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COMMUNICATION

In Vitro Evaluation of Dissolution Properties and Degradation Products of Omeprazole in Enteric-Coated Pellets

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ABSTRACT

This report describes results of an in vitro study in which capsules containing omeprazole in enteric-coated pellets from different Brazilian manufacturers were evaluated. The original product was the reference in comparison to three similar products (A, B, and C). Samples were submitted to severe conditions (40°C and 75% relative humidity during 120 days), and the tests performed were the omeprazole content, the percentage of omeprazole dissolved from the pellets, and the amount of H 238/85, its main degradation product. The data obtained suggest that these products could not be considered interchangeable. Differences in physical and physicochemical properties of products A, B, and C indicated that they did not maintain the required stability and that bioavailability might be affected by the poor dissolution of omeprazole from the pellets.

INTRODUCTION

Omeprazole is an antiulcer agent developed for short-term treatment of duodenal ulcers and for use in Zollinger-Ellison syndrome (1). It has been considered superior to cimetidine or ranitidine, H_2 histamine antagonists (2). It acts by the inhibition of the H^+/K^+-ATPase enzyme system at the secretory surface of the gastric parietal cells, suppressing gastric acid secretion (3).

Omeprazole is chemically stable and devoid of inhibitory activity at neutral pH. However, the compound is protonated at pH 5.0 and below and rapidly rearranges to two species, a sulphenic acid and a sulphenamide that reacts with sulfhydryl groups in the enzyme. Complete inhibition occurs when two reactive molecules derived from omeprazole are bound to each molecule of enzyme through disulfide linkages (1,3).

The protonation occurs inside the secretory canaliculi

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of the parietal cells (3), indicating that the drug dosage form should protect the omeprazole from gastric juice, permitting its dissolution in an adequate environment. Moreover, the drug's degradation can be avoided by technological means (4).

There are no references in the literature regarding the degradation products of omeprazole. Notwithstanding, specifications for individual and total impurities are presented in the third supplement of the *U.S. Pharmacopeia XXIII/National Formulary XXIII* (USP-NF) (5) as 0.3% and 1.0%, respectively.

Capsules containing enteric-coated pellets of omeprazole were developed by Hässle (Astra) and marketed in Sweden after 1988 (6). In Brazil, there are four omeprazole capsules considered as similar products that have the same active ingredient and dosage. However, they may exhibit differences in dissolution profile, bioavailability, and therapeutic efficacy due to the formulation and technology process used by each manufacturer (7).

From a public health point of view, these products should be interchangeable and have assured quality and clinical response (8). Thus, the aim of this study was to evaluate in vitro parameters related to quality and stability of the omeprazole capsules commercialized in the Brazilian market.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals and reagents were USP-NF grade. Omeprazole and 8-resp9-methoxy-1,3-dimethyl(1,2-thioxopyrido[1,2:3,4]imidazo-[1,2-a]benzimidazol-2(12*H*)-one (H 238/85) were furnished by Astra Hässle AB, Sweden. All substances were used without further purification.

Samples

Hard gelatin capsules containing 20 mg of omeprazole in enteric-coated pellets from four Brazilian manufacturers (original omeprazole-Astra as a reference and products A, B, and C). All of them were purchased from different drugstores throughout Brazil. As such, the samples had been subjected to various storage conditions.

The products were submitted to the same environmental conditions (40°C and 75% relative humidity) for 120 days. The samples were withdrawn from the stove at preestablished times (3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 36, 42, 60, 90, 120 days).

Experimental Design

At the beginning of the study ($t = 0$), the omeprazole content of capsules, the percentage of omeprazole dissolved from pellets, and the H 238/85 content were determined. Dissolution testing was also performed after 90 days; in addition, H 238/85 content was determined at $t = 60$ and $t = 90$ days.

