Chapter V
Size Exclusion Technology
(Non-Swelling)
Approach I

V.1 INTRODUCTION

The stomach anatomy and physiology constrain the parameters to be considered in development of gastro retentive dosage forms. Probably, the two most important features are their size and density.

- Size is especially important in designing in digestible solid dosage forms (single unit systems). The human pyloric diameter is 12 ± 7mm (Timmermans et al, 1993). It is open while the stomach is in a fasting state. The first mouthful thus passes directly into the duodenum, triggering closure of the pyloric sphincter. The pylorus then sorts the gastric contents, large particles being carried away by retrograde flow to the center of the stomach. Solids are evacuated by the pylorus slowly and regularly. Finally, indigestible materials, including solid pharmaceutical dosage forms, are evacuated by an Interdigestive Migration Myoelectric Complex (IMMC) peristaltic wave (Bernier et al, 1988). Particles with diameter < 7 mm are efficiently evacuated, and it is generally accepted that a diameter > 15 mm is necessary for useful prolongation of retention especially during the fasting state.

- Density determines the location of the system in the stomach. Systems with density lower than gastric contents can float to the surface, while high-density systems sink to bottom of the stomach. Both positions may isolate the dosage system from the pylorus (Dubernet et al, 2004).

V.2 MATERIALS

Table V. 1 List of Materials along with specifications and Manufacturer details

Ingredients	Specification	Manufacturer
Active		
Alfuzosin HCL	Ph.Eur	Torrent Pharmaceutical Ltd.
Excipients		
Colloidal Silicon Dioxide	NF	Degussa
Lactose Anhydrous (DCL 21)	NF	DMV international
Microcrystalline Cellulose (Avicel PH 102)	NF	FMC Biopolymer
Maize Starch	NF	Roquette
Povidone K-30	USP	ISP Technology
Talc	USP	Luzinac
Magnesium Stearate	NF	Ferro
Hydroxyproprl Cellulose 6 cps	USP	Dow chemicals
Ethyl cellulose (20 cps)	NF	Hercules
Ammonio methacrylate co-polymer Type A (Eudragit RS PO)	NF	Rohm -Gmbh
Ammonio methacrylate co-polymer Type B (Eudragit RL PO)	NF	Rohm -Gmbh
Triethylcitrate	NF	Cognis
Solvents		
Isopropyl Alcohol	USP	Finar
Acetone	NF	Finar
Methanol	NF	Finar

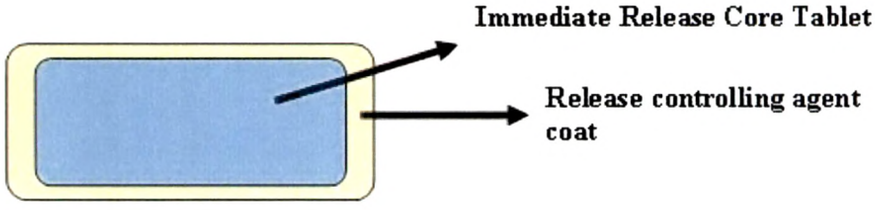
V.3 METHOD

Two formulation strategies were tried:

Approach IA

A pharmaceutical composition comprising of:

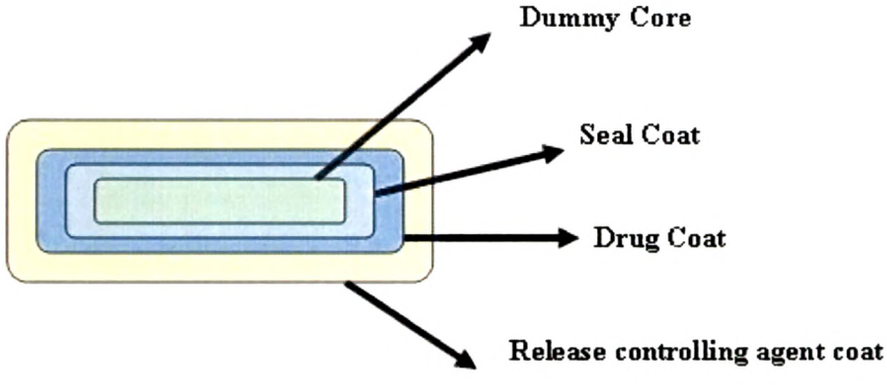
a) An immediate release core containing Alfuzosin HCL with water soluble excipients;
b) Core coated with a release controlling polymer.



Approach IB

A pharmaceutical composition comprising of:

a) A dummy core tablet;
b) Seal coating of the Core tablet;
c) Drug coating over seal coated tablet;
d) Coating of release controlling agent over drug coated tablet.



V.3.1 Approach IA

V.3.1.1 Preparation of tablet

V.3.1.1.1 Core tablet

Step 1: Mix Alfuzosin Hydrochloride and Colloidal silicon dioxide geometrically with maize starch and sift through 60# sieve.
Step 2: Sift Lactose anhydrous (DCL 21), Microcrystalline Cellulose (Avicel PH 102) and Povidone K-30 through 40# sieve.
Step 3: Mix materials of step 1 and step 2 in Conta blender for 10 min.
Step 4: Sift Talc and Magnesium Stearate through 60# sieve.
Step 5: Lubricate step 4 material with step 3 material in conta blender for 3 min.
Step 6: Compress lubricated blend of step 5 in compression machine fitted with 19.5 * 9.5 mm capsule shaped punches and appropriate dies.

V.3.1.1.2 Coated tablet

Step 7: Load the compressed tablets of step 6 in coating machine and pre heat the tablets.
Step 8: Prepare the coating suspension by dissolving Triethyl citrate in a mixture of Isopropyl alcohol and Acetone. Further disperse and dissolve Ammonio methacrylate copolymer type A and Type B (Eudragit RSPO and RLPO) in the solvent mixture and then disperse talc and Colloidal silicon dioxide (Syloid 244) in it by stirring.
Step 9: Spray the coating suspension prepared in step 8 on pre-warmed tablets of step 7 by maintaining appropriate temperature and pan rpm.
Step 10: Dry the coated tablet for 10 min and unload from pan.

V.3.1.2 Evaluation of formulations

V.3.1.2.1 Effect of different ratios of polymers

The polymers were tried in different ratios and their dissolution profile was evaluated in 0.01N HCl/paddle/50 rpm.

Table V. 2 Batches with different ratios of RSPO: RLPO

	B.No 005	B.No 008	B.No 12
RSPO:RLPO ratios	60:40	70:30	80:20

Weight gain of the polymer was kept constant at ~3.3% in all the three batches

V.3.1.2.2 Effect of Magnesium Stearate vs Colloidal silicon dioxide (Syloid 244) as antisticking agent in coating composition

Formulations were developed by adding magnesium stearate or colloidal silicon dioxide (syloid 244) in coating composition and their dissolution pattern in different media was studied.

V.3.1.2.3 Compatibility of magnesium stearate and colloidal silicon dioxide with Eudragit RSPO and RLPO with DSC

In order to investigate the cause of slower release profile with magnesium stearate, DSC studies were done. DSC pattern of the following samples were taken:

- Colloidal silicon dioxide (Syloid 244)
- Magnesium stearate
- Eudragit RSPO
- Eudragit RLPO
- Dry blend of Eudragit RSPO and Magnesium stearate
- Dry blend of Eudragit RLPO and Magnesium stearate
- Dry blend of Eudragit RSPO and Colloidal silicon dioxide
- Dry blend of Eudragit RLPO and Colloidal silicon dioxide
- Films containing Eudragit RSPO, RLPO , Magnesiun stearate, Triethyl citrate and talc (Sample A; composition mentioned below)
- Films containing Eudragit RSPO, RLPO , Colloidal silicon dioxide, Triethyl citrate and talc.(Sample B; composition mentioned below)

Table V. 3 Composition of Sample A and Sample B

S.No.	Ingredients	Sample A	Sample B
1	Ammonio methacrylate co-polymer Type A (Eudragit RS PO)	16.8	15.75
2	Ammonio methacrylate co-polymer Type B (Eudragit RL PO)	4.2	3.95
3	Triethylcitrate	4.2	3.8
4	Talc	4.9	6
5	Magnesium Stearate	1.9	NA
6	Colloidal silicon dioxide(Syloid 244)	NA	0.5
7	Isopropyl Alcohol	370	342
8	Acetone	380	228

V.3.1.2.4 Evaluation of Core Tablet and Polymer coated Tablets

Core tablets were evaluated for Length, Breadth, Thickness, Hardness, Average weight, Friability, Content uniformity and Dissolution profile.

Polymer Coated tablets were evaluated for Length, Breadth, Thickness, Average weight, Content uniformity, Related impurities, Assay, Water by KF and Dissolution profile.

V.3.2 Approach IB

V.3.2.1 Preparation of tablet

V.3.2.1.1 Core Dummy tablet

Step 1: Sift Lactose anhydrous (DCL 21), Microcrystalline Cellulose (Avicel PH 101), Maize Starch and Povidone K-30 through 40# sieve and mix in conta blender for 10 minutes.
Step 2: Sift Talc, Colloidal silicon dioxide and Magnesium Stearate through 60# sieve and lubricate with blend of step 1 in conta blender for 3 min.
Step 3: Compress lubricated blend of step 2 in compression machine fitted with 19.5 * 9.5 mm capsule shaped punches and appropriate dies.
Step 4: Load the compressed tablets of step 3 in coating machine and pre heat the tablets.

V.3.2.1.2 Seal Coat

Step 5: Seal coating solution was prepared by dissolving Triethyl citrate in mixture of Methylene Chloride and Methanol. Ethyl Cellulose (20 cps) was then dispersed and dissolved in solution prepared above.
Step 6: Spray the coating solution prepared in step 5 on pre-warmed tablets of step 4 by maintaining appropriate temperature and pan rpm.

V.3.2.1.3 Drug Coat

Step 7: Prepare the drug coating suspension by dissolving Triethyl citrate in a mixture of Methanol and Acetone. Further disperse and dissolve Alfuzosin Hydrochloride and Hydroxypropyl methyl cellulose (6 cps) in solution prepared above. Finally talc was dispersed in above solution.
Step 8: Spray the drug coating suspension prepared in step 7 on seal coated tablets of step 6 by maintaining appropriate temperature and pan rpm.

V.3.2.1.4 Polymer Coat

Step 9: Prepare the coating solution by dissolving Triethyl citrate in a mixture of Isopropyl alcohol and Acetone. Further disperse and dissolve Eudragit RSPO and RLPO in the solvent mixture and then disperse Talc in it by stirring.
Step 10: Spray the coating suspension prepared in step 9 on drug coated tablets of step 8 by maintaining appropriate temperature and pan rpm.
Step 11: Dry the coated tablet for 10 min and unload from pan.

V.3.2.2 Evaluation of formulations

V.3.2.2.1 Effect of different percentage of polymer coating

To study the effect of different percentage of polymer coating on dissolution profile, trials were taken and dissolution profile was evaluated in 0.01 N HCl /paddle/50 rpm/500 ml.

V.3.2.2.2 Evaluation of Finished formulation

Core tablets were evaluated for Length, Breadth, Thickness, Hardness, Average weight and Friability. Drug coated tablets were evaluated for Content uniformity and Dissolution in 0.01 N HCL. Polymer coated tablets were evaluated for Length, Breadth, Thickness, Average weight, Content uniformity, Related impurities, Assay , Water by KF and Dissolution profile in different media.

V.4 Gastric Residence Time

In order to study the gastric retention time in healthy volunteers, dummy formulation of B.No 23 of approach IA was prepared. The weight of the drug was compensated with Lactose which has solubility similar to that of Alfuzosin Hydrochloride.
Permission for the present study was obtained from the Institutional Ethics Committee.

V.4.1 Preparation of Barium Sulphate tablets

As Barium sulphate alone is not directly compressible, Barium sulphate was granulated with hydrogenated castor oil, which was dissolved in Methylene chloride and were dried. The dried granules were sized and then compressed in 16 station compression machine fitted with 1.3 mm round punches with suitable dies into tablets of total weight, 80 mg. These tablets were cut into two pieces each of 40 mg.

Two tablets each of 40 mg of Barium sulphate were placed inside the dummy core tablet formulation of B.No 23 and further coated with polymer coating as per the procedure mentioned above

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V.4.2 Protocol of the Gastric Residence Time study

Study Design:
The study was designed as Open label, single period, single treatment study in 4 healthy volunteers (2 for fasted study and 2 for fed study).

Selection of Study Population:
Healthy males 18 to 45 years of age were selected based on the inclusion and exclusion criteria given below.

Inclusion Criteria:
Volunteers must meet all of the following criteria in order to be included in the study:

- Sex: male
- Age: 18 - 45 years
- Healthy and willing to participate in the study
- Volunteer willing to adhere to the protocol requirements and to provide written informed consent.

Exclusion Criteria:

The volunteers will be excluded from the study based on the following criteria:

- Addiction to alcohol or history of any drug abuse
- Recent History of kidney or liver dysfunction
- Patients suffering from any chronic illness such as arthritis, asthma etc.

- HIV, HCV, HBsAg positive volunteers
- Volunteers suffering from any psychiatric (acute or chronic) illness requiring medications
- Administration of any investigational products in the period 0 to 3 months before entry to the study
- Existence of any surgical or medical condition, which, in the judgment of the *Chief Investigator and/or clinical investigator*, might interfere with the study or likely to compromise the safety of Volunteers
- Inability to communicate or co-operate due to language problem, poor mental development or impaired cerebral function

If some minor deviations as regards to laboratory results are detected, clinical investigator will asses their relevance to the purpose of the study and to the subject inclusion. As no blood sampling is planned for this study (only radiological imaging is planned), subjects with haematology and/or biochemistry and/or BMI outside the prescribed range may be included in the study.

In case of marked deviations of health status leading to Volunteer's non-inclusion in the study, wherever possible the Volunteer will be explained about the same with recommendation to visit his physician.

Food and fluid intake, drug administration

Eligible volunteers will undergo a brief clinical examination and vitals will be documented at BE Centre in TRC. Volunteers will be then taken to the study site where the volunteers enrolled for fed-state study will be served standardized high fat breakfast, half-an-hour before dosing. Dosing will be done with 200ml of water. The time gap between drug administrations to volunteers will be kept 1 minute. The test formulations must be swallowed whole without chewing. The volunteers for fasted state study would be administered the investigational product on empty stomach. Volunteers enrolled for fed study will be served lunch after 4.00 hours post dose.

X-Ray Exposures

The volunteers will undergo a series of X-Ray exposures. The time points for the same will be as follows;

Fasted state: 0, 0.33, 0.67, 1.0, 1.5, 2.5hrs (6 exposures)

Fed Study: 0, 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5hrs (8 exposures)

Additional exposures may be given on the discretion of the chief/clinical investigator.

Restrictions

The volunteer enrolled in a study would be confined in the study area (BE centre) overnight. The use of xanthine containing beverages (tea, coffee, cola drinks,) and foods

(chocolates) will be prohibited for 12 hours before check in and throughout their stay in study area at X-ray house.

The volunteers will be instructed to abstain from alcohol for 48 hours prior to and throughout the conduct of the study. He will be restricted from taking any medication (including over the counter products), throughout the study, unless authorized by the chief investigator.

The volunteers will be refrained from drinking water 1 hour before and 2 hours after dosing. The volunteer will be allowed to take 200ml of water with dosing. He will be asked to remain in sitting position for at least 7 hours post dose.

The volunteers will be restricted from doing any sort of stressful physical activity.

Radiation Safety data (www.radiologyinfo.org)

a) UMB radiation safety policy guidelines

All uses of radiation or radioactive materials in or on human research subjects must be specifically approved by the UMB Radiation Safety Committee. Proposed research studies will be reviewed with respect to the following radiation dose limit guidelines:

AGE OF SUBJECT	RADIATION DOSE LIMIT
Under 18 years	300 millirem (mrem) to any tissue in a 13 week period and 500 mrem annually
18 years or more	3,000 mrem to any tissue in a 13 week period and 5,000 mrem annually

b) FDA Regulatory Limits

FDA (Food and Drug Administration) radiation dose limits are applicable only to the use of "radioactive research drugs," as defined by the FDA regulations, in research studies with human subjects. Research protocols and Applications for Authorization that meet the criteria for use of "radioactive research drugs" must be approved by the UMB Radioactive Drug Research Committee, in addition to approval by the UMB Radiation Safety Committee.

AGE OF SUBJECT	RADIATION DOSE LIMIT
Under 18 years	300 (mrem) to the whole body, active blood-forming organs, lens of the eye and gonads from a single administration, and 500 mrem annually
18 years or more	3,000 mrem to the whole body, active blood-forming organs, lens of the eye and gonads from a single administration, and 5,000 mrem annually. 5,000 mrem to other organs from a single administration, and 15,000 mrem annually.

c) Other Radiation Dose Limits

NOTE: The following radiation dose limits are not applicable to human subjects in research studies. They are presented here for general information and guidance.

Occupationally Exposed Person (Radiation Worker)

AGE OF SUBJECT	RADIATION DOSE LIMIT
Under 18 years	125 mrem to any tissue in a 13 week period and 500 mrem annually.
18 years or more	3,000 mrem to any tissue in a 13 week period and 5,000 mrem annually.

Non-Occupationally Exposed Person ("Public")

AGE OF SUBJECT	RADIATION DOSE LIMIT
Any age	100 mrem per calendar year

Table V. 4 Comparisons of effective radiation dose with background radiation exposure for several radiological procedures described within the RadiologyInfo.org Web site

For this procedure:	Your effective radiation dose is:	Comparable to natural background radiation for:
Abdominal region		
Computed Tomography (CT)-Abdomen	10 mSv	3 years
Computed Tomography (CT)-Body	10 mSv	3 years
Computed Tomography (CT)-Colonography	5 mSv	20 months
Intravenous Pyelogram (IVP)	1.6 mSv	6 months
Radiography-Lower GI Tract	4 mSv	16 months
Radiography-Upper GI Tract	2 mSv	8 months
Central nervous system		
Computed Tomography (CT)-Head	2 mSv	8 months
Computed Tomography (CT)-Spine	10 mSv	3 years
Myelography	4 mSv	16 months
Chest:		
Computed Tomography (CT)-Chest	8 mSv	3 years
Radiography-Chest	0.1 mSv	10 days
Children's imaging		
Voiding Cystourethrogram	*5-10 yr. old:* 1.6 mSv	6 months
	Infant: 0.8 mSv	3 months
Face and neck		
Computed Tomography (CT)-Sinuses	0.6 mSv	2 months
Heart		
Cardiac CT for Calcium Scoring	2 mSv	8 months
Men's imaging		
Bone Densitometry (DEXA)	0.01 mSv	1 day
Women's imaging		
Bone Densitometry (DEXA)	0.01 mSv	1 day
Galactography	0.7 mSv	3 months
Hysterosalpingography	1 mSv	4 months
Mammography	0.7 mSv	3 months

V.5 Bio Study

Permission for the present study was obtained from the Institutional Ethics Committee.

