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Formulation and Evaluation of Serratiopeptidase Matrix Tablets

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A B S T R A C T

In the present research work, an attempt was made to prepare matrix tablets of serratiopeptidase which is a proteolytic enzyme isolated from the non pathogenic enterobacteria Serratia marcescens. Microbial enzymes have gained much popularity and they are economical and can be produced on large scale within the limited space and time. Serratiopeptidase as an immediate release dosage form irritates gastric mucosa in acidic environment and has a half life of 3-4 hrs. Due to the above limitations serratiopeptidase matrix tablets were prepared by using different hydrophobic and hydrophilic polymers like Ethocel (ethyl cellulose), Methocel (Hydroxy propyl methyl cellulose) in different ratios. Eight formulations (SP-1 to SP-8) were prepared using wet granulation method and evaluated for pre and post compressional parameters. Micromeritic properties showed poor flow properties for serratiopeptidase due to its amorphous nature when compared with the formulations SP-1 to SP-8 and acceptable resistance was shown by serratiopeptidase matrix tablets to withstand handling. According to *In-vitro* dissolution studies, SP-6 shows more retarding effect and thus found that T_{50} % value increases as concentration of ethyl cellose increases. Korsemeyer-Peppas release exponent (n) values of all serratiopeptidase matrix tablets are > 0.8 indicating drug diffusion is rapid due to swelling in the polymer (case 2 transport).

Keywords: Serratiopeptidase, Serratia marcescens, Matrix tablets, Ethyl cellulose, Hydroxy propyl methyl cellulose.

A R T I C L E I N F O

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1. Introduction

Enzyme is a biocatalyst which accelerates biochemical reactions and the sources of enzymes are microorganisms, higher plants and animals. Microbial enzymes have gained much popularity and there are of two enzymes, extracellular enzymes and intracellular enzymes [1,2]. Serratiopeptidase, also known as Serrapeptase or Serratiopeptidase, is a proteolytic enzyme isolated from the non pathogenic enterobacteria Serratiamarcescens. This enzyme was found naturally in the intestine of the silkworm, which is used by the silkworm to dissolve the cocoon and emerge as a moth [3-5].

A systemic review on serratiopeptidase has noted that there are limited adverse drug reactions reported, but they are used for both muscle and joint pain. One of the least complicated approaches to manufacture controlled release dosage forms involves the direct compression of blend of drug, retardant material and additives to formulate a tablet in which the drug is embedded in a matrix of the retardant. Matrix tablets is a promising approach for the establishment of extended release drug therapy, as tablets offer the lowest cost approach [6].

Serratiopeptidase is destroyed in the acidic environment of the stomach, and as such would require encapsulation in order to retain the bioactivity. Hence serratiopeptidase tablets were prepared using hydrophobic and hydrophilic polymers like ethyl cellulose, hydroxy propyl methyl cellulose in different ratios and to evaluate the matrix tablets by various pre and post formulation studies.

2. Materials and Methods

Serratiopeptidase was purchased from KP Labs, Hyderabad. Ethyl cellulose and Hydroxy propyl methyl cellulose was purchased from color on laboratories and all the other chemicals used were of analytical grade.

Method

The core tablets (average wt. 500mg) of Serratiopeptidase were prepared by wet granulation method. The composition of core tablet is given in table-1. Lactose was used as diluents, hydroxyl propyl methyl cellulose and ethyl cellulose were used as polymers. The materials were weighed, mixed and passed through a sieve to ensure complete mixing. The materials were mixed with starch mucilage and a cohesive mass if formed which was passed through sieve no #16. Then prepared granules were dried at 60^0C for 30 minutes. The tablets were prepared by compressing thoroughly using 13mm round, flat punches on 16 station tablet punching machine (cadmach).