Analytical Methodology

Assay

The content of omeprazole was determined using a technique modified from that described by Mathew et al. (9). A high-performance liquid chromatography (HPLC) system was used (Shimadzu LC-10AD equipped with a Rheodyne injector, Shimadzu 10AV multiple-wavelength detector and a Varian 4400 printer plotter). A Partisil ODS-3 column (Whatman™, 150 mm × 4.6 i.d., 5 µ) and a guard column µBondapack C18 of 10 µ (Waters™) were used. The mobile phase was 40% (v/v) acetonitrile and 25% (v/v) phosphate buffer solution (pH 7.6) in water. The flow rate was 1.0 ml/min, and the attenuation was 32 at 280 nm. The analyses were performed at room temperature with an injection volume of 20 µl.

An amount of the pellets corresponding to 20 mg of omeprazole was accurately weighed and stirred with 60 ml of phosphate buffer solution (pH 11.0) in an ultrasonic bath for 10 min to dissolve the pellets in a 100.0-ml volumetric flask. Ethanol 95% (v/v) was added (20 ml), and the mixture was stirred for an additional 5 min. The volume was completed with the same buffer, and the solution was then filtered through a Millipore™ PVDF 0.22 µ × 25 mm, discharging the first 10 ml. Next, 5.0 ml of this solution were diluted to 50.0 ml with water (protected from light, this solution was stable for 3 days).

The standard solution was prepared by dissolving 20 mg of omeprazole in 20 ml of ethanol 95% (v/v) in a 100.0-ml volumetric flask. The volume was completed with phosphate buffer solution (pH 11.0), and 5.0 ml were diluted to 50.0 ml with water and filtered through a Millipore™ PVDF 0.22 µ × 25 mm (this solution was protected from light and was stable for 3 days).

Dissolution Testing

Drug-release studies were carried out using the USP dissolution apparatus with the paddle method rotated at 100 rpm. In each study, six dosage forms were tested simultaneously. The temperature of the dissolution me-

dium was maintained at 37°C ± 0.5°C. Initially, the capsules were submitted to 500 ml of simulated gastric fluid without enzymes, prepared according to USP XXIII for 2 hr. Next, 400 ml of phosphate buffer solution (pH 7.6, ionic strength 0.1), equilibrated to 37°C, were added into the vessels. After another 30 min, 5.0 ml of each sample solution were withdrawn, filtered through Millipore™ AP 15–25 mm (glass microfiber) and transferred to a tube containing 1.0 ml of 0.3 M sodium hydroxide (this solution was protected from light and was stable for 3 days).

The standard solution was prepared by dissolving 20 mg of omeprazole in 10 ml of ethanol 95% (v/v) in a 100 ml volumetric flask. The volume was completed with a buffer solution prepared by the mixture of 100 ml of simulated gastric fluid and 80 ml of 0.235 M disodium hydrogen phosphate solution (pH 6.75–6.85). Next, 5.0 ml of this solution were diluted to 50 ml with the same buffer solution. The solution to be injected was prepared by diluting 5.0 ml with 1.0 ml of 0.3 M sodium hydroxide (this solution was protected from light and was stable for 3 days).

The chromatographic system used to analyze the dissolution testing samples was the same as the one described for the assay.

Degradation Products of Omeprazole

The content of H 238/85 was determined by high-performance liquid chromatography using the same apparatus described above. The column and the guard column were LiChrocart™ 125 mm × 4 i.d.–LiChrospher™ Si-60, 5μ, and LiChroCart™ 4 mm × 4 i.d.–LiChrospher™ Si-60, 5 μ, respectively. The mobile phase was prepared by diluting 5 ml of concentrate ammonia (relative density 0.91) with methanol to 100 ml. This solution was diluted with dichloromethane (25% v/v). The flow rate was 1 ml/min.

The samples were prepared by the following steps and then immediately injected: The contents of five capsules were ground in a mortar, and 0.13–0.14 g of the powder were transferred to a 100-ml volumetric flask (protected from light). The contents of the flask were then shaken for 5 min with 60 ml of mobile phase, diluting to the volume with the same solvent. The solution was filtered through a Millipore™ FH 0.22 μ × 25 mm.

The standard solution was prepared by dissolving 2.50 mg of H 238/85 in dichloromethane in a 100-ml volumetric flask. After that, 5.0 ml were diluted to 50.0 ml with the same solvent, and the solution to be injected was prepared by diluting 5.0 ml to 100.0 ml with the mobile phase.

