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Formulation and In-Vitro Evaluation of Gastroretensive Drug Delivery System of Ritonavir

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Abstract

The purpose of the research work was to develop a floating drug delivery system of Ritonavir in order to prolong the gastric residence time and increase its bioavailability. Gastroretensive retentive drug delivery system is designed with the aim to target the drug to its absorption site and to maintain the dosage form at that site for an extended period of time. To develop a floating Mucoadhesive tablet of ritonavir to prolong the gastric retention time for effective drug delivery system. Floating Mucoadhesive tablet of ritonavir was prepared successfully by direct compression method. Compatibility study of ritonavir with formulation ingredients was performed by DSC and FTIR results revealed that drug was compatible with all selected excipients. The present investigation was aimed to formulate floating drug delivery system using effervescent agent sodium bicarbonate and citric acid the tablet continuously floats for more than 12 h. Floating tablet was prepared using HPMC (K4M) carbopol 934.

Keywords: Gastroretensive, Mucoadhesive, floating drug delivery system, effervescent.

Introduction

Gastric retention is an approach for drug delivery in which initial part of GIT drugs that's were less soluble or get degraded in alkaline pH may be benefited from prolonged gastric retention increases bioavailability, decreases wastage of drugs, increases solubility of drug[1]. Drugs that have narrow absorption window in gastric intestinal tract will have poor absorption for these drugs. Gastro retentive drug delivery system several techniques are employed like low density, high density, raft system mucoadhesive system and in-situ gelling system[2]. Attempts have been made to be 8-10 hr. From mouth to colon, is relatively brief with considerable fluctuation. One of the important determinants of G.I transit is the residence time in the stomach. The oral controlled delivery of drugs having "absorption window" continually releasing the drug prior to absorption window for prolonged period of time, thus ensuring optimal bioavailability[3]. A floating dosage unit is useful for drugs acting locally in the proximal gastrointestinal tract. These systems are also useful for drugs that are poorly soluble or unstable in intestinal fluids. Floating tablets and Floating capsules are common examples of floating system[4].

Ritonavir (RN) is a protease inhibitor widely prescribed in antiretroviral regimen. It blocks the HIV protease,

thereby reducing the viral load in the infected individual. This drug is mainly suffers with low oral bioavailability due to degradation of RN by the Cytochrome P450-3A4 (CYP3A4) isoenzymes in the distal intestine, efflux of the absorbed drug by counter transporter proteins (mainly P-glycoprotein) present in the distal intestine and is unstable at alkaline pH. It shows pH-dependent solubility and solution stability[5]. Moreover, it is primarily absorbed from stomach and having short half-life (~3-5 hrs). Due to these characteristics, it was selected for the development of GRDDS[6].

Materials and Methods

Materials

Ritonavir was obtained as gift sample from Cipla Laboratories Ltd, Goa. Carbopol 940 and HPMC K4M were obtaied from Blue Cross Laboratory, Ltd, Nashik. Magnesium stearate , Talc, Lactose ect was purchased from Research-Lab Fine Chem.Industry, Mumbai.

Methods

Preformulation Study of Drug

Organoleptic Properties

The sample of Ritonavir was studied for Organoleptic characteristics such as color, odor and appearance

Melting Point

Melting point of Ritonavir was determined by taking a small amount of sample in a capillary tube closed at one end and placed in melting point apparatus. The melting point was noted in triplicate and average value was noted

Calibration curve of Ritonavir

The stock solution of drug was subsequently diluted with 0.1 N HCl to get 10µg/ml- 50 µg/ml. Then the absorbance of these dilute solutions was measured at a λ_{max} of 239 nm Calibration curve was performed in triplicate.

