Chapter IV
Preformulation

IV.1 INTRODUCTION

Preformulation is the first step in rational development of dosage forms of drug substance. Preformulation testing is defined as investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms that can be mass-produced.

IV.2 MATERIALS & METHOD

IV.2.1 Active Pharmaceutical Ingredient Characterization

Alfuzosin Hydrochloride was characterized for the following tests:

IV.2.1.1 Description

Physical appearance of Alfuzosin Hydrochloride was observed.

IV.2.1.2 Solubility

Solubility study of Alfuzosin Hydrochloride was performed at 37 ± 0.5 °C over a wide range of pH from 2.2 to 7.7.Along with it; solubility study in various organic solvents was also done.

IV.2.1.3 Hygroscopicity (Moisture sensitivity)

Alfuzosin Hydrochloride was kept in open Petri-dish maintaining a thin layer and was exposed to 40°C/75% RH in controlled humidity chamber for 3 months and water by KF (Karl Fisher method) and related Impurities (RI) were monitored.

IV.2.1.4 Density

IV.2.1.4.1 Bulk Density (ρb)

It is a measure used to describe a packing of particles or granules. Bulk density is important to determine the size of the equipment needed for handling and processing.

The equation for determining bulk density (ρ_b) is
ρb = m/ vb
where, m = mass of the substance
vb = bulk volume

IV.2.1.4.2 Tap density (ρt)

In this method powder was filled in a measuring cylinder. After that it was mechanically tapped on device. After 500 taps the volume was measured and ensured that the volume does not vary by more than 2%.The sample was weighed & put in equation given below:

ρt = m/ vt
where, vt = tapped volume

IV.2.1.5 Compressibility Index

Compressibility is indirectly related to the relative flow rate, cohesiveness and particle size distribution of the powder. Powders with compressibility values lesser than about 20% had been found to exhibit good flow properties. Tapped (ρ2) and Apparent (ρ1) bulk density measurements can be used to estimate the compressibility of a material.

Carr's Compressibility index (%) = (ρ2- ρ1) / ρ2 X 100.

IV.2.1.6 Particle Size and Shape Analysis

Various physical and chemical properties of drug substance are affected by their particle size distribution and shape. In case of highly soluble drugs, the particle size does not affect the dissolution. In solid dosage forms, particle size can affect the content uniformity and tablet characteristics like porosity and flow ability

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The particle size distribution of Alfuzosin Hydrochloride was determined by Malvern Analyzer (Malvern Mastersizer, MSS) and Microscopy was carried out by dispersing small amount of drug in liquid paraffin. Small amount of the dispersion was taken on slide. The dispersion was observed under microscope at 10x, 20 x and 50 x Magnification.

IV.2.1.7 Impurities in Alfuzosin Hydrochloride

Impurity profile of Alfuzosin Hydrochloride was evaluated same as method given in Ph.Eur monograph.

IV.2.1.8 Thermal analysis

The thermal analysis of Alfuzosin Hydrochloride (ALF/D450/IVB/41) was carried out on Mettler (DSC822e) Differential Scanning Calorimeter. The thermogram was recorded under nitrogen atmosphere at a heating rate of 100°C/min.

IV.2.1.9 FT-IR study

The FT-IR spectrum of Alfuzosin Hydrochloride (ALF/D450/IVB/41) was recorded by Bruker Tensor 27 FT-IR as dispersive IR (KBR + Powder).

IV.2.1.10 X-ray powder diffraction

The X-ray powder diffraction pattern of Alfuzosin Hydrochloride was recorded by PANanytical X'Pert PRO. Copper $K\alpha_1$ radiation was used (λ 1.5406) and the sample was scanned between 2-40 degree 2θ.

IV.2.2 Drug –Excipients compatibility Study

The importance of extensive excipient/drug substance compatibility studies is a topic of raging debate among preformulation scientists. Some excipients can be ruled out based on their chemical nature and an obvious incompatibility with the active pharmaceutical ingredient (API) of interest. Traditionally, formal excipient compatibility studies involve the preparation of known mixtures of drug and excipients. These binary mixtures are then stored under accelerated conditions and analyzed at specified times.

Table IV. 1 List of Alfuzosin Hydrochloride and excipients used for Compatibility study along with Manufacturers

S. No	Name of Excipient	Manufactured by
1.	Alfuzosin Hydrochloride	TRC, TPL
2.	Lactose Anhydrous (DCL 21)	DMV International
3.	Microcrystalline cellulose PH 102	FMC Biopolymers
4.	Dibasic calcium phosphate	Rhodia
5.	Mannitol (Pearlitol SD 200)	Roquette
6.	Pregelatinized starch	Roquette
7.	Maize starch	Roquette
8.	Polyvinyl Pyrrolidone K-30	BASF
9.	Talc	Pharmalinks
10.	Colloidal Silicon dioxide	Cabot Corporation
11.	Magnesium Stearate	Ferro, Belgium
12.	Eudragit RS PO	Rohm -Gmbh
13.	Eudragit RL PO	Rohm -Gmbh
14.	Hypromellose 2208 (K100MCR)	Dow
15.	Hydrogenated Castor Oil (Cutina HR PH)	Cognis
16.	Ethylcellulose 50cps	Dow
17.	Carbopol 971G	Noveon
18.	Hydroxypropyl Cellulose	Hercules
19.	Noveon AA1	Noveon
20.	Sodium Carboxy methyl cellulose	Hundae
21.	Polyethylene Oxide N-80	Dow
22.	Polyethylene Oxide N-10	Dow

Ratio of Alfuzosin Hydrochloride Ph.Eur. and excipients were decided based on the functional category of the excipient and Alfuzosin Hydrochloride Ph.Eur. potency in the dosage form. The required quantity of Alfuzosin Hydrochloride Ph.Eur. and the excipient were weighed and sifted through appropriate sieve and transferred to the flint glass vials. The vials were closed with previously washed and sterilized bromo butyl rubber stoppers and then sealed with aluminum cap.

The vials were kept under following storage conditions:

1. At 60 ± 2 ° C in open vials and

2. At 40 ± 2 °C and 75 ± 5 % RH condition both open and closed vials were kept but only open vials were analyzed.

3. Alfuzosin Hydrochloride, blends and the individual excipients were autoclaved at 121°C for 15 minutes and analyzed for physical and chemical tests.

4. Individual API, excipients and blend were kept as control under 2-8° C.

IV.2.3 Dissolution profile of Marketed preparation

Dissolution profile of Marketed formulation (Alfuzosin Hydrochloride Extended Release tablet 10 mg) was done in different media. Four media were selected for comparison of release profile based on the solubility and pharmacokinetics of the drug which are as follows:

1. 0.01 N HCl
2. pH 3.0 Acid- base buffer
3. pH 4.5 Acetate Buffer
4. pH 6.8 Phosphate buffer.

All the buffers were prepared as USP method and all reagent used were analytical reagent (AR) grade and solvents used were of HPLC grade.

IV.2.4 Permeability study in rat intestine

IV.2.4.1 Animals

Male Wistar rats weighing 275-325 g were used for the study. Permission for the present study was obtained from the Institutional Animal Ethics Committee. Rats were housed in cage at constant temperature (22 ± 2 °C) with free access to drinking water and food ad libitum. Rats were fasted for 24 hours prior to the start of the experiment with free access to 4 % dextrose solution.

IV.2.4.2 Perfusion Solution

Table IV. 2 Composition of Kreb's Ringer Bicarbonate Solution. (www.Sigmaaldrich.com)

Sr.no	Ingredients	MW	Gm/L	mmoles
1.	NaCl	58.5	7.0	120
2.	D-Glucose	180	1.8	10
3.	KCl	74.5	0.34	4.5
4.	MgCl2	95	0.0468	0.49
5.	Na2HPO4	142	0.18	1.8
6.	NaH2PO4	120	0.1	0.8

Kreb's Ringer Bicarbonate solution was used for intestinal perfusion and adjusted to pH 6.8 using 0.1 N HCl or 0.1 N NaOH.

IV.2.4.3 Drug Solution

Drug was dissolved in milli-Q water. Main stock solutions were prepared as 1mg/mL for both drug and phenol red. From the main stock solution, final drug perfusion solution was prepared as 20 µg/mL drug (for single-pass Intestinal Perfusion); 50 µg/mL drug (for closed loop method) and 5 µg/mL phenol red in Kreb's Ringer Bicarbonate Solution (pH=6.8). The final pH was adjusted to 6.8 (Drug Stock-DS) using 0.1 N HCl or 0.1 N NaOH and analyzed.

IV.2.4.4 Single-pass Intestinal Perfusion (SPIP) Technique in rats

In situ permeation studies were performed using established methods adapted from the literature (Doluisio et al., 1969; Mart'ın-Villodre et al., 1986; Sinko et al., 1995; Svensson et al., 1999). After fasting for 24 hrs, rats were anesthetized with an intraperitoneal (i.p) injection of urethane (1.25 g/kg). Upon verification of loss of pain reflex, laparotomy (midline abdominal incision) was performed. Small intestine was taken out of the peritoneal cavity on saline soaked cotton placed on the abdomen. Care was taken to avoid disturbance of blood and lymphatic circulation. In order to prevent enterohepatic recycling and the presence of bile salts in lumen, a small incision was made in the intestine at the angle of treitz (~3 cm after stomach) after tying the small blood vessels supply. A cannula was inserted and other part of cannula was attached to the peristaltic pump. A cut was made at the ileocaecal junction for collecting the perfusate. The animal was maintained at 37°C throughout the experiment by keeping it on the homoeothermic blanket. The exposed segment was covered with a cotton pad soaked in perfusion solution and then with aluminum foil to prevent evaporation of fluids. Initially, the intestinal segment was rinsed with kreb's Ringer Bicarbonate solution (37°C) until the outlet solution was clear (30 mins).The perfused kreb's solution was collected (Control Solution-CS). Test solution was prepared immediately before use by dissolving a fixed amount of Alfuzosin Hydrochloride in the vehicle solution (w/v). After dissolving the xenobiotic, the pH of the solution was checked and readjusted using 0.1 N HCl or 0.1 N NaOH when necessary. Thereafter, drug solution was perfused at a constant flow rate of 0.2 ml/min using a peristaltic pump. After 20 minutes, the intestinal perfusate (Test Sample-TS) were collected at 10 minute intervals for duration of 100 minutes. Samples were frozen immediately and stored at -70 °C until analysis. Finally, the length of intestinal segment was measured without stretching. At the end of the experiment, samples were collected from tubing of peristaltic pump at the flow rate of 0.2 ml/min to detect any adsorption in the tube (Adsorption sample-AS). Rats were euthanisized.

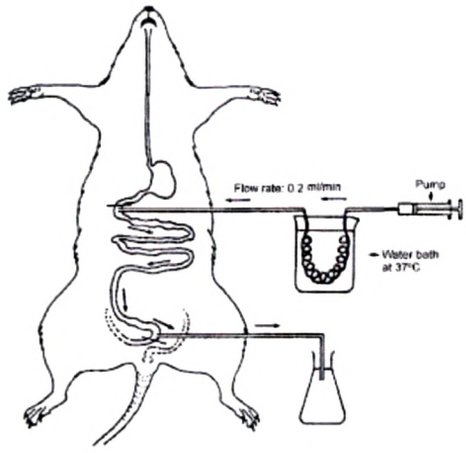


Figure IV. 1 Schematic diagram of Rat intestinal Perfusion Study

Figure IV. 2 Photograph of Single Pass Intestinal Perfusion in rat intestine

IV.2.4.4.1 Net Water Flux (NWF) Determination

Water reabsorption was characterized as an apparent zero-order process (Mart´ın-Villodre et al., 1986). Because water absorption and secretion during the perfusion may cause errors in the calculated effective permeability (Peff) values, a non-absorbable marker to correct water flux through the intestinal wall is needed (Crouthamel et al, 1983). For this purpose phenol red, as a non-absorbable marker, which was introduced in 1923(Gorham, 1923) was co-perfused with drug.

The absorption or secretion of water during the experiment was studied by correcting for change in phenol red area under curve (AUC).

Calculation of NWF (µl/h/cm) of the segment was done from intestinal perfusate samples collected over 10 to 100 minutes, the time required for achievement of steady state in peak area of phenol red. NWF values were determined by equation:

$$NWF = \frac{(1 - AUC\text{-}PRin / AUC\text{-}PRout)\, Qin}{L} \quad \text{---------- (1)}$$

where, AUC-PRin = AUC of Phenol Red of inlet solution

AUC-PRout = AUC of Phenol Red of outlet solution

Qin = Flow rate of perfusion solution entering the intestinal segment.

L = Length of perfused segment

A negative net water flux indicates loss of fluid from the mucosal side (lumen) to the serosal side (blood). A positive net water flux indicates secretion of fluid into the segment (Fagerholm et al., 1996).

IV.2.4.4.2 Drug Permeability Determination

Calculation of Peff was done from intestinal perfusate samples collected over 10 to 100 minutes. The Peff of drugs was calculated using equation according to the parallel tube model (Komiya et al., 1980, Levitt et al., 1988):

$$Peff = \frac{[-Qin.\ \ln (AUCout(corr)/AUCin)]}{A} \quad \text{---------- (2)}$$

where, AUCin = AUC (µg/mL) of drug in the entering solution,
AUCout(corr) = fluid transport corrected outlet AUC (µg/mL) of drug

$$AUCout(corr) = \frac{AUCout \,.AUC\text{-}PRin}{AUC\text{-}PRout}$$

A = mass transfer surface area (cm^2) within the intestinal segment assumed to be the area of cylinder ($2\pi rL$) with the length (L) measured after 100 mins and radius (r) of 0.18 cm (Kararli et al., 1995, Komiya et al., 1980).

It has been demonstrated that in humans at a Qin of 2-3 ml/min, Peff is membrane controlled. In the rat model the Qin is scaled to 0.2 ml/min, since the radius of the rat intestine is about 10 times less than that of human (Fagerholm et al., 1996).

During the experiment the effluent flow rate was stabilized after the first 3-4 fractions with the earlier fractions containing saline solution reminiscent from cleaning the intestinal lumen. Steady-state concentrations of phenol-red in effluents were achieved within 30 minutes at a perfusion flow rate of 0.2 ml/min .The steady –state values of the effective permeability coefficient of phenol red throughout the rat perfusion experiment were stable and showed low variability, indicating that the intestinal epithelial cells in the rat model possess normal mucosal transport mechanism and structural integrity. Based on these observations, we considered our in–situ perfusion model standardized for investigations of drug absorption.

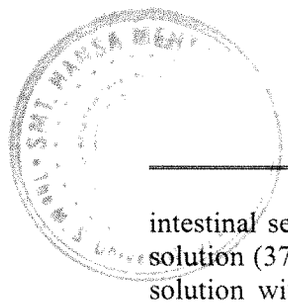
To have an average value of effective permeability, perfusate was collected for at least 100 minutes. Ratio of AUC for stock solution (SS) and adsorption sample (AS) were close to 1 indicating, stability of drug solution in Krebs ringer solution with no significant changes in inlet concentrations and no adsorption/absorption of the drug and phenol red to the polyethylene tube. Chromatograms of control samples (CS) depicted no peak for respective drug, indicating no contamination of Kreb's Ringer solution. Analysis of drug was not affected, as the retention time for Kreb's Ringer solution was different from the drug solution. Retention time for inlet solution (SS) and outlet solution (TS) was found to be same, indicating that unabsorbed drug remains unchanged in the outlet.

NWF was determined for observing water secretion and water absorption using equation-1 from Fagerholm et al, 1996. A negative net water flux indicates loss of fluid from the mucosal side (lumen) to the serosal side (blood). A positive net water flux indicates secretion of fluid into the segment (Fagerholm, et al., 1996). Standard deviation (STDEV) and Covariance (% CV) was determined to check the variability between the experiments calculated effective permeability was expressed as cm/sec x 10^{-4}. Steady state equilibrium was assumed to be attained when phenol red AUC became constant and average of P_{eff} values for that time duration is considered as final effective permeability of the drug.

IV.2.4.5 Close intestinal loop method in rats

Close intestinal loop method was done as described by Naruhashi et al, 2002 with slight modification.