The analyses were performed at room temperature with an injection volume of 40 μl. The attenuation was 64 at 280 nm.

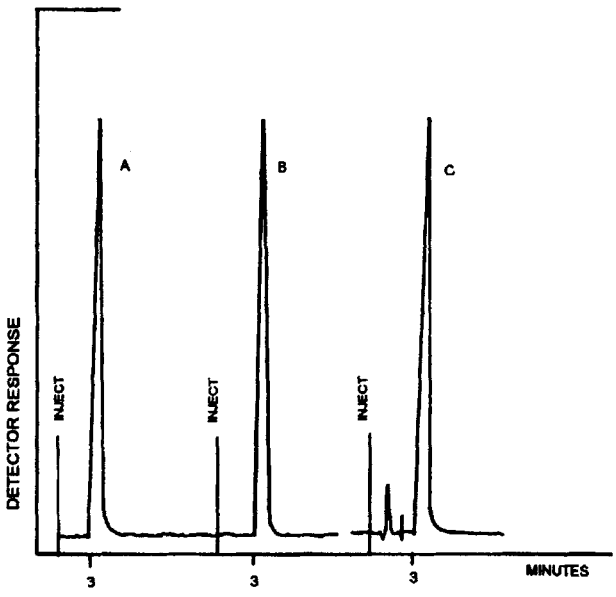

Figure 1. Chromatogram A is from a standard solution of omeprazole; B is from a capsule; and C is from dissolution testing. For chromatographic conditions, see text.

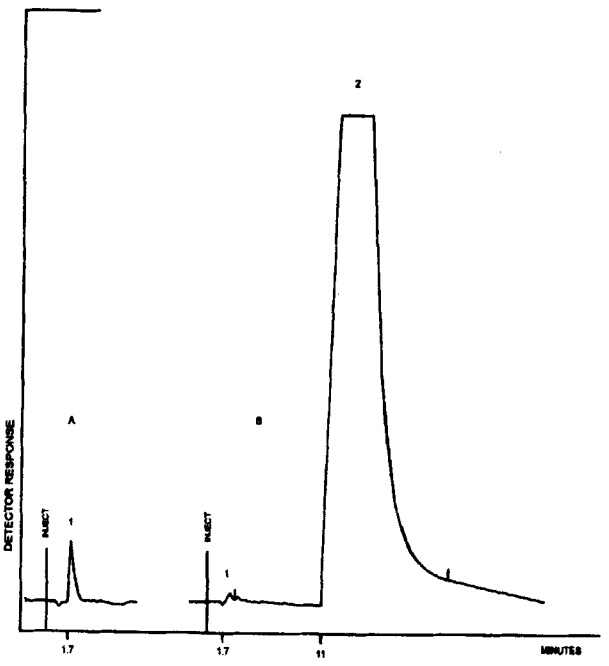

Figure 2. Chromatogram A is from a standard solution of H 238/85 (peak 1); B is from a capsule (H 238/85, peak 1; omeprazole, peak 2). For chromatographic conditions, see text.

Table 1

Omeprazole Content, Percentage of Omeprazole Dissolved from Pellets and H 238/85 Obtained from Capsules of Original Omeprazole-Astra and Omeprazole from Laboratories A, B, and C

	Time (Days)	Original Omeprazole (Astra)	A	B	C
Omeprazole content (mg/capsule)	0	19.85	21.80	21.49	23.30
	30	20.36	21.71	22.71	15.98
	60	19.81	20.47	20.42	14.69
	90	19.55	21.32	21.63	12.12
	120	19.21	20.79	21.94	8.47
Omeprazole dissolved (%)	0	85.23	85.59	88.73	80.81
	90	83.08	1.40	5.30	4.56
H 238/85 (%)	0	0.01	0.14	0.03	0.16
	60	0.10	10.15	0.11	0.46
	90	0.16	8.89	0.07	0.46

RESULTS AND DISCUSSION

A review of the published literature reveals few methods to quantify omeprazole in drug dosage forms. Only in the third supplement of the USP-NF XXIII there is a raw material monograph that contains methodologies and specifications for its physical and physicochemical characteristics, as well as chromatographic purity and assay (5). However, Mathew et al. developed a stability-indicating method for omeprazole. Their results suggest that it could be adequate to quantify omeprazole in capsules (9). This technique is based on reversed-phase HPLC.