Compatibility Study

Compatibility study was carried out by using Fourier Transform Infrared Spectrophotometer (BRUCKER). IR study was carried on pure drug. Physical mixture of drug and excipients were prepared and samples kept for 1 month at 40^0C. The infrared absorption spectrum of Ritonavir and physical mixture of drug and excipient was recorded using diamond disc

Differential Scanning Calorimetry:

The powdered sample (3 mg) was hermetically sealed in aluminum pans and heated at a constant rate 10^0C/min, over a temperature range of 30-300^0C with nitrogen flow rate of 30ml/min. Thermograms of the samples were obtained using differential scanning Calorimetry (DSC-60, Shimadzu, Japan). Thermal analysis data were recorded with Shimadzu software programs. Indian standard was to calibrate the DSC temperature and enthalpy scale

Preparation of Floating Mucoadhesive Ritonavir tablet by direct compression:

Floating Mucoadhesive tablets were prepared by direct compression method. The blended powder was evaluated for its pre-compression characteristics and then compressed on 10 station pilot press using 10 mm flat faced punches. The machine was adjusted to produce an approximate weight of 300 mg tablet.

Table No. 1: Composition of Formulation in Terms of Floating Mucoadhesivetablet as per factorial design (All values are expressed in mg)

Ingredients	Formulation code								
Quantity(mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Ritonavir	100	100	100	100	100	100	100	100	100
HPMC K4M	50	50	50	60	60	60	70	70	70
Carbopol 934	10	15	20	10	15	20	10	15	20
Sodium Bicarbonate	30	30	30	30	30	30	30	30	30
Citric acid	10	10	10	10	10	10	10	10	10
Talc	3	3	3	3	3	3	3	3	3
Mg Stearate	3	3	3	3	3	3	3	3	3
Spray driedLactose	94	89	84	84	79	74	74	69	64
Total Weight	300	300	300	300	300	300	300	300	300

Evaluation of floating Mucoadhesive tablets of ritonavir

Precompression Characteristics

Precompression evaluation includes measurement of Bulk Density, Tapped Density, Hausner's Ratio, and Compressibility Index of prepared formulations.

Bulk Density (BD)

An accurately weighed powder blend from each formula was lightly shaken to break any agglomerates formed and it was introduced in to a measuring cylinder. The volume occupied by the powder was measured which gave bulk volume. Bulk density (BD) of powder blends was determined.

Tapped density (TD)

An accurately weighed powder blend from each formula was lightly shaken to break any agglomerates formed and it was introduced into a measuring cylinder. The measuring cylinder was tapped until no further change in volume was noted which gave the tapped volume. The tapped densities (TD) of powder blends were determined.

Tapped Density = Total Weight of Powder / Total Weight of Tapped Powder

Compressibility Index

It is a simple index that can be determined on small quantities of powder. In theory, the less compressible a material the more flow able it is. The compressibility indices of the powder blends were determined using following formula.

Hausner's Ratio

Hausner's ratio greater than 1.25 is considered to be an indication of poor Flowability. A Hausner's ratio less than

1.12 indicates good flow while greater than 1.35 indicates poor flow.

Angle of repose (θ)

The angle of repose (θ) for powder was determined by placing the powder in a funnel. The tip of the orifice of the funnel was fixed from the ground horizontal surface at a height of 1cm and the powder were allowed to flow only under the force of gravity.

Evaluation of compressional characteristics floating tablets

Post compressional evaluation includes measurement of hardness, disintegration time, drug content, % friability, % swelling Index, and floating time of all prepared formulations.

Hardness test

The hardness of the tablets here was measured using Monsanto hardness tester (Cadmech). In this, was tablet is placed between the plungers, and was tightened from one end, and pressure required to break tablet diametrically was measured. The hardness was measured in terms of kg/cm.

Uniformity of Thickness

The uniformity of thickness was measured using Digital Vernier Calliper (Absolute Digimatic, Mitutoyo Corp., Japan). The average diameter and thickness of the tablet was calculated.