After fasting for 24 hrs, rats were anesthetized with an intraperitoneal (i.p) injection of urethane (1.25 g/kg). In order to prevent enterohepatic recycling and the presence of bile salts in lumen, a small incision was made in the intestine at the angle of treitz (~3 cm after stomach) after tying the small blood vessels supplied. Eight-centimeter closed loops each of duodenum and ileum were prepared by ligation at both ends. A cannula was inserted and other part of cannula was attached to the peristaltic pump. Cuts were made at the duodenum-jejunum and ileo-caecal junction for collecting the perfusate. The animal was maintained at 37°C throughout the experiment by keeping it on the homoeothermic blanket. The exposed segment was covered with a cotton pad soaked in perfusion solution and then with aluminum foil to prevent evaporation of fluids. Initially, the



intestinal segments (duodenum and ileum) were rinsed with kreb's Ringer Bicarbonate solution (37°C) until the outlet solution was clear (10 mins) and expelling the remaining solution with air pumped with a syringe. The perfused kreb's solution was collected (Control Solution-CS). Test solution was prepared immediately before use by dissolving a fixed amount of Alfuzosin Hydrochloride in the vehicle solution (w/v). After dissolving the xenobiotic, the pH of the solution was checked and readjusted using 0.1 N H Cl or 0.1 N Na OH when necessary.

Eight hundred microliters of Test solutions, including phenol red was administered into the loops as a bolus. The animal was kept on a warm plate at 37°C. After 15 min, the solution in the loop was collected and the loop was rinsed with kreb's solution to give a total volume of 3 ml. The concentration of Alfuzosin and phenol red in the effluent was measured by high-pressure liquid chromatography (HPLC) to estimate the remaining amount of Alfuzosin. The fraction of absorbed Alfuzosin was estimated from the difference between the dose administered initially and the amount remaining in the loops after 15 min.

IV.2.5 Analytical Methods

All the samples were analyzed for drug and phenol red AUC using validated high-performance liquid chromatography (HPLC).

Column: BDS, Hypersil (C8/C18), 150/250 X 4.6 mm, 5 µ particle size

Mobile phase: Ammonium acetate (10-50 mm)/KH2PO4 (20mm)/NaH2PO4 (10-50 mm):
Acetonitrile/Methanol

Flow rate: 1 mL/min

Retention time: 3-7 min

Injection volume: 40 µl Partial loop

Phenol red was detected at 438 nm

IV.3 RESULTS

IV.3.1 Description

Alfuzosin Hydrochloride is a white to off white powder.

IV.3.2 Active Pharmaceutical Characterization

IV.3.2.1 Solubility

Table IV. 3 Solubility study of Alfuzosin Hydrochloride at various pH at 37 ± 0.5 °C

pH	Solubility in mg/ml		Descriptive Term
	Literature value (Centre for Drug Evaluation and Research, 2003)	Experimental values	
2.2	176	167	Freely soluble
5.9	172	200	Freely soluble
6.6	235	111	Freely soluble
7.2	243	100	Freely soluble
7.22	234	NP	Freely soluble
7.37	179	NP	Freely soluble
7.7	109	125	Freely soluble

NP – Not performed.

Alfuzosin Hydrochloride was freely soluble in pH range from 2.2 to 7.7 and hence it was concluded as a highly soluble drug.

Table IV. 4 Solubility of Alfuzosin Hydrochloride in various solvents at ambient temperature

S.No.	Solvent	Solubility per gram of Alfuzosin Hydrochloride	Solubility Description
01	Methanol	~16 ml	Soluble
02	Ethanol	~746 ml	Slightly soluble
03	2- propanol	> 10,000 ml	Practically insoluble or insoluble
04	Acetonitrile	> 10,000 ml	Practically insoluble or insoluble
05	Ethyl acetate	> 10,000 ml	Practically insoluble or insoluble
06	Acetone	> 10,000 ml	Practically insoluble or insoluble
07	Methylene chloride	~ 8 000 ml	Very slightly soluble
08	Methanol: Methylene chloride (8:2)	~6 ml	Freely soluble
09	MeOH: Acetone (6:4)	~about 32 ml	Sparingly soluble
10	MeOH :IPA (6:4)	~66 ml	Sparingly soluble
11	Acetone :IPA (4:6)	~5.350 ml	Very slightly soluble

Alfuzosin Hydrochloride was found to be have good solubility in Methanol: Methylene Chloride (8:2) combination and in methanol. So for drug coating purpose, these solvents may be employed.

IV.3.2.2 Hygroscopicity (Moisture sensitivity)

Table IV. 5 Water by KF and Related impurities (RI) of Alfuzosin Hydrochloride kept in open at 40°C/75% RH

% Humidity	Water by KF (% w/w)	RI (%)	
		Single max	Total
Initial	0.8	0.01	0.01
After 3 Month at 75% RH /40°C	13.4	0.02	0.02

From above data it was concluded that Alfuzosin HCl picks up moisture when exposed to higher moisture conditions but it does not cause degradation as observed by related impurities.

IV.3.2.3 Bulk Density and Tap Density

The bulk density and tap density of Alfuzosin Hydrochloride API is presented in the following table.

Table IV. 6 Bulk Density and Tap Density of Alfuzosin Hydrochloride API

Batch No.	ALFU0500402	ALFU0500302
Bulk Density (g/cc)	0.21	0.20
Tapped Density (g/cc)	0.37	0.35

IV.3.2.4 Compressibility Index

Table IV. 7 Data of Carr's index of Alfuzosin Hydrochloride API

Batch No.	ALFU0500402	ALFU0500302
Carr's compressibility Index	43.2%	42.9%

From the compressibility index of above lots it was evident that Alfuzosin Hydrochloride exhibited poor flow properties and is not an ideal candidate for Direct Compression or there is a need for addition of a diluent with good flow properties and compressibility.

IV.3.2.5 Particle Size and Shape Analysis

Table IV. 8 Particle Size Distribution and Shape of Alfuzosin Hydrochloride

Alfuzosin Hydrochloride	Particle Size in µm (% of particles under size)			Particle shape
Lot No.	d10	d50	d90	
ALFU0500402	6.33	17.08	34.30	Irregular crystals
ALFU0500302	8.10	18.98	35.30	Irregular crystals

From above data it was observed that Alfuzosin Hydrochloride in all above batches exists as fine grade crystalline powder.

IV.3.2.6 Impurities in Alfuzosin Hydrochloride API

The results are given in table IV.9.

Table IV. 9 Impurity profile of Alfuzosin Hydrochloride

B No.	ALFU0500402	ALFU0500302
Impurity A	ND	ND
Impurity B	ND	ND
Impurity C	ND	ND
Impurity D	ND	ND
Impurity E	Below LOD	ND
Impurity F	ND	ND
Unknown Impurity	ND	ND
Total Impurity	0.03	0.02

Impurity profiling of the two batches indicate that there wasn't any significant impurity variation from batch to batch and all the known and unknown impurities were nearly in Not detected (ND) amount.

IV.3.2.7 Thermal analysis

The thermogram exhibited one endotherm at the peak temperature of 239.80^{0}C with onset temperature of 236.97^{0}C.

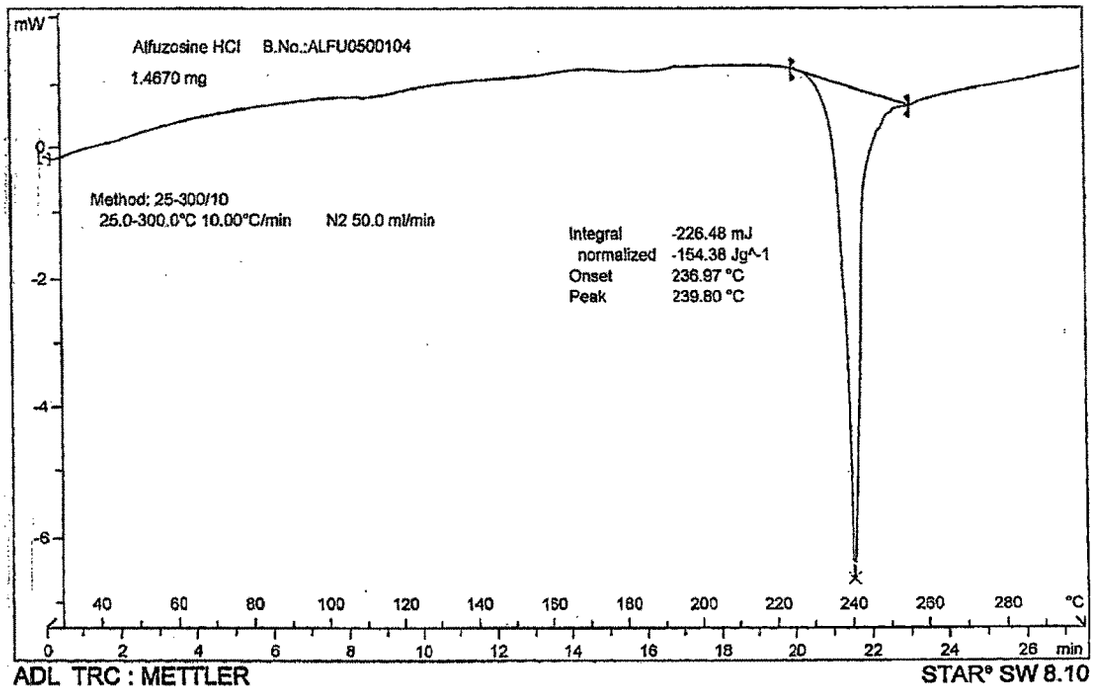


Figure IV. 3 DSC thermogram of Alfuzosin Hydrochloride

IV.3.2.8 FT-IR study

The assignments are given in the table IV.10.

Table IV. 10 Data of FT-IR of Alfuzosin Hydrochloride

Wave number (cm^{-1})	Assignment	Mode of vibration
3371,3141	N-H	Stretching
2937	Aliphatic C-H	Stretching
1658	-C = O	Stretching
1532	-N -- H (amide)	Bending
1601,1494	Aromatic –C = C	Stretching
1065	-C-O-C	Stretching
872	Aromatic –C-H	Bending

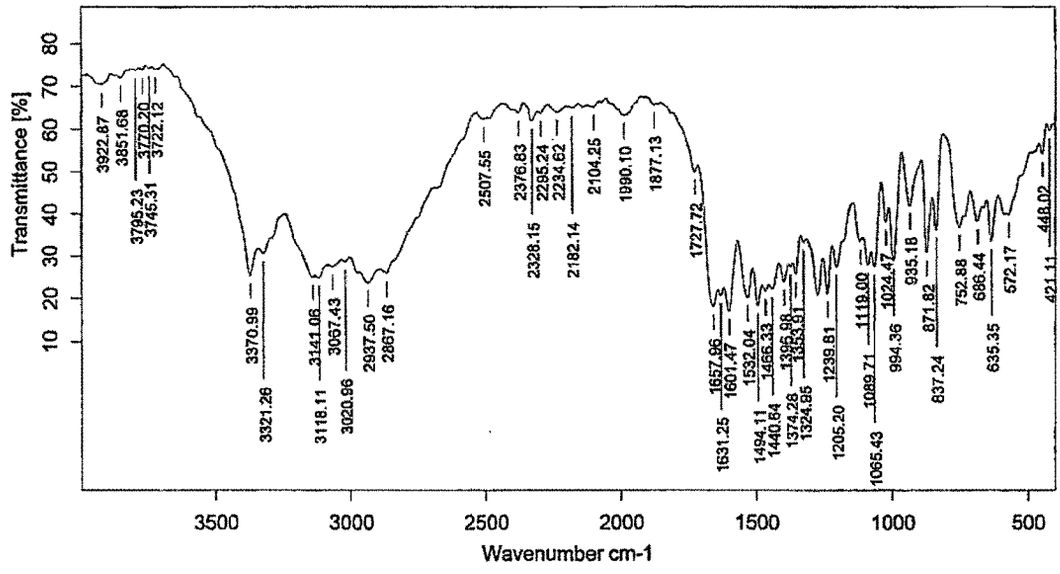


Figure IV. 4 FT-IR of Alfuzosin Hydrochloride

IV.3.2.9 X-ray powder diffraction

The results from X-ray diffraction pattern listed in the table IV.11.

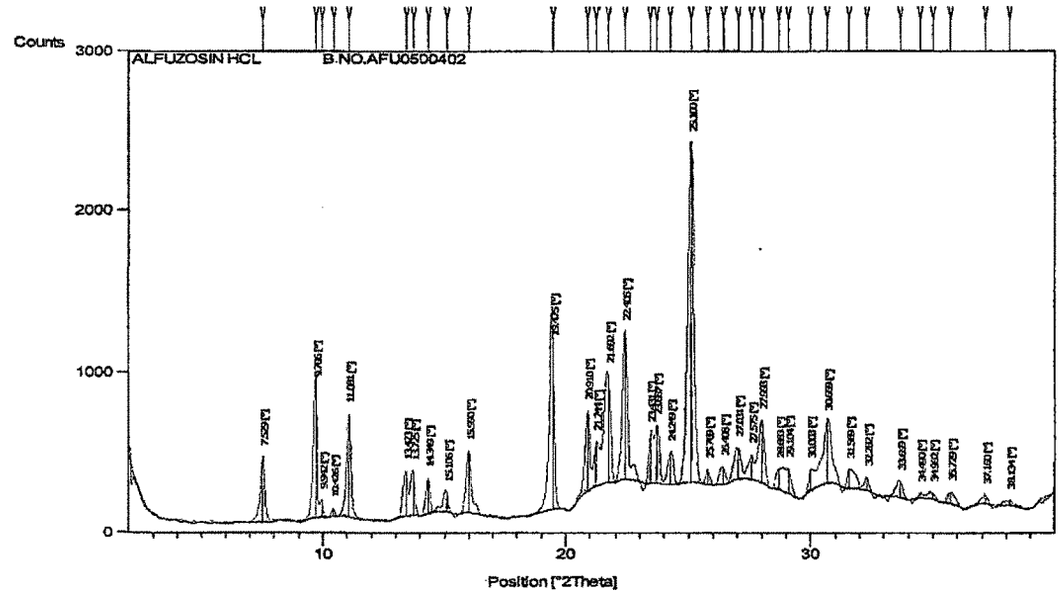


Figure IV. 5 X-Ray Diffractogram of Alfuzosin Hydrochloride

Table IV. 11 Data if X-Ray powder diffraction of Alfuzosin Hydrochloride

Pos. [°2 Th.]	d-spacing [A°]	Relative Intensity (%)
7.5292	11.74182	19.17
9.7055	9.11324	38.30
9.9421	8.89693	4.87
10.4257	8.48524	2.14
11.0805	7.98523	30.18
13.4225	6.59676	13.01
13.7249	6.45209	12.45
14.3491	6.17279	9.89
15.1060	5.86517	5.39
15.9898	5.54291	18.03
19.4747	4.55819	47.75
20.9100	4.24844	23.50
21.2443	4.18234	12.77
21.6916	4.09710	31.85
22.4055	3.96815	43.43
23.4305	3.79682	14.58
23.6870	3.75629	16.90
24.2489	3.67050	9.37
25.1002	3.54791	100.00
25.7691	3.45732	4.07
26.4081	3.37508	5.02
27.0308	3.29874	8.94
27.5753	3.23483	7.19
27.9932	3.18747	18.22
28.6835	3.11232	5.68
29.1037	3.06833	6.91
30.0028	2.97840	5.87
30.6587	2.91616	18.08
31.5689	2.83413	5.30
32.2824	2.77310	3.79
33.6594	2.66274	4.59
34.4905	2.60046	1.37
34.9817	2.56506	1.70
35.7286	2.51313	3.17
37.1600	2.41955	2.13
38.1336	2.35999	1.18

IV.3.2.10 TGA

A sharp decrease in weight loss of 3.0398% was observed at 200.021 °C.