In the present investigation, the method described by Mathew et al. (9) was modified to be used in common

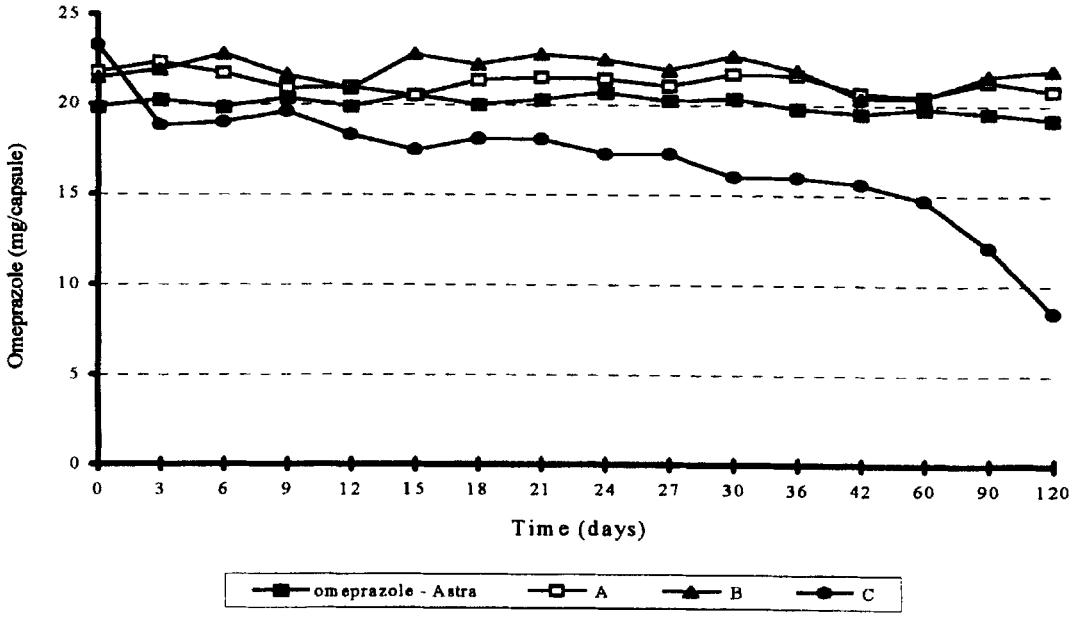

Figure 3. Variation of omeprazole content (mg/capsule) during the study (120 days) for the original omeprazole-Astra and for products A, B, and C.

