Stable and Bioequivalent Formulation Development of Highly Acid Labile Proton Pump Inhibitor: Rabeprazole

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Abstract Rabeprazole sodium is a Proton-Pump-Inhibitor (PPI), used for treatment of acidity by inhibiting H^+/K^+/ATPase pump at the gastric parietal secretary cells. The main challenge in the formulation of Rabeprazole is to prevent degradation of Active Pharmaceutical Ingredient (API) upon exposure to acidic environment or moisture. In order to prevent acid degradation of API in Gastro Intestinal Tract (GIT), tablets should be enteric coated to prevent its exposure to gastric acid. Coating of acid labile PPI with enteric coating acidic material may further cause the decomposition of acid labile PPI. In order to overcome this limitation, the core containing acid labile PPI and alkalizers was seal coated with water-insoluble polymer and non-hygroscopic alkalizer (stabilizer) and subsequently enteric coating was done on seal coated tablet, with a polymer to prevent its exposure to acidic environment of GIT and facilitate absorption through intestinal fluid. The optimized formulation was packed in Alu-Alu blister and charged for stability study at 40°C/75%RH. It was observed that over 6 months the formulation was stable with impurities in control. *In vivo* bio-equivalence study was performed, in which optimized formulation was found bio-equivalent with marketed reference formulation.

Keywords: Bio-equivalence, Enteric-coating, Rabeprazole, Seal-coating, Stability.

INTRODUCTION

Parietal cells of the stomach secret gastric acid in response to stimuli such as the presence of food in the stomach or intestine and the taste, smell, sight or even thought of food. Such stimuli result in the activation of histamine (H_2), acetylcholine (M_3) or gastrin (CCK_2) receptors located in the basolateral membrane of the parietal cells, which initiate signal transduction pathways resulting in activation of H^+/K^+/ATPase Pump (also known as Proton pump) consequentially inducing gastric acid secretion[1]. If this proton pump is inhibited by some means than gastric acid secretion will be reduced.

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Amongst all Proton-Pump Inhibitors (PPIs), Rabeprazole is the most potent acid secretion inhibitor during first day of dosing[2]. Rabeprazole is chemically 2-[[4-(3-methoxypropoxy)-3-methyl-pyridine-2-yl] methyl sulfinyl]-1H-benzimidazole[3], mainly used for treatment of acidity by decreasing acid secretion through inhibiting especially H^+/K^+/ATPase enzymatic system present on the secretary surface of gastric parietal cells[4]. Rabeprazole is a pro-drug that is converted to their active form in acidic environments. Rabeprazole is a weak base, and so specifically concentrates in the acidic secretory canaliculi of the parietal cell, where it is activated by a proton-catalysed process to generate a sulphenamide. As represented in Figure 1, the sulphenamide interacts covalently with the sulphydryl groups of cysteine residues in the extracellular domain of the H^+/K^+/ATPase enzyme - in particular Cys 813 - thereby inhibiting its acid secretary activity[5].

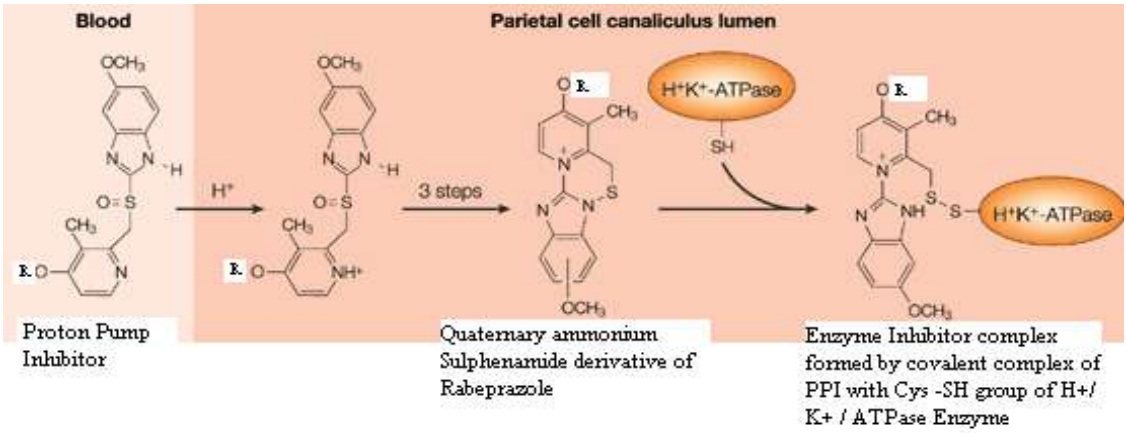

Fig. 1. Schematic representation of proton pump pnhibition by Rabeprazole (R= -$(CH_2)_3OCH_3$) in acidic secretary canaliculi of the gastric parietal cells[6] .