Evaluation

Pre Compression Parameters [7-9]

Drug and excipient compatibility study

FT-IR spectra were studied by Shimadzu 8400S, Japan FT-IR spectrometer. The samples (Serratiopeptidase and Excipients) were previously ground and mixed thoroughly with potassium bromide, an infrared transparent matrix, at 1:5 (Sample: KBr) ratio, respectively. The KBr discs were prepared by compressing the powders at a pressure of 5 tons for 5 min in a hydraulic press. The scans were obtained at a resolution of 4 cm^{-1}, from 4000 to 400 cm^{-1}. The formulations were evaluated for various physicochemical parameters including angle of repose, bulk density, tapped density, compressibility index and Hausner's ratio.

Bulk density

A 15 g quantity of the powder samples was placed in a 100 ml dry measuring cylinder and volume V_O, occupied by it, without tapping, was determined. The bulk density were calculated by following formula

Bulk density = W/Vo

Tapped density

The cylinder was then given 100 taps using tap density apparatus and the resulting volume, V_{100}, was noted determined. The tap density was calculated by using following formula.

Tapped density = W/V_{100}

Angle of repose

The fixed-funnel method was used to determine angle of repose. The granule formulation was carefully poured through a funnel until the apex of the conical pile just touched the tip of funnel. The height (h) of the pile of the powder and the radius (r) of its conical base were measured and applied to compute the angle of repose.

$\theta = \tan^{-1} h/r$

Carr's index (compressibility index)

Compressibility index is based on poured density and tapped density, carr's index was calculated by using this formula.

Carr's index (%) = poured density-tapped density/poured densityX100

Hausner's ratio

Hausner's ratio is defined as the ratio of tap density to bulk density of the prepared granules.

Hauser's ratio = TD/BD

HR <1.25 – indicates good flow property

HR >1.25 – indicates poor flow property

Post Compression Parameters [7-9]

The tablets were evaluated for hardness, thickness, friability, weight variation etc according to IP specifications.

Determination of drug content

Two tablets from each formulation were crushed to powder. Crushed powder were transferred into 100 ml flask and diluted to 100 ml water and stirred magnetically for 1 hr, centrifuged and filtered. 1 ml of this solution was taken and it was diluted to 100 ml with water and then absorbance was noted at 275 nm using UV-visible spectrophotometer.

***In-vitro* dissolution**

The release of serratiopeptidase from matrix tablets was determined by using dissolution apparatus (Lab India DS8000). This test was performed by using 900ml phosphate buffer 6.8 at 37^0C±0.5^0C temperature with 50 rpm. The sample was taken at every one hour intervals and the absorbance of the solution is measured at 275nm using UV visible spectrophotometer.

Mathematical Modeling for Drug Release Profile

The amount of serratiopeptidase released from the formulated tablets at different time intervals were fitted in

to several kinetic models such as Zero order kinetics, first order kinetics, Higuchi model and Korsemeyer-Peppas model to characterize mechanism of drug release.

3. Results and Discussion

From the FT-IR results it is evident that when serratiopeptidase fig-1 was compared with ethyl cellulose, HPMC and mixture fig- 2,3,4 there is no characteristic change in the peaks. These results confirm that there is no any chemical interaction between serratiopeptidase and excipients. Micromeritic properties showed poor flow properties for API due to its amorphous nature when compared with the formulations SP-1 to SP-8 and the results are tabulated in table no- 2.

Post-formulation parameters concluded that there should be certain amount of strength and resistance to friability for the tablet, so that tablet should not break during handling which also shows affect on dissolution. The hardness of serratiopeptidase matrix tablet ranges from 6.2 to 6.8 kg/cm^2. Friability ranges from 0.113% to 0.210%. This indicates that acceptable resistance is shown by serratiopeptidase matrix tablets to withstand handling and the results are given in table no – 3.