Friability Test

In this test 20 tablets were weighed and placed in a Roche Friabilator test apparatus, and then the tablets were subjected to rolling ad replaced shocks, resulting from free falls within the apparatus from the height of 6 inches. After 100 revolutions the tablets were removed, de-dusted and weighed again. The friability was determined as the percentage loss in weight of the tablets.

In-vitro Disintegration Time

Disintegration time was determined using USP disintegration apparatus with distilled water. The volume of medium was 900 ml and temperature were 37 ± 0.2^0C. The time in minutes taken for complete disintegration of the tablet with no palatable mass remaining in the apparatus was measured. To comply the test all tablets should disintegrate within 15 minutes.

Drug Content

Units were selected at random and drug content was determined as specified in monograph. The tablet preparation complies with the test, only if each individual content lies between 85 to 115% of the average content.

Swelling Index

The swelling properties of matrices containing drug were determined by placing tablet matrices in the dissolution test apparatus in 900 ml 0.1 N HCl at 37 ± 0.5^0C. The tablets were removed periodically from the dissolution medium and, after removing free water, the weight gain was measured.

Determination of Floating capacity

Three individual tablets from each formulation were put in an individual flask containing 400 ml of 0.1 N HCl solutions. Then note time in minutes for each tablet t go from the bottom to the top of the flask (floating lag time) and the time for which tablets constantly float on the water surface (duration of floating) were measured. The sample mean and standard

deviation were calculated.

Mucoadhesive Strength

Detachment Stress is the force required to detach the two surfaces of mucosa when a formulation/gel is placed between them". The detachment stress was measured by using a modified analytical balance.

In-Vitro Drug Release Studies

The samples were withdrawn at predetermined time points, diluted 10 times and were analyzed spectrophotometrically at 239 nm. In-Vitro drug release was performed for all prepared batches (F1-F9) and % cumulative drug release was computed.

The optimized formulation was subjected to stability study. These tablets were subjected for a period of three months as per ICH guideline at the 40^0C temperature and relative humidity 75% RH. The samples were withdrawn at, 1, 2, and 3 months for given temperature condition. The formulations were evaluated mainly for drug content and % drug release at the predetermined intervals.

Results and Discussions

Preformulation study

Organoleptic Properties

Stability study

Table No.2: Organoleptic Properties of Ritonavir

Identification test	Result of sample obtained	Reported standards
Colour	White	White
Odour	Odourless	Odourless
Melting point	126-128°C	126-132°C

All the physical properties of the drugs were within the limit of reported standards which assures the purity of the drug samples.

Solubility

Table No. 3: Solubility in different solvents

Sr. No	Solvent	Observation
1	Water	Insoluble
2	Methanol	soluble
3	0.1 N HCl	soluble

Calibration curve of Ritonavir

The stock solution of drug was subsequently diluted with 0.1 N HCl to get 10 µg/ml- 50 µg/ml.

Then the absorbance of these dilute solutions was measured at a λ_{max} of 239 nm Calibration curve was performed in triplicate.

Table No.4: Absorbance of Ritonavir in 0.1 N HCl at 239 nm

Sr. No.	Concentration (ppm)	Absorbance
1	10	0.160
2	20	0.324
3	30	0.489
4	40	0.638
5	50	0.812