Upon exposure to acidic or neutral environment or upon exposure to moisture Rabeprazole is converted to colored degradation products, as depicted in Figure 2[7]. So, it is recommended to formulate Rabeprazole as an enteric coated tablet to prevent decomposition of the API upon exposure to gastric acid. But most of the enteric coating materials are acidic in nature (e.g. Pthalate- Pthalic acid ester or Acrylate-Acrylic acid copolymers)[8], that may cause the decomposition of acid labile PPI. In present research work, to prevent the interaction of Rabeprazole with acidic enteric coating material, core tablet is seal-coated with water-insoluble polymer. Moreover, seal-coating provides a moisture barrier to core tablet[9].

Prior art search showed, a Japanese patent describing a method comprising intermediately coating the core containing PPI with water soluble polymer and then seal coated tablet was coated with an enteric coating polymer, but seal coating with water soluble polymer could not sufficiently extend the shelf-life of formulation[10]. An European patent application has disclosed a composition of Rabeprazole sodium with potassium hydroxide, sodium hydroxide and potassium carbonate, but these alkalizers are hygroscopic in nature and leading to degradation of Rabeprazole, because it absorbs water into the formulation[11]. Another excipient for stabilization of acid labile API is Magnesium oxide (MgO). Since MgO is very slightly soluble in purified water and its saturated solution yields a pH of about 10.3, it provides

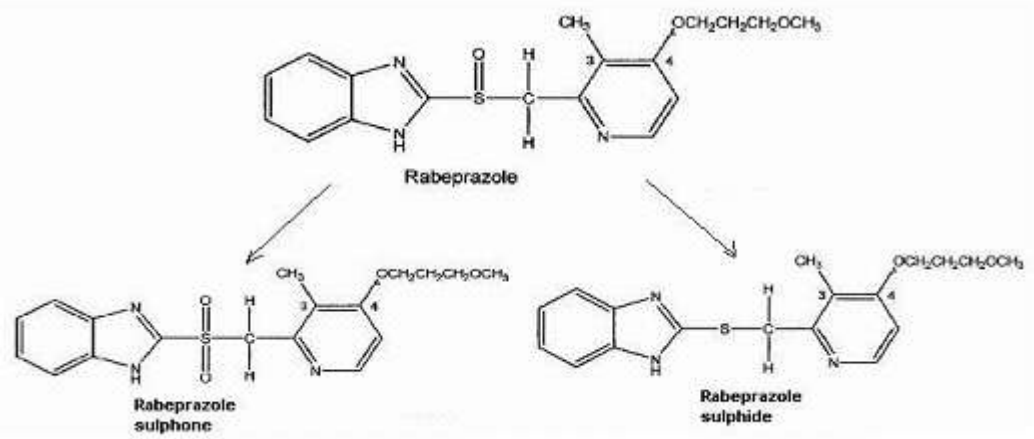

Fig. 2. Two major degradation products of Rabeprazole.

Table 1 Formulation ingredients of Rabeprazole tablet

Category	Ingredients	Application
Intra-granular ingredients	Rabeprazole sodium	Active Pharmaceutical Ingredient
	Mannitol	Bulking agent
	L HPC LH 21	Disintegrant
	Sodium hydroxide	Stabilizer (alkalizer)
	Absolute alcohol	Solvent
Extra-granular ingredients	Mannitol	Bulking agent
	Disodium hydrogen phosphate anhydrous	Stabilizer (alkalizer)
	L HPC LH 21	Disintegrant
	Purified Talc	Anti-adherant
	Magnesium stearate	Lubricant
Seal-coating ingredients	Ethyl cellulose 4 cps	Water- insoluble Polymer
	Light magnesium oxide	Stabilizer (alkalizer)
	Magnesium stearate	Anti-sticking agent
	Absolute alcohol	Solvent
Enteric-coating ingredients	Instacoat® Hydroxy Propyl Methyl Cellulose Pthalate (HPMCP)- IC EN 783	Enteric coating polymer
	Absolute alcohol	Solvent

alkaline pH to Rabeprazole micro-environment, which is most favorable for its stability[12].