Design

An Open-Label, Randomised, Two-way Crossover, Single Dose Study to Evaluate the Bioavailability of the Extended Release Test Formulation of Alfuzosin 10 mg Tablets Compared To An Equivalent Dose Of a Marketed Extended Release Formulation in 8 each for Fasted and Fed state, Healthy Adult Subjects.

Dosage

The subjects were dosed in the morning in both periods. In each period the volunteers were administered a single oral dose of either test product (one extended release tablet of Alfuzosin 10mg) or Marketed formulation (one tablet Alfuzosin ER 10mg) as per the randomization schedule with 240ml of water.

Washout Period

The washout period was ten days.

Fasting and Feeding Schedule

The volunteers checked in evening before the dosing day. Dinner was served to the Volunteers in both the periods. The subjects were fasted for at least 10 hours before dosing in both the periods. For the fed state the subjects were given high-fat breakfast half an hour before drug administration. The subjects were deprived of water for at least 1hr before dosing and 2 hours post dosing, after that water was permitted. The first main meal (lunch) of the dosing day was served four hours after drug administration and the subsequent meals (snacks, dinner, and breakfast) were served at approximately 8, 12, and 24 hours post dose in both the periods. The meals planned were identical for both the periods.

Chronogram for Collection of Samples

The blood samples were collected in a series of 16 x 6ml, from a secured peripheral venous access. The sampling time point relates to the drug administration time at following times Predose, 1.0, 2.0, 4.0, 6.0, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0, 12.0, 16.0, 20.0, 24.0, 48.0 hrs post dose in each study period.

Procedures for Sample Handling

The blood samples were collected in heparnised 10ml polypropylene tubes and centrifuged at 2000 RPM for 10 minutes. Plasma was separated in 5ml polypropylene tubes, which was subsequently stored at ≤-70°C till analysis.

V.6 Bio-Analytical Method For Estimation Of Alfuzosin In Human Plasma

LC-MS technique was used.

Bio-Analytical technique
LC-MS/MS technique was followed.

The summary of the chromatographic conditions and the detection parameters were as follows:

Instrument: Mass spectrometer (TSQ Quantum Finnigan)

Chromatographic Conditions

Chromatographic mode	:	Reversed phase	
Isocratic/gradient mode	:	Isocratic	
Column	:	*Brand*	Thermo Electron Corporation
	:	*Type*	Beta basic 8
	:	*Length x i.d (mm)*	100 x 4.6
	:	*Particle size (μ)*	5.0
Mobile phase	:	Acetonitrile: Methanol: Water (45: 45:10 v/v)	
		0.05 % Formic acid in water.	
Column temperature	:	45°C	
Flow rate	:	0.350 ml/min.	
Back pressure	:	80 bar (approximately)	
Retention time			:
Alfuzosin	:	2.45 min.	
Es-Citalopram(IS)	:	2.45 min.	
Run time	:	4.0 min.	

V.7 Development Of Bio Relevant Discriminatory Media

In order to find a correlation in vitro and in vivo, dissolution profile of B.No 23 along with Marketed Formulation was carried out in different media - pH 4.5 acetate buffer and pH 3.0 acid -base buffer.

V.8 RESULTS

V.8.1 Results of Approach IA

V.8.1.1 Effect of different Ratios of polymers

The polymers were tried in different ratios and their dissolution profile was evaluated in 0.01N HCl/paddle/50 rpm.

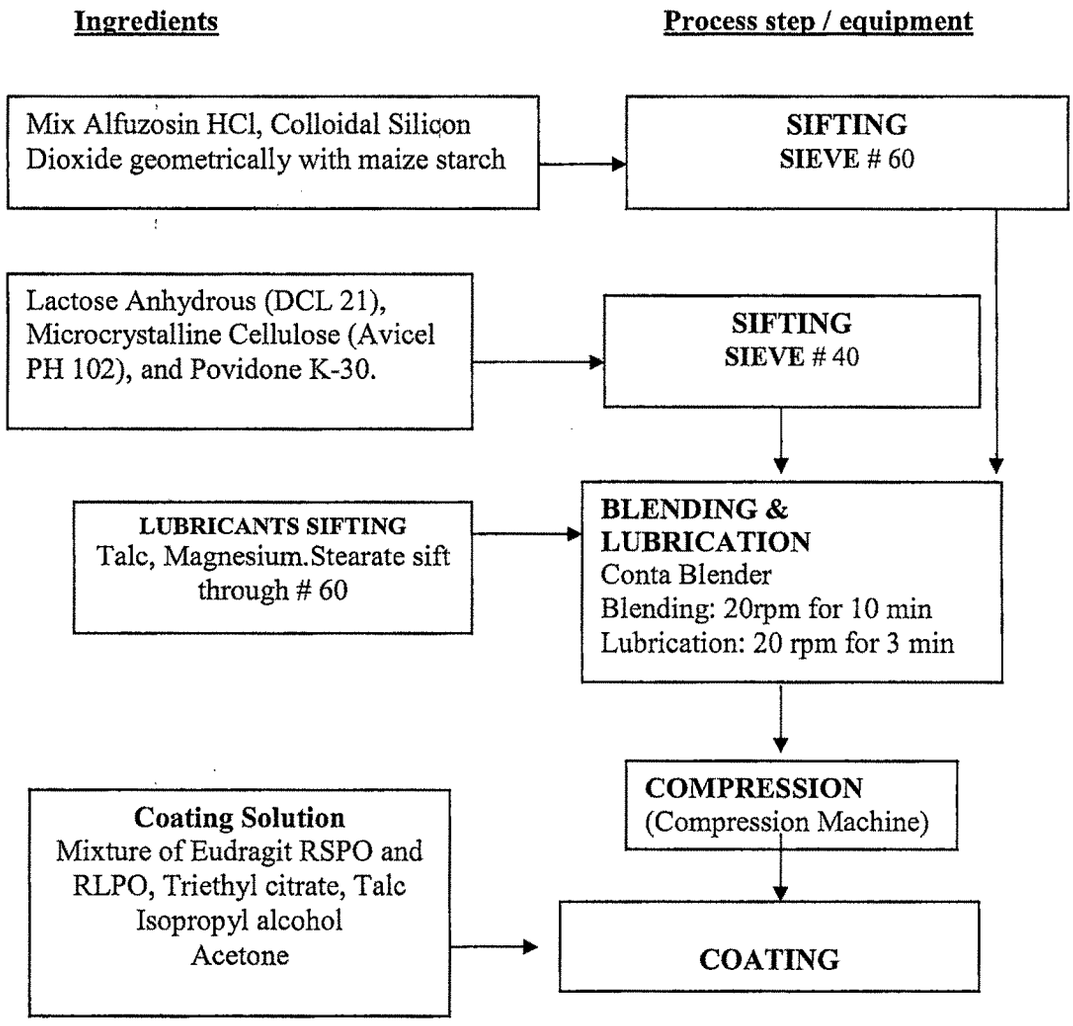
Table V. 5 Batches with different ratios of polymers

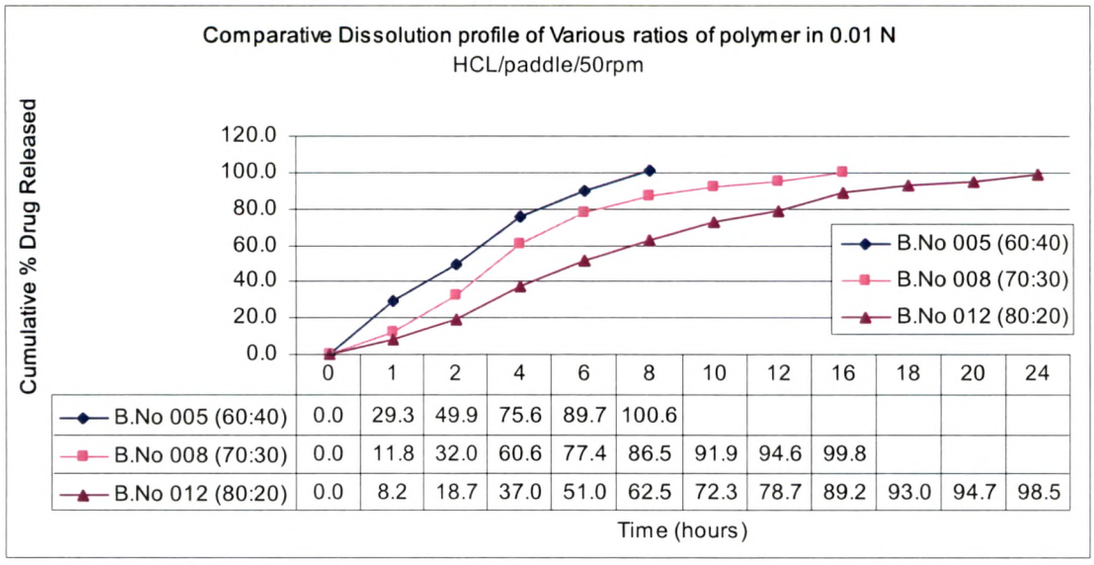
	B.No 05	B.No 08	B.No 12
RSPO:RLPO ratios	60:40	70:30	80:20

Table V. 6 Composition of Batches (B. No 05, B. No. 08, B. No. 12) with different ratio of polymers

Sr. No	Ingredients	Function	Qty / Tab. (In mg)		
			B. No 05	B. No 08	B. No 12
		CORE			
1	Alfuzosin HCL	Active	10.00	10.00	10.00
2	Colloidal Silicon Dioxide	Glidant	5.00	5.00	5.00
3	Lactose Anhydrous (DCL 21)	Diluent	453.00	453.00	453.00
4	Microcrystalline Cellulose (Avicel PH 102)	Diluent	340.00	340.00	340.00
5	Maize Starch	Diluent	130.00	130.00	130.00
6	Povidone K-30	Binder	32.00	32.00	32.00
7	Talc	Lubricant	20.00	20.00	20.00
8	Magnesium Stearate	Lubricant	10.00	10.00	10.00
	Core Tablet Weight		**1000.00**	**1000.00**	**1000.00**
		COATING			
9	Ammonio methacrylate co-polymer Type A (Eudragit RS PO)	Rate controlling polymer	21	24.5	28
10	Ammonio methacrylate co-polymer Type B (Eudragit RL PO)	Rate controlling polymer	14	10.5	7
11	Triethylcitrate	Plasticizer	7	7	7
12	Talc	Anti Tacking agent	8	8	8
13	Isopropyl Alcohol	Solvent	370	370	370
14	Acetone	Solvent	380	380	380
	Coated Tablet Weight		**1050.00**	**1050.00**	**1050.00**

Process Flow Diagram





	0	1	2	4	6	8	10	12	16	18	20	24
B.No 005 (60:40)	0.0	29.3	49.9	75.6	89.7	100.6						
B.No 008 (70:30)	0.0	11.8	32.0	60.6	77.4	86.5	91.9	94.6	99.8			
B.No 012 (80:20)	0.0	8.2	18.7	37.0	51.0	62.5	72.3	78.7	89.2	93.0	94.7	98.5

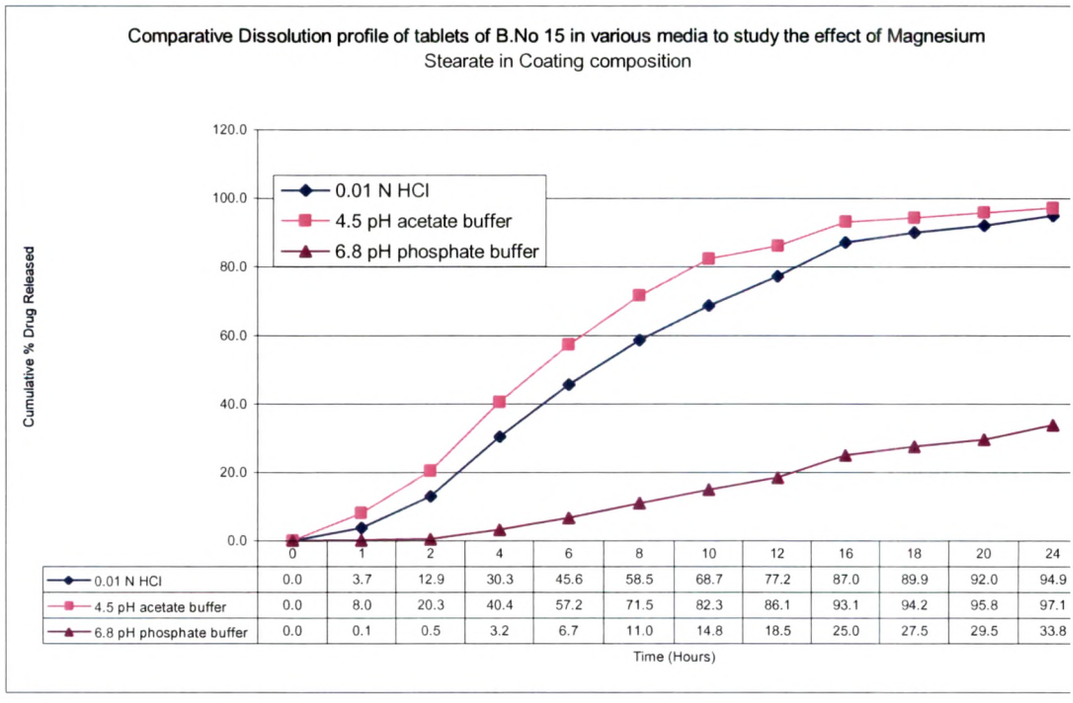
Figure V. 1 Dissolution profile of Batches with different ratio of polymers in 0.01 N HCL/paddle/ 50 rpm

As drug release profile was faster in 60: 40 and 70:30; so 80:20 was finalized for further development.

V.8.1.2 Effect of Magnesium Stearate used as antisticking agent in coating composition in various media in B.No.15

Table V. 7 Composition with and without magnesium stearate (B.No 15 vs B.No 17)

Sr. No	Ingredients	Function	Qty / Tab.(In mg)	
			B. No 15	B. No 17
	CORE			
1	Alfuzosin HCL	Active	10.00	10.00
2	Colloidal Silicon Dioxide	Glidant	5.00	5.00
3	Lactose Anhydrous (DCL 21)	Diluent	453.00	453.00
4	Microcrystalline Cellulose (Avicel PH 102)	Diluent	340.00	340.00
5	Maize Starch	Diluent	130.00	130.00
6	Povidone K-30	Binder	32.00	32.00
7	**Talc**	**Lubricant**	**20.00**	**20.00**
8	Magnesium Stearate	Lubricant	10.00	10.00
	Core Tablet Weight		**1000.00**	**1000.00**
	COATING			
9	Ammonio methacrylate co-polymer Type A (Eudragit RS PO)	Rate controlling polymer	16.80	16.80
10	Ammonio methacrylate co-polymer Type B (Eudragit RL PO)	Rate controlling polymer	4.20	4.20
11	Triethylcitrate	Plasticizor	4.20	4.20
12	Talc	Anti Tacking agent	4.90	4.90
13	Magnesium Stearate	Anti Tacking agent	1.90	NA
14	Isopropyl Alcohol	Solvent	370	370
15	Acetone	Solvent	380	380
	Coated Tablet Weight		**1032.00**	**1032.00**

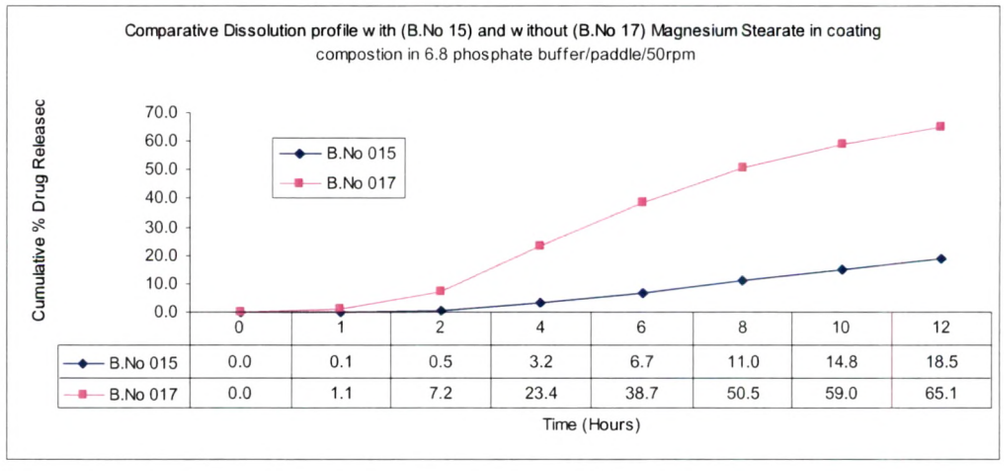


	0	1	2	4	6	8	10	12	16	18	20	24
0.01 N HCl	0.0	3.7	12.9	30.3	45.6	58.5	68.7	77.2	87.0	89.9	92.0	94.9
4.5 pH acetate buffer	0.0	8.0	20.3	40.4	57.2	71.5	82.3	86.1	93.1	94.2	95.8	97.1
6.8 pH phosphate buffer	0.0	0.1	0.5	3.2	6.7	11.0	14.8	18.5	25.0	27.5	29.5	33.8

Figure V. 2 Dissolution profile of B. No 15 (with magnesium stearate) in various media/paddle/50 rpm

Incomplete release was observed with Magnesium Stearate in polymer coating in 6.8 pH Phosphate buffer.