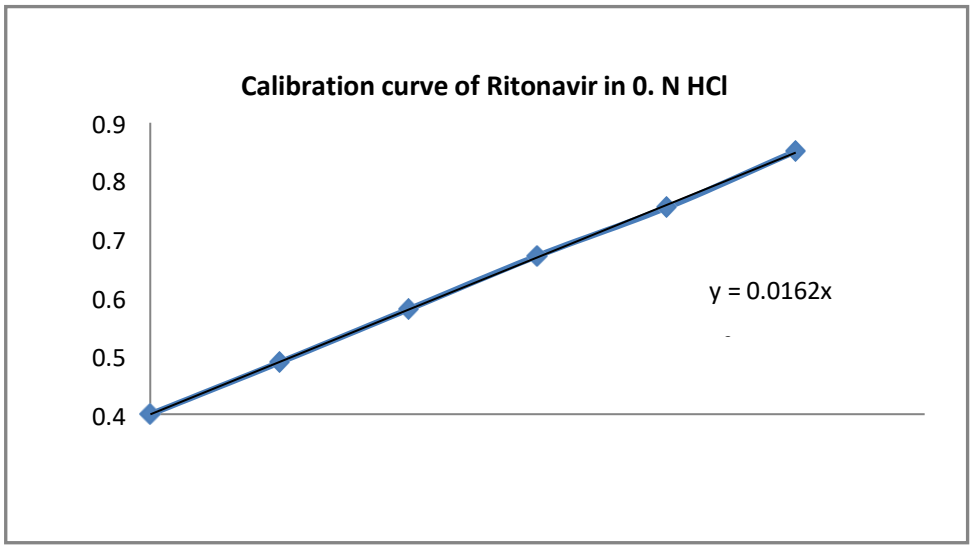

Figure No.1: Calibration curve of Ritonavir in 0.1 N HCl

Compatibility study

Fourier Transform Infrared Spectroscopy

Compatibility of the drug and excipients was confirmed by carrying out by studies like IR and DSC for pure drug and physical mixture of drug and polymers. The FTIR spectra of drug and its polymer mixtures were identical. In the IR spectral analysis of ritonavir exhibits all characteristic peaks. The characteristic absorption peaks of drug ritonavir was remained unchanged in drug-polymer admixture which indicates that there is no prominent chemical reaction between drug and polymer mixture, proving compatibility of drugs with selected excipients for the study.

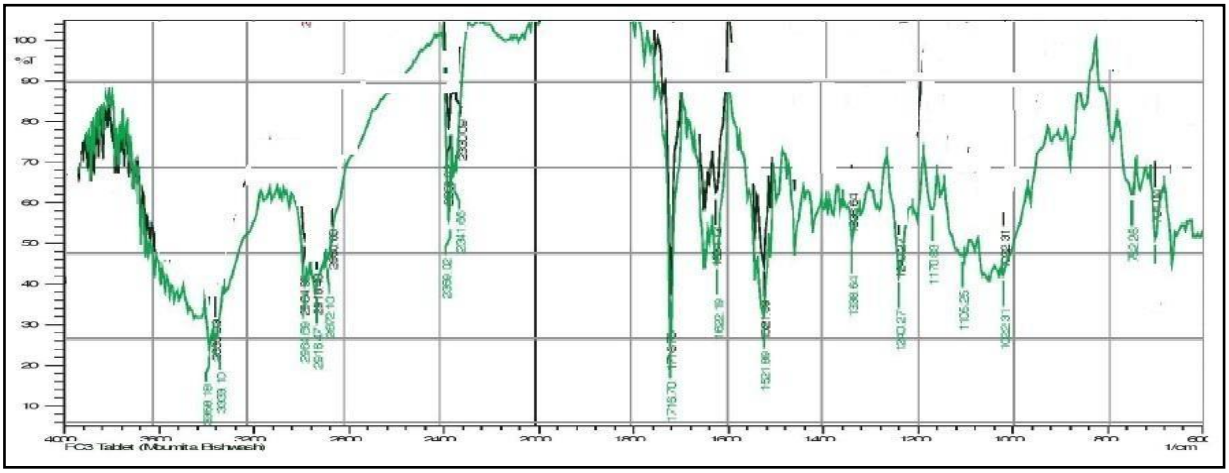

Figure No.2: IR Spectrum of Physical mixture of drug and excipients

Differential Scanning Calorimetry

The thermal behavior of drug and physical mixture of drug and polymer was studied by using DSC Thermogram. DSC thermogram of drug exhibited characteristic peak at 126.46^{0}C and physical mixture exhibited characteristic peak at 128.12^{0}C. DSC analysis was performed for pure ritonavir and physical mixture of drug with various excipients. Melting endotherm of drugs was well preserved in most of the cases as shown in figure 8.6 and 8.8 respectively. For physical mixtures, in all the cases melting endotherm of drug was well preserved with little or no change in enthalpy value of drug indicating compatibility of both drugs with selected excipients in the study. The polymers xanthan gum and carbopol 940P have been reported to be compatible with a number of drugs.