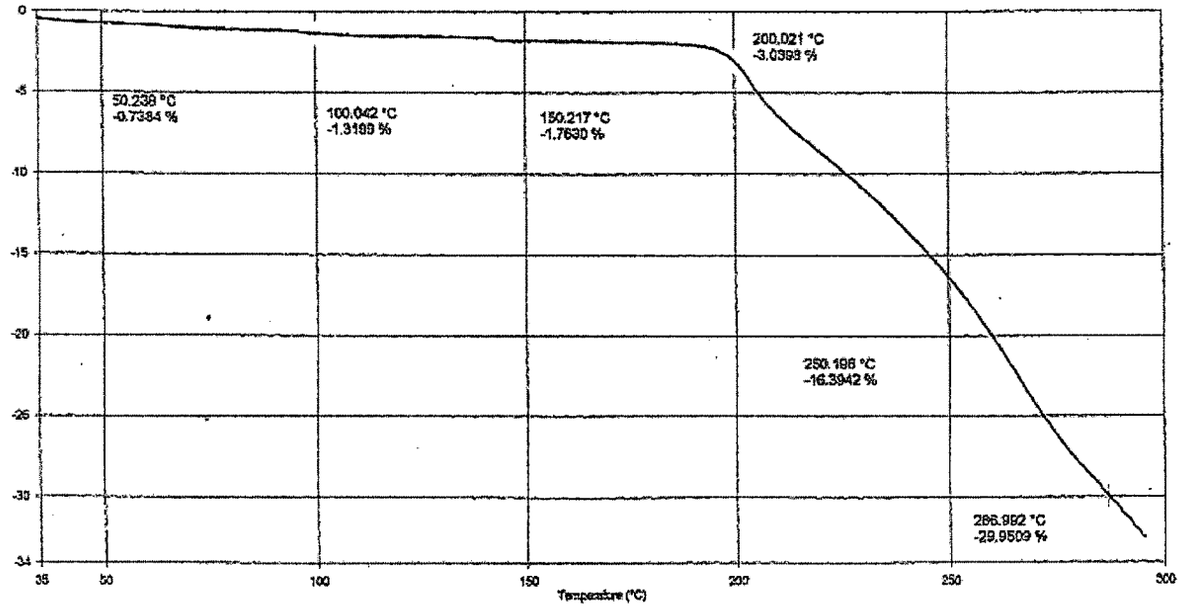


Figure IV. 6 TGA graph of Alfuzoisn Hydrochloride

IV.3.3 Drug –Excipients compatibility Study

Table IV. 12 Alfuzosin HCl -Excipients Chemical compatibility study (Autoclave)

S.NO	Name of the sample	API: Excp Ratio	Initial		Autoclaved	
			Related Impurities		Related Impurities	
			Single Max	Total	Single Max	Total
1	Alfuzosin HCL	-	0.010	0.010	0.010	0.030
2	Lactose anhydrous(DCI 21)	1:10	0.003 (IMP.E)	0.003	0.010	0.030
3	Microcrystalline Cellulose (Avicel PH 102)	1:10	0.003 (IMP.E)	0.003	0.010	0.030
4	Maize Starch	1:10	ND	ND	0.010(IMP.E)	0.040
5	Povidone K-30	1:4	0.010 (IMP.E)	0.010	0.030(IMP.E)	0.090
6	Talc	1:1	0.010 (IMP.E)	0.010	0.010(IMP.E)	0.030
7	Colloidal Silicon Dioxide	1:0.5	ND	ND	0.160	0.300
8	Magnesium .Stearate	1:1	0.005	0.010	0.020(IMP.E)	0.060
9	Eudraigit RS PO	1:2	0.010	0.010	0.010(IMP.E)	0.040
10	Eudragit RL PO	1:1	0.010	0.010	0.020	0.050
11	Dibasic calcium phosphate	1:10	0.010(IMP.E)	0.010	0.180	0.300
12	Mannitol (Pearlitol SD 200)	1:10	0.010(IMP.E)	0.010	0.010(IMP.E)	0.030
13	Hypromellose 2208 (K4M) CR premium	1:10	0.010(IMP.E)	0.010	0.020	0.040
14	Hydrogenated Castor oil	1:10	0.030	0.040	0.020(IMP.E)	0.050
15	Ethyl cellulose 10 cps	1:5	ND	ND	0.010(IMP.E)	0.040
16	Carbopol 971 G	1:10	ND	ND	0.020(IMP.E)	0.020
17	Hydroxy propyl cellulose	1:10	ND	ND	0.030(IMP.E)	0.080
18	Noveon AA1	1:10	0.010	0.020	0.020(IMP.E)	0.050
19	Sodium carboxy methyl cellulose	1:10	ND	ND	0.010	0.040
20	Polyethylene Oxide N-80	1:1	0.020(IMP.E)	0.030	0.040	0.050
21	Polyethylene Oxide N-10	1:1	0.010(IMP.D)	0.020	0.020	0.040

Table IV. 13 Alfuzosin HCl -Excipients Chemical compatibility study (60° C-Open)

S.NO	Name of the sample	API:Excp Ratio	Initial		15 days		1 Month	
			Related Impurities		Related Impurities		Related Impurities	
			Single Max	Total	Single Max	Total	Single Max	Total
1	Alfuzosin HCL	-	0.010	0.010	0.004(IMP.E)	0.01	0.01 (IMP E)	0.02
2	Lactose anhydrous(DCI 21)	1:10	0.003(IMP.E)	0.003	0.02(IMP.E)	0.02	0.02 (IMP.D)	0.04
3	Microcrystalline Cellulose (Avicel PH 102)	1:10	0.003(IMP.E)	0.003	0.01(IMP.E)	0.02	0.01 (IMP E)	0.02
4	Maize Starch	1:10	ND	ND	0.01(IMP.E)	0.02	0.01	0.030
5	Povidone K-30	1:4	0.010(IMP.E)	0.010	0.03(IMP.E)	0.06	0.04 (IMP.E)	0.09
6	Talc	1:1	0.010(IMP.E)	0.010	0.01(IMP.E)	0.03	0.01 (IMP E)	0.02
7	Colloidal Silicon Dioxide	1:0.5	ND	ND	0.02(IMP.E)	0.04	0.03 (IMP.E)	0.08
8	Magnesium Stearate	1:1	0.005	0.010	0.01(IMP.E)	0.02	0.01	0.03
9	Eudraigit RS PO	1:2	0.010	0.010	0.02(IMP.E)	0.03	0.02 (IMP.E)	0.03
10	Eudragit RL PO	1:1	0.010	0.010	0.01(IMP.E)	0.02	0.02 (IMP.E)	0.02
11	Dibasic calcium phosphate	1:10	0.010(IMP.E)	0.010	0.03(IMP.E)	0.07	0.03 (IMP.D)	0.06
12	Mannitol (Pearlitol SD 200)	1:10	0.010(IMP.E)	0.010	0.02(IMP.E)	0.04	0.02 (IMP E)	0.05
13	Hypromellose 2208 (K4M) CR premium	1:10	0.010(IMP.E)	0.010	0.01(IMP.E)	0.02	0.02 (IMP.E)	0.02
14	Hydrogenated Castor oil	1:10	0.030	0.040	0.02(IMP.E)	0.03	0.02 (IMP.E)	0.05
15	Ethyl cellulose 10 cps	1:5	ND	ND	0.01(IMP.E)	0.02	0.01 (IMP E)	0.02
16	Carbopol 971 G	1:10	ND	ND	0.02 (IMP.D)	0.02	0.05	0.06
17	Hydroxy propyl cellulose	1:10	ND	ND	0.03(IMP.E)	0.05	0.03 (IMP.E)	0.07
18	Noveon AA1	1:10	0.010	0.020	0.02(IMP.E)	0.06	0.07	0.14
19	Sodium carboxxy methyl cellulose	1:10	ND	ND	0.02	0.04	0.03	0.05
20	Polyethylene Oxide N-80	1:1	0.020(IMP.E)	0.03	NP	NP	0.02	0.07
21	Polyethylene Oxide N-10	1:1	0.010(IMP.D)	0.02	NP	NP	0.04	0.08

NP= Not performed

Table IV. 14 Alfuzosin HCl -Excipients Chemical compatibility study (40°C/75 % RH-Open)

S.NO	Name of the sample	API:Excp Ratio	Initial		15 Days		1month		3 month	
			Related Impurities		Related Impurities		Related Impurities		Related Impurities	
			Single Max	Total	Single Max	Total	Single Max	Total	Single Max	Total
1	Alfuzosin HCL	-	0.01	0.01	0.01(IMP.E)	0.01	0.01 (IMP E)	0.02	0.02 (IMP.E)	0.02
2	Lactose anhydrous(DCl 21)	1:10	0.003(IMP.E)	0.003	0.02(IMP.E)	0.03	0.02 (IMP.E)	0.04	0.05 (IMP. E)	0.07
3	Microcrystalline Cellulose (Avicel PH 102)	1:10	0.003(IMP.E)	0.003	0.01(IMP.E)	0.02	0.02 (IMP.E)	0.03	0.03 (IMP.E)	0.05
4	Maize Starch	1:10	ND	ND	0.01(IMP.E)	0.01	0.01 (IMP E)	0.02	0.01 (IMP E)	0.01
5	Povidone K-30	1:4	0.01(IMP.E)	0.01	0.06	0.15	0.08	0.23	0.08 (IMP.D)	0.25
6	Talc	1:1	0.01(IMP.E)	0.01	0.01(IMP.E)	0.01	0.01 (IMP E)	0.02	0.01 (IMP E)	0.01
7	Colloidal Silicon Dioxide	1:0.5	ND	ND	0.01	0.03	0.02 (IMP.D)	0.05	0.03 (IMP.D)	0.07
8	Magnesium .Stearate	1:1	0.005	0.01	0.01(IMP.E)	0.02	0.01 (IMP.D)	0.02	0.02 (IMP.E)	0.03
9	Eudraigit RS PO	1:2	0.01	0.01	0.02	0.03	0.02 (IMP.E)	0.03	0.02 (IMP.E)	0.01
10	Eudragit RL PO	1:1	0.01	0.01	0.02(IMP.E)	0.03	0.02	0.07	0.03 (IMP.E)	0.08
11	Dibasic calcium phosphate	1:10	0.01(IMP.E)	0.01	0.02(IMP.E)	0.03	0.02 (IMP.D)	0.04	0.02 (IMP.D)	0.06
12	Mannitol (Pearlitol SD 200)	1:10	0.01(IMP.E)	0.01	0.02(IMP.E)	0.02	0.01 (IMP E)	0.03	0.02 (IMP.E)	0.05
13	Hypromellose 2208 (K4M) CR premium	1:10	0.01(IMP.E)	0.01	0.01(IMP.E)	0.02	0.02 (IMP.E)	0.02	0.02 (IMP.E)	0.03
14	Hydrogenated Castor oil	1:10	0.01	0.03	0.02	0.03	0.01 (IMP E)	0.03	0.03 (IMP.E)	0.05
15	Ethyl cellulose 10 cps	1:5	ND	ND	0.01(IMP.E)	0.04	0.02 (IMP.E)	0.04	0.02 (IMP.E)	0.03
16	Carbopol 971 G	1:10	ND	ND	0.06 (IMP.D)	0.07	0.08 (IMP.E)	0.18	0.14 (IMP.E)	0.48
17	Hydroxy propyl cellulose	1:10	ND	ND	0.04(IMP.E)	0.06	0.05 (IMP.E)	0.1	0.07 (IMP.E)	0.11
18	Noveon AA1	1:10	0.01	0.02	0.04 (IMP.D)	0.13	0.08 (IMP.D)	0.19	0.12	0.23
19	Sodium carboxxy methyl cellulose	1:10	ND	ND	0.02(IMP.E)	0.05	0.07 (IMP.D)	0.19	0.03 (IMP.E)	0.05
20	Polyethylene Oxide N-80	1:1	0.02(IMP.E)	0.03	0.03(IMP.E)	0.04	0.03	0.04	0.04	0.06
21	Polyethylene Oxide N-10	1:1	0.01(IMP.D)	0.02	0.01(IMP.E)	0.04	0.03	0.05	0.04	0.08

Table IV. 15 Alfuzosin HCl -Excipients Chemical compatibility study (water content)

Sample	% water			
		60° C	40°C/75%RH	40°C/75%RH
			Closed	Open
	Initial	1 month	3 months	3 months
API(ALF02/08/03)	0.76	0.89	0.80	13.44
API + Lactose anhydrous	0.31	0.38	0.54	3.99
API + Hypromellose K 100 M CR	3.06	4.19	6.06	13.06
API + Povidone K-30	5.02	6.02	NP	16.73
API + Talc	0.42	0.53	0.62	7.20
API + Colloidal silicon Dioxide	0.96	1.10	1.63	9.55
API + Magnesium Stearate	1.01	1.19	3.87	9.95

Table IV. 16 Alfuzosin hydrochloride - Excipients physical compatibility study at 40°C/75%RH and 60°C

Sample	Initial Appearance	40°C/75%RH	60°C
API (ALF02/08/03)	White powder	White powder	White powder
API + Lactose anhydrous	White to off-white powder	White to off-white powder	White to off-white powder
API + Hypromellose K 100 M CR	White to off-white powder	White to off-white powder	White to off-white powder
API + Povidone K-30	White to off-white powder	White to off-white powder	White to off-white powder
API + Povidone K-90	White to off-white powder	White to off-white powder	White to off-white powder
API + Talc	White to off-white powder	White to off-white powder	White to off-white powder
API + Colloidal silicon Dioxide	White powder	White powder	White powder
API + Magnesium Stearate	White powder	White powder	White powder

The control samples at room temp condition were compared with samples stored for 1 month at 60°C and 3 months 40°C /75 % RH. No significant change was observed in the Physical appearance except moisture uptake in open condition at 40°C/75%RH. Despite significant increase in water content, degradation was not observed with the excipients studied.

Since the excipients used for formulation development were compatible and did not show any Physico-chemical changes, it was concluded that no incompatibility would be expected in finished formulation. Hence further trials were conducted using these excipients.

IV.3.4 Dissolution profile of Marketed preparation

Dissolution profile of Marketed formulation (Alfuzosin Hydrochloride Extended Release tablet 10 mg) was done in different media.

Table IV. 17 Dissolution profile of Marketed formulation in different media

Paddle 50 RPM Volume : 900ml	% Drug Release (Time in hrs)							
Media	1	2	4	6	8	12	20	24
0.01 N HCl	15.3	21.7	31.3	39.9	50.3	66.9	92.0	94.7
pH3.0 Acid-Base Buffer	12.9	17.9	25.7	32.3	38.4	49.8	71.0	80.0
pH 4.5 Acetate buffer	15.1	21.8	30.6	39.9	48.1	62.8	87.6	91.4
pH 6.8 Phosphate Buffer	11.9	16.3	26.2	31.8	38.4	48.1	64.2	72.8

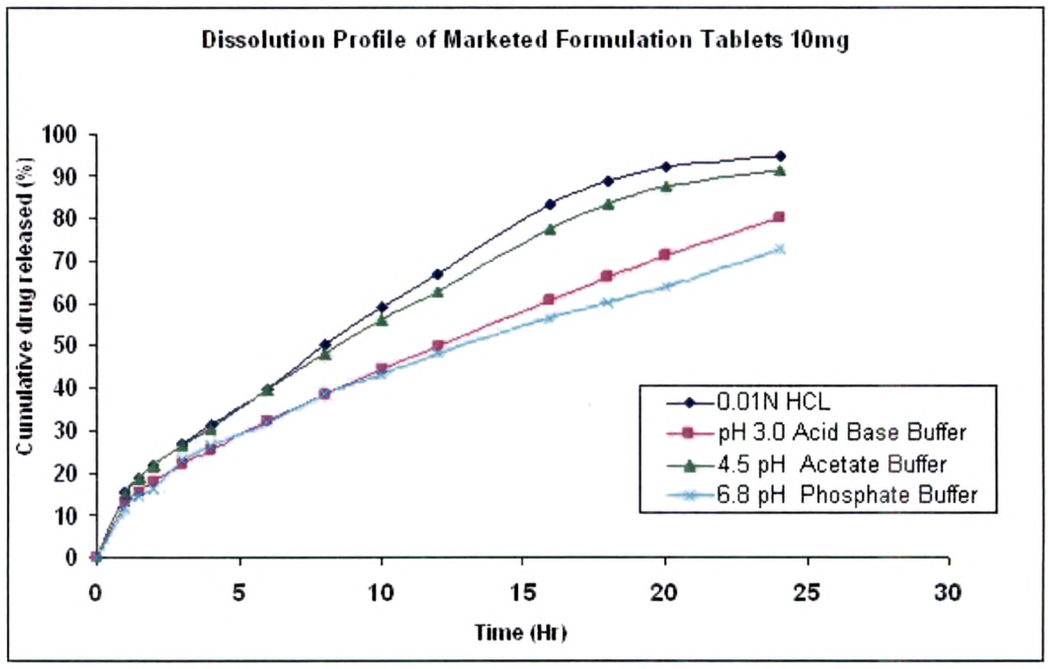


Figure IV. 7 Dissolution Profile of Marketed Formulation (Alfuzosin Hydrochloride Extended Release Tablet 10mg)

From dissolution study of marketed formulation, it was observed that the percentage of drug release was in the following order: 0.01N HCl > 4.5 pH acetate buffer> pH 3.0 acid base buffer > 6.8 pH phosphate buffer.