V.8.1.3 With and Without Magnesium Stearate (B.No 15 vs B.No 17)



	0	1	2	4	6	8	10	12
B.No 015	0.0	0.1	0.5	3.2	6.7	11.0	14.8	18.5
B.No 017	0.0	1.1	7.2	23.4	38.7	50.5	59.0	65.1

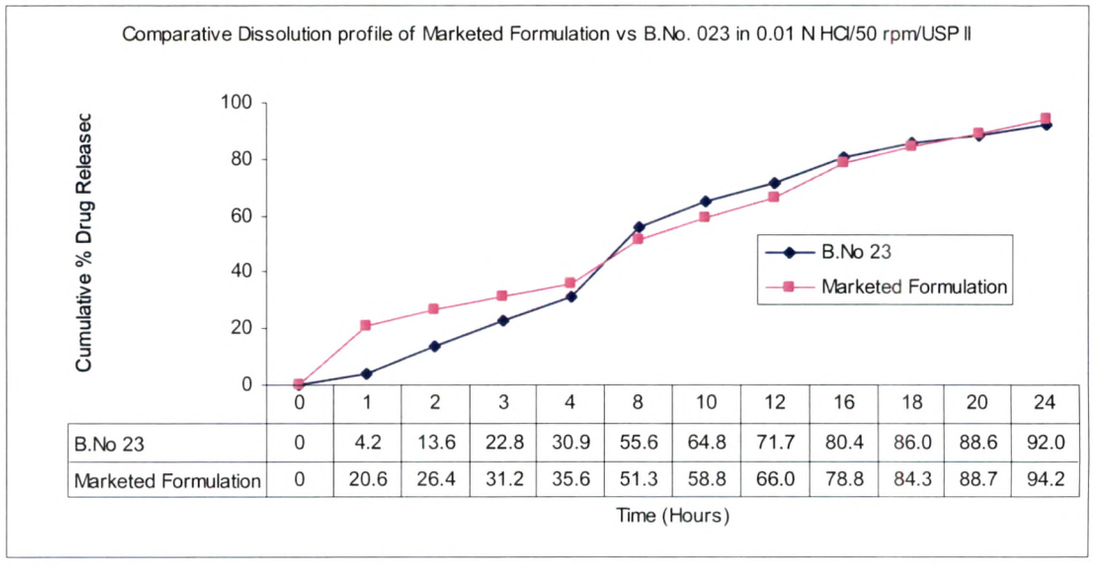
Figure V. 3 Dissolution profile with and without Magnesium stearte (B.No 15 vs B.No 17 in 6.8 pH phosphate Buffer/paddle/50 rpm.

As observed in the dissolution profile, B. No 17 (without magnesium stearate) showed a significantly faster dissolution profile than B. No 15 (without magnesium stearate). So, colloidal silicon dioxide was tried in B.No 23 as antisticking agent.

V.8.1.4 Effect of Colloidal silicon dioxide used as antisticking agent in coating composition in various media B.No. 23

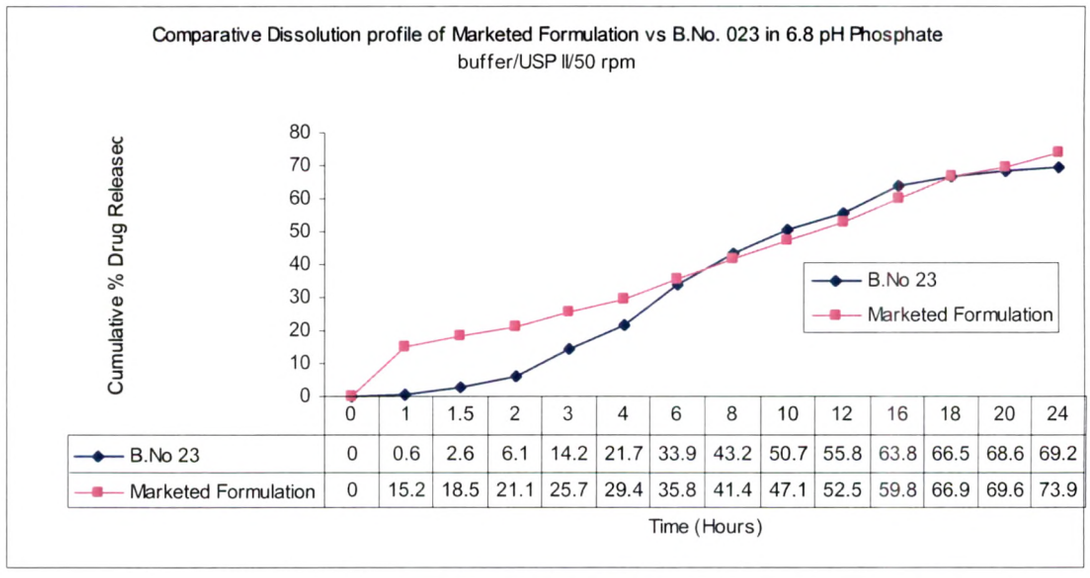
Table V. 8 Composition of Batch. No. 23 with colloidal silicon dioxide in coating composition

Sr. No	Ingredients	Function	Qty / Tab. (In mg)	% w/w
	CORE			
1	Alfuzosin HCL	Active	10.00	0.10
2	Colloidal Silicon Dioxide	Glidant	5.00	0.49
3	Lactose Anhydrous (DCL 21)	Diluent	453.00	43.98
4	Microcrystalline Cellulose (Avicel PH 102)	Diluent	340.00	33.01
5	Maize Starch	Diluent	130.00	12.62
6	Povidone K-30	Binder	32.00	3.11
7	Talc	Lubricant	20.00	1.94
8	Magnesium Stearate	Lubricant	10.00	0.10
	Core Tablet Weight		**1000.00**	
	COATING			
9	Ammonio methacrylate co-polymer Type A (Eudragit RL PO)	Rate controlling polymer	15.75	1.53
10	Ammonio methacrylate co-polymer Type B (Eudragit RS PO)	Rate controlling polymer	3.95	0.38
11	Triethylcitrate	Plasticizor	3.80	0.37
12	Talc	Anti Tacking agent	6.00	0.58
13	Colloidal Silicon Dioxide(Sylloid 244 FP)	Anti Tacking agent	0.50	0.0.5
14	Isopropyl Alcohol	Solvent	342.00	---
15	Acetone	Solvent	228.00	---
	Coated Tablet Weight		**1030.00**	



	0	1	2	3	4	8	10	12	16	18	20	24
B.No 23	0	4.2	13.6	22.8	30.9	55.6	64.8	71.7	80.4	86.0	88.6	92.0
Marketed Formulation	0	20.6	26.4	31.2	35.6	51.3	58.8	66.0	78.8	84.3	88.7	94.2

Figure V. 4 Comparative Dissolution profile of Marketed formulation vs B.No 23 in 0.01 N HCL/50 rpm/paddle



	0	1	1.5	2	3	4	6	8	10	12	16	18	20	24
B.No 23	0	0.6	2.6	6.1	14.2	21.7	33.9	43.2	50.7	55.8	63.8	66.5	68.6	69.2
Marketed Formulation	0	15.2	18.5	21.1	25.7	29.4	35.8	41.4	47.1	52.5	59.8	66.9	69.6	73.9

Figure V. 5 Comparative Dissolution profile of Marketed formulation vs B.No 23 in 6.8 pH phosphate Buffer/50 rpm/paddle

B.No 23 gave satisfactory dissolution profile in 0.01 N HCL and 6.8 pH phosphate buffer. So, it was taken for further study.

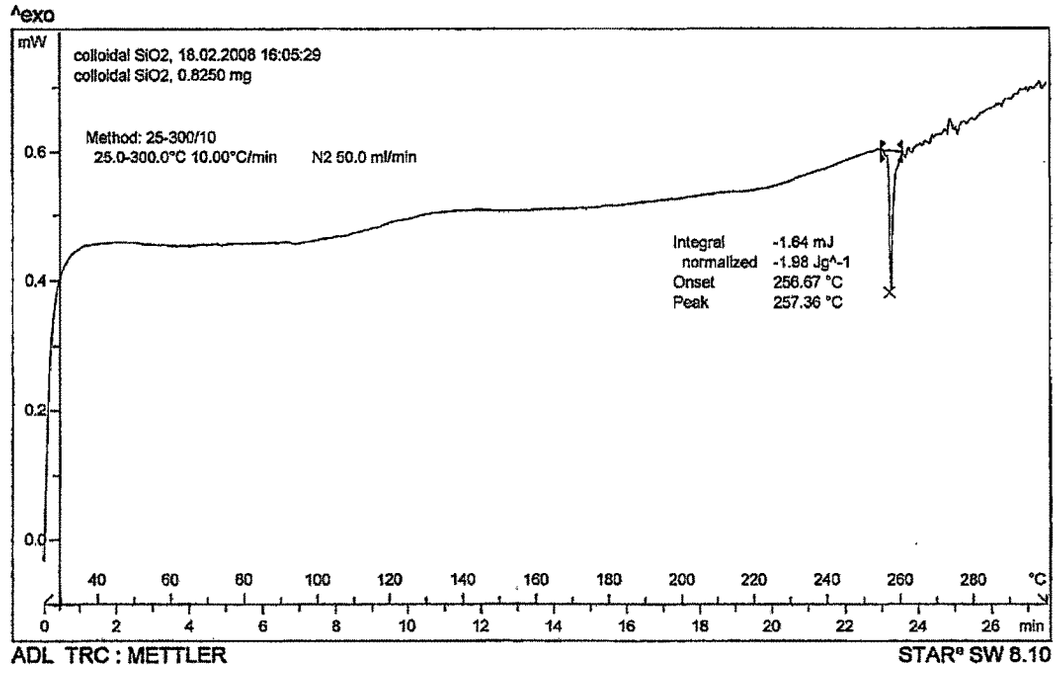
V.8.1.5 Summary of the DSC endothermic (-) peaks obtained with different samples

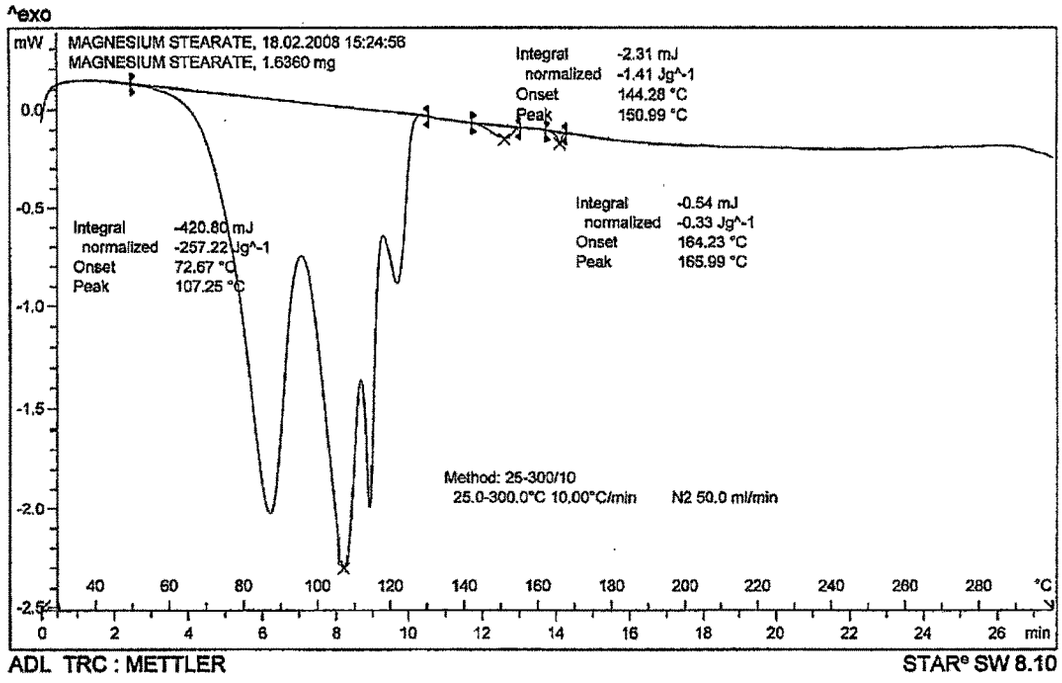
The following DSC pattern was obtained:

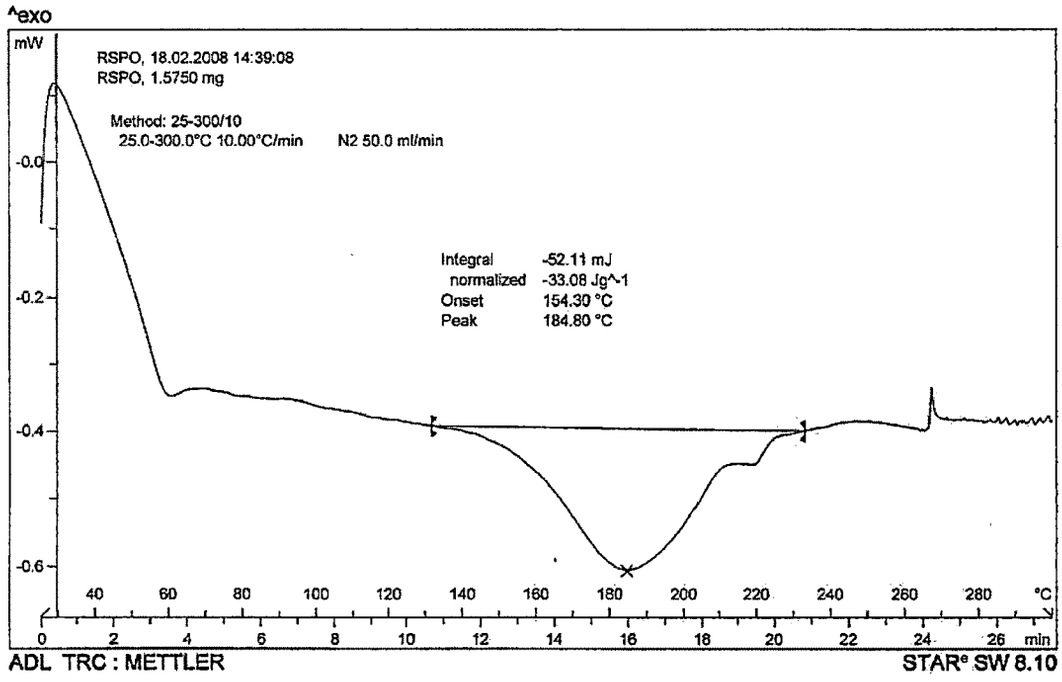
Table V. 9 Comparative endothermic peaks of the samples to study the compatibility of magnesium stearate and colloidal silicon dioxide with Eudragit RSPO and RLPO:

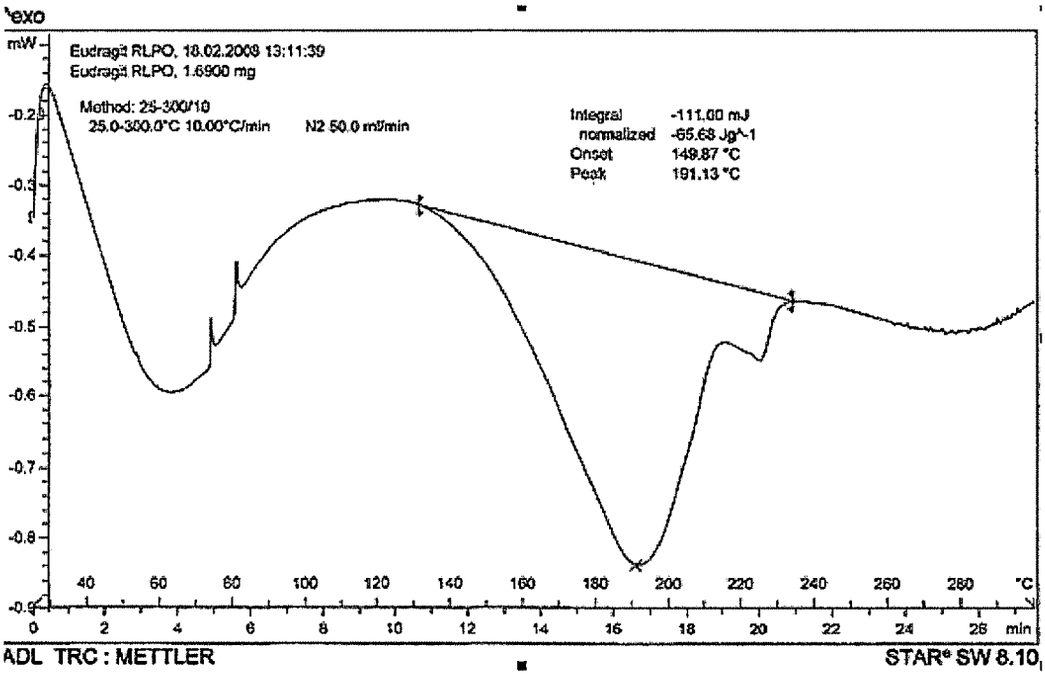
	Colloidal silicon dioxide(Syloid 244)	Magnesium stearate	Eudragit RSPO	Eudragit RLPO
Colloidal silicon dioxide (Syloid 244)	257.36	.....	.....	.....
Magnesium stearate	.....	107.25	.....	.....
Eudragit RSPO	.....	.....	184.80	.....
Eudragit RLPO	.....	.....	.....	191.13
Syloid + Eudragit RSPO	(A)	.....	180.80 [S(b)]	.....
Syloid + Eudragit RLPO	(A)	.....	.....	189.02 [S(b)]
Magnesium stearate+ Eudragit RLPO	.....	106.14 [R,S(b)]	.....	194.79 [S(f)]
Magnesium stearate+ Eudragit RSPO	.....	108.48 [R,S(f)]	184.99	.....
Sample A	.....	110.07[R,S(f)]	(A)	(A)
Sample B	(A)	.....	186.41[R,S(f)]	(A)

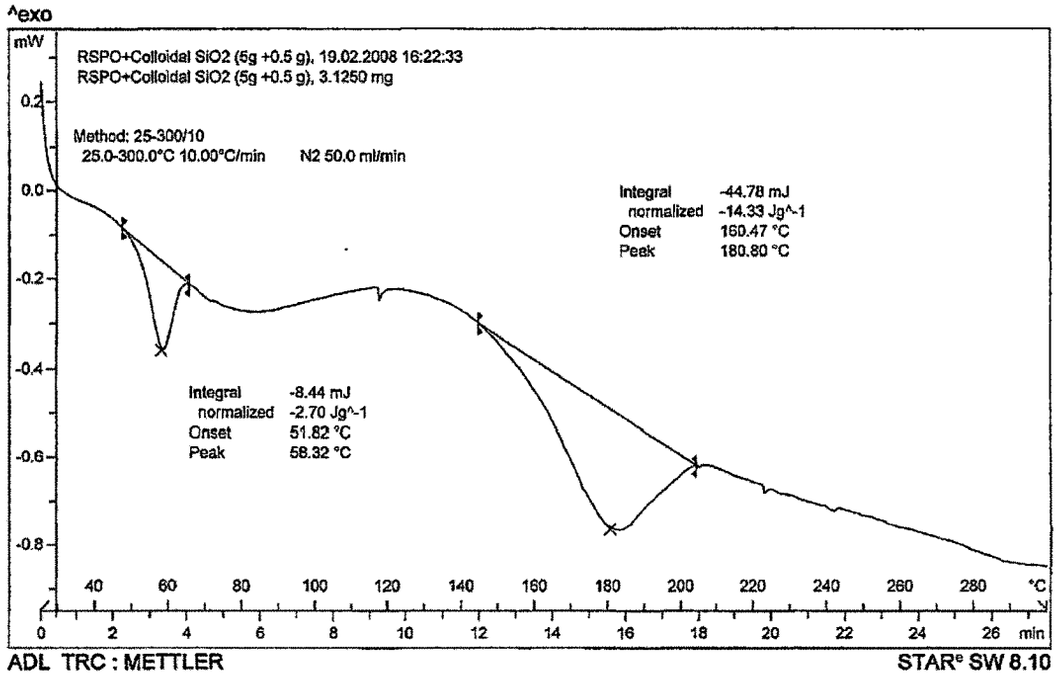
(A) Peak Absent
(P) Peak present
(R) Peak Retained
(S) Peak Shifted, Forward (f); Backward (b)