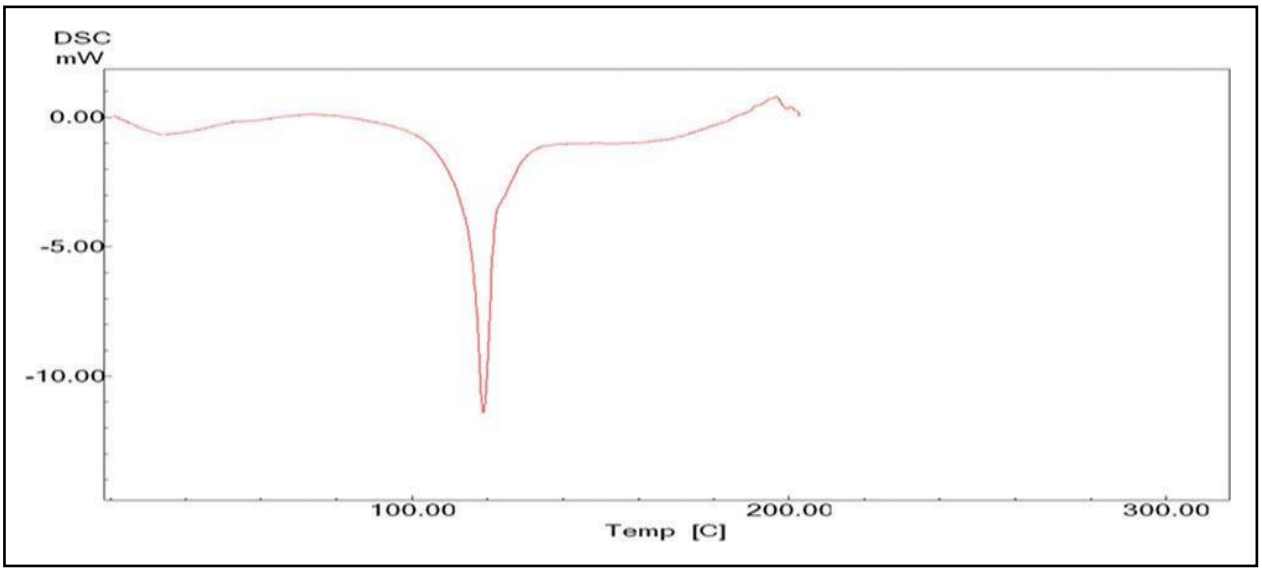

Figure No.3: DSC Thermogram of physical mixture of drug and excipients
Evaluation of Pre-compressed parameters

All formulations were studied for various rheological characteristics bulk density, true density, compressibility index, Hausner's ratio and angle of repose. The results of the studies indicated that the powder is blend is easily compressible.

Table No.5: Evaluation of precompression characteristics of floating tablets

Formulation code	Bulk density (gm/ml ±S.D.)	Tapped density (gm/ml ±S.D.)	Angle of Repose (θ±S.D.))	Compressibility Index (%±S.D.))	Hausner's ratio
F1	0.352±0.0040	0.416±0.0043	29.27±0.63	15.27±0.11	1.18±0.015
F2	0.365±0.0035	0.425±0.0042	28.62±0.57	14.01±0.10	1.16±0.005
F3	0.374±0.0032	0.412±0.0098	28.63±0.50	9.23±0.69	1.10±0.008
F4	0.387±0.0037	0.435±0.0026	26.57±0.56	11.02±0.55	1.12±0.006
F5	0.383±0.0032	0.442±0.0026	27.82±0.61	13.38±0.72	1.15±0.009
F6	0.361±0.0015	0.410±0.0025	27.64±0.54	12.03±0.24	1.13±0.003
F7	0.380±0.0036	0.459±0.0064	27.29±0.37	17.13±0.46	1.20±0.006
F8	0.376±0.0035	0.442±0.0060	29.35±0.52	14.80±0.16	1.17±0.024
F9	0.379±0.0021	0.441±0.0049	29.53±0.42	13.91±0.13	1.16±0.018