Table IV. 18 Comparative Dissolution profile of Marketed Formulation at various rpm

Paddle Volume : 900ml	% Drug Release (Time in hrs)						
Media	**1**	**2**	**6**	**8**	**12**	**20**	**24**
0.01 N HCl (100 rpm)	16.3	22.8	41.4	50.8	66.3	89.6	94.8
0.01 N HCl (50 rpm)	15.3	21.7	39.9	50.3	66.9	92.0	94.7

Dissolution profile was found to independent of paddle rpm.

IV.3.5 Permeability study in rat intestine

IV.3.5.1 Single-pass Intestinal Perfusion (SPIP) Technique in rats

IV.3.5.1.1 Net Water Flux (NWF) Determination

The results of net water flux values of Alfuzosin Hydrochloride determined in SPIP study are given in table IV.19.

Table IV. 19 Net water Flux values of Alfuzosin Hydrochloride in rat small intestine

Rat	AUC-PRin / AUC-PRout	Length of intestine (cm)	Flow rate(Qin) ml/min	NWF ml/cm/min	Average NWF
Rat 1	0.23758	73	0.2	0.002088813	0.0021
Rat 2	0.27430	70	0.2	0.002073438	

Standard Deviations (STDEV) was found to be 0.000011 and Covariance (%CV) was found to be 0.522 depicting low variability between the two experiments.

IV.3.5.1.2 Drug Permeability Determination

Table IV. 20 Results of Effective Permeability (Peff) of Alfuzosin Hydrochloride in rat small intestine

Rat	Peff ($x10^{-4}$ cm/sec)	Average Peff ($x10^{-4}$ cm/sec)
Rat 1	5.355	4.145
Rat 2	2.934	

Mean P_{eff} of two rats was found to be 4.145 x 10^{-4} cm/sec.

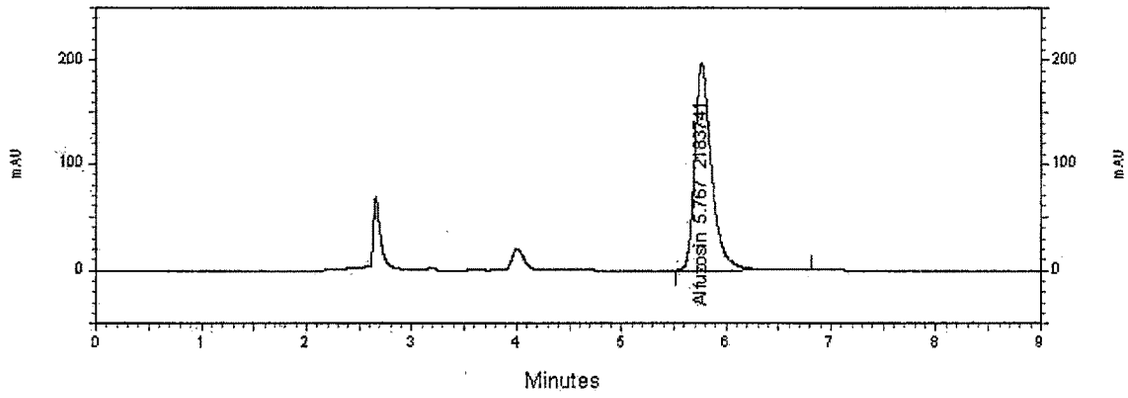


Figure IV. 8 Chromatogram of the stock solution (SS) of Alfuzosin Hydrochloride determined during SPIP study in rat 1.

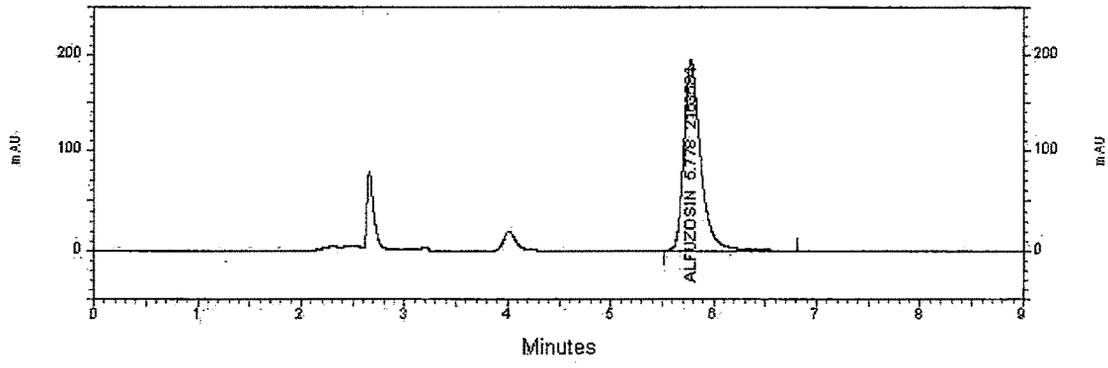


Figure IV. 9 Chromatogram of the adsorption sample (AS) of Alfuzosin Hydrochloride determined during SPIP study in rat 1.

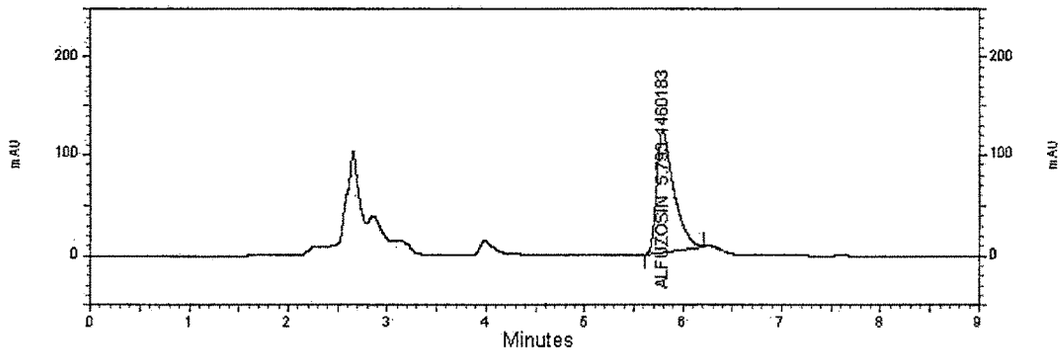


Figure IV. 10 Chromatogram of the Test sample (TS) of Alfuzosin Hydrochloride determined during SPIP study in rat 1.

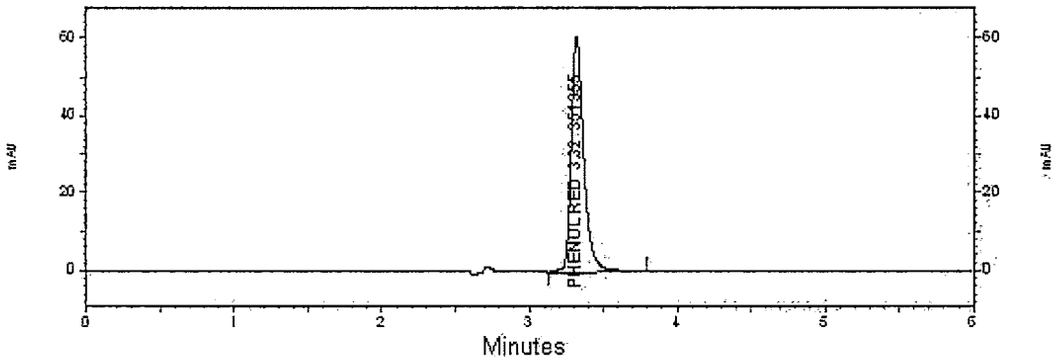


Figure IV. 11 Chromatogram of the stock solution (SS) of Phenol Red determined during SPIP study in rat 1.

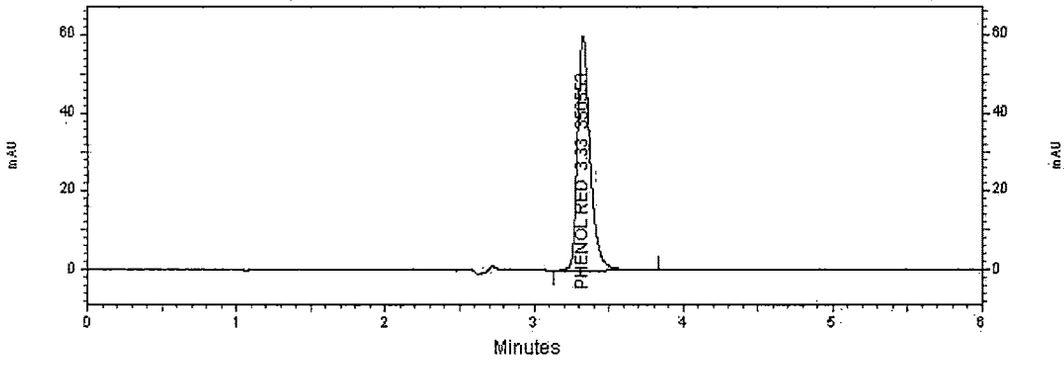


Figure IV. 12 Chromatogram of the adsorption sample (AS) of Phenol Red determined during SPIP study in rat 1.

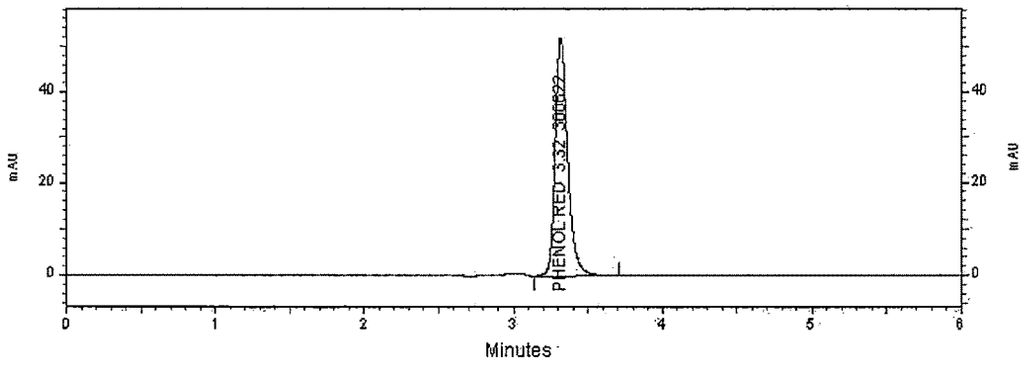


Figure IV. 13 Chromatogram of the Test sample (TS) of Phenol Red determined during SPIP study in rat 1.

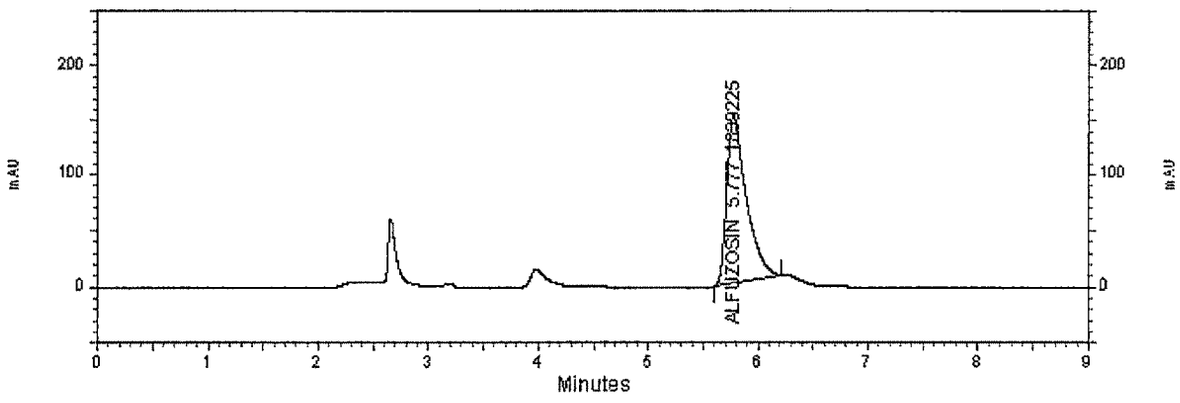


Figure IV. 14 Chromatogram of the stock solution (SS) of Alfuzosin Hydrochloride determined during SPIP study in rat 2.

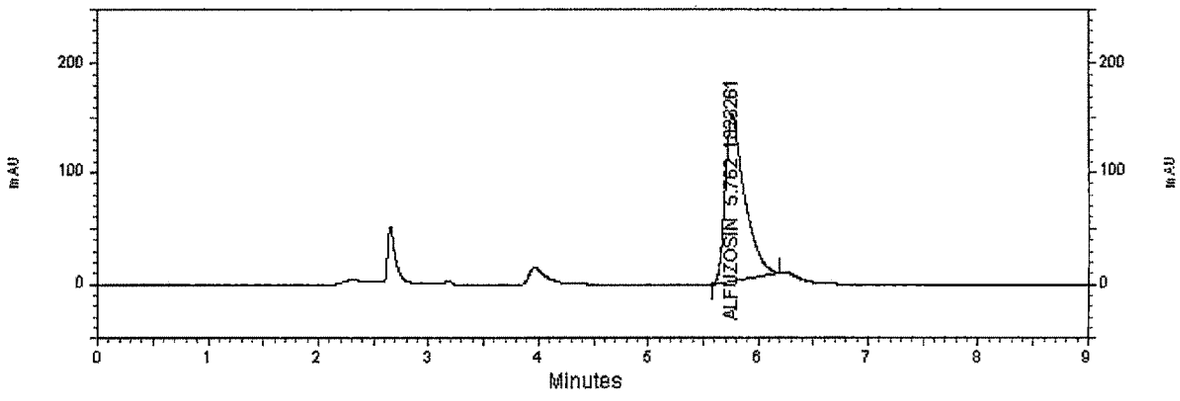


Figure IV. 15 Chromatogram of the adsorption sample (AS) of Alfuzosin Hydrochloride determined during SPIP study in rat 2.

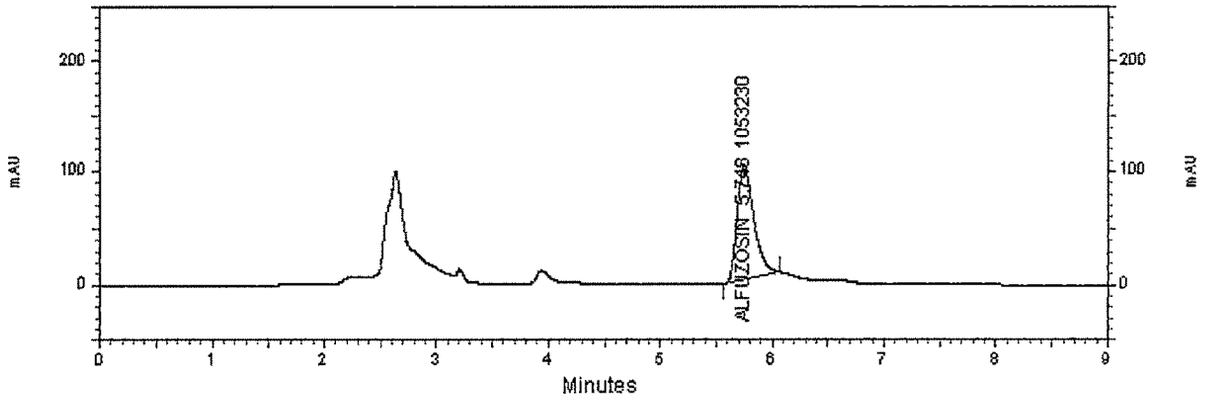


Figure IV. 16 Chromatogram of the Test sample (TS) of Alfuzosin Hydrochloride determined during SPIP study in rat 2.

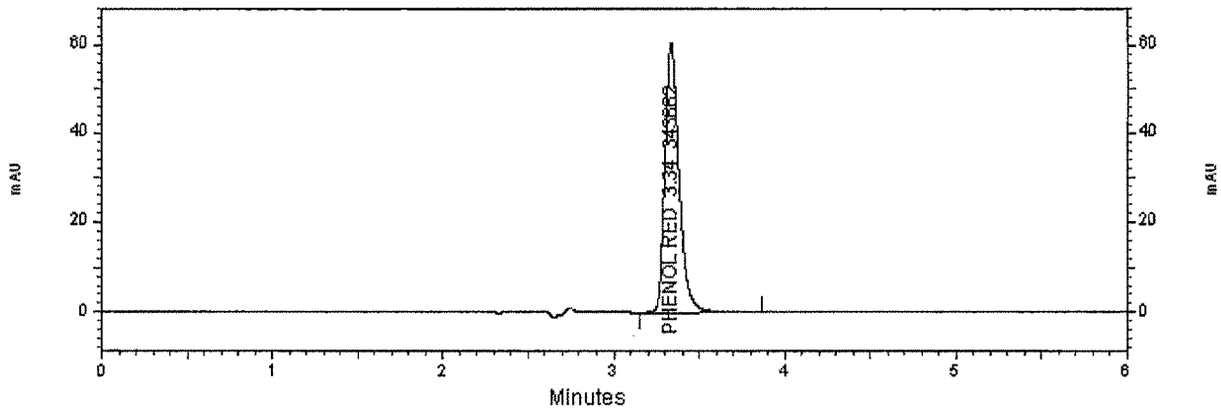


Figure IV. 17 Chromatogram of the stock solution (SS) of Phenol Red determined during SPIP study in rat 2.

