COMPARATIVE *IN VITRO* EVALUATION OF COMMERCIALLY AVAILABLE PANTOPRAZOLE TABLETS

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ABSTRACT
Comparative study on *in vitro* evaluations (hardness, friability, weight variation, assay, disintegration and dissolution tests) of marketed pantaprazole tablets (2 batches of each) from WHO GMP certified Nepalese companies (encoded with BP-02-A1, BP-02-A2), non-GMP certified Nepalese companies (encoded with BP-02-B1, BP-02-B2) and multinational companies (encoded with BP-02-C1, BP-02-C2) were done. The result of hardness, friability, weight variation, and assay and disintegration tests of all marketed products comply with pharmacopoeial limit. However, BP-02-A2 showed the fastest disintegration. Moreover, the comparison of percentage drug release of these companies on the basis of dissolution study demonstrated that BP-02-A2 (90 % drug release) complied best with standard RDRL protocol while BP-02-B2 (78% drug release) does not comply with above specification.

INTRODUCTION
Pantoprazole is proton pump inhibitor, which prevents the production of acid in the stomach.It reduces symptoms and prevents injury to the esophagus or stomach in patients with gastro esophageal reflux disease (GERD) or ulcers. Pantoprazole is also useful in conditions that produce too much stomach acid such as Zollinger-Ellison syndrome. Pantoprazole are found in the enteric coated tablet form. The drug binds irreversibly to the proton pumps, causing prolonged inhibition of gastric acid secretion.

The active ingredient in pantoprazole (pantoprazole sodium) delayed-release tablets is a substituted benzimidazole, sodium 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole sesquihydrate, a compound that inhibits gastric acid secretion. Its empirical formula is $C_{16}H_{14}F_2N_3NaO_4S$ x 1.5 H_2O, with a molecular weight of 432.4.
Pantoprazole sodium is a white to off-white crystalline powder, racemic and has weakly basic and acidic properties. It is freely soluble in water, very slightly soluble in phosphate buffer at pH 7.4, and practically insoluble in n-hexane. The stability of the compound in aqueous solution is pH-dependent [1] .

The purpose of this work is to compare some brands of pantoprazole tablets from WHO GMP certified, non-GMP certified (Nepalese) companies and multinational companies (Indian) on the basis of *in vitro* evaluation of tablets.

MATERIALS AND METHODS
Sample: Marketed Pantoprazole tablets (two batches of each) from WHO GMP certified Nepalese companies encoded with BP-02-A1, BP-02-A2, non-GMP certified Nepalese companies encoded with BP-02-B1, BP-02-B2 and multinational companies (Indian) encoded with BP-02-C1, BP-02-C2 were procured from the market.

Chemicals: Standard Pantoprazole was donated by National Healthcare Lab. (Chhata Pipra, Birgunj, Nepal) and all other reagents were of analytical grade and procured commercially.

Reagent: 0.1N HCl and phosphate buffer (pH 6.8) were used.

Equipments: Following equipments were used: Weighing balance (OHAUS, USA), Digital Vernier Caliper (Model no. CD-6" CS, Serial no.0202004, Mitutyo corporation, Japan), Hardness tester (Monsanto type, HICON, Grooves Enterprises, India), Friability Tester (HICON Pvt. Ltd., India), Tablet Disintegration Tester (HICON, Grooves Enterprises, India), Tablet Dissolution Tester-USP 24 (Model No. TDT-06P, Electrolab, India), pH meter (pH 211/Hanna Microprocessor pH meter), UV Spectrophotometer (Shimazdzu UV-1601).

Preparation of calibration curve: Standard solutions of different concentrations ranging from10 µg/ml to 80 µg/ml were prepared and concentration versus absorbance graph (standard calibration curve) was plotted. Unknown concentration of Pantoprazole was determined by using the equation of the standard curve.

In vitro evaluation: Hardness - The tablet hardness was measured by taking 10 tablet by the equipment mentioned above.

Friability: 20 tablets were selected randomly and weighed individually, then placed in the friability test apparatus. It was then operated for 100 revolutions. The tablets were then dusted, reweighed and percent loss was calculated.

Weight Variation: 20 tablets were selected randomly and weighed individually. The average weight was calculated and individual weight was compared to the average weight. The tablet passes the test if not more than two of the individual weights deviate from the average weight by more than ± 7.5% and none deviated by twice ± 7.5% [2].

Disintegration Test: The disintegration test was performed by taking 1 tablet in each of the six tubes of the basket. The apparatus was operated using 0.1N HCl as immersion fluid at 37 ± 2 °C for 2 hours. Then after, tablets were observed for any sign of disintegration, cracking or softening. Then, immediately tablets were taken outside and the immersion fluid was replaced with phosphate buffer, pH 6.8 and apparatus was operated on same condition for 1 hour.