Evaluation of precompression characteristics of floating tablets

The results of Hardness, Disintegration time, Drug content, Friability, Swelling index, Floating time all are summarized in the table given below:

Table No.6: Evaluation of precompression characteristics of floating tablets

Formulation code	Hardness (kg/cm^2) ± S.D.	Drug content (%) ± S.D.	Friability (%± S.D.)	Swelling index %	Thickness (mm)	Weight Variation mg
F1	3.42±0.058	88.35±0.040	0.166±0.033	34.07±0.67	3.76 ±0.26	298.13± 1.7
F2	3.51±0.074	89.00±0.027	0.219±0.047	40.73±0.74	3.87±0.15	299.81±0.01
F3	3.54±0.077	98.42±0.018	0.296±0.081	51.55±0.89	3.98±0.21	300.07±0.01
F4	3.32±0.055	91.69±0.029	0.341±0.181	42.22±0.89	3.91±0.41	298.3±0.023
F5	3.53±0.050	90.61±0.010	0.368±0.041	43.70±0.67	3.99±0.68	299.19±1.69
F6	3.58±0.079	95.53±0.017	0.372±0.028	44.88±0.44	3.90±0.12	298.12±0.16
F7	3.56±0.085	93.22±0.023	0.511±0.026	46.07±0.67	3.90±0.49	300.8±0.018
F8	3.57±0.05	92.65±0.030	0.534±0.33	47.25±2.10	3.91±0.16	299 ± 0.018
F9	3.77±0.011	95.14±0.025	0.610±0.23	47.40±0.68	3.93±0.08	300.35±0.15

In-Vitro Floating duration

Table No.7: Floating duration time and Floating lag time

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Floating time (hr.)	12	12	12	12	12	12	12	12	12
Floating lag time (sec)	45	55	33	100	98	114	100	95	97

The developed optimized formulation met all the pre-requisite to become a floating mucoadhesive tablet, swelled and floated instantaneously at the acidic condition of the stomach within 33 seconds.

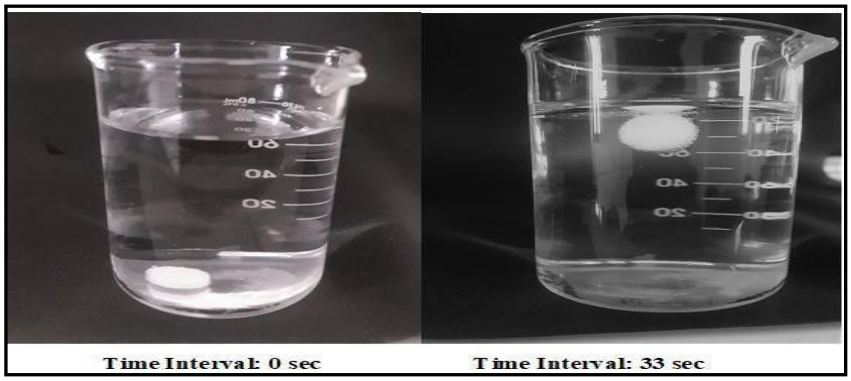

Figure No.4: Floating lag time of optimized tablet formulation

Mucoadhesive Strength

Table No.8: Mucoadhesive Strength and Force of tablet

Formulation code	Mucoadhesive Strength (gm)	Mucoadhesive force (dyne)
F1	10.15 ± 0.56	0.5657
F2	11.03 ± 0.02	0.6147
F3	20.05 ± 0.01	1. 1175
F4	12.50 ± 0.02	0.6967
F5	16.21 ± 0.07	0. 9035
F6	13.18 ± 0.01	0. 7346
F7	18.17 ± 0.05	1. 0127
F8	17.17 ± 0.01	0.9569
F9	16.85 ± 0.01	0.9391