MATERIALS AND METHODS

Materials

Rabeprazole sodium (Cadila Pharmaceuticals Limited, India), Mannitol (Roquette, France), Low substituted Hydroxy propyl cellulose (L-HPC, Shinetsu Chemicals, Japan), Sodium hydroxide (Cento Pharma, India), Disodium hydrogen phosphate anhydrous BP (Sujata Chemicals, India), Ethyl cellulose (Colorcon Asia pvt ltd., India), Light magnesium oxide BP (SCORA, France), Purified Talc BP (Luzenac Pharma, USA), Magnesium stearate BP (Ferro Synpro VG, Ohio), Methylene chloride BP (Reliance chemicals, India), Absolute alcohol BP (Shree Chalthan Vibhag Khand Udyog Sahakari Mandli Ltd., India). All other reagents were of analytical grade and they were used as received from manufacturer or supplier. Applications of the entire active ingredient as well as inactive ingredients (excipients) are mentioned in Table 1.

Experimental Methods

Rabeprazole is moisture sensitive API, hence all the processing steps including granulation, compression and coating were carried out at 30º±5ºC and 30±5% Relative Humidity (RH). Due to light sensitivity of Rabeprazole, processing was carried out under the light of sodium vapor lamp.

Preparation of core tablet

Intra-granular and extra-granular material as enlisted in Table 2 were accurately weighed and sifted through 40# sieve. Intragranular material was mixed in the dry state in Rapid Mixer Granulator (RMG- Sigmagran®) for 10 minutes at slow speed of impeller. Weighed sodium hydroxide was completely dissolved in absolute alcohol with continuous stirring. This binding solution was slowly added to pre-mixed intra-granular material in RMG mentioned above and mixed at 75 RPM of Impeller speed for 5 minutes. Then prepared wet mass was dried in Fluidized Bed Dryer (FBD-Retsch® TG-200) at inlet temperature of 50ºC-60ºC and outlet temperature

Table 2 Formulations details of Rabeprazole Tablets, 20mg (core and coated tablet)

Formulations	Rab 1	Rab 2	Rab 3	Rab 4	Rab 5	Rab 6
Intra-granular Ingredients (mg)						
Rabeprazole sodium	20.00	20.00	20.00	20.00	20.00	20.00
Mannitol	76.10	76.10	66.10	71.10	76.10	76.10
L-HPC	5.00	10.00	15.00	15.00	20.00	15.00
Sodium hydroxide	0.40	0.40	0.40	0.40	0.40	0.40
Absolute alcohol	0.50	0.50	0.50	0.50	0.50	0.50
Extra-granular Ingredients (mg)						
Mannitol	15.00	15.00	25.00	20.00	15.00	15.00
L-HPC	20.00	15.00	10.00	10.00	5.00	10.00
Disodium hydrogen phosphate	5.00	5.00	5.00	5.00	5.00	5.00
Purified talc	5.00	5.00	5.00	5.00	5.00	5.00
Magnesium stearate	3.00	3.00	3.00	3.00	3.00	3.00
Total weight of core tablet	**150.00**	**150.00**	**150.00**	**150.00**	**150.00**	**150.00**
Seal-coating Ingredients (mg)						
Ethyl cellulose 4cps	0.96	0.96	0.96	0.96	0.96	0.96
Light Magnesium oxide	0.77	0.77	0.77	0.77	0.77	0.77
Magnesium stearate	0.77	0.77	0.77	0.77	0.77	0.77
Absolute alcohol	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Total weight of Seal coated tablet	**152.50**	**152.50**	**152.50**	**152.50**	**152.50**	**152.50**
Enteric-coating Ingredients (mg)						
Instacoat® Hydroxy Propyl Methyl Cellulose Pthalate (HPMCP)	15.00	15.00	15.00	15.00	15.00	15.00
Absolute alcohol	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Final weight	**167.50**	**167.50**	**167.50**	**167.50**	**167.50**	**167.50**

Abbreviations: Rab No.= Rabeprazole tablet formulation number

of 40ºC-45ºC for approximately 50 minutes, until LOD not more than 2.0% w/w at 105ºC for 4 minutes was achieved.