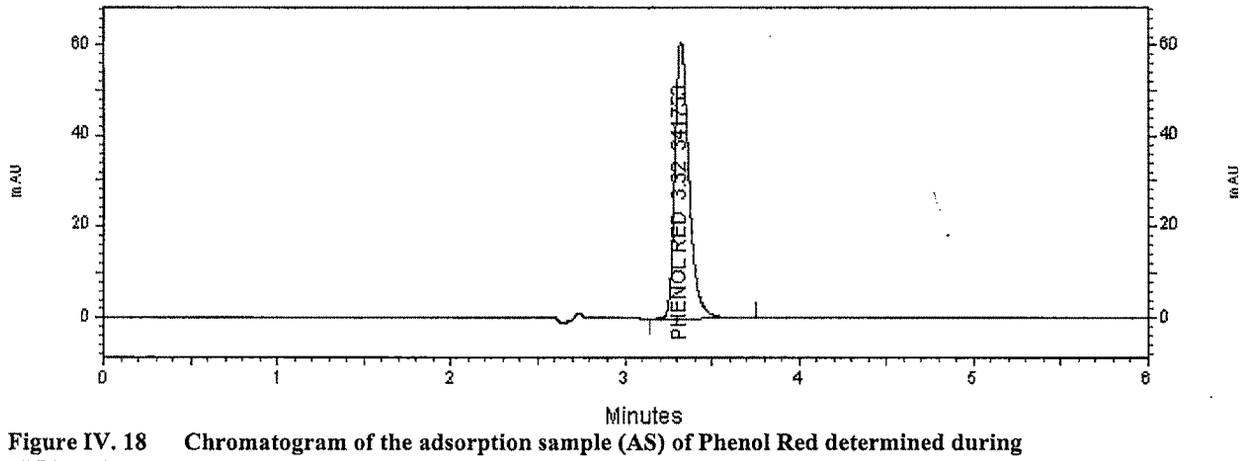


Figure IV. 18 Chromatogram of the adsorption sample (AS) of Phenol Red determined during SPIP study in rat 2.

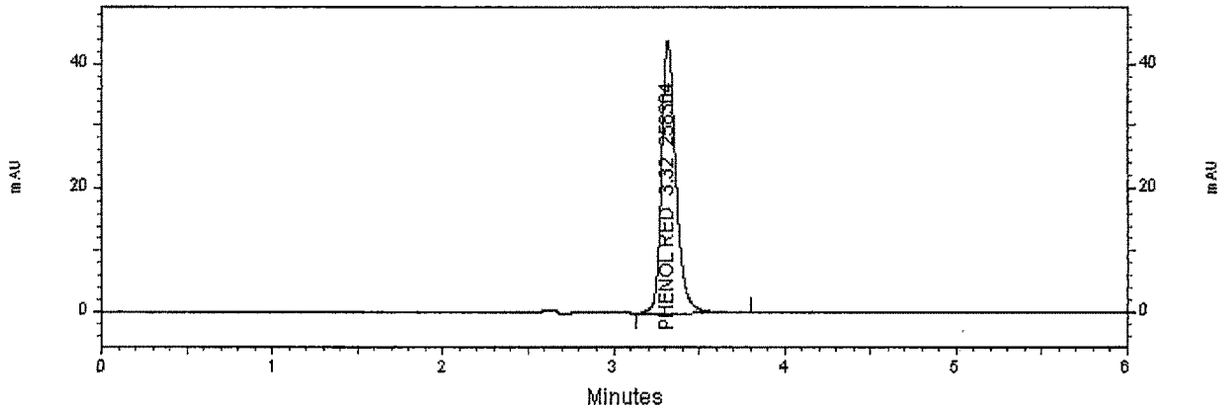


Figure IV. 19 Chromatogram of the Test sample (TS) of Phenol Red determined during SPIP study in rat 2.

IV.3.5.2 Closed intestinal loop method in rats

Table IV. 21 Comparative percentage of Alfuzosin Hydrochloride absorbed in duodenum and ileum of rat intestine

% Absorbed			
	Duodenum	Ileum	Difference
Rat 1	32.10	61.93	29.83
Rat 2	11.10	42.77	31.67
Average	21.60	52.35	
STDEV	14.85	13.55	
CV	68.75	25.88	

Table IV. 22 Comparative percentage of disappearance of Phenol Red in duodenum and ileum of rat intestine

% Disappeared		
	Duodenum	Ileum
Rat 1	18.57	10.20
Rat 2	18.36	1.23
Average	18.47	5.72
STDEV	0.15	6.34
CV	0.80	110.98

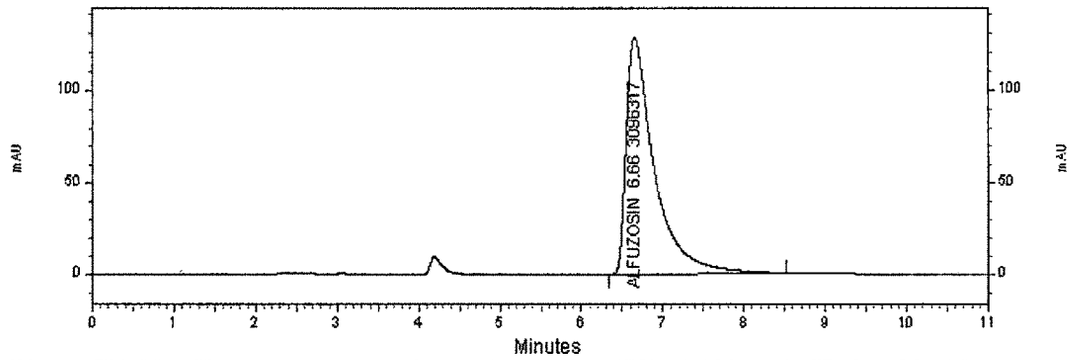


Figure IV. 20 Chromatogram of the stock solution (SS) of Alfuzosin Hydrochloride determined during closed loop method in rat 1.

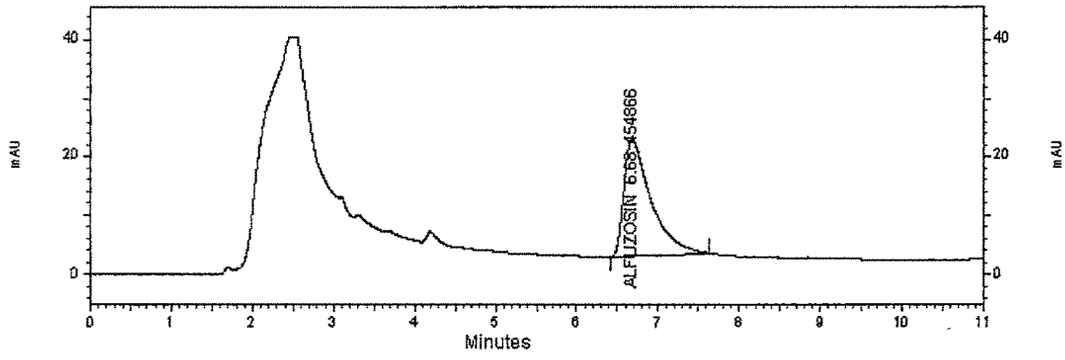


Figure IV. 21 Chromatogram of the Test sample (TS) of Alfuzosin Hydrochloride in duodenum determined during closed loop method in rat 1.

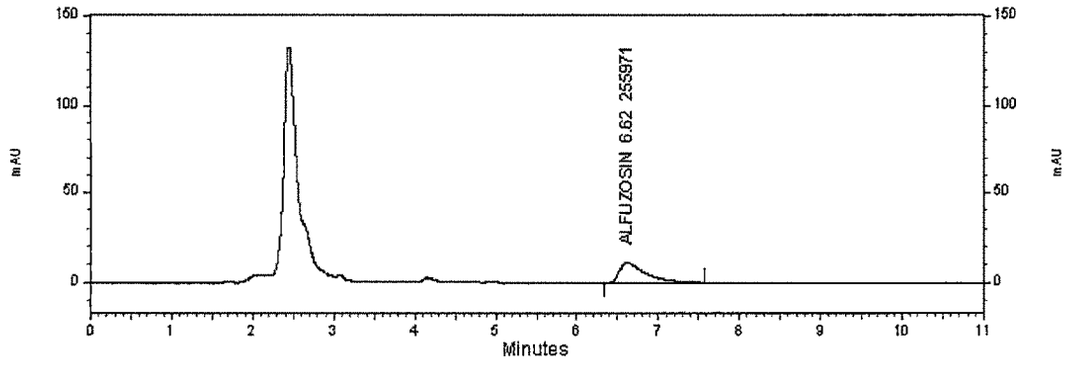


Figure IV. 22 Chromatogram of the Test sample (TS) of Alfuzosin Hydrochloride in ileum determined during closed loop method in rat 1.

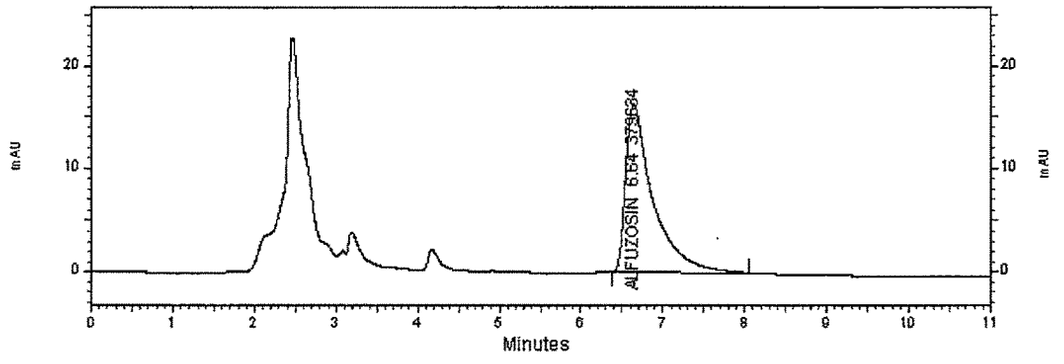


Figure IV. 23 Chromatogram of the Test sample (TS) of Alfuzosin Hydrochloride in duodenum determined during closed loop method in rat 2.

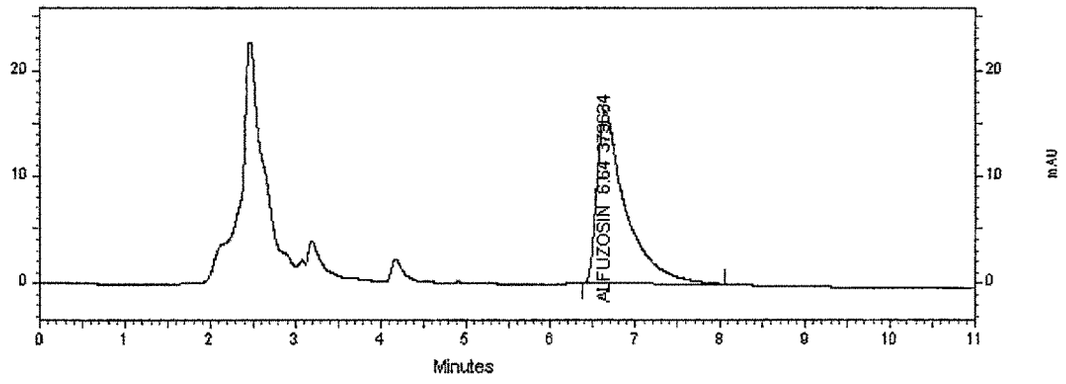


Figure IV. 24 Chromatogram of the Test sample (TS) of Alfuzosin Hydrochloride in ileum determined during closed loop method in rat 2.

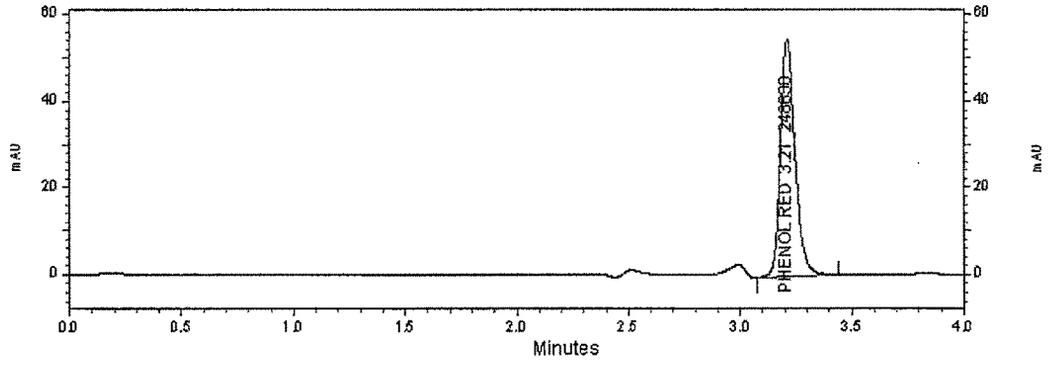


Figure IV. 25 Chromatogram of the stock solution (SS) of Phenol Red determined during closed loop method in rat 1.

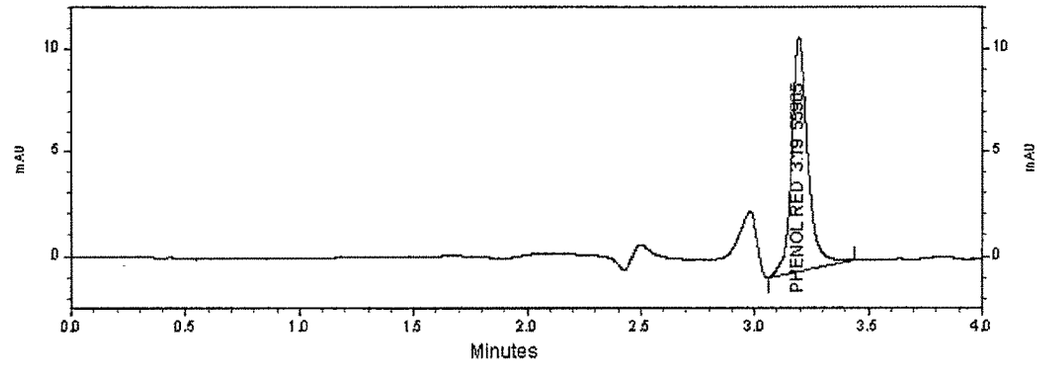


Figure IV. 26 Chromatogram of the Test sample (TS) of Phenol Red in duodenum determined during closed loop method in rat 1.

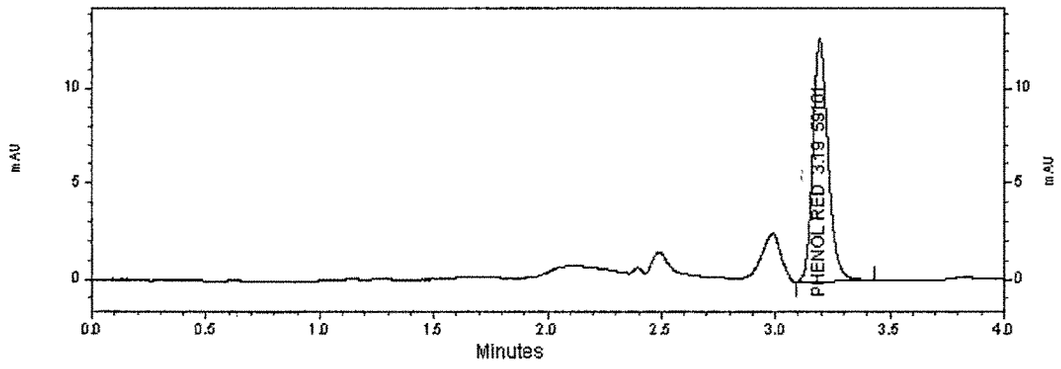


Figure IV. 27 Chromatogram of the Test sample (TS) of Phenol Red in ileum determined during closed loop method in rat 1.