Assay: 20 tablets were weighed and powedered. 35 mg of pantoprazole sodium equivalent to pantoprazole was weighed and dissolved in ethanol to produce, the solution was filtered. 1 ml of sample was taken and dissolved 50 ml volumetric flask. Absorbance was measured at 289 nm using Shimadzu UV Spectrophotometer model UV 1601 and percent purity was determined. This method was as per Royal Drug Research Laboratory (RDRL, Bijulibazar, Kathmandu, Nepal) protocol [3].

Dissolution Tes: The dissolution test was conducted using simulated gastric fluid (0.1N HCl) and intestinal fluid (phosphate buffer, pH-6.8) as dissolution medium. Using simulated gastric fluid, 900 ml of 0.1N HCl was placed in the vessel and allowed to come to 37 ± 0.5 °C. Then, pantoprazole tablets were placed in six baskets, one in each basket and stirrer was rotated at 100 rpm for 2 hrs. After 2 hrs, the medium was thrown to observe the integrity of coating layer of tablets. The coating layer was found to remain intact. Immediately, same tablets were placed in 900 ml of phosphate buffer (pH-6.8) at same rotation speed and

temperature as mentioned above for 1 hr. After 15, 30 and 45 min, sample of 5 ml was pipetted out and same volume of fresh phosphate buffer was added to keep volume of the dissolution medium constant. The sample was diluted to 15 ml and the absorbance was measured at 289 nm and calculation was done using Lambert beer's law. Similarly, the absorbance of known concentration of standard solution of pantoprazole was measured and percent drug release was calculated as per RDRL protocol [3].

Statistical analysis: The results are expressed as mean and standard deviation.

RESULTS AND DISCUSSIONS

Detail result about in vitro evaluations (hardness, friability, weight variation, disintegration, assay, dissolution tests) of marketed pantoprazole tablets is given in Table 1.

Hardness: The hardness of the tablets was tested with tablet hardness tester varied from 5.45 + 0.41 to 9.6+ 1.42 kg.

Friability: Friability test was performed by Roche type friabilator. The weight losses of the tablets were 0.17% to 0.29% and from 0 to 0.03% for WHO GMP certified and non-GMP certified companies (Nepal) respectively while the rage for multinational companies of India was from 0 to 0.21%. However, the range was within pharmacopoeial limit [2].

Weight variations: The content is calculated from the average weight of each brand. Based on the content of pantoprazole tablets, the average weight required to get the content. It is called as range (standard) for 100 % value. Then on this basis, the maximum tolerance weight (105 %) and minimum tolerable weight (95%) was calculated to evaluate the weight variation result. The weight variation tolerances of tablets differ depending on average tablet weight. The result of weight variation showed that all batches of companies encoded BP-02-A1, BP-02-A2 (WHO GMP certified company of Nepal), BP-02-B2 (non-GMP certified companies, Nepal) as well as multinational companies encoded with BP-02-C1, BP-02-C2 fall within 7.5% tolerance except one non-GMP certified company (Nepal) encoded with BP-02-B1, which come within 5% tolerance. All companies satisfy USP specification [2].

Disintegration test: Disintegration test was done using 0.1N HCl and phosphate buffer (pH 6.8). Neither of the tested tablets disintegrated up to 2 hrs in 0.1N HCl nor there was any sign of cracking or softening. But in phosphate buffer (pH 6.8), all tablets disintegrated at different time. WHO GMP certified company (Nepal), encoded BP-02-A2 showed the fastest disintegration, the disintegration time were 4.85±0.42 min, 5.4±0.63 min respectively for two batches. The disintegration time of BP-02-A1 was 4.85±0.42 min, 5.76±0.92 min respectively for batches 1 & 2. The disintegration time for (non-GMP certified companies, Nepal) BP-02-B1, were 11.84±2.32 min, 10.23±1.47 min respectively for two batches. The disintegration times of BP-02-B2 were 8.32±1.6 min, 7.42±0.32 min respectively for two batches. The multinational companies (India) BP-02-C1 demonstrated the slowest disintegration. The disintegration times were 13.63±1.23 min, 12.21±1.32 min respectively. The disintegration times for BP-02-C2 were 10.21±0.26 min, 10.21±0.26 min respectively for two batches. The specification for the disintegration of enteric coated tablet in phosphate buffer (pH 6.8) is 1 hour according to B.P. [4].

Assay: The content of pantoprazole tablets was found ranging from 36.44 mg to 39.1mg of the stated amount (40mg per tablet).