Dried granules were sifted through multi-mill (Cadmill®, Cadmach Machinery Co. Ltd.) equipped with 1.5-5.0 mm screen at 500-600 RPM and collected in polyethylene lined drum. The dried milled granules were transferred to double cone blender and extra-granular material were added to it and mixed for 10 minutes in same blender. The prelubricated blend was ready for lubrication. For lubrication magnesium stearate was added in double cone blender and mixed at 10 RPM for 5 minutes. Sampling was done to check the uniformity of the blend. The final blend was compressed into core tablet using round shaped plain standard concave die-punches set of 9/32" dimension in rotary tablet press (RIMEK®, India).

Seal - coating of core tablet

Seal coating serves as a separating layer to prevent interaction between acid labile Rabeprazole sodium and acidic enteric coating material. In addition, it also acts as a moisture barrier to core tablet. Seal coating materials were dispensed as mentioned in Table 2 and dissolved in absolute alcohol. This was stirred for 45 minutes till homogeneous suspension was obtained that was sifted through 60# screen. Seal coating of core tablets was done in 24" auto coater (Ganson Auto

Table 3 Coating parameters

Coating parameters	Set limits
Pan speed	2-8 RPM
Inlet temperature	50ºC ± 5ºC
Outlet temperature	40ºC ± 5ºC
Tablet bed temperature	30ºC ± 5ºC
Peristaltic pump speed	5-10 RPM
Compressed air pressure	2-4 kg/cm^2
Atomizing air pressure	2-4 kg/cm^2
Spray rate	100-150 gm/minute

Coater® GAC-275, India) using the same solution as per set parameters, as mentioned in Table 3.

Enteric - coating of seal-coated tablet

Enteric coating of the seal coated tablet was done to prevent release of Rabeprazole into gastric acidic media as Rabeprazole is unstable at gastric pH. For enteric coating, accurately weighed HPMC Pthalate as mentioned in Table 2 was slowly added into absolute alcohol and mixed for 45 minutes, subsequently passed through 100# screen. Seal coated tablets were preheated in coating pan for 10 minutes at 40ºC -45ºC. The tablets were coated in 24" auto coater (Ganson Auto Coater® GAC-275, India) to achieve 3% weight gain. After enteric coating (up to 3% weight gain), average weight gain and average thickness was checked for 20 tablets. Enteric coated tablets were dried to achieve constant weight gain. The pictorial diagram of enteric coated tablet of Rabeprazole is displayed in Figure 3.

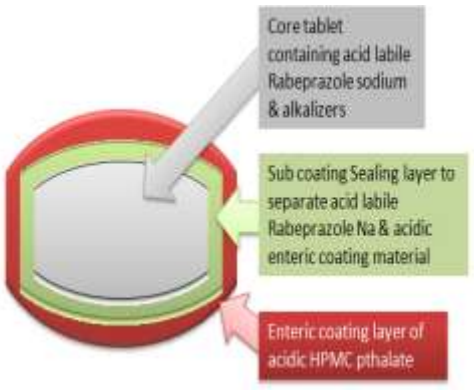

Fig. 3 Schematic representation of enteric coated Rabeprazole sodium tablet.

In Process Quality Control (IPQC) testing of Rabeprazole tablets

Enteric coated tablets of Rabeprazole were subjected to IPQC tests that includes appearance, dimension (diameter and thickness), hardness, friability, disintegration time; during compression and coating as per Pharmacopoeia[13]. The formulations were also assessed by content uniformity test and dissolution testing by USP Type I Basket apparatus at 50 RPM in 900 ml of 0.1 N HCl for 120 minutes and afterwards in Phosphate buffer of pH 7.4 for 45 minutes[14].