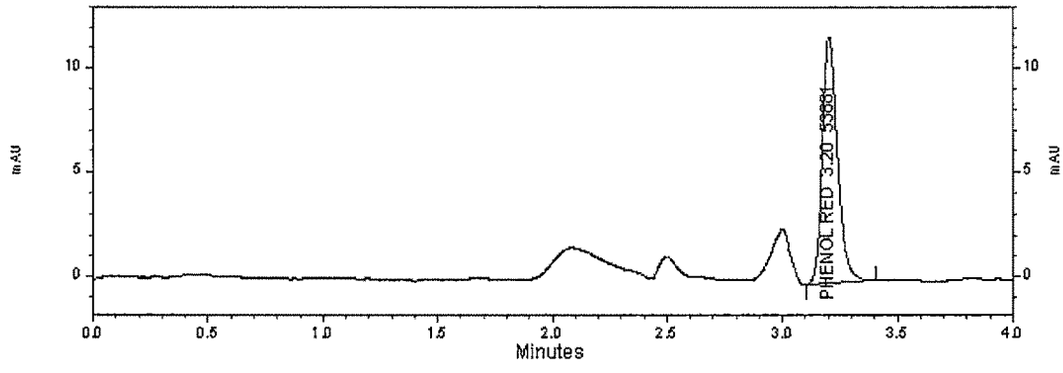


Figure IV. 28 Chromatogram of the Test sample (TS) of Phenol Red in duodenum determined during closed loop method in rat 2.

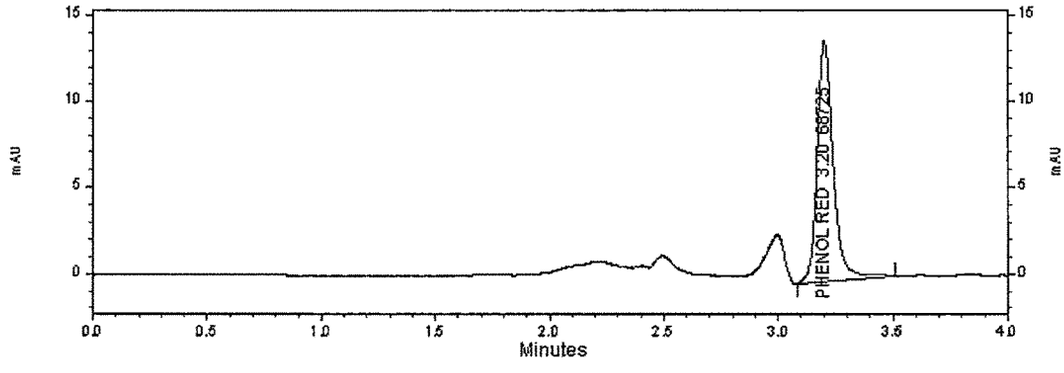


Figure IV. 29 Chromatogram of the Test sample (TS) of Phenol Red in ileum determined during closed loop method in rat 2.

IV.4 DISCUSSION

Alfuzosin was freely soluble at all pH. It exists in different polymorphic forms like anhydrous, dihydrate, trihydrtae, and tetrahydrate form (Borrega and Kitamura, 1996). Results of X-Ray, FT-IR, DSC and TGA studies confirmed that Alfuzosin Hydrochloride used in the present study used was pure anhydrous form.

The phenomenon of a substance passing through the intestinal wall (intestinal permeability) was initially noted in the middle of the seventeenth century by the observation of the passage of a dyed substance with a molecular mass of 262 daltons in the small intestine in dogs (Musgrave ,1901)

Justification for use of single pass intestinal study in rats:
The intestinal permeability is the propensity of a compound to move across the epithelial barrier of the intestine. *In vitro* and *in situ* absorption models, such as *in situ* perfusion of rat intestine, Caco-2 cell monolayer model, and excised intestinal segments in the Ussing chamber, are commonly used to investigate transport mechanism, classify permeability and *in vivo* absorption of drugs in humans (Amidon et al, 1995; Lennernas et al, 1997). Comparisons between human *Peff* data and these preclinical permeability models showed that they could be used to investigate and classify passive transport with a high accuracy (Lennernas, 1997). Recently, a GI-transit-absorption model (GITA) has been developed to analyze and predict drug absorption, and there is some evidence that this model is among the various models, cell-based assays using Caco-2 and MDCK cell lines are commonly used to predict passive absorption and the results obtained are greatly affected by experimental parameters such as pH (Yamashita et al, 2000). In contrast, *in situ* approaches provide experimental conditions closer to what is encountered following oral administration, with a lower sensitivity to pH variations due to a preserved microclimate above the epithelial cells (Hogerle et al, 1983; Shiau et al, 1985). These techniques maintain an intact blood supply to the intestine, and can be used to estimate the impact of clearance pathways, such as enzymes and transporters, that are present in the gut. Moreover, drug permeability (Ungell et al, 1998), expression of drug metabolizing enzymes and transporters have been shown to vary along the intestinal tract (Hakkak et al, 1993; Zhang et al, 1996; Makhey et al, 1998), which can be investigated using intestinal perfusion of the various regions. In addition, it was recently reported that oral drug absorption in rats and humans is very similar (Chiou et al, 1998). Thus, it is likely that the intestinal perfusion conducted in rats may give a better prediction of the fraction of oral dose absorbed in humans than in in- vitro models.

Single-pass perfusion studies are generally accepted for estimating intestinal absorption. The perfused rat small intestine preparation proves to be a useful tool for examination of the effect of the absorptive, secretory and metabolic activities of the intestine on drug bioavailability (Kisielinski et al, 2002; Doherty et al, 2000). Also other authors cited that the human in vivo permeabilities can also be predicted using preclinical permeability models, such as in situ perfusion of rat small intestine (Lennernäs, 1998; Cong et al, 2001). Other results obtained in this laboratory suggested that the single-pass perfusion technique of small intestine in rats appears to be a suitable technique for specifying

between passive transporting mechanisms as well as the carrier-mediated absorptive processes (Kuneš et al, 2005).

Single pass intestinal perfusion study showed that net water flux was positive with 0.0021 ml/cm/min. Similar values in this range were obtained for other compounds studied with the lab model established in our lab. The net water flux values obtained with different compounds varying in molecular weight, solubility and permeability were found in the range of 0.0004 to 0.0018 ml/cm/min.

Mean permeability study for Alfuzosin Hydrochloride was found to be 4.145×10^{-4} cm/sec. Mean permeability values were also calculated for a range of compounds varying in molecular weight, solubility and permeability and in order to establish a model for prediction of human oral absorption (HOA), the SPIP (Single pass intestinal perfusion) Peff (effective permeability) values were correlated with reported HOA. A plot was drawn of the reported fraction absorbed following oral administration in human (HOA) of the different compounds against the permeability values obtained after in-situ single pass intestinal perfusion in rat intestine. Sigmoidal relationship was obtained between percentage of fraction absorbed(% Fa) and Log Peff values with regression coefficient as 0.8856 which is near to 1, indicating a good correlation between percentage fraction absorbed (% Fa) and Log Peff values. A non –linear regression curve fit showed that the training set Peff data from experimental model could be described by a sigmoidal function:

$$\% \text{ HOA} = 100/(1+(10^{\log \text{Peff}50\%}/10^{\log \text{Peff}})^{\text{Slope}})$$

where, Peff 50% is the Peff at 50% HOA and Peff is the experimental value obtained and slope is that obtained from the sigmoidal curve.

Putting Log Peff values obtained for Alfuzosin Hydrochloride in the above equation, percentage of HOA was found to be 55 % and 43 % with an average of 49%. Reported value for HOA for Alfuzosin Hydrochloride are 64% for 5 mg capsule or solution (Centre for Drug Evaluation and Research, 2003). Similarity of 76.56% was found between predicted value and reported value of HOA for Alfuzosin HCl.

Based on the model established in our lab, compounds were classified into different grades for absorption, namely High, Moderate and Poor.

Table IV. 23 Grading system based on various compound analyzed

Grade	HOA (% Fraction)
Poor	0-30
Moderate	31-70
High	>71

Based on this Alfuzosin HCL was categorized under moderate absorption drug.

Although much of the data available in the literature predict higher absorption of Alfuzosin HCl in the proximal part of small intestine (Maggi et al., 2000; Andrieu et al., 1995) but there wasn't any study conducted to support the data. Hence, single intestinal perfusion study was conducted to determine the permeability of the drug and closed loop method was employed to know the site of absorption. Our results showed that Alfuzosin had moderate permeability and was more rapidly absorbed through the ileum than duodenum. This data is in accordance with that obtained by Haddouche et al., 1996; done using Ussing Chamber.

Haddouche et al, used strips of different segments of rat intestinal membrane (duodenum, jejunum and ileum) by using Ussing chamber to determine segmental absorption and found that Steady-state of mucosal to serosal fluxes in the various intestinal regions was observed 40 min after introduction of Alfuzosin in the luminal fluid. Marked differences in transport rate of Alfuzosin between different segments of the intestine were noticed by them (Table IV.24).

Table IV. 24 Unidirectional mucosal to serosal fluxes of unlabelled Alfuzosin

Intestinal segment	Flux (nmol/cm^2 per hour)
Duodenum	6.37 ±1.01
Jejunum	8.59 ± 1.47
Ileum	14.20 ± 1.31
Colon	5.43 ± 1.17

Transepithelial passage decreased in the order ileum > >jejunim~duodenum~ colon, and paralleled hydroelectric permeability (conductance) in duodenum, jejunum and ileum

Furthermore results of our study showed that loss of phenol red in duodenum was more than in ileum. In order to measure the drug loss, a non absorbable marker such as PEG 4000, phenol red or insulin is often added to the drug solution to assess the net intestinal water transport (Doluisio et al., 1969; Sinko et al., 1995). However Gabus- Sannie and Buri (1987) have shown that traces are partially absorbed or adsorbed. However in our case high amount may be due to higher adsorption in villi of duodenum which are more abundant in duodenum than in ileum.

The mechanism responsible for faster absorption rate in ileum than in duodenum has not been predicted. Here we have made an attempt to predict a possible hypothesis responsible for this behavior.

Factors effecting permeability

The permeability to the membrane, luminal drug concentration and the residence time are the major determining factors for drug absorption after oral administration. Although some reports have investigated the transit of luminal contents in detail (Murata et al., 1987; Kimura and Higaki, 2002), there are few reports that demonstrate the relation between the permeability and the luminal concentration of drugs in each segment of the

GI tract. Let us look at factors which effect the permeability of a compound through the intestinal membrane.

Pore radius and Surface area of the paracellular pathway

It has been reported that the permeability of drugs is generally higher in the upper portion of the small intestine than in the lower one. Usually, the permeability of the colonic membrane is considered to be much lower than that of small intestine (Palm et al., 1996; Artursson et al., 1993). This has been proposed to be due to a decreased total surface area of the membrane and increased tightness of the epithelium (e.g. higher electrical resistance) in the colonic region (Thomson et al., 1986; Curran et al, 1960). In order to elucidate the difference in paracellular permeability, pore radius of the junctional pathway in each region of intestinal membrane was estimated by Yoshie et al. (2006) from the permeability ratio of mannitol and urea by using Renkin equation. Since single-pass perfusion method sometimes fails to detect the permeability of poorly absorbable drugs such as mannitol, the loop method was applied by them to measure the permeability of mannitol and urea. They found that permeability of mannitol and urea in the colon was significantly lower (0.019 and 0.191 respectively) than that in the jejunum (0.053 and 0.536 respectively) or ileum (0.042 and 0.616 respectively)

However, the calculated values of the pore radii in the jejunum, ileum and the colon were found by Yoshie et al. to be 6.19, 5.31 and 6.23 A°, respectively indicating that the pore radius of the paracellular pathway was almost similar in all segments. The difference in villous surface area of three segments was calculated by them from reports of Mayhew (1988) and Collett et al. (1997). An amplification ratio in different segments i.e. jejunum, ileum and colon was found to be 7.84, 3.55 and 1.9 respectively.

They found that villous structure of the jejunum amplifies the area for four-folds compared to the colon and two-folds to the ileum. Following the order of epithelial surface area, drug permeability in the colon must be markedly lower than that in upper small intestine. However when regional permeability of five drugs; atenolol, metoprolol, Naproxen, antipyrine and griseofulvin was accessed by closed loop method by Yoshie et al (2006), only two drugs showed the significantly lower permeability in the colon and the differences of the permeability were only 1.4-fold. When comparing the permeability in the jejunum and ileum, no significant differences were observed between all drugs investigated by them (Table IV.25).

Table IV. 25 Physico- chemical properties and permeability of drugs at various regions in rat intestine

				Permeability (X 10^{-4} cm/sec)		
Drug	Molecular weight	pK_a	log D (6.5)	Jejunum	Ileum	Colon
Atenolol	266.38	10.8	-2.34	0.295 ±0.062	0.244±0.096	0.207±0.038
Metolprolol	267.41	10.08	-1.08	1.521 ±0.213	1.603±0.414	1.006±0.389
Naproxen	230.28	4.06	0.83	0.964 ±0.388	1.382±0.445	1.409±0.200
Antipyrine	188.25	1.6	1.79	1.496 ±0.025	1.124±0.180	1.078±0.141
Griseofulvin	352.79	Neutral	2.88	0.909 ±0.293	1.243±0.293	1.296± 0.094

These findings have clearly demonstrated that the surface area is not a main factor to cause the regional differences in drug permeability. As one of the reasons to explain this discrepancy, it appears that the lipophilic drugs (highly permeable drugs) can permeate the intestinal membrane rapidly through the villous tips thus the contribution of total surface area to the regional difference in drug permeability becomes smaller. A similar finding has previously been reported in which the absorptive surface area was compared between the intestinal membrane and Caco-2 monolayers (Artursson et al., 2001). In the case of hydrophilic drugs (poorly permeable drugs), they might diffuse farther toward the crypt regions before permeation and the differences in the surface area can be more significant. Therefore, the lower permeability of mannitol and urea in the colon should be attributable not to the tighter structure of the junctional pathway, but to the less number of pore associated with a smaller surface area of the epithelial membrane. In this meaning, colonic epithelium is regarded as relatively tight, having the higher electric resistance and low paracellular permeability (Curran and Schwartz, 1960; Ma et al., 1991, 1995).

In our experiment, we have taken same length of duodenum and ileum i.e. 8 cm but as villi in duodenum are more than in ileum i.e. despite of having larger surface area in duodenum, permeability of Alfuzosin, a hydrophilic drug, through ileum was more than that in duodenum. So, the reason for high surface area and more pores can't be the mechanism for high permeability of Alfuzosin through ileum. Also other authors have reported pore radius to be different in different segments of the intestine. Trans epithelial electrical resistance (TEER) increases as the width of tight junctions decrease and the tight junction width has been determined to be 0.75–0.8 nm in jejunum, 0.3–0.35 nm in ileum, and 0.2–0.25 nm in colon (Balaji et al,2001)

Regional membrane fluidity

It has been suggested that the lipid composition in the intestinal membrane could be a factor to regulate the fluidity of the membrane and thereby affect the transcellular transport characteristics (Meddings and Theisen, 1989; Brasitus and Schachter, 1984). Many groups have previously demonstrated regional differences in the lipid composition and membrane fluidity. The study of Garriga et al. (2002) in chicken small intestine, Ibrahim and Balasubramanian (1995) in monkey small intestine and Dudeja et al. (1989) in human small intestine showed that the lower portion of the small intestine is more fluid than the upper.

Yoshie et al (2006) measured membrane fluidity by using the fluorescence depolarization method in rat intestinal BBMVs (Brush Bordered membrane vesicles).They found that both polarization and anisotropy were highest in duodenum, followed by the order of jejunum> ileum > colon; thus clearly indicating that the cellular membranes from proximal intestine were less fluid than distal one and hence they predicted that higher permeability of naproxen in the colon might be due to higher membrane fluidity .

Similar might be the case for our model drug, Alfuzosin also. Alfuzosin is a low molecular weight (389.46 for Alfuzosin and 425.9 for Hydrochloride) and mildly lipophilic solute (log $D_{octanol/water}$ = 1.5 at pH 7.40. So, it might be possible that Alfuzoin

get dissolved in ileum more than duodenum (due to higher membrane fluidity in ileum compared with duodenum) and that might be the reason for higher permeability in ileum than duodenum. This factor might contribute only partly as Alfuzosin had been reported to be absorbed by paracellular pathway by Haddouche et al. (1996).