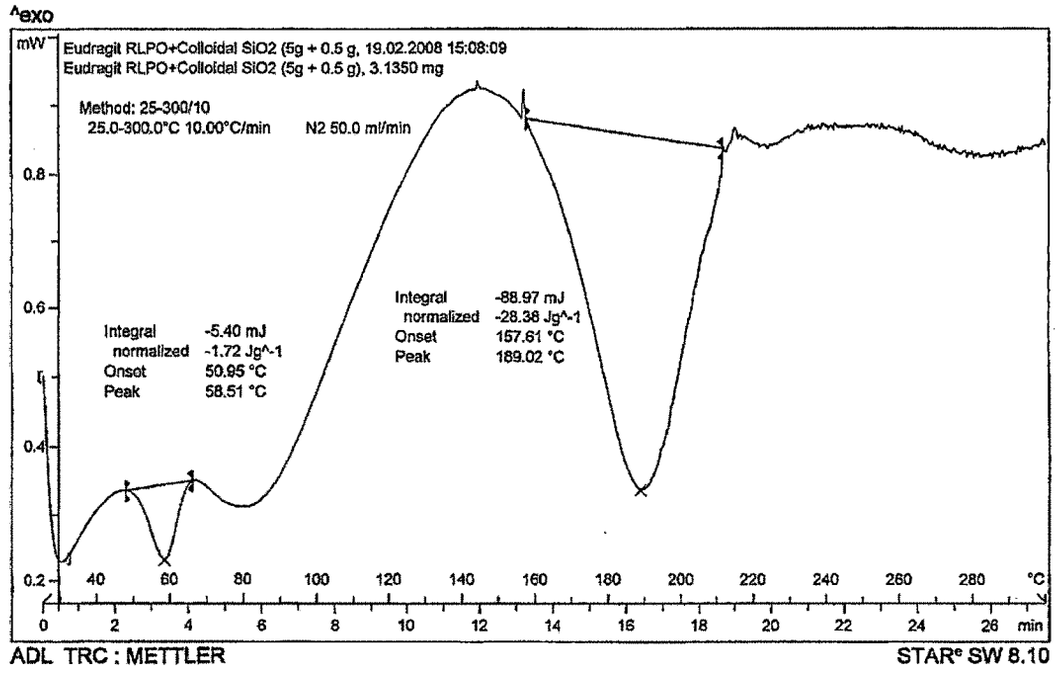

^exo
mW
colloidal SiO2, 18.02.2008 16:05:29
colloidal SiO2, 0.8250 mg
Method: 25-300/10
25.0-300.0°C 10.00°C/min N2 50.0 ml/min
Integral -1.64 mJ
normalized -1.98 Jg^-1
Onset 256.67 °C
Peak 257.36 °C
0.6
0.4
0.2
0.0
40 60 80 100 120 140 160 180 200 220 240 260 280 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 min
ADL TRC : METTLER
STARe SW 8.10

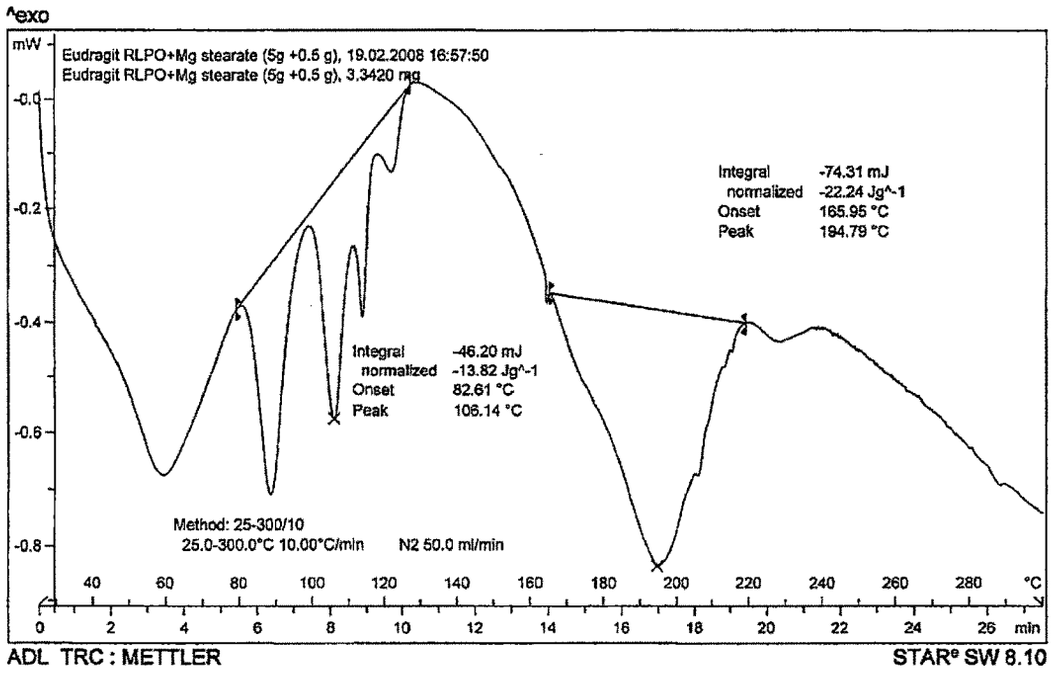

^exo
mW
MAGNESIUM STEARATE, 18.02.2008 15:24:56
MAGNESIUM STEARATE, 1.6360 mg
Integral -2.31 mJ
normalized -1.41 Jg^-1
Onset 144.28 °C
Peak 150.99 °C
Integral -420.80 mJ
normalized -257.22 Jg^-1
Onset 72.67 °C
Peak 107.25 °C
Integral -0.54 mJ
normalized -0.33 Jg^-1
Onset 164.23 °C
Peak 165.99 °C
Method: 25-300/10
25.0-300.0°C 10.00°C/min N2 50.0 ml/min
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
40 60 80 100 120 140 160 180 200 220 240 260 280 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 min
ADL TRC : METTLER
STARe SW 8.10

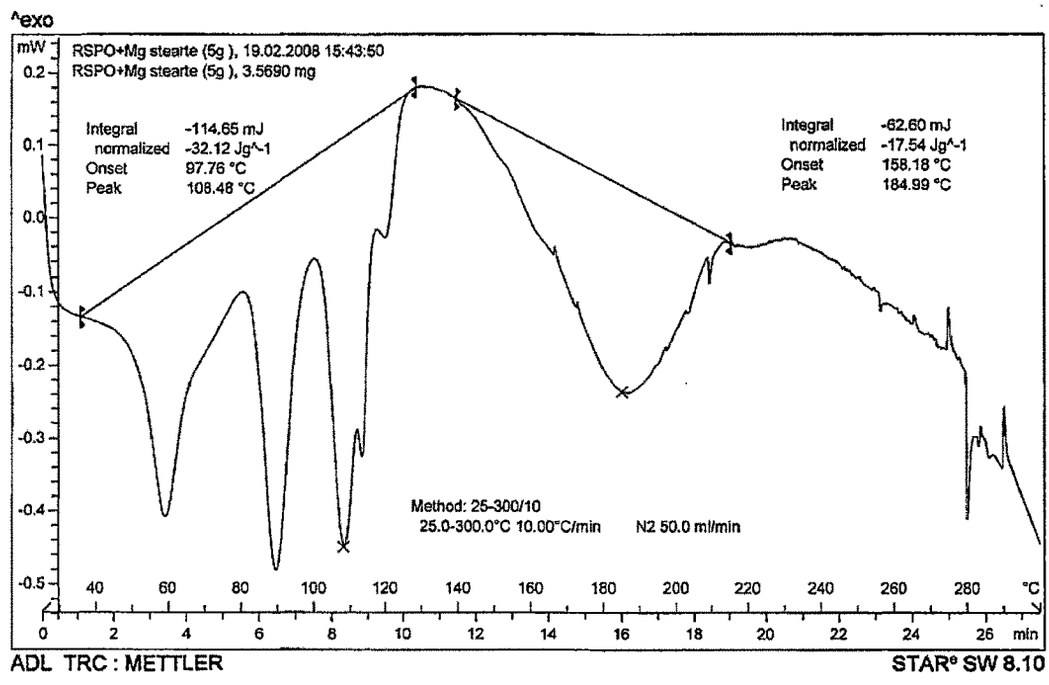

^exo
mW
RSPO, 18.02.2008 14:39:08
RSPO, 1.5750 mg
Method: 25-300/10
25.0-300.0°C 10.00°C/min N2 50.0 ml/min
Integral -52.11 mJ
normalized -33.08 Jg^-1
Onset 154.30 °C
Peak 184.80 °C
-0.0
-0.2
-0.4
-0.6
40 60 80 100 120 140 160 180 200 220 240 260 280 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 min
ADL TRC : METTLER
STARe SW 8.10

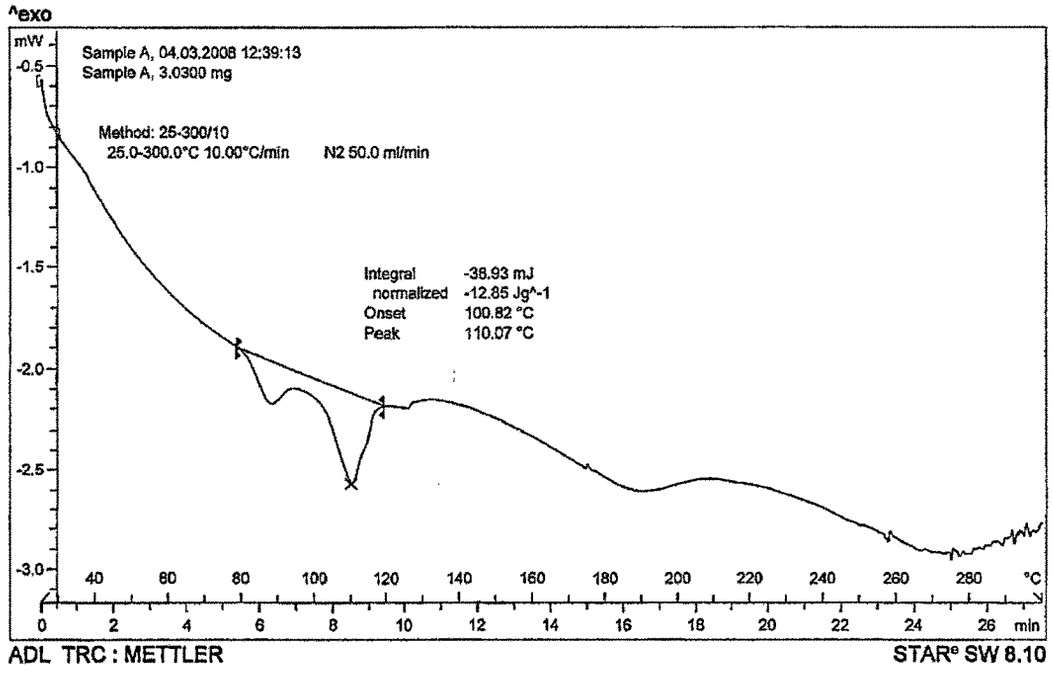

^exo
mW
Eudragit RLPO, 18.02.2008 13:11:39
Eudragit RLPO, 1.6900 mg
Method: 25-300/10
25.0-300.0°C 10.00°C/min N2 50.0 ml/min
Integral -111.00 mJ
normalized -65.68 Jg^-1
Onset 149.87 °C
Peak 191.13 °C
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
40 60 80 100 120 140 160 180 200 220 240 260 280 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 min
ADL TRC : METTLER
STARe SW 8.10

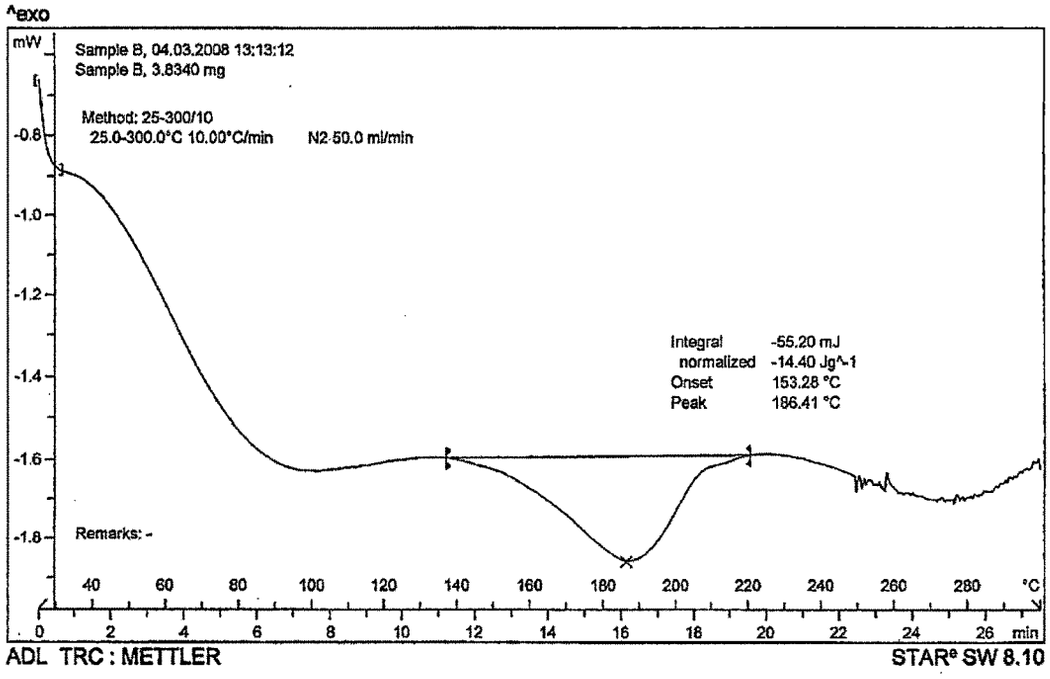

^exo
mW
RSPO+Colloidal SiO2 (5g +0.5 g), 19.02.2008 16:22:33
RSPO+Colloidal SiO2 (5g +0.5 g), 3.1250 mg
Method: 25-300/10
25.0-300.0°C 10.00°C/min N2 50.0 ml/min
Integral -44.78 mJ
normalized -14.33 Jg^-1
Onset 160.47 °C
Peak 180.80 °C
Integral -8.44 mJ
normalized -2.70 Jg^-1
Onset 51.82 °C
Peak 58.32 °C
0.2
0.0
-0.2
-0.4
-0.6
-0.8
40 60 80 100 120 140 160 180 200 220 240 260 280 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 min
ADL TRC : METTLER
STARe SW 8.10


^exo
mW
Eudragit RLPO+Colloidal SiO2 (5g + 0.5 g, 19.02.2008 15:08:09
Eudragit RLPO+Colloidal SiO2 (5g + 0.5 g), 3.1350 mg
Method: 25-300/10
25.0-300.0°C 10.00°C/min N2 50.0 ml/min
Integral -88.97 mJ
normalized -28.38 Jg^-1
Onset 157.61 °C
Peak 189.02 °C
Integral -5.40 mJ
normalized -1.72 Jg^-1
Onset 50.95 °C
Peak 58.51 °C
0.8
0.6
0.4
0.2
40 60 80 100 120 140 160 180 200 220 240 260 280 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 min
ADL TRC : METTLER
STARe SW 8.10


^exo
mW
Eudragit RLPO+Mg stearate (5g +0.5 g), 19.02.2008 16:57:50
Eudragit RLPO+Mg stearate (5g +0.5 g), 3.3420 mg
Integral -74.31 mJ
normalized -22.24 Jg^-1
Onset 165.95 °C
Peak 194.79 °C
Integral -46.20 mJ
normalized -13.82 Jg^-1
Onset 82.61 °C
Peak 106.14 °C
Method: 25-300/10
25.0-300.0°C 10.00°C/min N2 50.0 ml/min
-0.0
-0.2
-0.4
-0.6
-0.8
40 60 80 100 120 140 160 180 200 220 240 260 280 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 min
ADL TRC : METTLER
STARe SW 8.10


^exo
mW
RSPO+Mg stearte (5g), 19.02.2008 15:43:50
RSPO+Mg stearte (5g), 3.5690 mg
Integral -114.65 mJ
normalized -32.12 Jg^-1
Onset 97.76 °C
Peak 108.48 °C
Integral -62.60 mJ
normalized -17.54 Jg^-1
Onset 158.18 °C
Peak 184.99 °C
Method: 25-300/10
25.0-300.0°C 10.00°C/min N2 50.0 ml/min
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
40 60 80 100 120 140 160 180 200 220 240 260 280 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 min
ADL TRC : METTLER
STARe SW 8.10


^exo
mW
Sample A, 04.03.2008 12:39:13
Sample A, 3.0300 mg
Method: 25-300/10
25.0-300.0°C 10.00°C/min N2 50.0 ml/min
Integral -38.93 mJ
normalized -12.85 Jg^-1
Onset 100.82 °C
Peak 110.07 °C
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
40 60 80 100 120 140 160 180 200 220 240 260 280 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 min
ADL TRC : METTLER
STARe SW 8.10


^exo
mW
Sample B, 04.03.2008 13:13:12
Sample B, 3.8340 mg
Method: 25-300/10
25.0-300.0°C 10.00°C/min N2 50.0 ml/min
Integral -55.20 mJ
normalized -14.40 Jg^-1
Onset 153.28 °C
Peak 186.41 °C
Remarks: -
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
40 60 80 100 120 140 160 180 200 220 240 260 280 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 min
ADL TRC : METTLER
STARe SW 8.10

V.8.1.6 Evaluation of Tablet

V.8.1.6.1 Evaluation of Core Tablet

Table V. 10 Physico-chemical parameters of core tablet of Approach IA of B.No 23

S.No.	Physical / Chemical Parameter	B.No 23
	Physical Parameter	
1	Length (mm)	19.5
2	Breadth (mm)	9.5
3	Thickness (mm)	6.14 (5.9-6.3)
4	Hardness (N)	196 (184-208)
5	Average weight (mg)	1003.30 (1001.5-1005.4)
6	Friability (%)	0.27
	Chemical Parameter	
7	Content Uniformity (%)	99.02 ± 1.19
8	Dissolution profile (0.01 N HCl/50 rpm/500 ml) 10 min 15 min 30 min 45 min 60 min	 73.3 ± 9.73 81.3 ± 3.60 85.8± 2.51 98.3± 1.92 98.5 ± 1.41