In Vitro drug release studies

The dissolution studies were carried out for all nine formulations (i.e., F1 to F9)

Table No.9: Percent Cumulative drug release of different Formulations (F1-F9)

Time (hr.)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	6.36 ±1.98	6.64 ±5.95	7.57 ±2.15	9.12 ±2.42	7.78 ±2.06	8.84 ±1.90	8.06 ±2.02	4.90 ±2.10	5.70 ±1.53
2	17.29 ±2.38	21.68 ±1.93	19.27 ±2.25	15.79 ±1.74	16.43 ±2.83	16.67 ±2.32	15.58 ±4.11	10.15 ±2.04	10.57 ±2.08
3	25.42 ±2.06	27.57 ±1.83	25.14 ±2.05	25.34 ±2.31	19.06 ±1.99	23.18 ±2.501	17.46 ±2.05	19.25 ±2.07	12.86 ±2.84
4	35.88 ±2.52	31.58 ±2.54	32.99 ±2.21	31.70 ±1.58	21.81 ±2.61	29.81 ±2.49	18.03 ±2.56	24.45 ±2.83	23.46 ±2.01
5	39.84 ±1.87	39.84 ±2.22	40.14 ±1.94	42.31 ±2.41	25.34 ±1.91	36.72 ±1.92	22.24 ±1.93	30.41 ±1.95	30.41 ±1.90
6	46.40 ±2.02	48.55 ±2.39	48.55 ±2.11	47.24 ±2.15	28.29 ±2.15	38.05 ±1.9	33.08 ±2.07	37.90 ±1.90	39.25 ±1.89
7	55.16 ±2.10	57.31 ±2.62	57.39 ±1.99	53.01 ±1.94	31.84 ±1.95	45.69 ±2.34	36.87 ±2.00	45.43 ±1.43	47.58 ±4.95
8	61.10 ±2.04	65.66 ±2.19	68.37 ±2.04	62.00 ±2.17	43.97 ±2.06	49.72 ±2.06	45.22 ±2.15	53.01 ±2.11	50.86 ±2.47
9	64.98 ±2.07	73.26 ±2.04	75.24 ±2.54	69.41 ±2.00	54.99 ±2.00	65.32 ±1.98	53.29 ±2.01	62.43 ±1.92	55.16 ±1.81
10	71.44 ±2.64	80.21 ±2.03	82.71 ±2.36	76.94 ±1.86	64.87 ±1.63	76.74 ±2.45	65.36 ±2.56	70.44 ±1.36	59.30 ±1.56
11	76.73 ±2.42	79.53 ±2.69	86.63 ±1.96	79.53 ±2.62	82.36 ±1.25	83.18 ±2.48	70.61 ±2.00	75.90 ±2.33	65.37 ±2.06
12	84.53 ±2.48	84.48 ±2.09	98.35 ±2.08	82.72 ±2.08	89.63 ±1.98	90.82 ±2.63	86.43 ±2.53	79.87 ±1.88	72.97 ±1.32

The drug release shows that as the concentration of polymer goes on increasing the drug release also goes on decreasing and as well as time for drug release will be more sustained or release time will also go on increasing, but we want more and optimize release at 12 h., it was shown by F3 batch 98.35 ±2.08. Hence, F3 batch was taken as optimize formulation due to highest drug release up to 12 hr.

