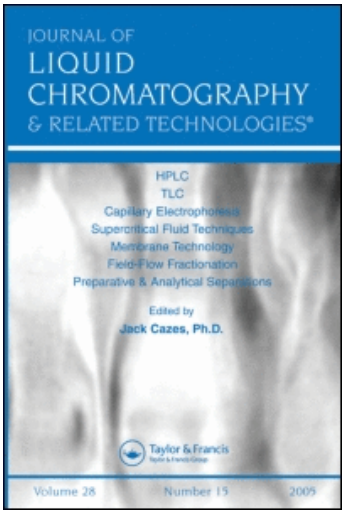

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A Fast, Validated HPLC Method Applied to the Dissolution Test of Gastro-Resistant Capsules of Pantoprazole Pellets

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A Fast, Validated HPLC Method Applied to the Dissolution Test of Gastro-Resistant Capsules of Pantoprazole Pellets

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Abstract: A fast and simple liquid chromatographic method has been developed for determination of pantoprazole in delayed release gastro-resistant capsules employing a reverse-phase column, mobile phase consisting of acetonitrile-water (75:25), flow rate 1.0 mL min^{-1} and 290 nm detection. Selectivity, accuracy, linearity, intra- and inter-day precision were evaluated. The method is not affected by the pellets excipients or induced acid degradation products. Linearity ranged from 2.0–18.0 μg mL^{-1}. The relative standard deviation (RSD) for intra-day and inter-day precision varied from 0.92–2.00%. Capsules analysis resulted in 93.48–105.15% of the labeled pantoprazole amount. Recoveries after spiking pellets with pantoprazole ranged from 96.27–102.87%.

Keywords: Pantoprazole, Liquid chromatography, Quality control, Gastro-resistant pellets, Dissolution, Capsules

INTRODUCTION

Substituted benzimidazoles, later known as proton pump inhibitors, PPIs[1] have widely been used as anti-ulcer drugs[2] with high and long lasting anti-secretory activity. It suppresses gastric acid secretion by specific inhibition of the H^+/K^+ ATPase enzyme system at the secretory surface of the gastric parietal cell. All PPIs are prodrugs such as omeprazole.[3]

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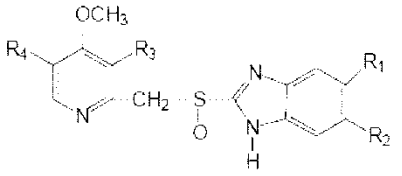

Compound	R1	R2	R3	R4
Omeprazole	OCH_3	H	CH_3	CH_3
Lansoprazole*	H	H	CH_3	H
Pantoprazole	OCF_2H	H	OCH_3	H

*the substituent in 4-position of the pyridine is OCH_2CF_3 instead of OCH_3 as for the other compounds.

Figure 1. Chemical structures of anti-ulcer benzimidazole derivatives.

Omeprazol has successfully been introduced in the clinic in 1989.[1] Its monograph can officially be found in the British Pharmacopoeia, BP2005,[4] in the United States Pharmacopeia, USP29,[5] and in the European Pharmacopeia, EP2005, supplement 5.2.[6] USP29[5] includes the monograph for delayed release capsules, lansoprazole and for omeprazole. Omeprazole capsules with gastro-resistant pellets is also available in the Mexican Pharmacopeia.[7] In both drug monographs, the apparatus paddles and the two stage acid and buffered media are required for the dissolution test. Pantoprazole official monographs are not found in USP29 or other compendia, to our knowledge. The uniformity of quality assessment of pantoprazole may be limited due the lack of official pharmacopeial monographs in its pharmaceutical dosage forms. On the other hand, methods for quantification of pantoprazole and other benzimidazole derivatives, like omeprazole and lansoprazole (Figure 1), in pharmaceutical formulations, have been reported. Some of them were based on UV-Vis,[2,8,9] and first order UV derivative spectrophotometric methods,[10] isocratic reversed-phase high performance liquid chromatography (RP-HPLC) with UV detection[11,12] in which the majority of mobile phases are a mixture of acetonitrile and phosphate buffer, pH 6.0,[13] pH 7.0,[11] or pH 7.4.[12] Differential pulse voltammetry[14] and analysis of pantoprazole enantiomers in plasma and serum with chiral stationary phases have also been reported.[15,16]