In-vitro dissolution studies showed that, with increase in the hydrophobic polymer (ethyl cellulose), the percent drug release has been retarded, shown in table-4 and fig no -5. For all the formulations the dissolution was conducted for twelve hours and among all the formulations, SP-6 showed optimum release profile indicating it to be the best formulation in present research. The stability tests were conducted on SP-6 and the formulation was analyzed for its organoleptic properties, moisture content and dissolution profile.

The results showed that the colour and gross nature of tablets was slightly changed for batch-3 which is kept at 60°C/80%RH. Changes was not seen in batch-1 & 2 which is kept at 25°C/60%RH & 40°C/70%RH. The percentage of moisture content of SP-6 showed high values when the tablets are kept outside the container at room temperature and relative humidity. The results are given in table-6,7,8. SP-6 released at a faster rate when stored at 60°C/80%RH which may be due to the polymer relaxation, and the results are given in table no-4 and fig-5. Different model dependent approaches (Zero order, First order, Higuchi, Korsemeyer-Pappas plots) were performed for all matrix tablets.

The results of these models follow Korsemeyer-Peppas model as “best fit model” follows diffusion mechanism. This is due to previously proved fact depending on R^2 value obtained from model fitting. From the results, SP-6 shows more retarding effect and thus found that T_{50} % value increases as concentration of EC increases. Korsemeyer-Peppas release exponent (n) values of all serratiopeptidase matrix tablets are greater than 1 indicating drug diffusion is rapid due to swelling in the polymer (case 2 transport).