Dissolution test: Dissolution test, as per RDRL protocol was carried out in six tablets of each batch. The percentage drug release was analyzed in both 0.1 N HCl and in phosphate buffer (pH 6.8) and shown in fig 1. The percent drug release from all companies (WHO GMP certified, non-GMP certified and multinational companies) in 0.1 N HCl were less than 10% and were in range of 0.85% to 3.2%.

In phosphate buffer, the percent drug release was significantly higher. The percent drug release from both batches of (WHO GMP certified company) BP-02-A1 was 83.85±1.46 and 82.0±1.02 and from BP-02-A2 was 83.97±0.86 and 90.02±3.17 respectively. The percent drug release from both batches of (non GMP certified company) BP-02-B1 was 78.07±1.23 and 82.49±0.97 and from BP-02-B2 was 82.42±0.56 and 81.02±0.23 respectively. The percent drug release from both batches (multinational companies, India) BP-02-C1 was 86.42±1.12 and 85.3±1.22 and from BP-02-C2 was 87.32±1.23 and 85.42±1.42 respectively.

The possible reason for the difference in dissolution rate may be due to difference in particle or surface area of the drug particles [5]. The fast rate of dissolution may be due to the inclusion of such disintegrant by manufacturer which does not swell but exert its disintegrating action by capillary action because capillaries are likely to be formed and liquid is drawn up through these capillaries at very rapid rate. It ruptures the intergranular bond and the granules are thrown apart with resultant breaking of tablets [6].

CONCLUSION

The comparision of percentage drug release of these companies on the basis of dissolution study demonstrate that BP-02-A2 (90 % drug release) complied best with standard RDRL protocol while BP-02-B2 (78% drug release) does not comply with specification.

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Table 1- In vitro evaluation of marketed Pantoprazole tablets

Classification of company for the test	**WHO GMP certified Companies of Nepal**				**Non-GMP certified Companies of Nepal**				**Multinational Companies**			
Company coded with	**BP-02-A1**		**BP-02-A2**		**BP-02-B1**		**BP-02-B2**		**BP-02-C1**		**BP-02-C2**	
Batch no (From Mfd date (Mar '05-Feb '06)	Batch 1 Sep '05	Batch 2 Nov '05	Batch 1 Oct '05	Batch 2 Feb '06	Batch 1 Jan '06	Batch 2 Feb ' 06	Batch 1 '05-Jul	Batch 2 '06-Jan	Batch 1 '05-Sep	Batch 2 '05-Nov	Batch 1 '05-Mar	Batch 2 '05-Sep
Expiry date	'07-Aug	'07-Oct	'07-Sep	'08-Jan	'08 -Dec	'09 -Jan	'07 -Jan	'07-Jun	'07-Aug	'07-Oct	Mar '08	Sep '08
Hardness (kg/cm2)	5.45± 0.41	6.15 ± 1.04	5.5± 0.76	6.7 ± 1.14	8.5 0.± 0.35	7.5 ± 0.47	7.32± 1.42	6.21± 1.02	8.5 ± 1.02	9.6 ± 1.42	6.97± 0.63	8.54 ± 1.32
Friability (%)	0.29	0.23	0.21	0.17	0.01	0	0.03	0	0	0.12	0.14	0.21
DT (30mins)	4.85± 0.42	5.76 ± 0.92	4.85 ± 0.42	5.4± 0.63	11.84± 2.32	10.23 ± 1.47	8.32± 1.6	7.42± 0.32	13.63 ± 1.23	12.21± 1.32	10.21± 0.26	11.04 ± 0.97
Average weight (mg)	172± 2.33	173 ± 2.21	232± 2.65	230± 3.12	472 ±4.21	468 ± 4.85	202± 2.23	204 ±4.34	196 ± 2.87	195 ±3.23	151 ± 2.65	153 ± 3.1
7.50%	184.9	185.97	249.4	247.2	(+5%=495)	(+5%= 491.4)	217.15	219.3	210	209.6	168.77	164.47
-7.50%	159.1	160.02	214.6	212.7	(-5%= 448.4)	(-5%= 444.6)	186.85	188.7	181.3	180.37	139.67	141.52
Pass	20	20	20	20	20	20	19	20	20	20	20	20
>107.5%	0	0	0	0	>105%= 0	>105%= 0	1	0	0	0	0	0
<92.5%	0	0	0	0	<95%=0	<95%=0	0	0	0	0	0	0
Assay (100+-10%)	91.12	93.55	95.27	97.75	91.31	93.87	94.72	96.93	95.42	93.9	96.24	95.36