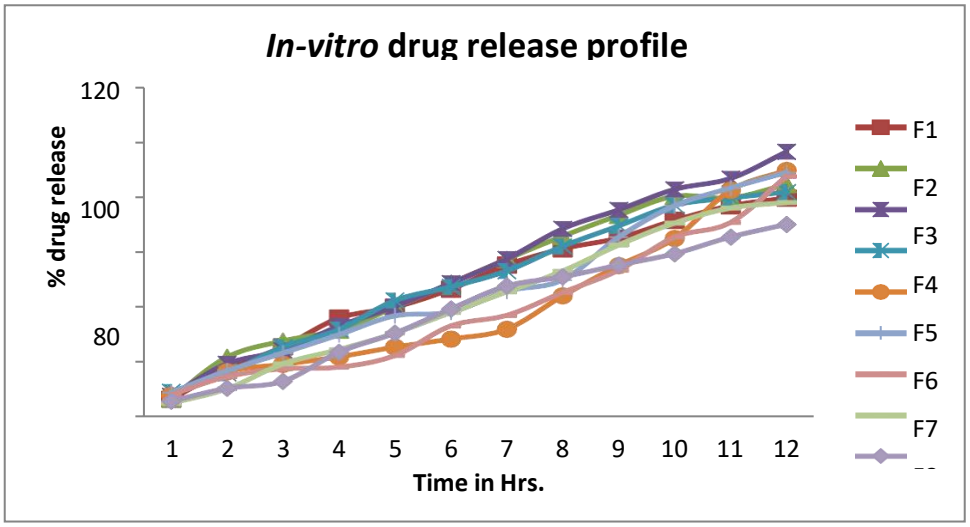

Figure No.5: Dissolution Profile of Formulation

Batches (F1-F9)8.5.1: F3 and Marketed Formulation

Table No.10: Percent cumulative drug release of F3 and Marketed Formulation

Time (hrs.)	% drug release	
	F3 Batch	Marketed formulation
1	7.57	5.56
2	19.27	13.63
3	25.14	21.69
4	32.99	28.96
5	40.14	37.42
6	48.55	50.56
7	57.39	46.31
8	68.37	55.23
9	75.24	66.41
10	82.71	77.13
11	86.63	82.29
12	96.35	87.66

Stability Studies

The selected formulation F3 was wrapped in aluminum foil and stored at 40± 2ºC and % RH 75% ± 5% temperature for 3 months. After 3 months the formulation F3were evaluated for the hardness, drug content and *in-vitro* % drug release. It was observed that there was no significant variation in the physical appearance, average weight, hardness and loss of drying after placing the tablets at various temperature and humidity conditions for a period of 3 months. Also, the cumulative % drug release data showed that each of the formulation released a drug amount, within the limits laid down as per the ICH guidelines for stability studies.

Table No.11: Stability study for optimized formulation F3 at 40±2ºC+75% RH

Frequency of testing	Drug content (% ± S.D.)	Mucoadhesive strength (gm± S.D.)	% Drug release at 12 h (% ± S.D.)
	Formulation F3		
0	98.68±0.26	20.61±1.02	99.10±1.75
8 days	98.42±0.10	20.12±1.23	98.20±0.99
15 days	99.52±0.25	20.10±1.12	99.74±1.74
1 month	98.25±0.10	20.00±0.98	98.45±1.35
2 months	98.16±0.56	21.10±1.04	99.12±2.15
3 months	98.02±0.45	21.31±1.12	97.46±1.14

Conclusion

Floating Mucoadhesive dosage forms have long been employed to improve the bioavailability of drugs undergoing significant hepatic first-pass metabolism. Ritonavir has been selected as model drug because it exhibits pharmacokinetic and pharmacochemical properties justified for floating mucoadhesive drug delivery. Experiments were conducted to investigate the influence of various polymers like HPMC K4M and Carbopol 934 on mucoadhesive strength and release kinetics of floating mucoadhesive tablets of Ritonavir. *In-Vitro* dissolution studies were conducted in apparatus II (using paddle) at 50 rpm for 12 h. The data was statistically analyzed and mechanism of drug release kinetics studied.

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