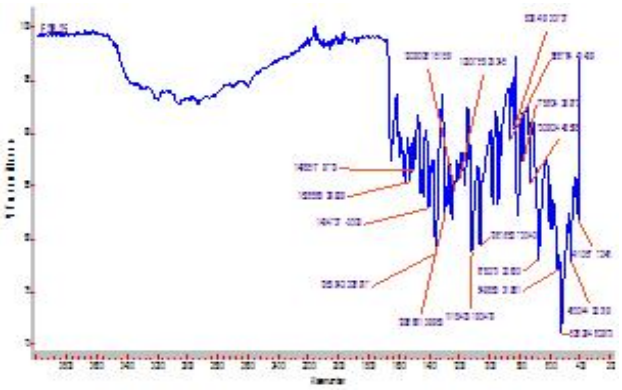

Figure 1: FT-IR Spectra of Serratiopeptidase pure drug

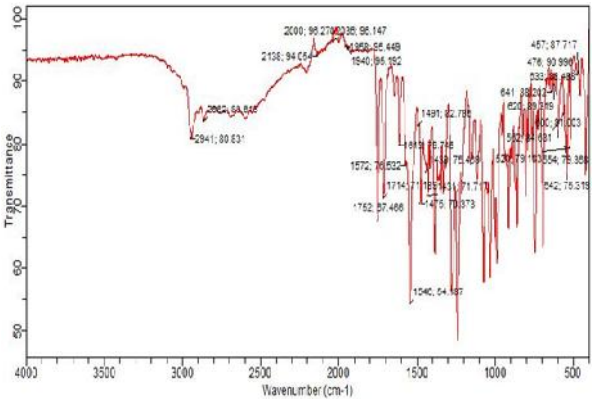

Figure 2: FT-IR spectra of Ethyl Cellulose

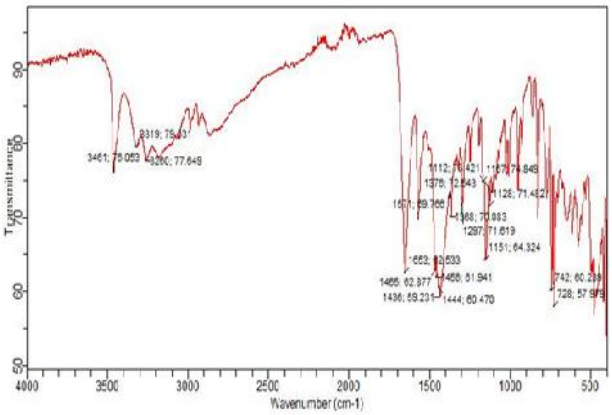

Figure 3: FT-IR spectra of HPMC

Figure 4: FT-IR spectra of Polymer Mixture + Drug

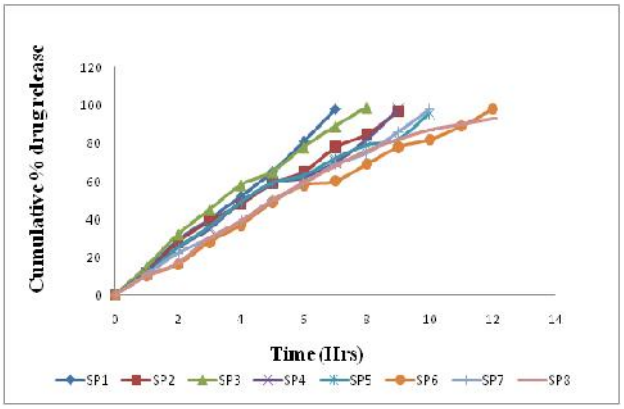

Figure 5: Cumulative % Drug release for SP1-SP8

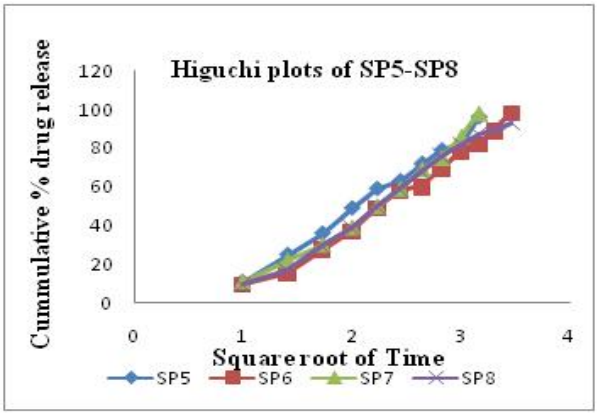

Figure 9: Higuchi plots of SP5-SP8

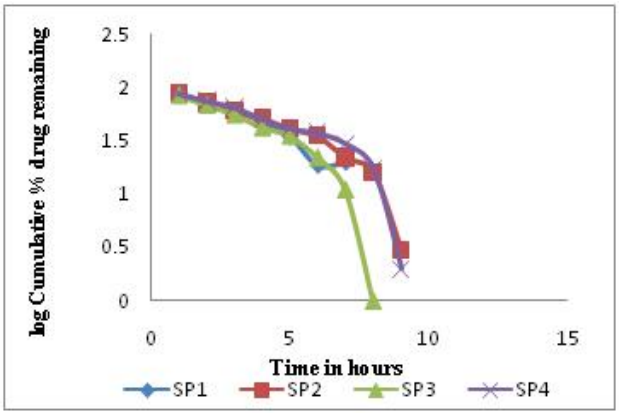

Figure 6 First order plots of SP1-SP4

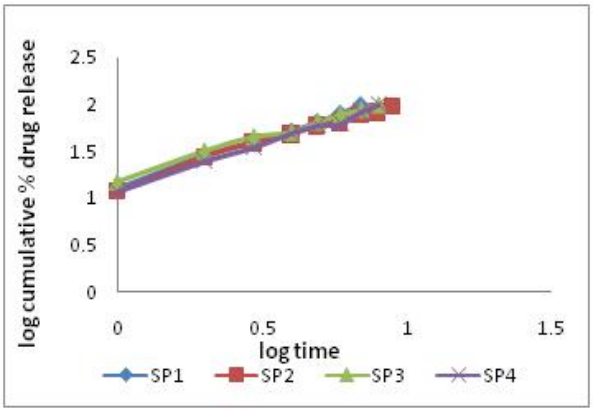

Figure 10: Peppa's plots of SP1 to SP4

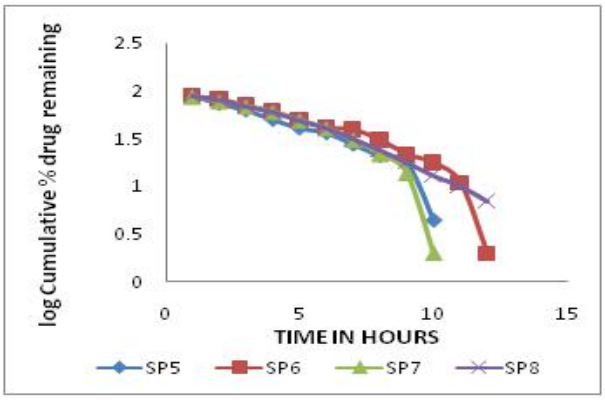

Figure 7: First order plots of SP5-SP8

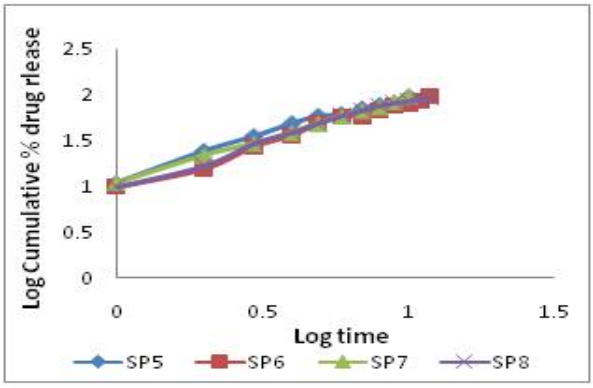

Figure 11: Peppa's plots of SP5-SP8

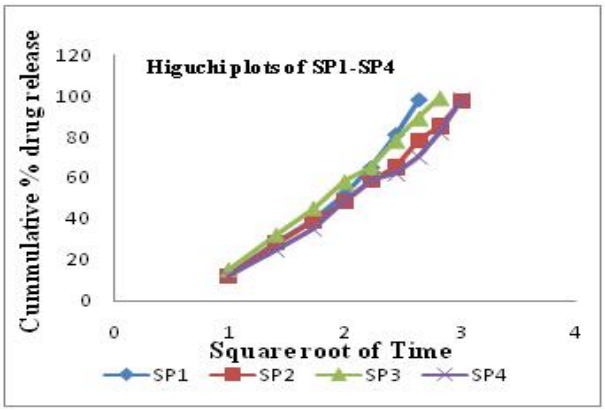

Figure 8: Higuchi plots of SP1-SP4

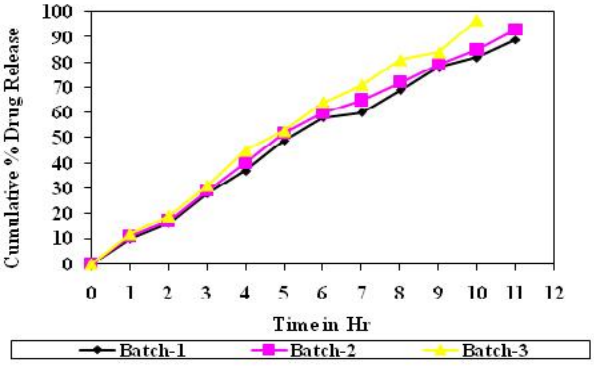

Figure 12: Stability studies of *in-vitro* dissolution plot of SP-6

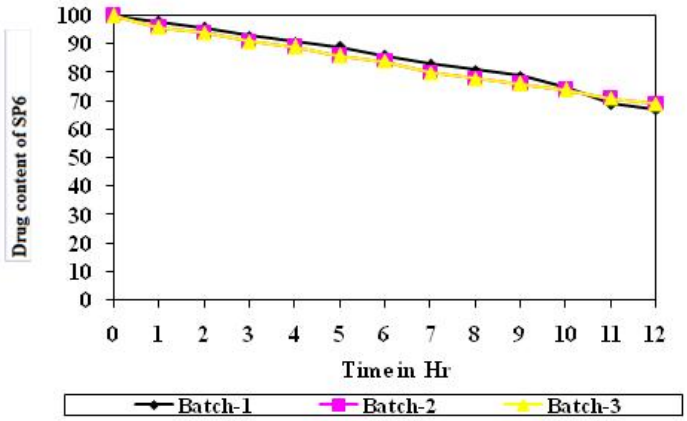

Figure 13: Drug content of SP- 6 - Stability data