Stability study of Rabeprazole tablets

Final optimized formulation was packed in Alu-Alu blister (cold formed foil: made up of 25 micron OPA Film /Adhesive/45 micron Aluminium foil/ Adhesive/60 micron PVC film) and charged for accelerated stability study for 6 months at 40°±2°C and 75%±5% RH[15] in stability chamber (Thermolab®, India). During stability study, Assay as well as % Related Substances (RS) were determined at 1 Month, 3 Months and 6 Months.

Bio-Equivalance (BE) study

In vivo bioavailability study of Rabeprazole tablets was performed for optimized stable formulation of Rabeprazole tablets to determine the rate and extent of drug absorption into blood from drug released from enteric coated tablets. A randomized, two treatment, two-period, two sequence, single dose, two way crossover bioavailability study on Rabeprazole sodium 20 mg tablet of Cadila Pharmaceuticals Ltd., India (Test Drug Product) compared with that of Pariet® 20 (Rabeprazole sodium 20 mg) tablet of Janssen Pharmaceutica[16], Belgium (Reference Drug Product) in 23 healthy, adult, male human subjects under fasting conditions was carried out. Rabeprazole sodium was extracted from human plasma by a Liquid-liquid extraction by using 5 mL Tertiary Butyl methyl ether as an extracting solvent[17]. The study protocol was approved by ethical committee and subjects were duly informed.

The pharmacokinetic parameters were calculated for Rabeprazole sodium 20 mg Tablet using WinNonlin-Pro® Software 4.0.1 version (Pharsight Corporation, USA)[18]. Descriptive statistics (Mean, SEM) for Rabeprazole sodium 20

Table 4 Results of IPQC testing of all formulations of Rabeprazole tablets

IPQC Parameters	Rab 1	Rab 2	Rab 3	Rab 4	Rab 5	Rab 6
Diameter (mm.)	7.15 ± 0.40	7.13 ± 0.60	7.12 ± 0.20	7.13 ± 0.10	7.15 ± 0.10	7.14 ± 0.20
Thickness (mm.)	3.62 ± 0.30	3.63 ± 0.10	3.63 ± 0.40	3.66 ± 0.10	3.65 ± 0.50	3.65 ± 0.30
Friability (%w/w)	0.21	0.13	0.31	0.36	0.42	0.09
Hardness (Kg/cm^2)	7-8	9-10	8-9	8-9	9-10	9-10
Disintegration test profile						
a) Coating layer removal time (minutes.)	24	36	37	32	34	29
b) Complete disintegration time (minutes)	57	54	46	53	53	48
Dissolution test profile						
Percentage drug release						
a) 0.1 N HCL within 02:00 hrs	00	00	00	00	00	00
b) pH 8.0 Tris HCl buffer after 2:00 hrs						
1) 02:10	02	03	06	08	07	14
2) 02:20	24	29	32	36	34	37
3) 02:30	43	48	67	63	59	68
4) 02:40	74	79	81	79	76	91

mg Test and Reference Tablets was calculated for all pharmacokinetic parameters.

RESULTS AND DISCUSSION

In Process Quality Control (IPQC) Tests

The results of in process quality control tests, are listed in Table 4. Hardness of all the formulations lies within the range of 5-8 kg /cm^2, suggested that the product was firm enough to withstand handling without breaking, chipping or crumbling and not so hard that the disintegration time was unduly prolonged. All the formulations passed in friability test, because % weight loss was within Pharmacopoeial limit. In *in vitro* disintegration test, enteric coating layer was remained intact in 0.1N HCl for 2 hrs. Enteric coating layer of tablet started to imbibe the alkaline media of Tris-HCl buffer of pH 8.0 and completely removed approximately at 30 minute and afterwards tablets were completely disintegrated within 50 minutes.

Stability study

Results of stability study are summarized in Table 5, the results showed that level of known impurities was below 0.5% and are acceptable as per ICH guidelines[19]. Furthermore, assay results of Rabeprazole suggested that Rabeprazole remained stable in sealed Alu-Alu blister packing in the optimized formulation for 6 months in stability study at 40°C/75%RH.