Pantoprazole is more stable in neutral and alkaline conditions than omeprazole and lansoprazole, however, it is unstable in acidic medium.[11,17] Hence, it is commercialized as enteric coated tablets containing pantoprazole sodium sesquihydrate (equivalent to 40 mg of pantoprazole). In Brazil, compounding pharmacies manufacture pantoprazole capsules by filling capsule dosage forms with commercially available gastro-resistant pellets as a substitute to high cost industrial products.

The purpose of this work is to present a fast alternative high performance liquid chromatographic method using isocratic mode, which can either be

applied to pantoprazole assay or content uniformity, as well as to a dissolution test of capsules containing gastro-resistant pellets.

EXPERIMENTAL

Materials

Pantoprazole sodium sesquihydrate working standards claimed purity of 99.85% (Hetero Drugs, India, supplied by Baldacci, São Paulo, Brazil) and three pellets batches (P1, P2, P3) produced in India and acquired from three different suppliers (Deg, Extracaps and Galena, São Paulo, Brazil) were used. The pellets label claimed to contain pantoprazole sodium sesquihydrate equivalent to 15% w/w of pantoprazole. Five capsules batches (C1, C2, C3, C4, C5) containing gastro-resistant pellets, equivalent to 40 mg of pantoprazole, were provided by different local compounding pharmacies located in Minas Gerais, southeast of Brazil. Acetonitrile was HPLC grade. Other reagents (hydrochloric acid, phosphoric acid, sodium laurylsulphate, sodium phosphate monobasic, and sodium hydroxide) were analytical grade. Distilled or ultrapurified water was used when necessary (Milli-Q-Plus, Millipore, Bedford, MA, USA).

Equipment and Analytical Conditions

An HP1100 series chromatograph (Agilent, Palo Alto, CA, USA) equipped with a quaternary pump, automatic injector, and a diode array detector (DAD) module was used coupled to a column oven (CH-500, Eppendorf, Madison, WI, USA). Internal ChemStation software version 07.01 was used for data acquisition. The method was performed using a LiChrospher® RP-18 column (Waters, Milford, MA, USA), 250 mm × 4 mm i.d. and 5 μm particle size (Merck, Germany), maintained at 30°C. The diode array detector was set at 290 nm and the automatic injector fitted at 20 μL. A mobile phase consisting of a mixture of acetonitrile and water (75:25) was used at a flow rate of 1.0 mL min^{-1}. A 72RL Hanson Research dissolution test system was employed with the basket apparatus for pellets or capsules, according to USP29 general methods, dissolution test <711> and drug release <724>.

All standard and sample solutions were filtered through a 0.45 μm filter (Sartorius, Germany).

Standard Solutions

Standard stock solutions were prepared by dissolving pantoprazole sodium sesquihydrate in 0.1 M sodium hydroxide to obtain 1.0 mg mL^{-1} of pantoprazole. Further dilutions were prepared to 0.1 mg mL^{-1} with phosphate buffer

pH 6.8 and to 10 $\mu g\ mL^{-1}$ with a mixture of acetonitrile-water (50:50). Phosphate buffer pH 6.8 was prepared by mixing 19 volumes of 0.1 M hydrochloric acid with 17 volumes of concentrate phosphate buffer pH 11 (sodium phosphate monobasic, 16.35 g; sodium hydroxide, 7.05 g, and sodium laurylsulphate, 3.0 g in water to make 1000 mL). The pH was adjusted to 6.8 with phosphoric acid 20% (v/v) or sodium hydroxide 40% (w/v).