Haddouche et al. found that after complete inhibition of active ionic movements by cell poisons (i.e. depletion of cell energetics by concomitant blockade of glycolysis by 2-deoxyglucose and mitochondrial electron transport chain by sodium azide) lead to the loss of viability but was not associated with a decreased transport rate for Alfuzosin. In fact a marked rise in drug transport, basically attributable to the increase in paracellular permeability was found. The strong correlation between P app values of Alfuzosin and D-mannitol, a paracellular probe, support the view that diffusion of Alfuzosin mainly occurs through this pathway. D-glucose, which triggers loosening of tight -junctions (Madara and Pappenheimer, 1987) was found to increase significantly tissular conductance and the mucosal to serosal flux of D-mannitol but its effect on Alfuzosin flux was not significant.

Also pKa of Alfuzosin is 8.13 and at pH 7 which is that of ileum, 93% of the drug will be in ionized form whereas at pH less than 5.3 which is that in duodenum, 100% of the drug will be in ionized form. Though the contribution of unionized form of Alfuzosin is less, it might be additional factor responsible for greater membrane permeability in ileum as compared to duodenum.

Effect of mucus layer

The villi and microvilli structures are found in highest density in the duodenum followed by jejunum, and then in ileum, and in lower density in a short section of the proximal colon (Kararli, 1998; Durgin and Hanan, 2004). The microvilli have glycoproteins (the glycocalyx) protruding into the luminal fluid. There is residual negative charge in the glycoproteins. Some cells in the monolayer are known as goblet cells whose function is to produce the mucus layer that blankets the glycocalyx. The mucus layer is composed of a high-molecular-weight (HMW) (2 x 10^6 Da) glycoprotein, which is 90% oligosaccharide, rich in sialic acid residues, imparting negative charge to the layer (Madara , 1991). The glycocalyx and the mucus layer make up the structure of the unstirred water layer (UWL) (Shiau et al, 1985). The thickness of the UWL is estimated to be 30–100 mm in vivo, consistent with very efficient stirring effects (Lennerna¨s, 1998). In isolated tissue (in the absence of stirring), the mucus layer is 300–700 mm thick (Shiau et al, 1985).

It has been suggested that the mucus layer at the surface of the intestinal epithelium has only a minor function as a barrier (Winne and Verheyen, 1990), but others suggest that it could function as a rate limiting barrier against the absorption of highly permeable drugs (Nimmerfall and Rosenthaler, 1980; Smithson et al., 1981). The thickness of the mucus layer varies in different luminal segments. Szentkuti et al. have demonstrated that mucus layer is thicker in the upper intestine than in the lower or colon in fasted rat (Szentkuti and Lorenz, 1995). In a study conducted by Yoshie et al (2006), after removing the mucus layer by dithiothreitol, jejunal permeability of griseofulvin but not of naproxen

was significantly enhanced to the same level with that in the ileum and colon, suggesting the significant role of mucus layer on the proximal-intestinal absorption of griseofulvin.

Alfuzosin HCl ionizes into cationic Alfuzosin as soon as it dissolves in GI (gastro intestinal) fluids and due to negative charge, Alfuzosin might form a complex with gycocalyx which is more in duodenum due to higher gylocoproteins in duodenum than in ileum. This might be responsible for slower diffusion rate through duodenum than ileum.

GI transit Rates

The gastrointestinal (GI) absorption of orally administered drugs is determined by not only the permeability of GI mucosa but also the transit rate in the GI tract. It is well known that the gastric emptying rate is an important factor affecting the plasma concentration profile of orally administered drugs, and the intestinal transit rate also has a significant influence on the drug absorption, since it determines the residence time of the drug in the absorption site. The reason why the residence time is also a critical factor for drug absorption is that there is the site difference in absorbability for some drugs. Kimura and Higaki (2002) developed the GI-Transit-Absorption Model (GITA Model) to analyze and predict the drug absorption kinetics by taking into account both the two factors, *i.e.* GI transit and drug absorbability including its site difference. They found that the GI transit rate *(ki)* value decreased along the intestinal tract from upper (28.75 h^{-1}) to lower (0.46 h^{-1}) region and the transit rate differed considerably in each segment, although it depended on the estimated intestinal length. Especially, the transit rates in the upper segments such as duodenum and upper jejunum were extremely high, but the ones in the lower segments such as upper and lower ileum were very low (Table IV.26)

Table IV. 26 Gastrointestinal Transit rate constant ((ki) for each segment

Method	k_i Value (h^{-1})							
	Stomach	Duodenum	Upper jejunum	Lower jejunum	Upper ileum	Lower ileum	Caecum	Large intestine
GITK Model	2.03	28.75	18.07	4.21	1.16	0.46	NP	NP

Similar tendency in GI transit has been reported by Marcus and Lengemann, 1962.

Kimura and Higaki (2002) found that theophylline was rapidly absorbed from the GI tract. Theophylline is characterized as a well-absorbable drug without first-pass elimination (Ogiso et al, 1993). Theophylline disappeared rapidly from the GI lumen and only a little amount reached the ileum. Substantial absorption rates of theophylline were found to be much larger in the upper segment than those in the lower segments, and the duodenum, upper jejunum and lower jejunum largely contribute to the absorption of theophylline. On the other hand, a large amount of ampicillin, a poorly absorbable drug, can reach the lower intestine and almost 70% of the dose can enter the cecum. From the absorption rate profile for ampicillin in each segment they found that the site of the largest absorption was clarified to be the lower jejunum. Although the residence time was

longer and the drug amount was larger in the lower intestine compared to the upper segments, the actual absorption rate was not the largest because of a small absorption rate constant *(ka)* value in the ileum. They also studied the substantial contribution of each segment to the cephalexin absorption *in-vivo* with *ki (gastro intestinal transit rate constant)* and *kai* (absorption rate constant*)* values of each segment. The *kai* values of cephalexin obtained by an *in-situ* loop method were larger in the upper small intestine than in the lower region, indicating that the preferential absorption site was considered to be the upper part as has been reported previously (Maekawa et al, 1977).

However, as the residence time in the upper small intestine is very short, they found that the substantial absorption site of cephalexin *in-vivo* was shifted to below lower jejunum in the fasted state. They concluded that the transit rate, or the residence time, in each site could change the substantial absorption site *in-vivo*.

Based on the transit time distribution for seven SI (small intestine) compartments, Yu et al (1996) developed a compartmental absorption and transit (CAT) model to simulate the rate and extent of drug absorption .On analysis of more than 400 human SI transit times, a log normal distribution with mean SI transit time of 3.3 h was obtained (Yu et al, 1996). By integrating the transit of material through the SI as represented by various numbers of compartments and plotting the cumulative amount that reaches the colon, Yu determined that seven equal transit time small intestine compartments gave the best fit to the observed cumulative frequency distribution. The seven-compartment transit model may be visualized as having the first compartment representing the duodenum, the second half of the first compartment, along with the second and third representing the jejunum, and the rest representing the ileum. They found that corresponding transit times in the duodenum, jejunum, and ileum were 14, 71, and 114 min.

In our experiment Alfuzosin was incubated into duodenum and ileum for same time period i.e. 15 min. So, the possibility that transit time plays a role in more absorption from ileum than duodenum can be ruled out. Although in-vivo it might be an important factor responsible for higher drug absorption as Alfusoin is absorbed by paracellular pathway as discussed above. Thus drugs that are absorbed mainly by paracellular pathway require greater residence time for absorption as their absorption is solely by diffusion process and paracellular route is estimated to be about 0.01% of the total available surface area of the intestine (Lennerna¨s et al, 1994,1995; Fagerholm at al, 1995; Madara and Pappenheimer ,1987; Nellans ,1991).

Cations:

For passive diffusion, the size and shape of a drug molecule, its acid and base dissociation constants, and the pH of the gastrointestinal tract all influence the absorption rate coefficient for specific regions of the GI tract. Pade and coworkers measured the Caco2 cellular permeability for a diverse set of acidic and basic drug molecules at two pH values (Pade and Stavchansky, 1997). They concluded that the permeability coefficients of the acidic drugs was greater at pH 5.4, whereas that of the basic drugs was greater at pH 7.2 and the transcellular pathway was the favored pathway for most drugs, probably due to its larger accessible surface area. The paracellular permeability of the

drugs was size and charge dependent. The permeability of the drugs through the tight junctions decreased with increasing molecular size. Further, the pathway also appeared to be cation-selective, with the positively charged cations of weak bases permeating the aqueous pores of the paracellular pathway at a faster rate than the negatively charged anions of weak acids or neutral molecules (Adson et al., 1994; Karlsson et al., 1994). . Thus, the extent to which the paracellular and transcellular routes are utilized in drug transport is influenced by the fraction of ionized and unionized species (which in turn depends upon the pK_a of the drug and the pH of the solution), the intrinsic partition coefficient of the drug, the size of the molecule and its charge. Furthermore it has been reported that cations are generally more easily transported through the tight junctions than are non ionic species or anions (Rojanasakul et al., 1992; Adson et al., 1994; Hamilton et al., 1987).

Since Alfuzosin exists in 100% ionized form (cationic form) below pH 5.5 i.e. in duodenum and 99.3% at pH 7 which is pH of ileum; it should have been more absorbed from duodenum than ileum. But as discussed above, due higher complexation of cationic Alfuzosin with anionic sialic acid in duodenum than ileum, Alfuzosin might be diffusing at a faster rate in ileum than duodenum.

Solvent Drag

Water flux across the intestinal epithelium occurs via both transcellular and paracellular routes. Although the relative magnitude of the flux through each route is not known, it has been suggested that a significant fraction is paracellular (Chang and Rao, 1994). Intestinal absorption of small paracellular marker molecules has been shown to increase in direct proportion to the net water absorption, suggesting that absorption could be mediated by ''solvent drag'' in the fluid absorbed through the paracellular pathway. (Pappenheimer and Reiss, 1987; Krugliak et al., 1989). Paracellular absorption by ''solvent drag'' has been suggested to be an important component for intestinal absorption of many hydrophilic drugs and peptides (Nellans, 1991; Leahy et al., 1994; Pappenheimer et al., 1997).

Permeability of the intestinal mucosa to water and electrolytes is high in the duodenum and jejunum. It decreases along the length of the intestine until permeability in the ileum is low. The tight junctions between intestinal epithelial cells are the site of most permeation by water and electrolytes. In the duodenum and jejunum they are "leaky" tight junctions. However, the ability to absorb water does not decrease, but water flows across the epithelium more freely in the proximal compared to distal gut i.e. water can move out from intestinal lumen to blood or from blood to intestinal lumen because the effective pore size is larger. The distal intestine actually can absorb water better than the proximal gut. The observed differences in permeability to water across the epithelium is due almost entirely to differences in conductivity across the paracellular path as the tight junctions vary considerably in "tightness" along the length of the gut. Grim, 1962, studied the water and electrolyte flux rates in duodenum, jejunum, ileum and colon in 6 canine model and found that the net flux rate of water in the duodenum was negative (-0.2 microliters per minute per square centimeter of mucosal area), as might have been

anticipated in what is essentially a secreting part of the small intestine. In the jejunum and ileum, the net flux rates were positive and nearly equal (0.2 microliters per minute per square centimeter of mucosal area). That the absorption of water from the colon (1.1 microliters per minute per square centimeter of mucosal area) was much more efficient than from the small intestine is indicated by the net flux rate, which was more than 5 times greater than in jejunum and ileum.

Grim, 1962, also found that the net flux rate of sodium in the duodenum was negative (-0.026 microequivalents per minutes per square centimeter of mucosal area), as was the case for water. At the other levels of the intestine there was a positive net flux rate or a positive absorption of sodium which increased aborally, the highest value being reached in the colon (0.155 compared with 0.028 and 0.040 in jejunum and ileum respectively).

Similar results were obtained with chloride net flux rates (-0.020, 0.033, 0.061, 0.2225 microequivalents per minutes per square centimeter of mucosal area for duodenum, jejunum, ileum and colon respectively).

A hypoosmolar solution at the luminal side of the intestinal epithelium stimulates water absorption which might, in parallel, increase the uptake of compounds that are freely or partly transported with water (solvent drag) (Ma and Krugliak,1996; Cox et al, 1996; Ochsenfahrt and Winne, 1974; Karino et al, 1982; Powell,1987; Hunt et al, 1992; Krugliak et al, 1994). It has been suggested that the transport route is paracellular for both intestinal water and hydrophilic compounds under conditions of so-called convective water transport, but conclusive evidence is lacking (Ma and Krugliak,1996; Cox et al, 1996; Krugliak et al, 1994; Pappenheimer and Reiss,1987; Fine et al,1993,1994; Schwartz,1995).In a number of in vivo studies in humans, Hans Lennerna's have stimulated water absorption using hypotonic solutions (170-180 mosm/L), but the jejunal Peff was not increased for hydrophilic drugs with molecular weights between 225 and 348 Da (terbutaline, atenolol, and enalaprilat) (Lennerna¨s et al, 1994; Lennerna¨s,1995; Fagerholm et al, 1995; Nilsson et al, 1994; Fagerholm et al, 1999). These results suggested that quantitative paracellular absorption does not occur for any compound greater than 200 Da in the proximal human small intestine in vivo (Lennerna¨s et al, 1994; Lennerna¨s, 1995; Fagerholm et al, 1995; Nilsson et al, 1994; Fagerholm et al, 1999). This is consistent with the small area of the paracellular route, which is estimated to be about 0.01% of the total available surface area, and suggests molecular/pore size ratios greater than unity ((Lennerna¨s et al, 1994; Lennerna¨s,1995; Fagerholm et al, 1995; Madara and Pappenheimer,1987; Nellans,1991). However, water transport might affect drug absorption in more distal regions of the small intestine, as suggested for talinolol in an open perfusion study of the human ileum (Gramatte et al, 1994).

Na^+ ions in the lumen of the ileum are exchanged for H^+ ions while Cl^- is exchanged for $HCO3^-$ at the same time. The H^+ ions combine with $HCO3^-$ to yield $H2O^+ CO^2$, which diffuse out of the lumen. Most Na^+, Cl^- and, by subsequent osmosis, H_2O is absorbed by this electroneutral transport mechanism (Despopoulos and Silbernagl, 2003).

As described above, duodenum and jejunum are leaky tight junction through which water can flow in either direction and as per Eugene Grim net flux of water, sodium and chloride is more in ileum than in duodenum and jejunum, so this might be the most relevant and most powerful hypothesis for higher diffusion rate of Alfuzosin in ileum than duodenum.

Also as water is more absorbed more ileum than duodenum, so concentration of Alfuzosin in ileum will be more than in duodenum. The rate at which the substance diffuses inward is proportional to the concentration of molecules on the outside and because this concentration determines how many molecules strike the outside of the membrane each second. Conversely, the rate at which molecules diffuse outward is proportional to their concentration inside the membrane. Therefore, the rate of net diffusion into the cell is proportional to the concentration on the outside minus the concentration on the inside, or:

Net diffusion $\mu = (Co - Ci)$

in which Co is concentration outside and Ci is concentration inside. As Alfuzosin is absorbed by paracellular pathway, so the concentration gradient established by more water absorption in ileum might be responsible for higher diffusional rate than in duodenum.

Aquaporins

Aquaporins (AQPs) are membrane water channel proteins expressed in various tissues in the body. A substantial body of data has been published about the molecular genetics, structure, localization and developmental expression of the mammalian water channels (aquaporins); however, there is relatively little information about their importance in mammalian physiology.

Early perfusion studies suggested that proximal segments of small intestine have higher osmotic permeability than distal segments (Fordtran *et al.* 1965). The duodenum and proximal jejunum have been proposed to be highly water permeable to permit rapid osmotic equilibration of intestinal contents (Hindle & Code, 1962; Soergel *et al.* 1963; Powell & Malawer, 1968). The small intestine contains a highly convolved leaky epithelium with low electrical resistance and low reflection coefficients for small solutes. Rapid water movement across the epithelium in small intestine is generally believed to occur by a paracellular pathway. This concept was supported by the finding of low osmotic permeability of brush border membrane vesicles from small intestine (Worman & Field, 1985; van Heeswijk & van Os, 1986); however, these studies did not consider possible differences in water permeability in cell membranes derived from different segments of the small intestine. Recent data indicate expression of at least two different aquaporins in small intestine epithelia (Table_IV.27). Zeuthen and coworkers have proposed the interesting hypothesis that Na^+-glucose and other cotransporters in enterocytes are able to transport water actively and each turnover of the transporter is proposed to carry hundreds of water molecules and thus accomplish isosmolar fluid

transport (Zeuthen & Stein, 1994; Loo *et al.* 1996; Meinild *et al.* 1998). Although there is evidence in an oocyte expression system for coupled water transport by the Na^+-glucose transport protein (SGLT1) and other cotransporters, the possibility of water transport by these proteins in small intestine is so far unproven.