Dissolution (%drug release)												
On 0.1N acid (2hr)	2.8 ± 0.32	2.11 ±0.2	0.9 ±0.12	1.07 ±0.57	3.2 ± 1.34	2.21 ±1. 01	2.41 ±0.45	2.01 ±0.82	1.71 ±0.73	1.5 ±0.21	0.85 ±0.54	0.32 ±0.17
On 6.8 buffer												
15mins	29.97 ±1.76	32.34 ±1.23	35.71 ±1.34	42.01 ±1.23	54.52 ±3.45	52.21 ±1.11	42.32 ±0.1	38.21 ±0.23	65.32 ±2.23	67.32 ±1.31	61.53 ±1.21	58.43 ±1.45
30mins	60.13 ±2.36	61.56 ±2.34	66.97 ±2.21	76.54 ±2.34	65.53 ±2.12	69.65 ±0.88	69.11 ±0.46	57.34 ±0.85	78.65 ±1.23	75.32 ±1.67	79.32 ±0.87	74.45 ±2.12
45mins	83.85 ±1.46	82.32 ±1.02.	83.97 ±0.86	90.02 ±3.17	78.07 ±1.23	82.49 ±0.97	82.42 ±0.56	81.02 ±0.23	86.42 ±1.12	85..3 ±1.22	87.32 ±1.23	85.42 ±1.42

Fig1
Percent drug release from marketed Pantoprazole tablets in Phosphate buffer, pH 6.8 (n=6)

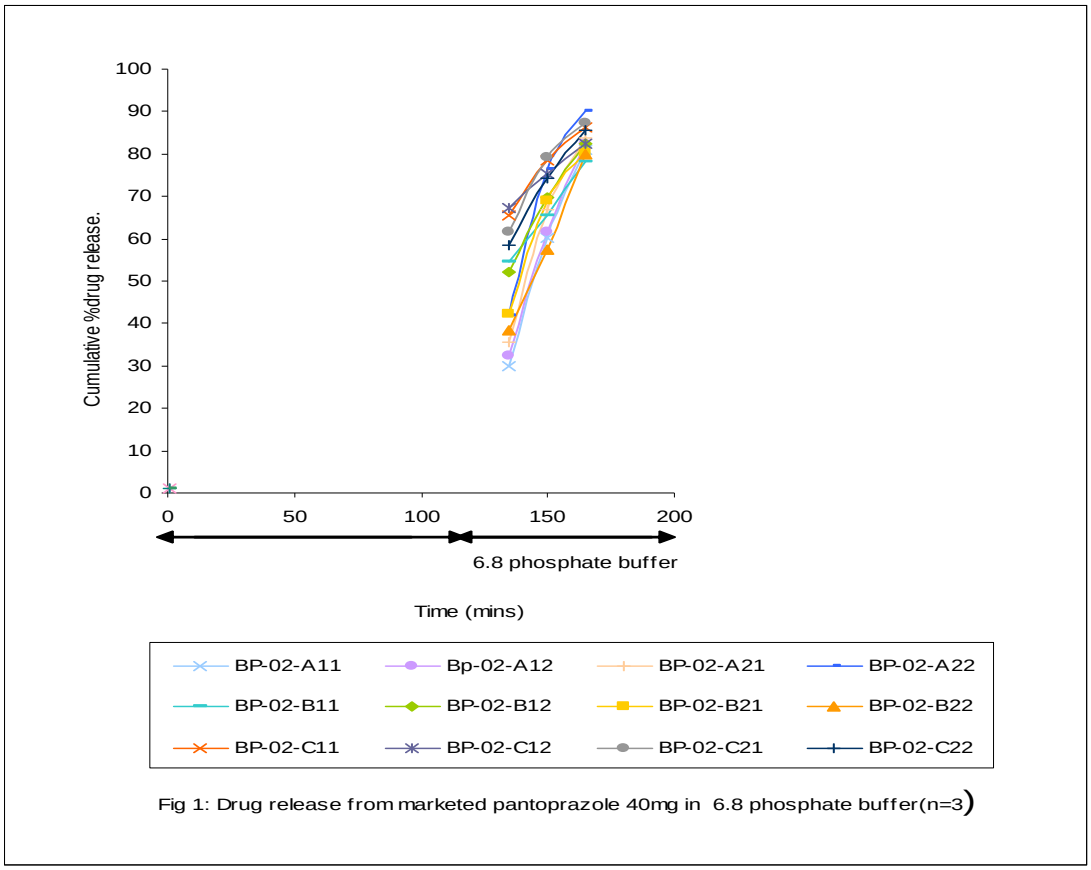

Fig 1: Drug release from marketed pantoprazole 40mg in 6.8 phosphate buffer(n=3)