V.8.1.6.2 Evaluation of Polymer Coated Tablet

Table V. 11 Physico-chemical parameters of polymer coated tablets of Approach IA of B.No 23

S.No.		B.No 23
	Physical Parameter	
1	Length (mm)	19.7
2	Breadth (mm)	9.6
3	Thickness (mm)	6.30 (6.1-6.4)
4	Average weight (mg)	1056 (1047-1060)
	Chemical Parameter	
5	Content Uniformity	99.4 ± 0.7
6	Related Impurities (%) Single unknown Total Impurities	 0.02 0.03
7	Assay (%)	99.1
8	Water By KF (% w/w)	3.82

V.8.2 Results of Approach IB

V.8.2.1 Formulation Composition with Approach IB

Table V. 12 Composition with Approach IB of B. No. 02

Stage	Ingredients	mg/tab
Core Tablet	Lactose Anhydrous	514.00
	Maize Starch	215.00
	Microcrystalline Cellulose(Avicel pH 101)	387.00
	Polyvinyl pyrrolidone K-30	42.00
	Talc	24.00
	Magnesium stearate	12.00
	Colloidal silicon dioxide	6.00
	Total wt. of tablet	**1200**
Seal coat	Ethyl cellulose (20 cps)	17.00
	Triethyl citrate	3.40
	Talc	3.60
	Acetone	148.00
	Methanol	131.00
	Weight Gain	**2.51%**
Drug layering	Alfuzosin HCL	10.00
	HPMC 6 cps	29.09
	Triethyl citrate	6.97
	Talc	11.45
	Acetone	q.s.
	Methanol	q.s.
Polymer coat	Eudragit RSPO	18.18
	Eudragit RLPO	1.00
	Triethyl citrate	2.66
	Talc	4.16
	Isopropyl alcohol	q.s.
	Acetone	q.s.
Total Weight of the Tablet		**1307.51**

V.8.2.2 Effect of different % coating with 95:5 (1.13 % vs 2.1%)

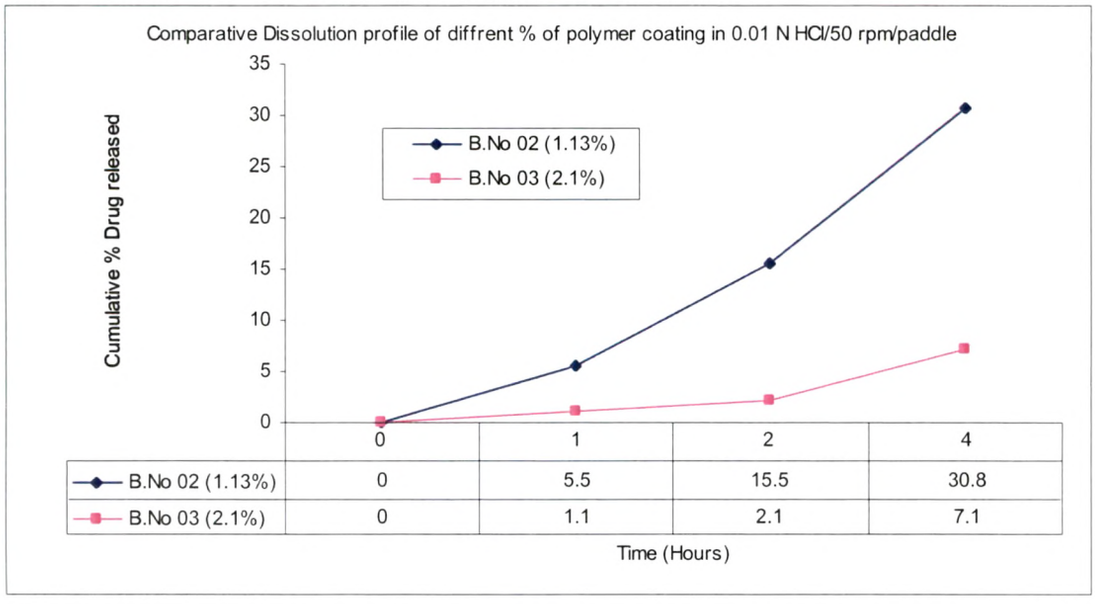


Figure V. 6 Comparative Dissolution profile of B.No 02 (1.13%) vs B.No 03 (2.1%) in 0.01 N HCl/50 rpm/paddle

B.No 03 release profile was significantly slower than that of B.No 02.So B.No 02 was used for further study.

V.8.2.2.1 Evaluation of Core Dummy Tablet

Table V. 13 Physical parameters of core tablet of Approach IB of B.No 02

S.No.	Physical Parameter	B.No 02
1	Length (mm)	19.5
2	Breadth (mm)	9.5
3	Thickness (mm)	7.32 (7.25-7.40)
4	Hardness (N)	245 (228-257)
5	Average weight (mg)	1210 (1190-1250)
6	Friability (%)	0.34

V.8.2.2.2 Evaluation of Drug Coated Tablet

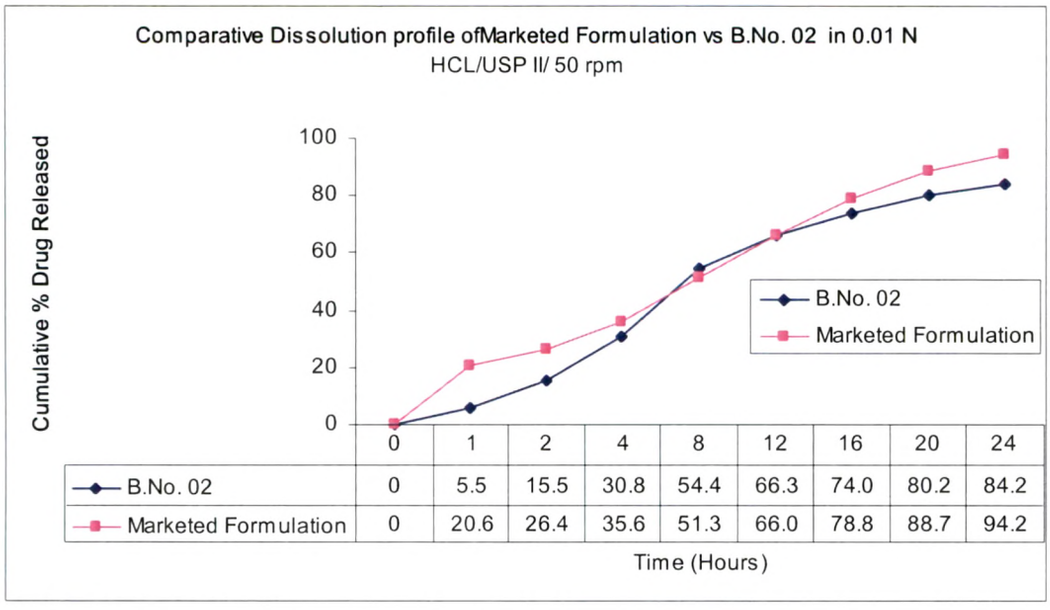
Table V. 14 Chemical parameters of Drug coated tablets of Approach IB of B.No 02

S.No.	Chemical Parameter	B.No 02
1.	Content Uniformity	102.94 ± 7.79
2.	Dissolution profile (0.01 N HCl/50 rpm/500 ml) 30 min 60 min	 102.2 ± 4.29 103.2 ± 4.87

V.8.2.2.3 Evaluation of Polymer Coated Tablet

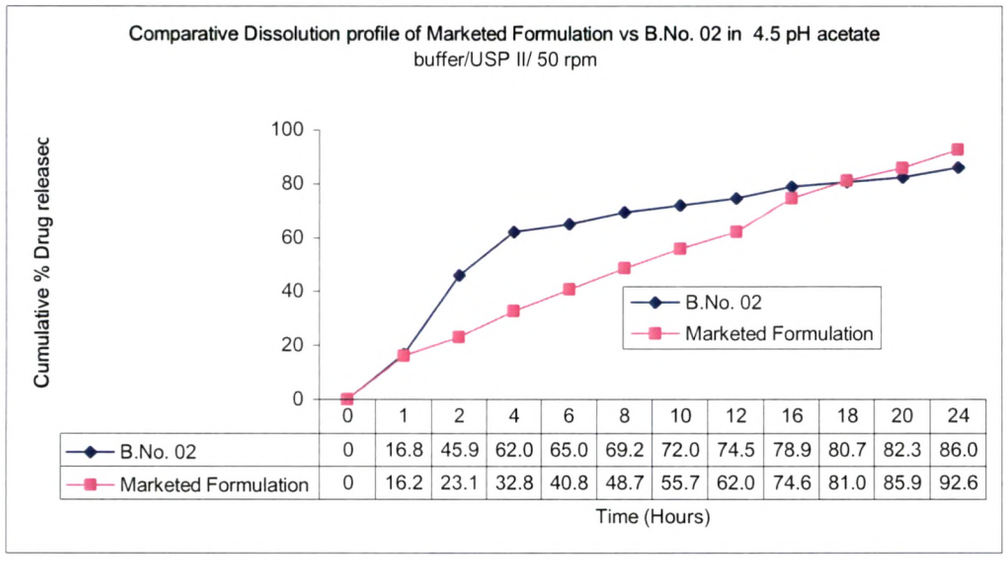
Table V. 15 Physico-chemical parameters of polymer coated tablets of Approach IB of B.No 02

S.No.		B.No 02
Physical Parameter		
1.	Length (mm)	19.9
2.	Breadth (mm)	9.8
3.	Thickness (mm)	7.62 (7.58-7.75)
4	Average weight (mg)	1317 (1295-1340)
Chemical Parameter		
5.	Content Uniformity	99.13 ± 0.78
6.	Related Impurities (%) Single unknown Total Impurities	 0.02 0.07
7.	Assay (%)	100.54
8.	Water By KF (% w/w)	3.95



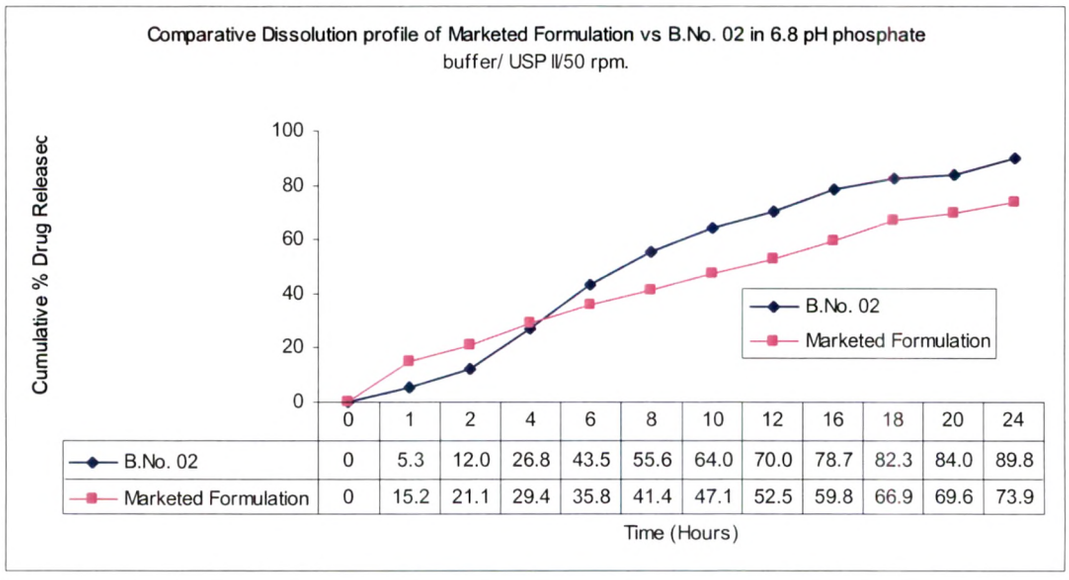
	0	1	2	4	8	12	16	20	24
B.No. 02	0	5.5	15.5	30.8	54.4	66.3	74.0	80.2	84.2
Marketed Formulation	0	20.6	26.4	35.6	51.3	66.0	78.8	88.7	94.2

Figure V. 7 Comparative Dissolution profile of B.No 02 vs Marketed Formulation in 0.01 N HCl/50 rpm/paddle



	0	1	2	4	6	8	10	12	16	18	20	24
B.No. 02	0	16.8	45.9	62.0	65.0	69.2	72.0	74.5	78.9	80.7	82.3	86.0
Marketed Formulation	0	16.2	23.1	32.8	40.8	48.7	55.7	62.0	74.6	81.0	85.9	92.6

Figure V. 8 Comparative Dissolution profile of B. No 02 vs Marketed Formulation in 4.5 pH acetate buffer/50 rpm/paddle



	0	1	2	4	6	8	10	12	16	18	20	24
B.No. 02	0	5.3	12.0	26.8	43.5	55.6	64.0	70.0	78.7	82.3	84.0	89.8
Marketed Formulation	0	15.2	21.1	29.4	35.8	41.4	47.1	52.5	59.8	66.9	69.6	73.9

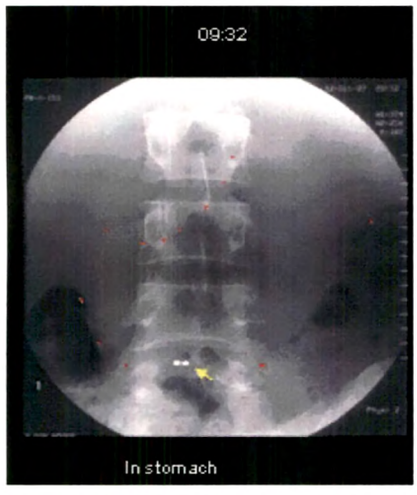
Figure V. 9 Comparative Dissolution profile of B.No 02 vs Marketed Formulation in 6.8 pH phosphate Buffer/50 rpm/paddle

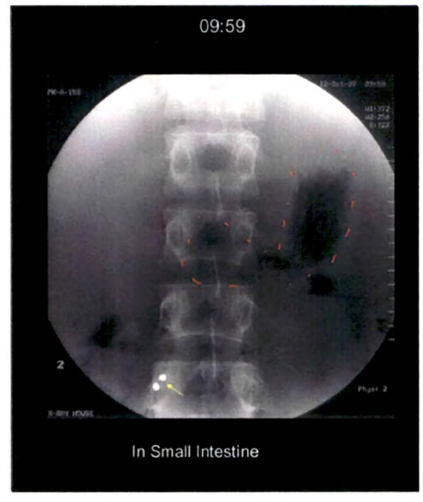
Approach IA and IB had similar dissolution profile but due to ease of formulation, Approach IA was finalized for further study.

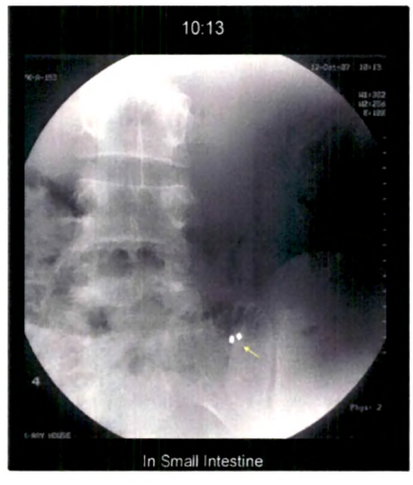
V.8.3 Gastric Retention time

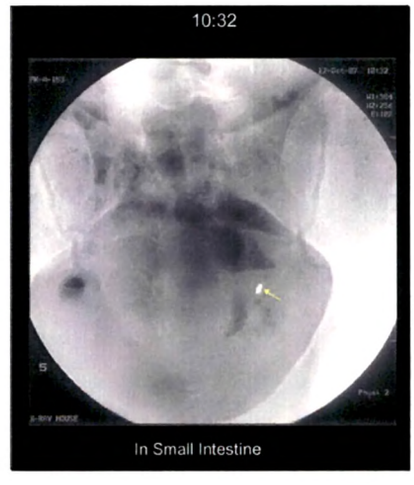
Table V. 16 Gastric Retention time of Dummy tablets of B.No 23 of Approach IA in Healthy volunteers under Fasted condition

	Size Exclusion	
Time (min)	**PK-A-15**	**PK-E-108**
0	Stomach	Stomach
20	Small Intestine	Small Intestine
40	Small Intestine	Small Intestine
60	Small Intestine	Small Intestine
90	Small Intestine	Small Intestine
150	Small Intestine	Small Intestine