Table 1: Formulation Table for Serratiopeptidase Matrix Tablets

S.NO	Formulation ratio in (mg)	SP1	SP2	SP3	SP4	SP5	SP6	SP7	SP8
1	Drug	30	30	30	30	30	30	30	30
2	Lactose	363.35	323.35	388.35	378.35	348.35	298.35	338.35	308.35
3	Ethyl cellulose	40	80	-	-	40	80	40	80
4	HPMC	-	-	15	25	15	25	25	15
5	Talc	25	25	25	25	25	25	25	25
6	Magnesium stearate	16.65	16.65	16.65	16.65	16.65	16.65	16.65	16.65
7	Starch	25	25	25	25	25	25	25	25
8	Total	500	500	500	500	500	500	500	500

DRUG-Serratiopeptidase (SP), HPMC-Hydroxy propyl methyl cellulose, EC- Ethyl Cellulose.

Table 2: Pre compression (Micromeritic) parameters of Serrtiopeptidase matrix tablets

F. Code	Derived properties		Flow properties		
	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle of repose (Degree)	Carr's index (%)	Hausner's ratio (%)
SP-1	0.606	0.625	18.05	3.0	1.0
SP-2	0.520	0.540	17.74	2.6	1.2
SP-3	0.460	0.512	18.77	9.3	1.1
SP-4	0.540	0.555	24.44	2.6	1.0
SP-5	0.571	0.588	19.54	2.8	1.0
SP-6	0.540	0.555	19.79	2.6	1.3
SP-7	0.444	0.454	19.49	2.3	1.4
SP-8	0.476	0.526	20.80	9.4	1.1