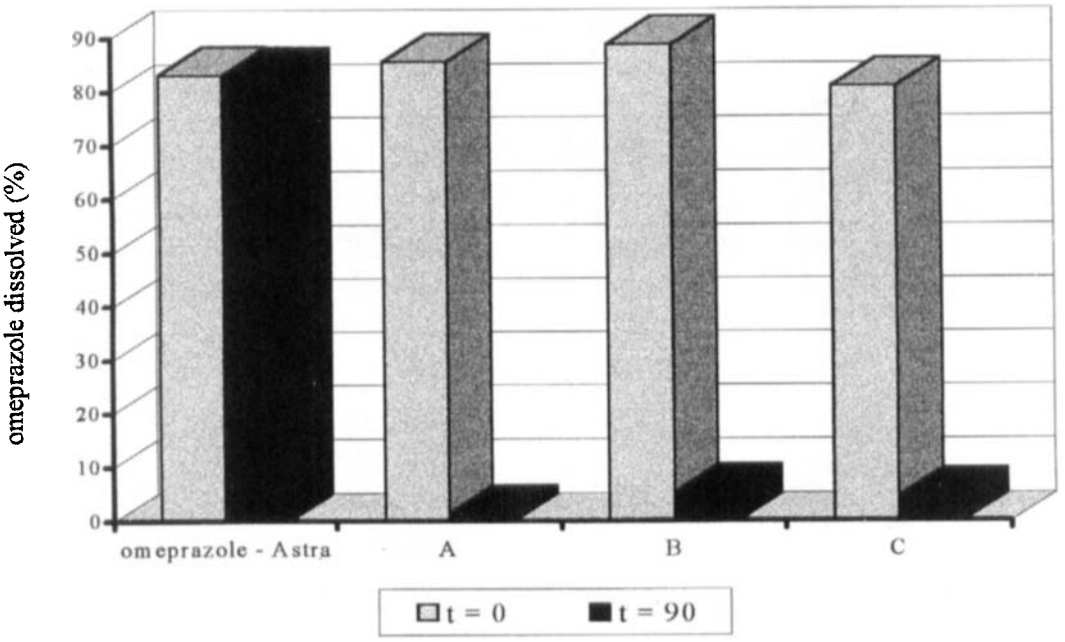

Figure 4. Percentage of omeprazole dissolved from the pellets for the original omeprazole-Astra and products A, B, and C.

quality control laboratories. The HPLC assay method adapted to the omeprazole assay was sensitive and precise with a relative percentage standard deviation of 0.25% based on six readings. The linearity was obtained with a correlation coefficient of 0.9988 for a concentration range of 10–50 μg/ml. The omeprazole retention time in this study was very similar to that obtained by Mathew et al. There were no interferences from the excipients (Figs. 1A and 1B).

Drug dissolution testing should be an integral part of pharmaceutical development and routine quality control monitoring of drug-release characteristics (10). In this research, dissolution testing of omeprazole capsules was conducted using the paddle method as described in the USP-NF XXIII, with acid and buffer stages. As expected, the pellets did not disintegrate in simulated gastric fluid. However, following the addition of the buffer solution, their disintegration was complete at the beginning of the study ($t = 0$). Immediately after the withdrawal of dissolution medium samples, 1.0 ml of sodium hydroxide 0.3 M was added to avoid drug degradation. With these conditions, the retention time was identical to that obtained in the assay (Fig. 1C).

H 238/85 is one of the omeprazole degradation products identified by Hässle Astra AB. To detect and quantify this impurity, the HPLC system used in the present investigation was similar to that described by Andersson et al. (11). These authors quantified several omeprazole metabolites in plasma or urine samples, which had a chemical structure similar to H 238/85. The chromatographic conditions were adequate to separate the compounds with a relatively short retention time (Figs. 2A and 2B).

Table 1 summarizes the results of the omeprazole content, the percentage of omeprazole dissolved from the pellets, as well as the percentage of H 238/85 for the reference (original omeprazole-Astra, A, B, and C products).

In relation to omeprazole content, product C had 23.30 mg/capsule (116.50%) at $t = 0$. However, at the end of the study, this value was reduced to 42.35% (8.47 mg/capsule). Product C is the only one that showed a marked variation in the amount of drug (Fig. 3).

For drug dissolution testing, the data showed no significant variations among the products ($t = 0$). The percentage of omeprazole released from the pellets was 85.23%, 85.59%, 88.73%, and 80.81% for the reference and products A, B, and C, respectively (Table 1). Notwithstanding, the pattern of drug release was drastically different after 90 days, as these values were reduced to 83.08%, 1.40%, 5.30%, and 4.56%, respectively (Fig. 4).

It is quite difficult to explain these findings without the knowledge of the whole formulation and technology