Bio-Equivalence (BE) Study

Pharmacokinetic parameters (C_{max}, T_{max}, K_{el}, $t_{1/2}$, AUC_{0-t} and $AUC_{0-\infty}$) for both test and reference drug products are summarized in Table 6. In this study, both formulations under the study (i.e. Test and Reference) follow the same pattern in absorption, metabolism and elimination phases for Rabeprazole sodium 20mg tablet.

The relative bioavailability was assessed by ratio of C_{max} and $AUC_{0-\infty}$ values. The ratios of least square Means of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were

Table 5 Results of stability testing of optimized formulation at 40°C/75%RH

Test conditions / Level of impurity	Rabesulphone (%)	Rabesulphide(%)	Max. Ind.	Total Impurity (%)	Assay (%)
Initial	0.26	0.07	0.06	0.47	99.40
1 Month	0.41	0.11	1.61	2.62	100.60
3 Months	0.46	0.12	1.43	2.53	99.30
6 Months	0.48	0.14	1.46	2.38	99.10

Table 6 Summary of pharmacokinetic parameters of Rabeprazole test (Cadila) and reference (Pariet) tablets

Pharmacokinetic parameters	Rabeprazole sodium 20mg Tablet	
	CADILA Test Tablet	EISSAI Pariet® Reference Tablet
C_{max} (ng/ml)	340.279 ± 27.319	322.689 ± 29.877
T_{max} (hr)	2.000 ± 0.213	2.304 ± 0.208
K_{el} (hr^{-1})	0.812 ± 0.087	0.761 ± 0.085
$t_{1/2}$ (hrs)	1.114 ± 0.133	1.329 ± 0.211
AUC_{0-t} (ng/ml/hr)	561.767 ± 67.273	552.823 ± 67.350
$AUC_{0-\infty}$ (ng/ml/hr)	621.907 ± 69.078	622.299 ± 66.486

All values represent Mean ± SEM of twenty three subjects.

101.27%, 100.33% and 99.77% respectively for generic Rabeprazole 20mg tablet of Cadila Pharmaceuticals Ltd., India compared with that of Pariet 20mg (Rabeprazole sodium 20mg) tablet of Janssen Pharmaceutica, Belgium.

Since the 90% CI for AUC, C_{max} ratios were falling within the 80-125% for Rabeloc 20mg (Rabeprazole sodium 20mg) tablet with Pariet 20mg (Eisai Co. Ltd. Tokyo) tablet, it was concluded that the generic Rabeprazole 20mg tablet of Cadila Pharmaceuticals Ltd., India is bioequivalent to Pariet 20mg Tablet of Janssen Pharmaceutica, Belgium after single dose administration in healthy human volunteers.

CONCLUSION

The main challenge in the formulation of Rabeprazole tablets was the degradation of Rabeprazole upon exposure to acidic environment which results into high impurity level. Thus, to prevent exposure of Rabeprazole in gastric acidic environment enteric coating was done and to prevent interaction between Rabeprazole and acidic enteric coating material, seal coating was done over the core tablet.

Another challenge in the formulation of Rabeprazole tablets was to match rate and extent of bioavailability of test product with Reference product i.e. Pariet®. To achieve the same rate and extent of bioavailability as Pariet®, formulation containing different ratios of intragranular and extragranular diluent and disintegrant were prepared as mentioned in Table 2.

Among all six formulations, formulation VI passed in all IPQC parameters and it showed similar disintegration and dissolution profile as shown by Pariet®. Formulation VI did not release Rabeprazole in gastric acidic pH and released 91% of Rabeprazole in 45 minutes at intestinal alkaline pH. During stability testing, Rabeprazole remained stable upto 6 Months at 40°C/75%RH with impurity levels within limits. In formulation VI, the 90% CI for AUC and C_{max} ratios were falling within the range of 80-125% for Rabeloc 20mg (Rabeprazole sodium 20mg) tablet with Pariet® 20mg (Eisai Co. Ltd. Tokyo) tablet. So from above

data, it can be concluded that Rabeprazole 20mg tablet of Cadila Pharmaceuticals Ltd., India is stable and bioequivalent to reference product i.e. Pariet® 20mg tablet of Janssen Pharmaceutical, Belgium in terms of both rate and extent of bioavailability after single dose administration in healthy human volunteers.

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