Assay and Content Uniformity Preparation

For the pantoprazole assay, the contents of ten capsules of each batch were weighed and pulverized in a metallic mortar. Pellets, equivalent to 50 mg of pantoprazole, were transferred to a 50 mL volumetric flask with the aid of 25 mL 0.1 M sodium hydroxide, sonicated for 5 min, and mechanically shaken for 15 min. The volume was completed with the same solvent and the mixture filtered through a paper filter. The filtrate was additionally diluted in phosphate buffer pH 6.8 to reach a theoretical concentration of 100 $\mu g\ mL^{-1}$ of pantoprazole and, subsequently, to 10 $\mu g\ mL^{-1}$ with a mixture of acetonitrile-water (50:50).

For content uniformity, a capsule dosage form (amount of pellets equivalent to 40 mg of pantoprazole) was used, and proceeded as aforementioned to obtain theoretical concentration of 8 $\mu g\ mL^{-1}$.

A blank solution was prepared by diluting 1 mL of 0.1 M sodium hydroxide to 10 mL with phosphate buffer pH 6.8 and by diluting 1 mL of the previous solution to 10 mL with acetonitrile-water (50:50).

Dissolution Test Samples

The dissolution test was performed according to USP29 general methods, <724> and <711> using baskets, 100 rpm agitation speed, and 60 min for the two stages, acid (500 mL 0.1 M hydrochloric acid), and buffered (475 of remaining 0.1 M hydrochloric acid added of 425 mL of phosphate buffer concentrate pH 11, with pH adjusted to 6.8). After the acid stage, 25 mL were withdrawn from the vessel and a 2 mL aliquot was used for sample dilutions in a 10 mL volumetric flask with phosphate buffer pH 6.8. A further dilution was prepared by diluting 5 mL of the previuos solution to 10 mL with acetonitrile-water (50:50), to obtain a theoretical concentration of 8 $\mu g\ mL^{-1}$. Thereafter, buffer stage sample solutions were prepared by diluting 2 mL of dissolution medium to 10 mL with acetonitrile-water (50:50), to obtain a theoretical concentration of 8.4 $\mu g\ mL^{-1}$.

Acid Degraded Pantoprazole Solution

Empty capsules ($n = 2$) were filled with pantoprazole sodium sesquihydrate equivalent to 40 mg of pantoprazole. The capsules were transferred to the

basket apparatus of the dissolution system, immersed in 500 mL 0.1 M hydrochloric acid medium, 37°C, and submitted to 100 rpm speed for one hour. An aliquot was withdrawn and diluted with phosphate buffer pH 6.8 to reach 16 $\mu g\ mL^{-1}$ of pantoprazole. The solution was further diluted to 8 $\mu g\ mL^{-1}$ with a mixture of acetonitrile-water (50:50).

Method Validation

The method was validated according to the International Conference on Harmonization Guidelines.[18]

Selectivity was evaluated by comparing the chromatograms of pantoprazole standard, acid degraded pantoprazole, and pellet solutions. The UV spectra obtained from each peak were also compared.

Linearity was verified using five standard solutions (2, 6, 10, 14, and 18 $\mu g\ mL^{-1}$) in three consecutive days. All standard solutions were prepared in triplicate and five injections of each solution were performed. The least squares method was used for statistical evaluation.[19] A common standard curve using the data from the three days' curves was constructed.

The precision of the method applied to the pellets was studied for the repeatability (intra-day) and intermediate precision (inter-day). Solutions were prepared at 10.0 $\mu g\ mL^{-1}$ in sextuplicate with five injections of each solution in three consecutive days.

Accuracy determination was performed by the standard addition to the pellets (P1, P2, P3) in triplicate. Sample solutions were prepared, as described previously for assay preparation, except at 5.0 $\mu g\ mL^{-1}$ (50% of the assay concentration). Aliquots of standard solutions were added, so that 75%, 100%, and 150% of the assay concentration (10.0 $\mu g\ mL^{-1}$) was obtained. Pantoprazole recovery was calculated by the formula $\%R = [(C_T - C_A)/C_R] \times 100$ (in which, C_T is total concentration in spiked pellet solution; C_A is concentration in nonspiked pellet solution; C_R is concentration of standard solution).