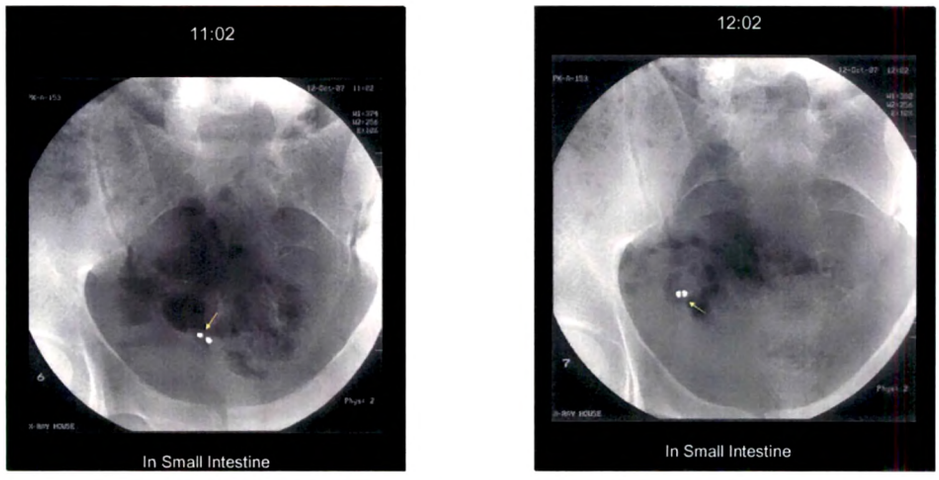
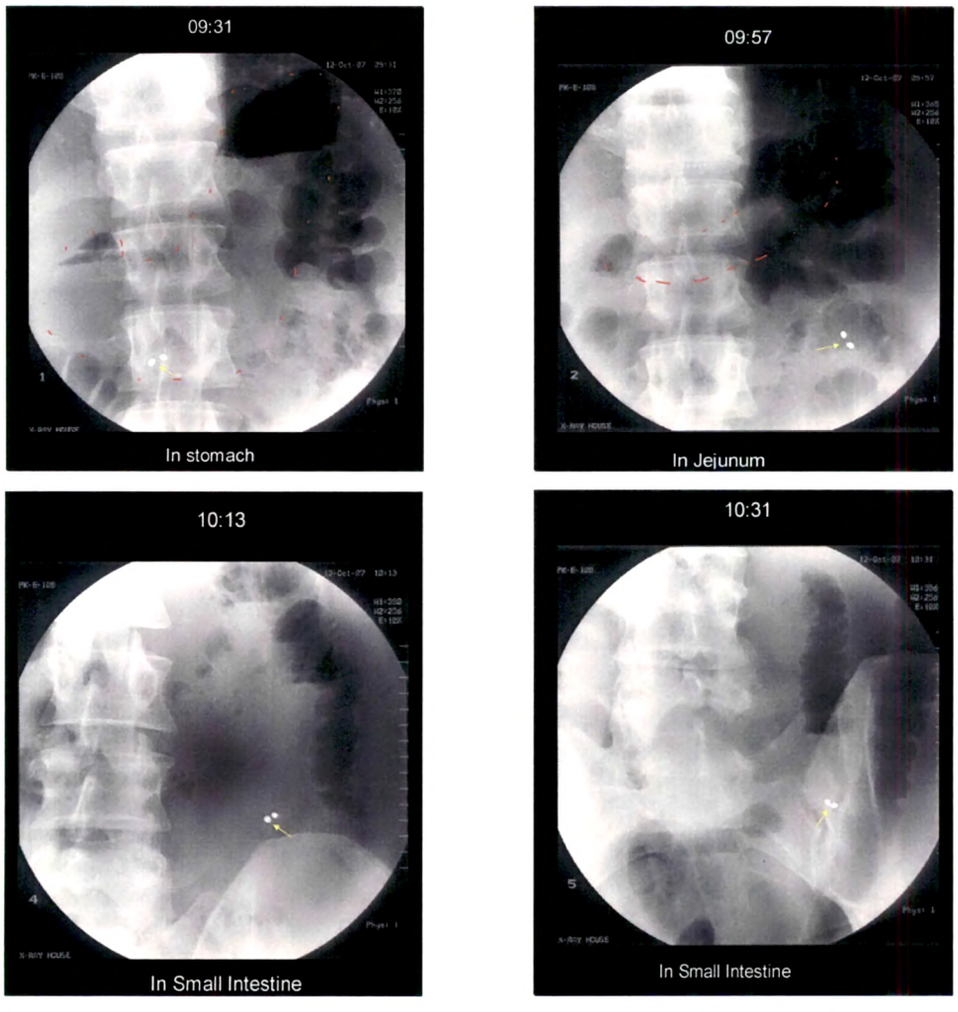
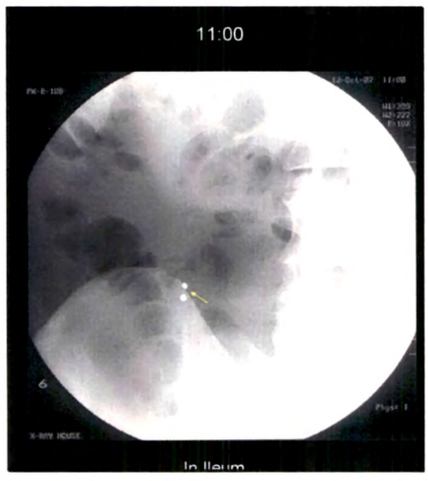


Figure V. 10 Gastric Retention time of Dummy tablets of B.No 23 in Healthy volunteer (PK-A-153) under Fasted condition





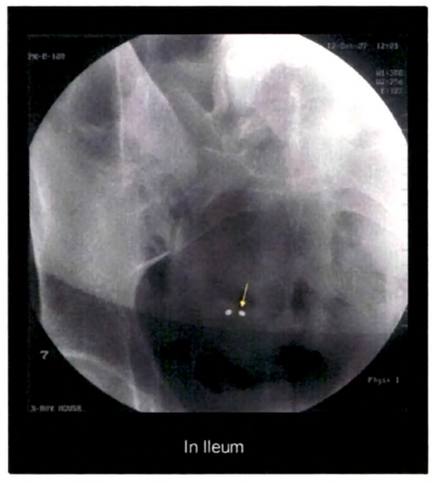
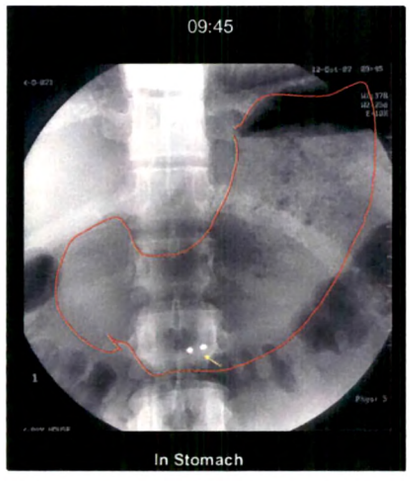
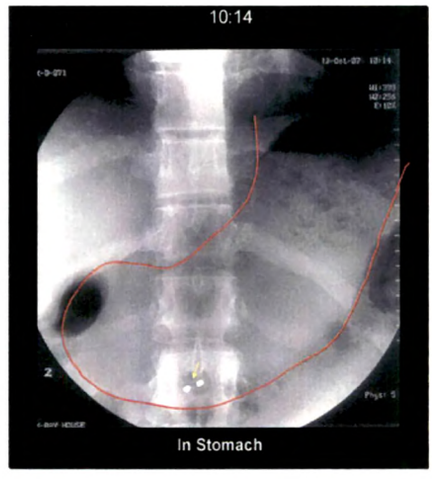


Figure V. 11 Gastric Retention time of Dummy tablets of B.No 23 in Healthy volunteer (PK-E-108) under Fasted condition

Table V. 17 Gastric Retention time of Dummy tablets of B.No 23 in Healthy volunteers under Fed condition

	Size Exclusion	
Time (min)	**PK-B-071**	**PK-B-653**
0	Stomach	Stomach
0.5	Stomach	Stomach
1.5	Stomach	Stomach
2.5	Stomach	Stomach
3.5	Stomach	Stomach
4.5	Stomach	Stomach
5.5	Small Intestine	Stomach
6.5	Small Intestine	Stomach





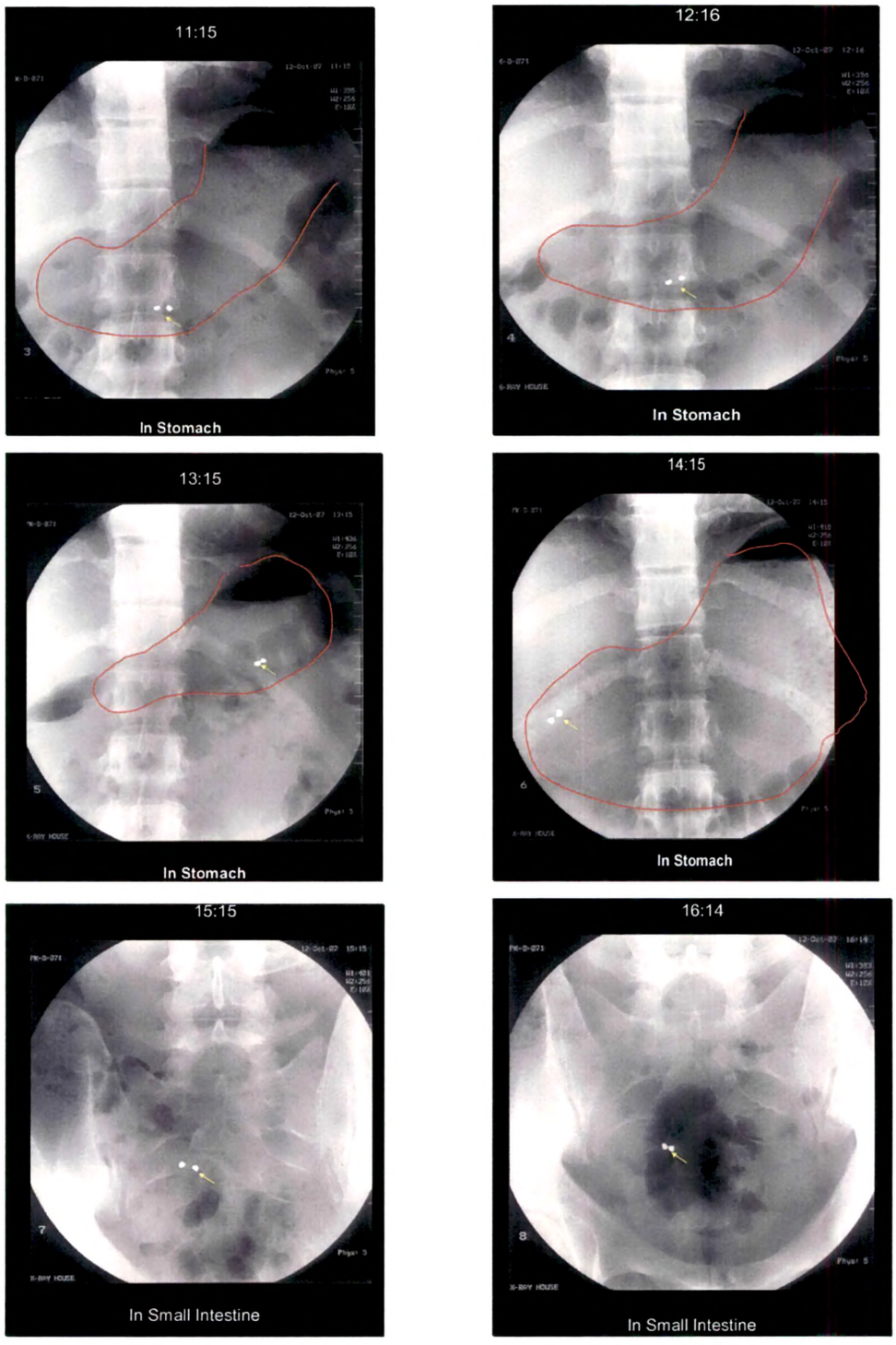
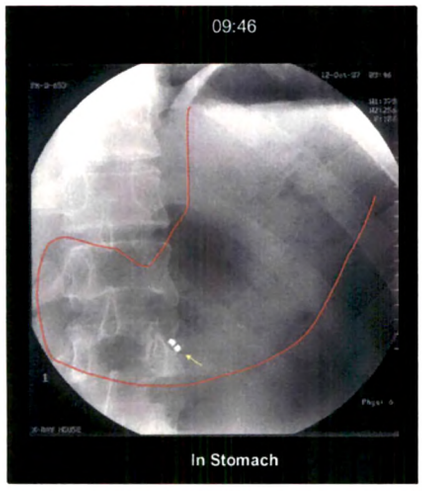
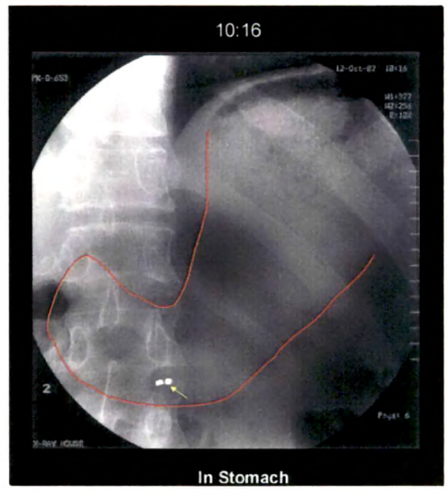
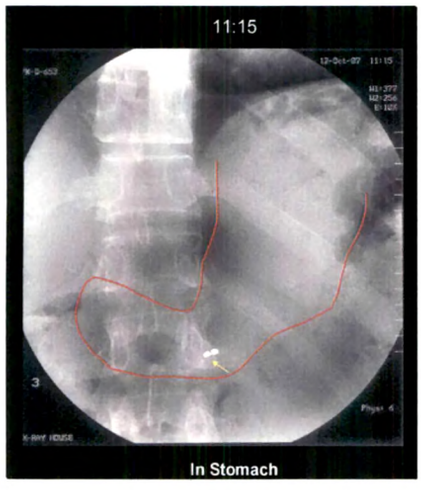
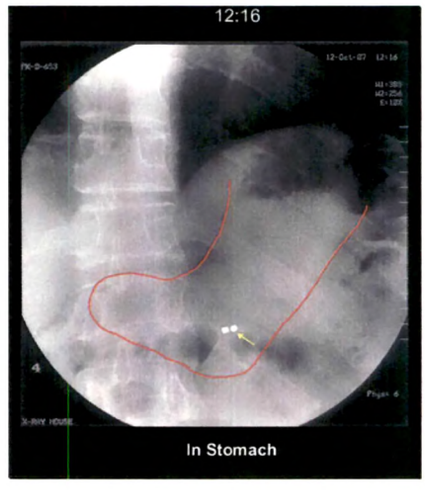


Figure V. 12 Gastric Retention time of Dummy tablets of B.No 23 in Healthy volunteer (PK-B-071) under Fed condition


09:46
In Stomach


10:16
In Stomach


11:15
In Stomach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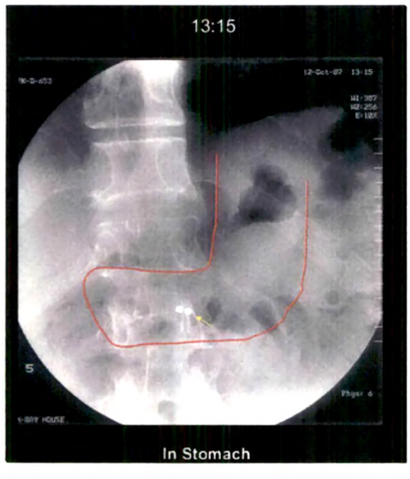
In Stomach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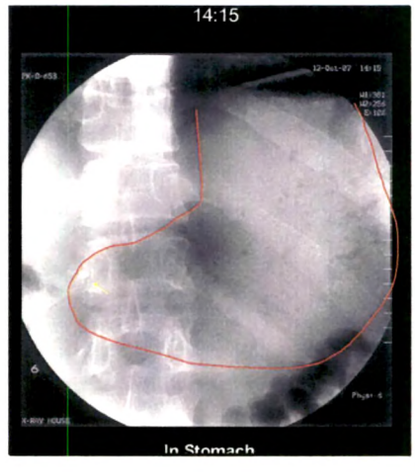
In Stomach


14:15

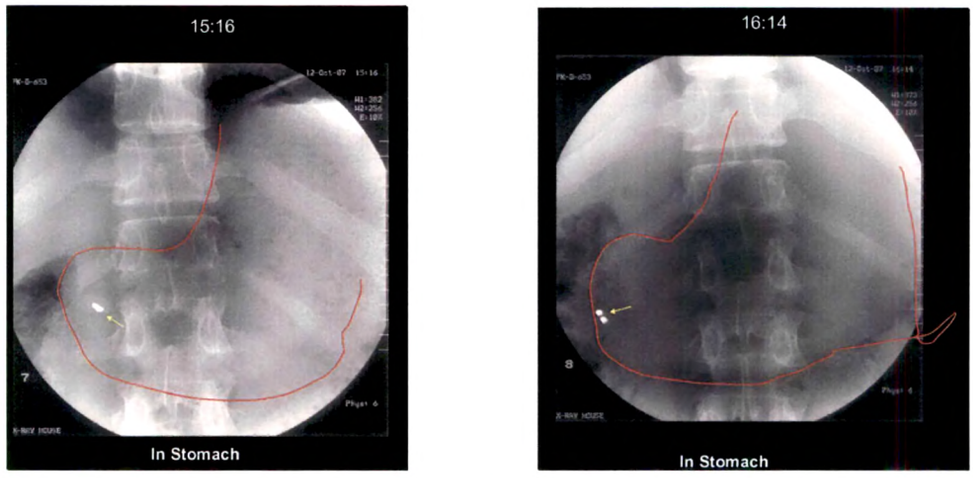


Figure V. 13 Gastric Retention time of Dummy tablets of B.No 23 in Healthy volunteer (PK-B-653) under Fed condition

In the fasted state, the tablets emptied within 20 min interval in both the volunteers studied but remained in small intestine till the time of observation i.e. 2.5 hrs.

In the fed state, in one of the volunteer, the tablet emptied from the stomach between 4.5 – 5.5 hours but in second volunteer, tablet remained in the stomach till the study duration i.e. 6.5 hours post dose. Further investigation of gastric retention time was impeded due to limitation of number of human X-Ray exposures.

In both the studies, fasted and fed, none of the tablet disintegrated during the time interval studied as observed in X-Ray photographs. Two mini-tablets of barium sulphate (40 mg each) embedded in the core tablet of the placebo formulation of B.No. 23 remained in close integrity with each other throughout the study period. Also no discomfort or adverse effect was reported by any volunteer during the study.

V.8.4 Bio Results

Table V. 18 Mean plasma concentration (ng/mL) of B.No 23 and Marketed formulation under fasting and Fed state (N=8 volunteers)

Time (Hrs)	Fasted		Fed	
	TEST Plasma concentration (ng/mL) [Mean ± SD]	REFERENCE Plasma concentration (ng/mL) [Mean ± SD]	TEST Plasma concentration (ng/mL) [Mean ± SD]	REFERENCE Plasma concentration (ng/mL) [Mean ± SD]
0.0	0.00±0.00	0.00±0.00	0.03±0.01	0.00±0.00
1.0	0.02±0.00	3.84±1.34	0.03±0.00	2.75±1.91
2.0	0.00±0.00	4.57±1.60	0.00±0.00	3.98±1.63
4.0	0.01±0.01	7.94±1.85	0.00±0.00	7.28±1.99
6.0	0.00±0.00	7.96±2.31	0.03±0.00	8.49±3.94
7.0	0.03±0.02	7.19±1.94	0.01±0.00	8.21±3.95
7.5	0.02±0.02	6.83±1.91	0.00±0.00	8.99±4.71
8.0	0.03±0.01	6.71±2.01	0.00±0.00	9.69±5.13
8.5	0.03±0.02	6.45±1.96	0.00±0.00	9.16±4.90
9.0	0.03±0.02	6.23±1.82	0.00±0.00	8.30±4.35
10.0	0.04±0.03	5.87±1.79	0.01±0.00	7.42±3.57
12.0	0.04±0.03	5.93±2.24	0.02±0.00	6.04±1.91
16.0	0.36±0.33	4.79±1.83	0.22±0.30	4.11±0.60
20.0	1.34±0.85	3.60±1.86	0.28±0.00	2.19±0.51
24.0	1.25±0.91	3.18±2.20	2.54±0.00	1.35±0.44
48.0	0.00±0.27	0.00±0.00	0.41±0.00	0.00±0.00

Table V. 19 Summary of Pharmacokinetic Results of marketed Formulation under Fasting and Fed condition (N=8 volunteers)

Parameters	Unit	Fasted	Fed
		Alfuzosin ER (Reference) (Mean ± SD)	Alfuzosin ER (Reference) (Mean ± SD)
Cmax	ng/ml	7.723 ± 3.966	8.822 ± 1.880
Tmax	Hours	4.50± 2.56	6.25 ± 2.72
AUC_{0-t}	ng*h/mL	106.19± 49.56	156.17 ± 66.68
AUC_{0-inf}	ng*h/mL	111.41± 50.21	165.98 ± 62.10
THALF	Hours	5.11± 0.97	6.73 ± 1.15
MRT_{0-t}	Hours	10.58± 2.76	12.72 ± 3.36
MRT_{0-inf}	Hours	11.76± 2.52	14.66 ± 2.98

As plasma concentrations of B.No 23 (test formulation) were very low, therefore summary results of pharmacokinetic parameters of reference marketed formulation are only given.