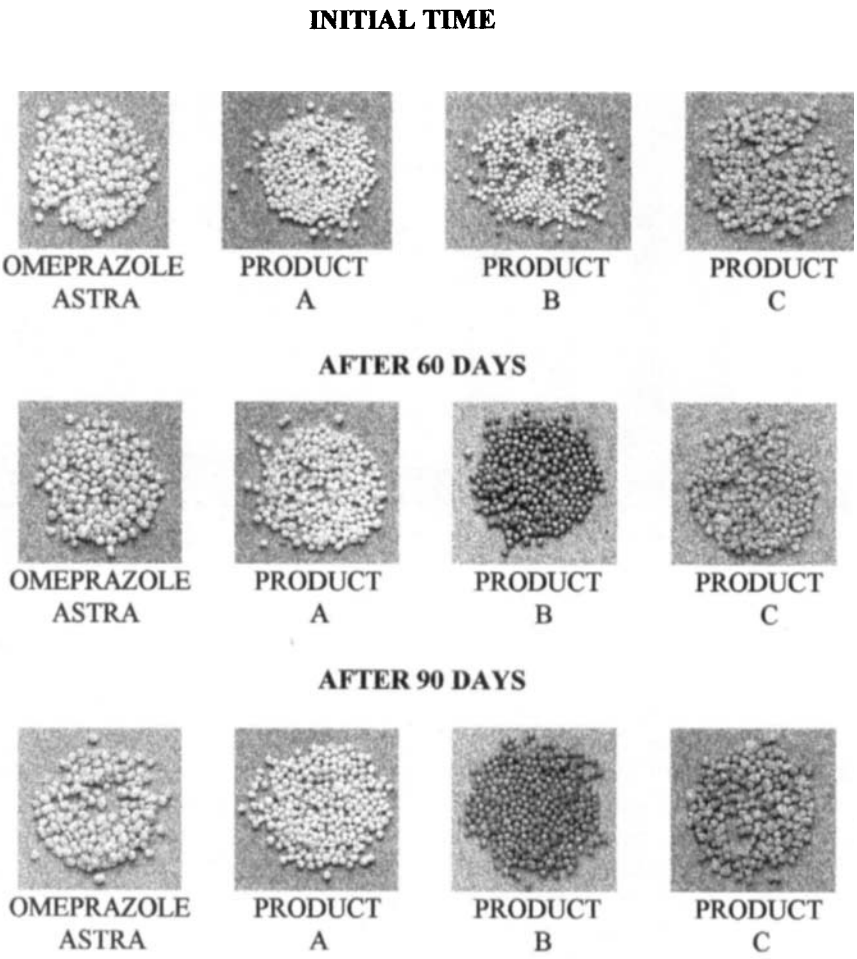

Figure 5. Aspect of the enteric-coated pellets containing omeprazole at beginning of the study and after 60 and 90 days.

processes used by the different manufacturers. It is suspected that the disintegration and dissolution characteristics of the drugs were affected by the polymers used to obtain the gastric resistance of the pellets (4).

At the beginning of the study, the concentration of H 238/85 drug products was lower than 0.2% for all four products studied. While the reference product and product B did not overcome this value after 60 and 90 days, products A and C did, with product A presenting an increase of about 75 times the initial value (Table 1).

The shape of the pellets was different for each product (Fig. 5), varying from spherical to oblong shapes. Products B and C presented some colored pellets (without active ingredient) that disintegrated in simulated acid gastric.

Color alteration of pellets that contained omeprazole was observed during the study (Fig. 5). This fact could be related to enteric-coated film, with alteration of the dissolution properties as discussed previously. On the other hand, there was no correlation between this alteration and the increase of H 238/85 as products B and C did not present a significant increase of this impurity, but had their dissolution characteristics severely affected (Table 1).

The original omeprazole-Astra product showed no significant alteration of omeprazole content, percentage of drug dissolved from the pellets, and percentage of H 238/85 when subjected to study conditions. However, the same did not occur with the others. In spite of maintaining drug content after 120 days, products A and B did not dissolve more than 1.40% and 5.30% of the

omeprazole, respectively. Product C showed the worst performance because the omeprazole content was reduced drastically, and its drug dissolution was smaller than 5.0% (although its H 238/85 content did not go over 0.5%). Considering that only dissolved drug can be absorbed from the gastrointestinal tract, the bioavailability and therapeutic efficacy of these products could be affected.

CONCLUSIONS

An important implication of this study is that the products investigated cannot be considered interchangeable. Differences in physical and physicochemical properties of products A, B, and C after being submitted to 120 days of severe temperature and relative humidity conditions indicate that they did not maintain the required stability parameters. These products might also have their bioavailability affected by poor dissolution of the drug from the pellets.

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