Table 3: Post compression parameters of Serratiopeptidase matrix tablets

F. Code	Thickness Mean±SD(mg)	Hardness (mm)	Friability (%)	Weight variation Mean±SD(mg)	Drug Content Mean±SD (mg)
SP-1	2.21±0.3	6.8	0.148	497±0.5	98.2±0.6
SP-2	2.34±0.5	6.5	0.158	498±0.6	98.4±0.8
SP-3	2.49±0.1	6.7	0.210	499±0.3	99.1±0.4
SP-4	2.51±0.2	6.8	0.148	496±0.8	96.3±0.3
SP-5	2.54±0.6	6.3	0.113	497±0.7	97.1±0.1
SP-6	2.49±0.3	6.4	0.144	496±0.7	99.1±0.7
SP-7	2.39±0.5	6.9	0.168	499±0.3	98.2±0.6
SP-8	2.28±0.2	6.2	0.179	483±0.2	99.4±0.2

Table 4: *In- vitro* Drug Release profile of Serratiopeptidase Matrix tablets

Time (hrs)	Cumulative % drug release							
	SP-1	**SP-2**	**SP-3**	**SP-4**	**SP-5**	**SP-6**	**SP-7**	**SP-8**
1	13	12	15	12	11	**10**	11	10
2	29	28	32	25	25	**16**	22	17
3	40	39	45	35	36	**28**	30	30
4	52	48	58	49	49	**37**	39	39
5	65	59	65	59	59	**49**	50	50
6	81	65	78	62	63	**58**	59	59
7	98	78	89	70	72	**60**	69	68
8	-	85	99	82	79	**69**	75	76
9	-	97	-	98	82	**78**	86	82
10	-	-	-	-	96	**82**	98	87
11	-	-	-	-	-	**89**	-	90
12	-	-	-	-	-	**98**	-	93

