidelines using selected Didanosine - loaded HPMC micro particles for a period of 3 months. The microparticles were periodically evaluated for drug content and in vitro drug release. The evaluated parameters did not show any significant change during the time course of storage confirmed that the prepared Didanosine-loaded HPMC microparticles

CONCLUSION

At this juncture it has been concluded that the design and the evaluation of Didanosine in HPMC microparticles has been found satisfactory and conducive. As the drug release profile of the Didanosine has been considerably tremendous and vivid. The further studies on the prepared novel formulation have been suggested for the efficacy of the drug in various animal models.

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