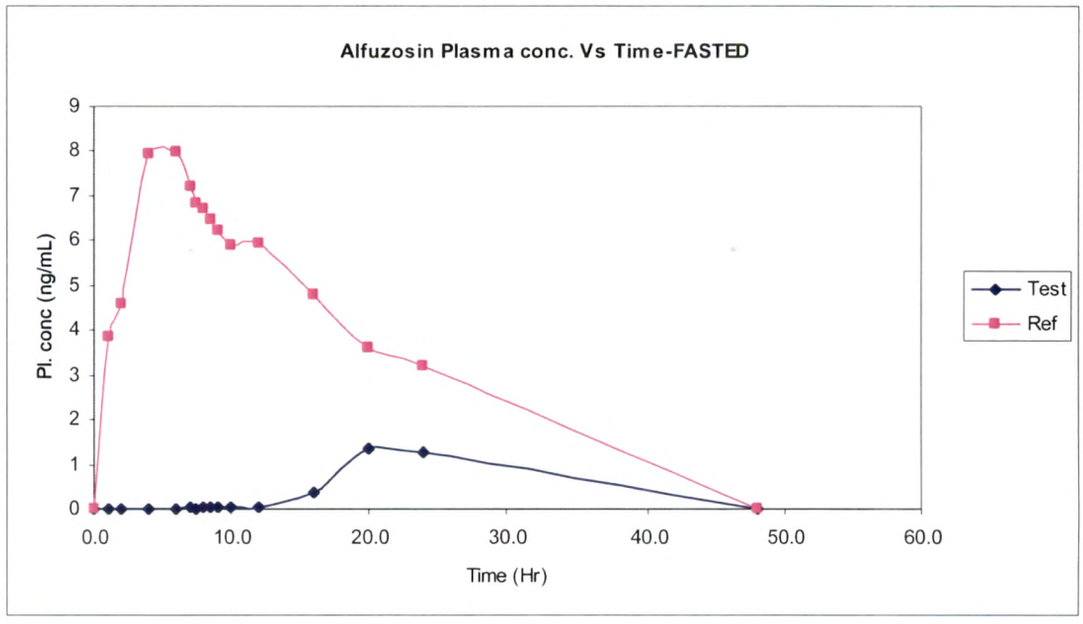


Figure V. 14 Mean plasma concentration vs time curve of B.No 23 (Test) vs Marketed Formulation (Reference) in Fasted state (N=8)

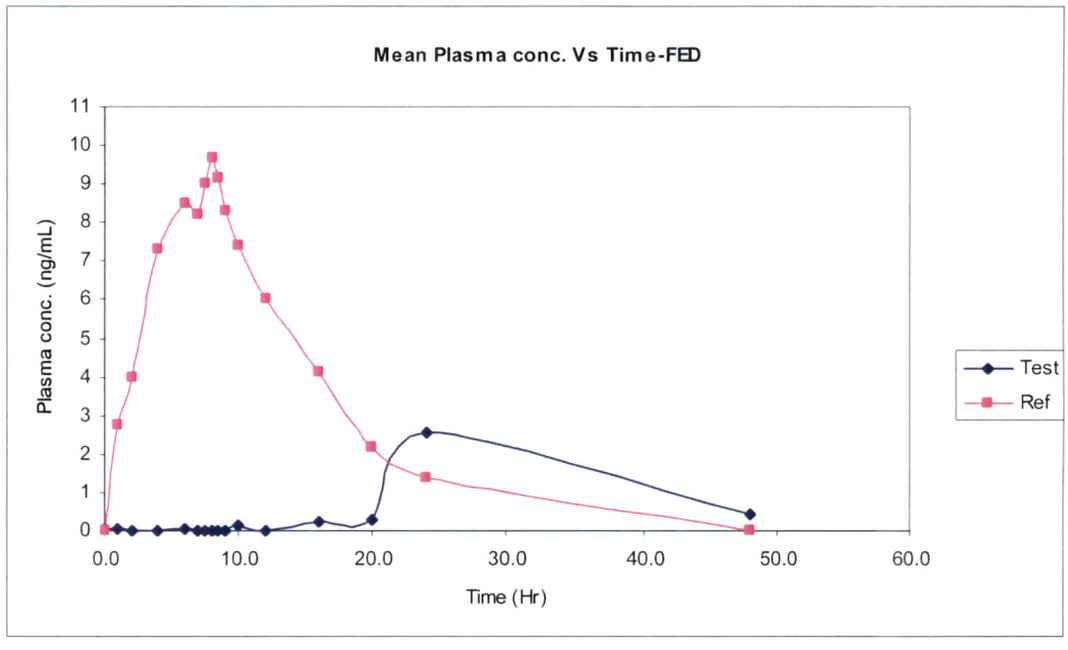
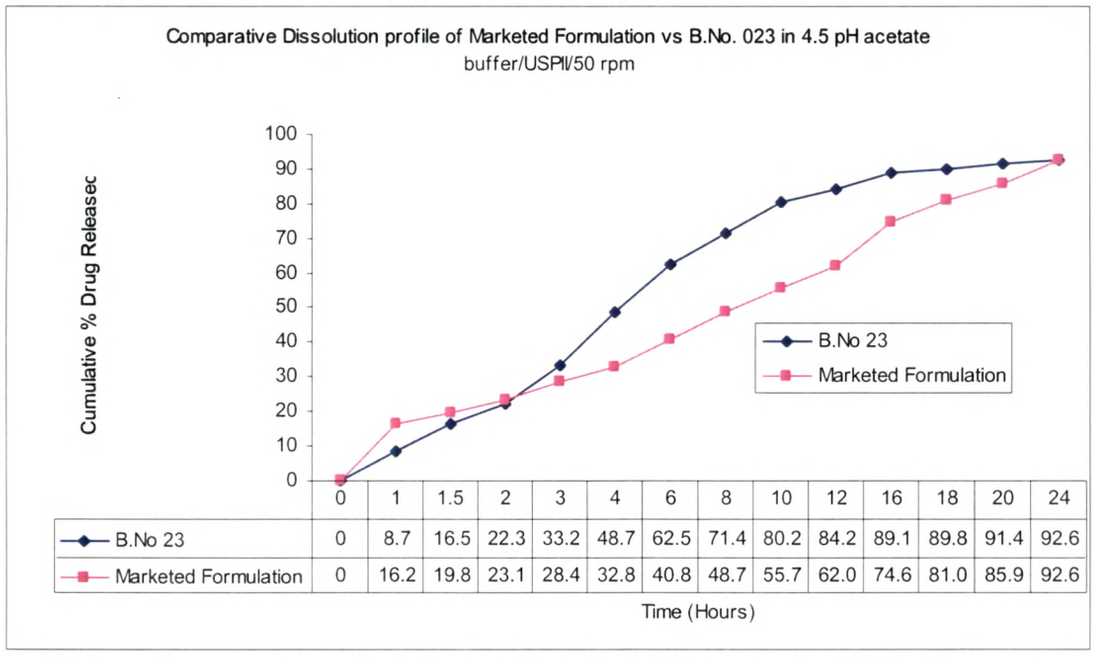


Figure V. 15 Mean plasma concentration vs time curve of B.No 23 (Test) vs Marketed Formulation (Reference) in Fed state (N=8)

In both the fasted and fed condition, there was a lag time in absorption and only small peak was observed after 20 hrs in fasted state and after 24 hours in fed state. The plasma levels obtained in the test subjects were not significant for pharmacokinetic and statistical analysis.

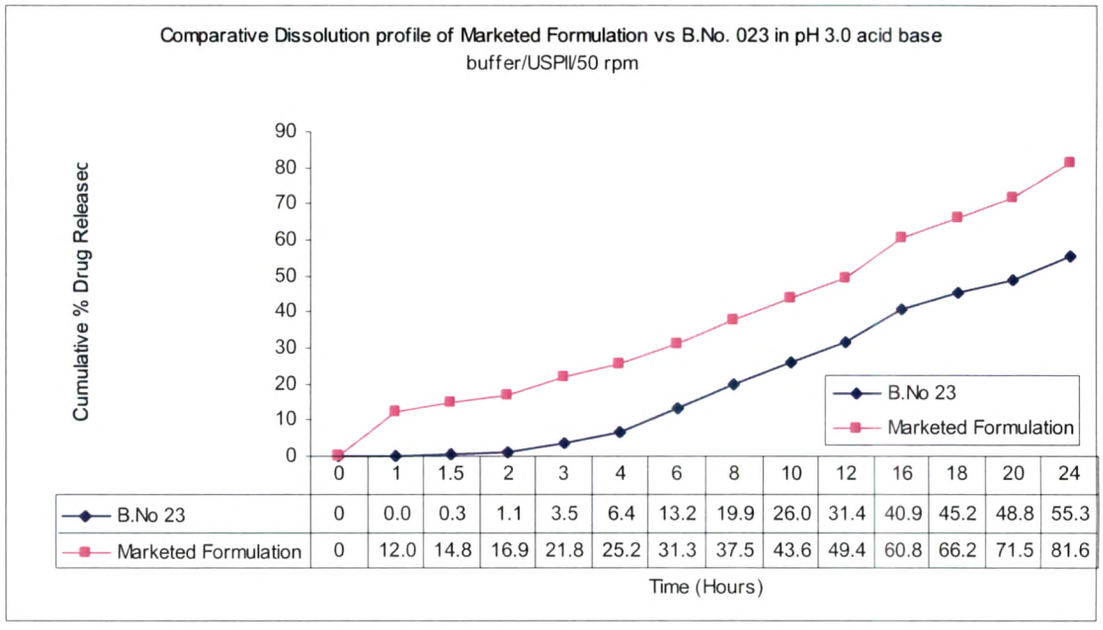
Therefore the bioavailability was not assessed between the two formulations (marketed and test) of Alfuzosin ER 10 mg under fasting and fed state. This may be possibly due to inadequate drug release from the test formulation, whereas with reference formulation the observed pharmacokinetic values were comparable with that of the literature under similar experimental conditions (UroXatral® (alfuzosin) package insert, 2003). In order to access the possible causes of this unusual behavior, in-vitro testing of the formulation was carried out in other medias so that an IVIVC could be established.

V.8.5 Post Bio IVIVC



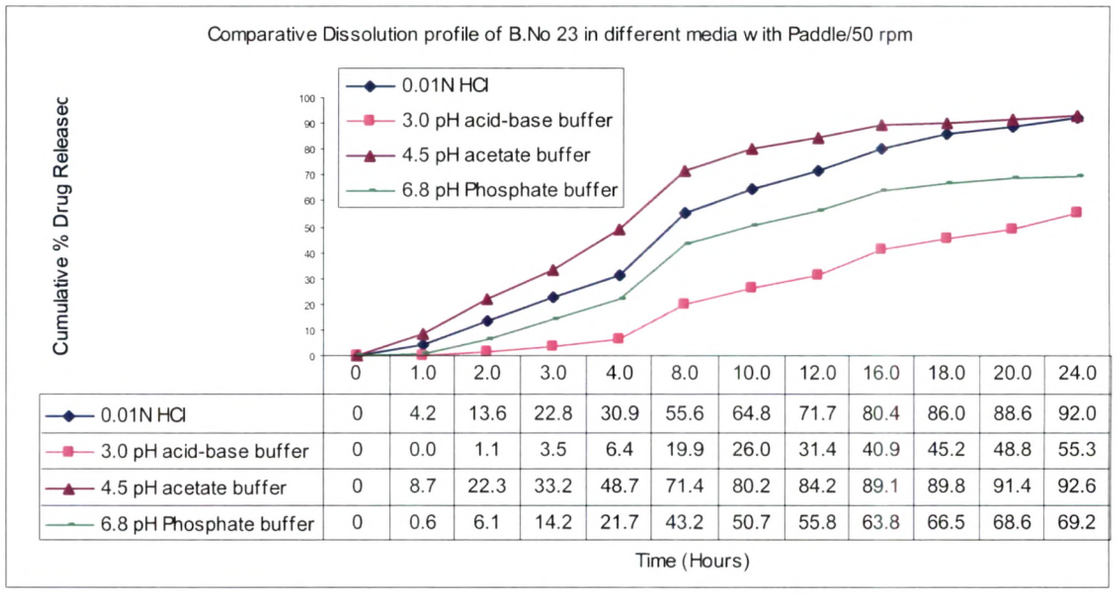
	0	1	1.5	2	3	4	6	8	10	12	16	18	20	24
B.No 23	0	8.7	16.5	22.3	33.2	48.7	62.5	71.4	80.2	84.2	89.1	89.8	91.4	92.6
Marketed Formulation	0	16.2	19.8	23.1	28.4	32.8	40.8	48.7	55.7	62.0	74.6	81.0	85.9	92.6

Figure V. 16 Comparative Dissolution profile of B.No 023 vs Marketed Formulation in 4.5 pH acetate Buffer/50 rpm/paddle



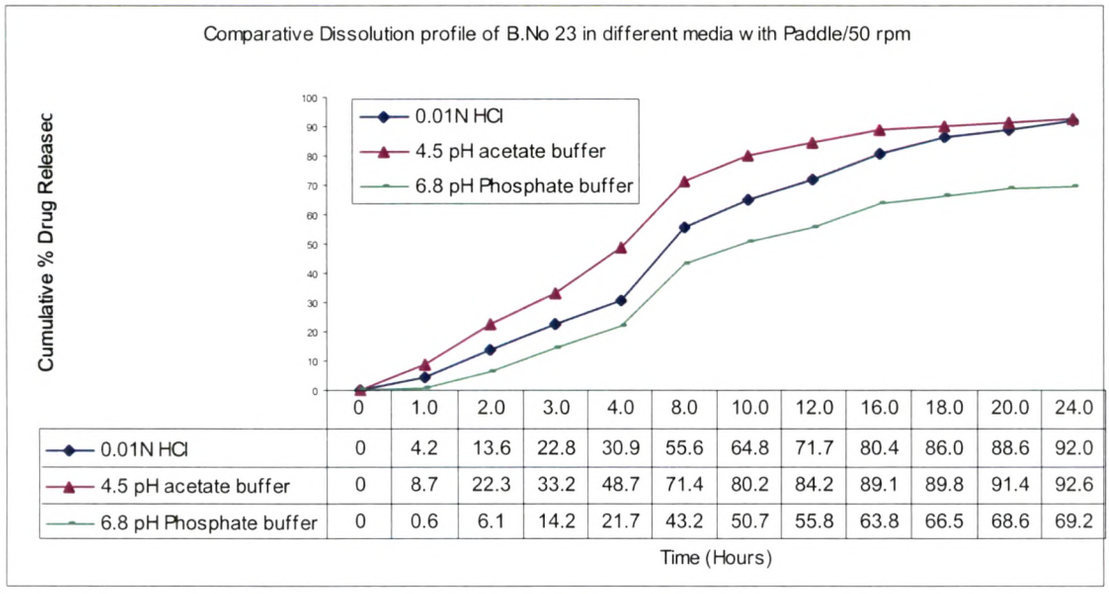
	0	1	1.5	2	3	4	6	8	10	12	16	18	20	24
B.No 23	0	0.0	0.3	1.1	3.5	6.4	13.2	19.9	26.0	31.4	40.9	45.2	48.8	55.3
Marketed Formulation	0	12.0	14.8	16.9	21.8	25.2	31.3	37.5	43.6	49.4	60.8	66.2	71.5	81.6

Figure V. 17 Comparative Dissolution profile of B.No 023 vs Marketed Formulation in pH 3.0 acid base Buffer/50 rpm/paddle



	0	1.0	2.0	3.0	4.0	8.0	10.0	12.0	16.0	18.0	20.0	24.0
0.01N HCl	0	4.2	13.6	22.8	30.9	55.6	64.8	71.7	80.4	86.0	88.6	92.0
3.0 pH acid-base buffer	0	0.0	1.1	3.5	6.4	19.9	26.0	31.4	40.9	45.2	48.8	55.3
4.5 pH acetate buffer	0	8.7	22.3	33.2	48.7	71.4	80.2	84.2	89.1	89.8	91.4	92.6
6.8 pH Phosphate buffer	0	0.6	6.1	14.2	21.7	43.2	50.7	55.8	63.8	66.5	68.6	69.2

(a)



	0	1.0	2.0	3.0	4.0	8.0	10.0	12.0	16.0	18.0	20.0	24.0
0.01N HCl	0	4.2	13.6	22.8	30.9	55.6	64.8	71.7	80.4	86.0	88.6	92.0
4.5 pH acetate buffer	0	8.7	22.3	33.2	48.7	71.4	80.2	84.2	89.1	89.8	91.4	92.6
6.8 pH Phosphate buffer	0	0.6	6.1	14.2	21.7	43.2	50.7	55.8	63.8	66.5	68.6	69.2

(b)

Figure V. 18 Comparative Dissolution profile of B.No 023 in various media/50 rpm/paddle

V.9 DISCUSSION

Results of dissolution profile with various ratios of polymers showed that dissolution profile in 0.01 N HCl decreased with increase in concentration of Eudragit RSPO i.e. ammoniomethacrylate copolymer type A. The number of quaternary ammonium ions in Eudragit RSPO are less as compared to Eudragit RLPO. So swelling of RSPO is less than that of RLPO and this was responsible for decrease in release profile with increase in Eudragit RSPO.

It was found that dissolution profile of B.No 17 (without Magnesium stearate) was significantly faster than B.No 15 (with Magnesium Stearate)in 6.8 pH phosphate buffer.This was further confirmed by dissolution profile of B.No 23 in which colloidal silicon dioxide (Syloid 244) was used as antisticking agent. This indicated that Magnesium stearate might be incompatible with the polymers used for polymer coating (Eudragit RSPO and RLPO).

Reason for this was confirmed with DSC studies. DSC pattern showed that with binary mixture of RSPO or RLPO with Magnesium stearate there was no shift or disappearance of peak but when films were casted as sample A (with magnesium stearate) and Sample B (with colloidal silicon dioxide), dried and DSC done, surprisingly incompatibility was observed between RSPO and RLPO with Magnesium stearate. The absence of peak for RLPO in casted films of sample A and sample B might be due to low quantity of polymer. Following literature data also support our observation:

According to Handbook of Pharmaceutical excipients "dispersions of Eudragit L30D, L100-55 and RS 30D are incompatible with magnesium stearate (Rowe et al, 2003).