Table 5: Mathematical Modelling for serratiopeptidase matrix tablets

Formulation code	Correlation Co-efficient Values (R^2)				Diffusion Exponent value(n)
	Zero order	First order	Higuchi	Korsemeyar peppas	
SP1	0.99	0.92	0.96	0.99	1.01
SP2	0.99	0.85	0.97	0.98	0.91
SP3	0.99	0.89	0.99	0.99	0.88
SP4	0.97	0.91	0.97	0.99	0.98
SP5	0.98	0.83	0.99	0.98	0.89
SP6	0.97	0.86	0.98	0.98	0.94
SP7	0.97	0.91	0.97	0.99	0.99
SP8	0.99	0.91	0.99	0.98	0.94

Table 6: Stability testing (moisture Content) Batch-1 (SP-6)

Sample No	Initial weight	Final weight (g)	Difference (g)	Percentage of moisture (%)
1	0.523	0.536	0.013	2.42%
2	0.520	0.534	0.014	2.62%
3	0.518	0.532	0.014	2.63%
Avg				2.55%

Serratiopeptidase tablets stored in container at room temperature and humidity for 12 weeks

Table 7: Stability testing (moisture Content) Batch-2 (SP-6)

Sample No	Initial weight	Final weight (g)	Difference (g)	Percentage of moisture (%)
1	0.513	0.536	0.023	4.29%
2	0.510	0.534	0.024	4.47%
3	0.512	0.538	0.026	4.83%
Avg				4.53%

Moisture content of serratiopeptidase tablets stored in container at 40 ± 2^0c and 75% $\pm$ 5% RH for 12 weeks

Table 8: Stability testing (moisture Content) Batch-3 (SP-6)

Sample No.	Initial weight	Final weight (g)	Difference (g)	Percentage of moisture (%)
1	0.510	0.540	0.030	5.55%
2	0.515	0.543	0.028	5.15%
3	0.512	0.543	0.031	5.70%
Avg				5.46%

Moisture content of serratiopeptidase tablets stored outside container at room temperature and humidity for 12 weeks

Table 9: Stability studies in-vitro dissolution profile of SP-6

S.NO	Medium	Time	% Drug release of SP-6		
			Batch-1 (25^0c/60%RH)	Batch-2 (40^0c/70%RH)	Batch-3 (60^0c/80%RH)
1	6.8 pH Phosphate buffer	1	10	11	12
2		2	16	17	19
3		3	28	29	31
4		4	37	40	45
5		5	49	52	53
6		6	58	60	64
7		7	60	65	71
8		8	69	72	81
9		9	78	79	84
10		10	82	85	97
11		11	89	93	-
12		12	98	98	-

Table 10: Drug content of SP- 6 - Stability data

Weeks	Drug content of sp-6		
	Batch-1 (25^0c/60%RH)	Batch-2 (40^0c/70%RH)	Batch-3 (60^0c/80%RH)
1	98	96	96
2	96	94	94
3	93	91	91
4	91	89	89
5	89	86	86
6	86	84	84
7	83	80	80
8	81	78	78
9	79	76	76
10	75	74	74
11	69	71	71
12	67	69	69

4. Conclusion

From the present research it was concluded that the formulation, SP-6 (Drug with 16% ethyl cellulose and 5 % HPMC) of serratiopeptidase has achieved 98% drug release for 12 Hrs. Results indicated that, drug release has been retarded with increase in the concentrations of hydrophobic polymer (Ethyl cellulose), with increase in concentration of hydrophilic polymer (HPMC) the drug release has been completed within 7 hrs. The above formulation may also decrease the gastric irritation and may improve patient compliance with reduction in dosage frequency.

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