Table IV. 27 Expression of mammalian aquaporins in gastrointestinal tissues (Tonghui and Verkman; 1999)

AQP	Species	Tissue	Cell type	Method
AQP1	Human	Liver	Epithelium of intrahepatic bile duct	IH
			Endothelium of peribiliary capillaries	IH
		Gallbladder	Neck epithelium	IH
		Pancreas	Exocrine acinar cells	IF
		Colon	Crypt epithelium	IF
	Rat	Salivary gland	Capillary endothelium	IH, IG
		Liver	Cholingiocytes	IH
		Pancreas	Capillary endothelium	IH
		Oesophagus	Lymphatic endothelium	IH
		Small intestine	Endothelium of central lacteal	IH
		Colon	Lymphatic endothelium	IH
AQP3	Human	Colon, liver, pancreas and small intestine	?	NB
	Rat	Colon	Villus epithelium	IH
		Oesophagus	Squamous epithelium	IH
AQP4	Human	Stomach	Parietal cells and chief cells	IH
	Rat	Salivary gland	Epithelium of excretory duct	IH
		Stomach	Parietal cells	IH
		Ileum	Crypt epithelium	IH,IS
		Colon	Villus epithelium	IH
AQP5	Rat	Salivary gland	Secretory epithelium	IH
			Secretory lobule	IS
AQP7	Human	Small intestine	?	NB
	Rat	Small intestine	?	NB
AQP8	Rat	Salivary gland	Acinar cells	IS
		Liver	Hepatocytes	IS
		Pancreas	Acinar cells	IS
		Jejunum	Villus epithelium	IS
		Colon	Villus epithelium	IS
	Mouse	Liver, pancreas and colon	?	NB
		Salivary gland	?	RT-PCR
AQP9	Human	Liver	?	NB
	Rat	Liver	Hepatocytes	IS

Abbbreviations: IH, immunohistochemistry; IF, immunofluorescence; IG, immunogold; IS, *in situ* hybridization; NB, Northern blot; RT-PCR, reverse transcription-polymerase chain reaction.

Rapid water movement across the plasma membranes of cells is mediated by the membrane water channel proteins, aquaporins (AQPs). Matsuzaki et al, 1999 found that along the small intestine, AQP3 was hardly detected in the duodenum and jejunum. In the ileum, weak labeling for AQP3 was detected at the basolateral membranes of absorptive epithelial cells located in the villus tip.

Recently it has been postulated that water may be actively absorbed from small intestine through transporter proteins named aquaporins. As Alfuzosin Hydrochloride is highly soluble in water at all pH and more aquaporins reside in ileum, this might be another reason for higher absorption rate through ileum.

P gp

In 1976, Juliano and Ling found a correlation between the degree of drug resistance of Chinese hamster ovary cells and the presence of a 170 kDa membrane glycoprotein. The glycoprotein appeared to be unique to mutant cells displaying altered drug permeability, so they named it P-glycoprotein where the"P" stands for "permeability" (Juliano & Ling 1976). Pgp is a phosphoglycoprotein belonging to the ATP (adenosine triphosphate)-binding cassette (ABC) transporter super-family. ABC transporters have a highly conserved ATP binding region, which is characteristic for these transporters.

The function of Pgp in the intestine in not fully understood but it has been suggested that intestinal efflux transport by Pgp and other efflux proteins can affect the rate and extent of drug absorption and metabolism in rat and human intestine (Gan et al 1996; Terao et al 1996; Lown et al 1997; Kruijtzer et al 2002). Mechanism of multidrug resistance of P-gp is the ATP dependent multidrug efflux pump. Probably, there are three modes. One mode thinks P-gp combines with drug at once when drug diffuse into the cell membrane, and ATP provide the energy, then P-gp pump drug from the cell. One mode believes most lipophilic anticancer drug, which get through the cell membrane very slowly, combine with P-gp in the lipidic dual molecular layers, and are pumped from the cells when they doesn't get into the cells, what is called hydrophobic vacuum pump. The other mode suggests the inside cell are pumped by P-gp into the inside membrane lumen firstly, then pumped out of the cell (Hall et al, 1999).

In the intestine of human, P-glycoprotein is located almost exclusively within the brush border on the apical (luminal) surface of the enterocyte, and other normal tissues such as the liver, brain, kidney, and so on also express P-glycoproteins.

Expression level of P-gp in each small intestine varies with maximal expression in ileum and moderate express in duodenum and jejunum, and then a decrease in expression through the proximal and distal colon. Although the length of rat small intestine, the P-gp

mediated Rho 123 transport showed a regimal variation, and the transport rate in the ileum was about 2.5-to 5.0- fold higher than other regions (Yumoto et al, 1999).

One determinant for interaction with Pgp is the relative hydrophobicity of the interacting molecule. Substrates for Pgp have a partition coefficient (octanol/water) of approximately +1 or greater (Hunter & Hirst 1997). Pgp transports large hydrophobic, uncharged or slightly positively charged molecules, although it seems as if some hydrophilicity is needed (Sarkadi & Muller 1997; Stein 1997). Alfuzosin being a mildly lipophilic compound and exists in vivo mainly in cationic sate, might not be a substrate for Pgp.

Also as determined by Haddouche et al, concentration – dependence of alfuzosin was determined in ileum at luminal concentrations ranging from 0.1 to 5 mM and found that $Jm_{\rightarrow s}$(mucosal to serosal flux) increased linearly as a function of the concentration suggesting that the mechanism of intestinal passage of Alfuzosin is purely diffusional and is not a P glycoprotein substrate.

Also as per NDA 21-287(Centre for drug evaluation and research, 2003), pharmacokinetics of marketed formulation of Alfuzosin Extended release tabletsformulation is dose proportional over the range of 7.5 to 40 mg. Had it been a substrate to P-glycoprtein, pharmacokinetics would not have been linear.

CYP

Cytochrome P450 3A4 (CYP3A4), the major phase I drug metabolizing enzyme in humans, and P-glycoprotein (P-gp),the MDR1 gene product, are present at high concentrations in villus -tip enterocytes of the small intestine and share a significant overlap in substrate specificity. A large body of research both in vitro and in vivo has established metabolism by intestinal CYP3A4 as a major determinant of the systemic bioavailability of orally administered drugs.

There is a first pass extraction progress when drugs orally absorbed, which are metabolized by the microsomal cytochrome superfamily of enzymes. The cytochrome P450 are responsible for the majority of the oxidative metabolism of drugs.
The site of such first pass oxidative metabolism has usually been considered to be the liver because of its site, its relatively high level of P450 activity, and its anatomic location relative to the site absorption. However, recent studies indicate that CYP3A4 metabolic activity in the intestinal mucosa may substantially contribute to the over all first pass effect.
The expression of CYP3A4 in the intestine is site dependent that means the concentration of CYP3A4 is different in different portion. Abnormally CYP3A4 is greatest in the duodenum and lowest in the ileum. That is to say, the upper small intestine (duodenum and proximal jejunum) serves as the major site for intestinal first pass metabolism of drugs (Paine et al, 1993), De Waziers et al, 1990, have found the express level of CYP3A4 in duodenum is higher than that in liver.
Based on the in vitro and in vivo metabolism studies (UroXatral® (alfuzosin) package insert ,2003), Alfuzosin undergoes metabolism by the following pathways:

- Oxidation of the tetrahydrofuran ring, resulting in the formation of SL80.0306 and SL 80.0363.
- O-demethylation of the dimethoxyquinazoline ring, resulting in the formation of SL80.0037 and SL80.0018.
- N-dealkylation of the aliphatic chain, resulting in the formation of SL79.0723 and SL79.0724.
- N-demethylation of alfuzosin, resulting in the formation of SL85.0090.
- Hydroxylation of the parent compound, resulting in the formation of Met-A, Met-B and Met-D.

Although it has been reported that Alfuzosin undergoes O-demethylation, furan ring cleavage and N-dealkylation reactions in rat (Mas-Chamberlin et al., 1984), Abdenour Haddouche et al in his study reported lack of first-pass metabolism of Alfuzosin in the various intestinal regions and concluded that permeation mechanism occurs mainly through the paracellular route and Cyp is not involved in intestinal metabolism of Alfuzosin. Also as discussed above pharmacokinetics of Marketed formulation of Alfuzosin Extended Release Tablet is dose proportional in the range of 7.5 to 30 mg. Therefore, the possibility of Alfuzosin Hydrochloride being a substrate for CYP in small intestine is nullified.

IV.5 CONCLUSION

The results of the present study investigated confirmed the results of Haddouche et al., (1996) that Alfuzosin is found to be absorbed through ileum at a faster rate than in duodenum which is against much of literature claiming that higher absorption of Alfuzosin HCl occurs in the proximal part of small intestine (Maggi et al., 2000; Andrieu et al., 1995). Although Haddouche et al., 1996 studied by using Ussing chamber, we had utilized in –situ method (closed loop) which better predicts in vivo conditions.

Although Alfuzosin is absorbed by paracellular pathway as determined by Haddouche et al but it has a molecular weight of 384 and paracellular pathway is said to impermeable to compound above molecular weight of 200 daultons. In reality, molecular radii rather than molecular weight is the determinant factor responsible for absorption. This is based on the observation made by various authors in various studies as under:

Various studies have attempted to correlate the molecular weight of various hydrophilic molecules with their percentage of absorption (Hamilton et al., 1987; Arturrson et al., 1993; Chadwick et al., 1977). However it appears that the permeability of these molecules is not simply inversely proportional to their molecular weight or molecular radius but depends on the molecular structure of these molecules (Hamilton et al., 1987; Arturrson et al., 1993).

Polyethylene Glycol (PEG) has been used extensively as a marker of intestinal permeability since the 1970s (Chadwick et al, 1977) although in most studies it has been treated as a single molecular weight marker and its polydispersed nature was ignored. Some investigators have questioned the suitability of PEG as a paracellular probe. For example, the intestinal permeation rate of PEG 400 is significantly higher than mono-, di-, and trisaccharide markers of comparable molecular weight (Maxton et al, 1986). The higher than expected permeation of PEG 400 compared with other markers of similar molecular weight is almost certainly due to its smaller molecular size. A recent study using monodispersed PEG oligomers showed PEG 194 permeation to be sixfold greater than mannitol in cell monolayers despite being of comparable molecular weight (Artursson at al, 1993). However, the findings of the present study suggest this can be explained by the smaller hydrodynamic radius of PEG 194 (3.2 Å) compared with mannitol (4.1 Å). A relatively small difference in the radii of the probes will have profound effects on permeability particularly where they lie close to the radius of the paracellular pore.

As Alfuzosin was found to be more rapidly absorbed from ileum than in duodenum, there was no hypothesis proposed for that. We have looked at various reasons responsible for effecting permeability and found that "solvent drag theory" as most appropriate hypothesis to justify our results.

As it was confirmed that Alfuzosin was absorbed more rapidly through ileum, question arised whether it is a suitable candidate for gastro retentive system. Justification for this was found on the basis of absorption and dissolution number.

Lobenberg and Amidon, 2000, have summarized the relationships between dose, dissolution characteristics, drug solubility, and drug absorption properties. These relationships can be described as follows:

1. Absorption number (An) = $(P_{eff}/R) \bullet \langle Tsi \rangle$,

where R is the gut radius and <Tsi> the residence time of the drug within the intestine.

2. Dissolution number (Dn) = $(3D/r^2) \bullet (C_s/\rho) \bullet \langle Tsi \rangle$,

where D is the diffusivity of the dissolved drug, ρ is the density of the dissolved drug, C_s is the drug solubility, and r is the initial radius of the drug particle.

3. Ratio of dose to dissolved drug (D0) = $M/V_0/C_s$
where M is the dose of the drug and V_0 is the volume of fluid consumed with the dose.

The fraction of drug absorbed is closely related to the drug's effective permeability across mucosal cells. If the Peff of a drug is less than 2x 10^{-4}cm/s, then drug absorption will be incomplete, whereas complete absorption can be expected for substances whose Peff exceeds this value. For poorly soluble drugs, critical variables include the volume of the intestinal fluids, GI pH, and GI transit time (where adequate time is needed to dissolve poorly soluble materials). For these lipophilic compounds, food and bile salts may increase drug solubility.

Class I compounds are highly permeable and readily go into solution (Dn > 1). In this case, the fraction absorbed (F) can be expressed as follows:

$F = 1 - \exp(-2An)$.

For these agents, as "An" increases, the fraction of drug absorbed increases, with 90% absorption (highly permeable compounds) occurring when An = 1.15. Referring back to the equation for An, we see that F can be affected by a change in the compound's membrane permeability, the gut radius of the host, or the intestinal transit time. Based on these factors alone, it is evident that differences in GI physiology due to factors such as disease, age, or animal species can alter the value of An and, therefore, the fraction of drug absorbed.

For Class II drugs (high permeability, low solubility), Dn < 1. In these cases, the relationship between D0 and Dn is critical for determining the fraction of drug absorbed, and the rate of drug dissolution tends to be the rate-limiting step. Accordingly, anything that increases the rate and extent of in vivo dissolution will also increase the bioavailability of that compound.
In PharmPK discussion, 2006, calculations for dose number, dissolution and absorption number for Alfuzosin were done by the equations which are as under. Also Alfuzosin was compared with Metoprolol which has properties similar to that of Alfuzosin.

Alfuzosin:

log D(7.4) = 1.89
Base pKa = 8.13
Exlog P = 1.4
Intrinsic solubility = 0.438 mg/mL
Peff = 0.27 x 10^-4 cm/s (human jejunal effective permeability (Peff)
Dose number = 0.83 small intestine (Dose / Amount dissolved in 250 mL water at intrinsic solubility)
Dissolution number = 43.7 (mean small intestine transit time / mean dissolution time)
Absorption number = 0.54 (mean small intestine transit time / mean absorption time)

Metoprolol:

log D(7.4) = 0.26
Base pKa = 9.7
Exlog P = 1.88
Intrinsic solubility = 9.2 mg/mL
Peff = 0.94 x 10^-4 cm/s (human jejunal effective permeability (Peff))
ExPeff = 1.3 x 10^-4 cm/s
Dose number = 0.0026 (Dose / Amount dissolved in 250 mL water at intrinsic solubility)
Dissolution number = 5x10^-3 (mean small intestine transit time / mean dissolution time)
Absorption number = 1.88 (mean small intestine transit time / mean absorption time)

According to Kasim et al, 2004, having a log P less than the log P for metoprolol would be sufficient to categorize the molecule as being low permeability. However in this case the log P values of Alfuzosin and Metoprolol are nearly equal and by virtue of its higher pKa , metoprolol has a much lower distribution coefficient at pH 7.4. Therefore, on the basis of log P or log D alone the categorization of high or low permeability might be difficult.

However, permeability depends on more than just lipophilicity. Hydrogen bonding is a key parameter in determining how fast a molecule can passively cross through a biological membrane. Alfuzosin ($C_{19}H_{27}N_5O_4$, Molecular weight (Mwt) 389.46 g/mol) has five nitrogen's and four oxygen's all of which can participate in H-bonding to the polar head groups of a phospholipid bilayer. Even though Alfuzosin does not break any of Lipinski's rules (> 5 hydrogen bond donors, > 500 MWt, > 5 log P, > 10 # N and O) it does have an estimated human effective permeability that is much lower than the experimental Peff for Metoprolol. As mentioned above, the Absorption Number (small intestinal transit time / small intestinal absorption time) calculated by GastroPlus (Simulations Plus, Inc.) is 0.54 for Alfuzosin and 1.88 for Metoprolol. Therefore, the mean time for absorption of Alfuzosin would be longer than the mean small intestinal transit time. On the basis of the low estimated Peff and low absorption number one would

have to put Alfuzosin into the low permeability class. The Dissolution Numbers and Dose Numbers for Alfuzosin and Metoprolol would definitely put them both in the high solubility category. Based on above equations and justification, Alfuzosin according to Biopharmeceutical classification system should fall under Class III (high solubility, low permeability).

So definitely prolonging the residence time in the stomach and providing a release profile where dissolution rate is equivalent to absorption rate will improve the bioavailability of the drug by increasing the small intestine transit time. Therefore, our selection of Alfuzosin as model drug for Gastroretentive drug delivery is appropriate and well justified.

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