Alavi et al (2002) in their screening studies for choice of excipients found that magnesium stearate was incompatible with anionic polymers (attributed to reaction of magnesium ions with the -CHO carboxylic group of the polymer (Eudragit L 100, S 100).

Results of dissolution profile with Approach IB showed release profile similar to Approach IA but as the process was lengthy and cumbersome, Approach IA was used for further studies.

As plasma concentration of Alfusozin was below level of quantification in the test treatment group, the Pharmacokinetic parameters in that group could not be computed. Hence the pharmacokinetic profile of the two formulations of extended release Alfuzosin 10 mg (test and reference formulation) under fasted and fed state could not be compared.

All volunteers were monitored for adverse events as specified in the protocol. No serious or severe adverse events were reported during this study. Two volunteers complained of headache during period-II and period-I respectively. These adverse events were not clinically significant and no treatment was given for the same.

Alfuzosin was measured by using a validated LCMS method. For analysis of Alfuzosin the internal standard used was Es-citalopram. The calibration curves were linear from 0.15 ng/ml to 50.0 ng/ml for Alfuzosin.

Although the results of study of gastric retention in fasting state showed that the formulation emptied from the stomach within 20 min interval in both the volunteers studied ~~from the stomach~~ and remained in small intestine till the time of observation i.e. 2.5 hrs but in vivo no significant plasma concentration were detectable and showed a minor peak after 20 hrs.

In the fed state also in gastric retention study, in one of the volunteer (PK-B-071), the tablet emptied from the stomach between 4.5 – 5.5 hours but in second volunteer (PK-B-653), tablet remained in the stomach till the study duration i.e. 6.5 hours post dose but in vivo no significant plasma conc. was detectable and showed a minor peak after 24 hrs.

In vitro dissolution study in 0.01 N HCL and 6.8 pH phosphate buffer data did not show any correlation with in vivo bioequivalence data. This may be possibly due to inadequate drug release from the test formulation in -vivo, whereas with reference formulation the observed pharmacokinetic values were comparable with that of the literature under similar experimental conditions (UroXatral® (alfuzosin) package insert, 2003). Post bio investigation showed that dissolution data at pH 3.0 acid -base buffer showed a lag time which was observed in vivo as well. So, it may be concluded that with this formulation pH 3.0 acid base media is most bio-relevant media. A small peak observed after 20 and 24 hrs in fasted and fed state might be due to small amount of drug released in colon where a small amount of absorption can occur. Following literature data supports the results of our study:

PMMA (polymethyl methacrylate) had been reported to be a model substrate for compounds which have problem in wetting because its surface free energy is a reasonable estimate for hydrophobic compounds (Luner et al, 1996). Luner et al (2001) evaluated the wetting behavior of PMMA through determination of contact angle and surface tension using various solutions representing the fasting and fed states. They found that synthetic surfactants, depending on both their surface tension and concentrations, provide greater wetting for given surface relative to bile salt-lipid solutions representative of physiologic conditions. Conversely, in vivo fluids, as modeled by the bile salt-lipid solutions used in their study may not wet surfaces like PMMA as readily as some surfactant solutions.They concluded that slower dissolution of substrates with similar surface energy may take place in vivo with surfactants like Triton X-100 and sodium dodecyl sulphate. They also found that wetting was much better in fed state solutions as compared to fasted state.

With polymer-coating for sustained release oral dosage forms, drug release through the polymer membrane is diffusion controlled (Sutter at al, 1988). The diffusion is thereby dependent on the membrane permeability, which is seen as directly related to the water uptake or swelling, respectively, of the polymer membrane (Sutter at al, 1988). This hydrogel hypothesis is widely applicable for swellable polymers like cellulose derivates

(ethyl cellulose, cellulose acetate) (Lippold et al,1989; Bindschaedler et al,1987; Lippold et al,1981), however, in the case of the cationic polymethacrylate Eudragit RS and Eudragit RL, a more complex drug release mechanism may exist. Eudragit RLPO and RSPO are water insoluble, swellable film forming polymers based on neutral methacrylic acid esters with a small proportion of trimethylammonioethyl methacrylate chloride. Eudragit RLPO and RSPO are copolymers of acrylic and methacrylic acid esters with a low content in quaternary ammonium groups. The ammonium groups are present as salts and make the polymers permeable. With Eudragit RLPO, the molar ratio of quaternary ammonium groups to the neutral ester group is 1:20, with Eudragit RSPO this ratio is 1:40. Since quaternary ammonium groups determine the swellability of films and their permeability to water, dissolved salts and medicinal substances, Eudragit RLPO, which contains more of these groups, forms highly permeable films with little delaying action. By contrast, and owing to the reduced content in quaternary ammonium groups, films of Eudragit RS swell less easily and are slightly permeable to active ingredients.

The permeability of the films of Eudragit RSPO and RLPO and the release behavior of controlled release drug formulations with these films have been reported to be pH-independent (Lehmann, 1999; Eudragit, technical information), if the drug shows pH-independent solubility. Due to quaternary ammonium groups (QAG), the degree of ionization of the polymer should not be affected by the pH within the physiological pH range. In our study, surprisingly, a pH-dependent drug release was observed. As observed in preformulation study (Chapter IV), solubility of Alfuzosin HCL is pH independent, so pH-dependent release profile may solely be due to polymer coating of RSPO and RLPO.

Results of dissolution profile of our study (B. No. 23) followed the following order in different buffer media: 4.5 pH acetate buffer> 6.8 pH phosphate buffer> pH 3.0 acid base buffer.

Similar observation of pH-dependent release profile with different buffer species have been made by other workers as well who had formulated drugs (with pH independent solubility) along with Eudragit RSPO and RLPO polymers. Following are some of the studies done:

Bodmeir et al (1996) studied the influence of Buffer species and strength on Diltiazem HCl release from beads coated with aqueous dispersions of Eudragit RS, RL 30D. They found that the drug release in different media was in the following order: pH 5.0 acetate > pH 3.5 formate > pH 7.4 phosphate buffer>0.1 M HCl.

Knop (1996) also studied the effect of Eudragit RS 30 D on theophylline coated pellets and found that drug release in the different media was in the following order: formate buffers>phosphate buffers>citrate buffers> all buffers containing chloride ions.

Wagner et al (2002) studied theophylline release from Eudragit RD 30D coated micro tablets and found that dissolution was in the following order with following anionic species: acetate media > succinic acid > disuccinate >sulfate> nitrate.

In order to explain the observed "pH-dependent" drug release behavior, emphasis was shifted from pH-considerations to the influence of the anionic buffer species present in the dissolution media. An ion exchange mechanism was used to explain the drug release from the coated beads. Eudragit RS and RL contain 33 and 66 mole of quaternary ammonium group per mole of polymer (Okar, 1982). The dissociation of these quaternary ammonium groups in aqueous media is responsible for the hydration and swelling of the polymer coating or films. The anionic counter ions of the quaternary ammonium groups are chloride ions. With ion exchange resins, ions are bound to an insoluble cross linked polymer resin carrying oppositely charged functional groups such as quaternary ammonium groups. The affinity of ions to ion exchange resins is characterized by the ion selectivity coefficient. Accordingly, the degree of hydration and swelling of the resins is affected by this interaction (Kunin, 1958; Helfferich, 1962) .Applying this concept to the present study, the chloride counter ions of the quaternary ammonium groups (QAG) in Eudragit RS/RL could be replaced by the buffer anions of the dissolution medium during dissolution studies. The degree of hydration and swelling and subsequently the drug release was governed by the interaction between the cationic groups and the counter ions.

Bodmeier et al. found that Eudragit RSPO and RLPO polymers can act as a strong basic anion exchanger, and drug release was discovered to be inversely proportional to the selectivity coefficient of the anion species toward the QAG (Bodmeier et al, 1996). The selectivity coefficients of the buffer anions for anion exchangers was found in following order: chloride > formate > acetate by Kunin, 1958 and Helfferich, 1962. A larger selectivity coefficient indicates a strong interaction between the fixed groups and the counterions and therefore a lesser degree of hydration and swelling; a slower drug release is expected. They found that dependent on the attraction of the anion toward the QAGs, a water flux was induced by back and forth exchanging anions. Strong attraction (nitrate, sulfate) resulted in a low water flux while weak attraction resulted in a high flux (acetate, succinic acid).

According to Wagner et al. (2002), monovalent anions comprising a small hydrodynamic radius (r_h) and, hence, a small hydrodynamic molar volume ($V_{h,m}$), should have an obstructive effect on the drug release through Eudragit RS 30 D membranes compared with anions with large hydrodynamic radius and volume, which displayed a release-enhancing effect.

Falkenhagen et al., 1960; Dorfner, 1991, concluded that the extent and rate of the ion exchange was however controlled by the excess of anions in the media according to the mass balance, the mean residence time of an individual anion before being replaced by another one of the same species should be in the same order of magnitude as the attraction of the anion toward the QAG or the selectivity coefficient. In other words, anions displaying a strong attraction toward the QAG were supposed to have a long residence time at the QAG before being replaced, while anions of weak interaction forces should result in short residence times. Anion-specific residence times, however, would indicate a dynamic process of anions going back and forth to the QAG. In other words the oscillation of anions around the QAG was inducing a water flux.

As discussed above, release profile in 0.01 N HCL and pH 3.0 acid- base buffer should have been similar, instead, a faster dissolution profile was observed in 0.01 N HCl as compared to pH 3.0 acid- base buffer. This may be attributed to higher osmotic pressure exerted on polymer coated films of Eudragit RSPO and RLPO by more of chloride ions in 0.01 N HCL as compared to pH 3.0 acid -base buffer. This would have resulted in higher exchange of chloride ions, higher swelling and subsequently higher dissolution in 0.01 N HCl as compared to pH 3.0 acid-base buffer.

Dissolution profile in Approach IA was more effected by buffer anion species as compared to Approach IB. Approach IA consisted of Eudragit RSPO : Eudragit RLPO ::8:2 whereas in Approach IB Eudragit RSPO : Eudragit RLPO ::95:0.5 i.e. Numbers of quaternary ammonium ions were less in Approach IB as compared to Approach IA.

In other words, higher the QAG density the more likely the exchanges of an anion from one QAG to another, enhancing the water flux.

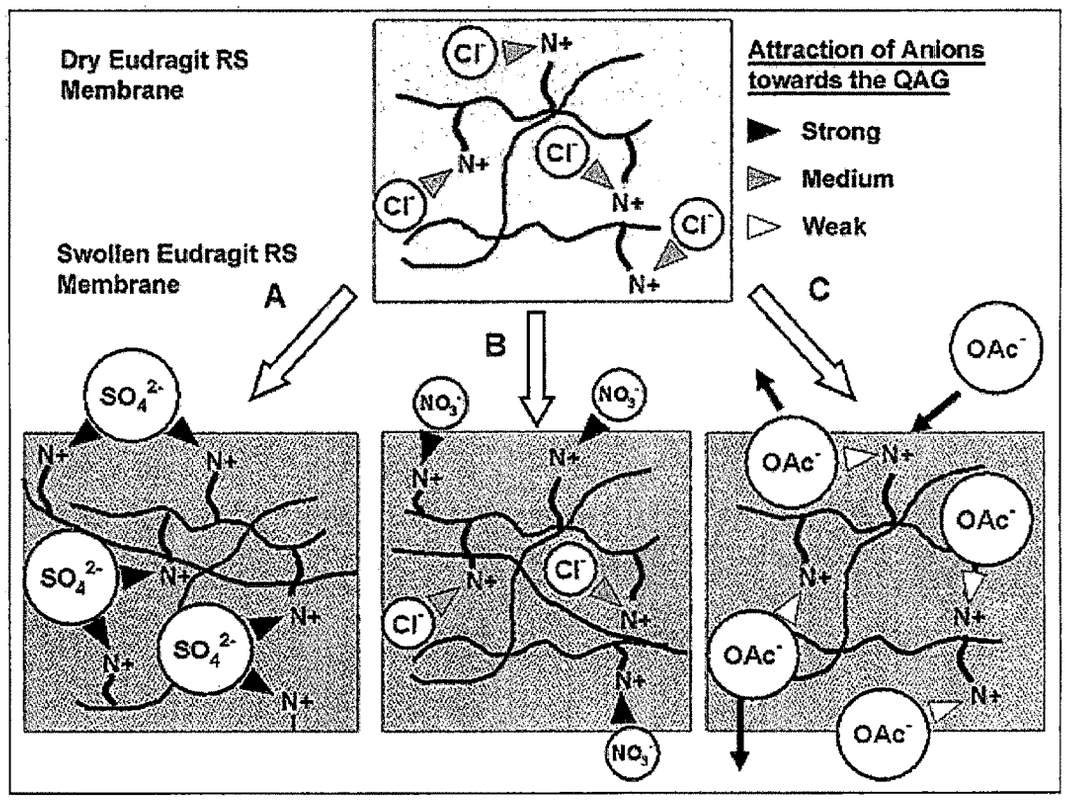


Figure V. 19 Scheme of interaction of Eudragit RS with various anion species (a = Cross linking: decelerated oscillation, hindered water flux, low permeability; b = Surface sealing: decelerated oscillation, hindered water flux, low permeability; c = "Active water carrier": permanent oscillation, induced water flux, high permeability).

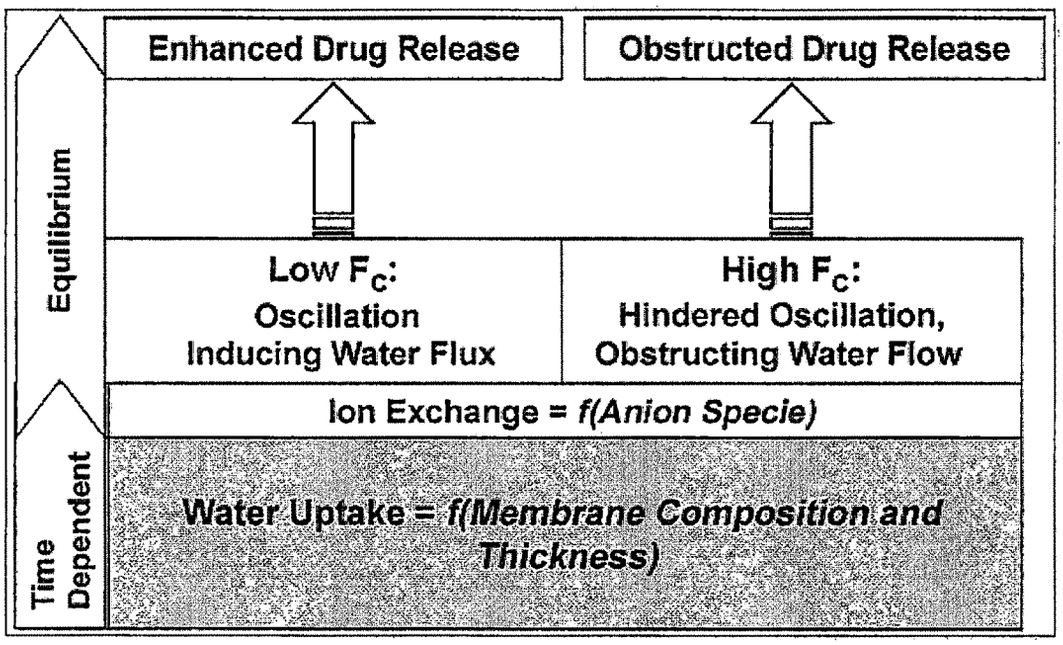


Figure V. 20 Drug release mechanism of Eudragit RS membranes (Fc = Coulomb force)

V.10 CONCLUSION

When non disintegrating dosage forms, like other indigestible solids, are administered in the fasting state, they typically are not retained in the stomach for over 2 hour due to the inter- digestive migratory motor complex (IMMC). On the other hand, in the fed stomach the gastric retention time (GRT) of non disintegrating dosage form depends mostly on the dosage form size as well as the composition and the caloric value of food; indigestible spheres smaller than 1 mm in diameter freely pass into the intestine, often at rates faster than solid nutritive food (Dressman et al, 1998). Spheres with diameters of 1–2.4 mm pass with the calorie-containing components of a solid meal (Hasler, 1995).

In general, the GRT of dosage forms and in particular large dosage forms is longer in the fed state in comparison to the fasting state. Large dosage forms are retropelled from the pyloric-antrum for further digestion and evacuation at the end of the fed state, or are retained until the arrival of the subsequent 'housekeeper wave'. In such cases, the GRT is a function of the length of the digestive process. Thus theoretically, continuous feeding can prolong GRT of the dosage form for more than 24 hour (Read et al, 1987).

Efforts were made to identify a cut-off size above which the dosage forms will be retained in the stomach for prolonged periods of times. Large dosage forms, such as 13 mm diameter non disintegrating tablets, were retained in the stomach for 171± 29 min, almost an hour more than 7-mm tablets, after a light breakfast of 360 kcal (Khosla et al, 1990).

The 'housekeeper wave' does not always completely clear the stomach from non disintegrating dosage forms (Wilding et al, 2000). For instance, a radiotelemetric capsule for pH measurements ('Heidelberg capsule', 25 x 8 mm, length x diameter) was randomly retained in the stomach of one healthy subject from a group of eight for over 12 hour. During that time three 'housekeeper waves' were recorded (Coupe et al, 1991).

Gardner et al (1985) studied the gastric residence time (GRT) of an orally administered, non digestible, pH-sensitive, radiotelemetric device (Heidelberg capsule) in 6 subjects. The GRT of the Heidelberg capsule was compared with the half-emptying time ($t_{1/2}$) of diethylene triamine penta acetic acid labeled with technetium 99m after a 4 ml/kg liquid fatty meal. They found that mean (+/-SD) GRT (4.3 +/- 1.4 h) was significantly (p less than 0.001) longer than the mean $t_{1/2}$ (1.1 +/- 0.3 h); the GRT was prolonged compared with the $t_{1/2}$ in each subject.

Similar results from gastric retention study were obtained in our study. But in in-vivo study, plasma concentrations were very low in both fasted and fed state. This was attributed due to lag time in drug release as shown by post bio studies in pH 3.0 acid-base buffer which was found to be bio-relevant discriminatory media.

It was also further concluded that Eudragit RSPO and RLPO, although are pH independent polymers but are effected by anions in the buffer media. So, a proper choice of media selection for in-vitro studies is critical in using these polymers for formulation

development. From the results of our study we also concluded that magnesium state is incompatible with Eudragit RSPO and RPLO and care should be taken while preparing films of these polymers with Magnesium stearate.

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