Assay, Content Uniformity, and Dissolution Test

The RP-HPLC validated method was applied to all five capsule batches (C1-C5) for determination of pantoprazole.

RESULTS AND DISCUSSION

Preliminary tests were accomplished using reverse-phase methods previously described, which employed mobile phases composed of acetonitrile and phosphate buffer, pH 7.0 (40:60),[11] or acetonitrile and phosphate buffer, pH 7.4 (2:1),[12] at high flow rates of 1.6 $mL\ min^{-1}$ and 2.0 $mL\ min^{-1}$,

respectively. In the present work, a LiChrospher RP-18 column was used. As a high backpressure can damage the column, 1.0 mL min^{-1} flow rate was selected for column safety. However, standard pantoprazole peaks presented asymmetry, in both mobile phase conditions. The acetonitrile and methanol (50:50) mobile phase described for pantoprazole determination in a Supelcosil C18 column[14] was tested, yielding a symmetric peak, however, the retention time presented very close to the void volume of the column, due to the higher eluent force of the mobile phase. Thereafter using a LiChrospher RP-18 column, methanol was replaced by water to increase pantoprazole retention time and different proportions of acetonitrile and water were tested (60:40; 65:35; 70:30; and 75:25). It was observed that the peak symmetry was augmented by the increasing of the acetonitrile proportion. It was possible to obtain a symmetric peak of pantoprazole with a small retention time of approximately 2 min using 75% of acetonitrile.

For the pellets solution, the typical chromatogram (Figure 2) reveals occurrence of the pantoprazole symmetrical peak at about 2 min. The UV spectrum (insert) obtained in any point of the peak was coincident with that of the pantoprazole standard. Therefore, selectivity was inferred by the resolution of the pantoprazole peak and by the corresponding ultraviolet spectrum. The method also showed selectivity regarding the acid degradation products of pantoprazole, as can be seen in Figure 3. In the representative chromatogram, the pantoprazole peak is absent and four other peaks can be observed, with retention times around 1.6, 2.7, 3.1, and 3.2 min, respectively. Three of these peaks presented different UV spectra from that of pantoprazole, as shown in Figure 3. The last two peaks show identical UV spectra.

The standard calibration curves, obtained in three different days showed good linearity in the range from 2 to 18 mg mL^{-1}. No significant difference was found between the three curves by ANOVA statistical analysis ($p < 0.01$). A common regression equation could be established ($y = -2.9036 + 46.72x$). Correlation (r) and determination coefficients (r^2)

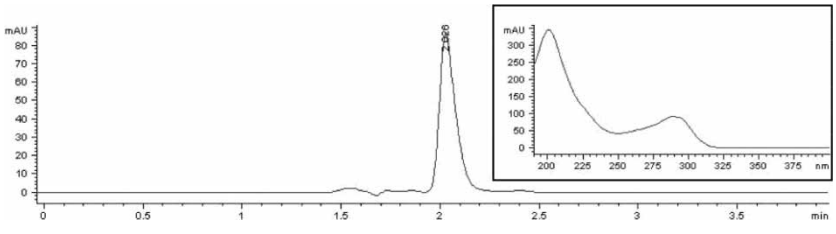

Figure 2. Typical chromatogram resulting from the assay or dissolution test of solutions of capsules containing gastro-resistant pellets of pantoprazole (10 μg mL^{-1}). The insert shows the ultraviolet spectrum of the peak of pantoprazole, eluted at around 2 min. RP-HPLC conditions: C_{18}, 250 mm × 4 mm column, acetonitrile-water (75:25) mobile phase, 1 mL min^{-1} flow rate, UV detection λ 290 nm.

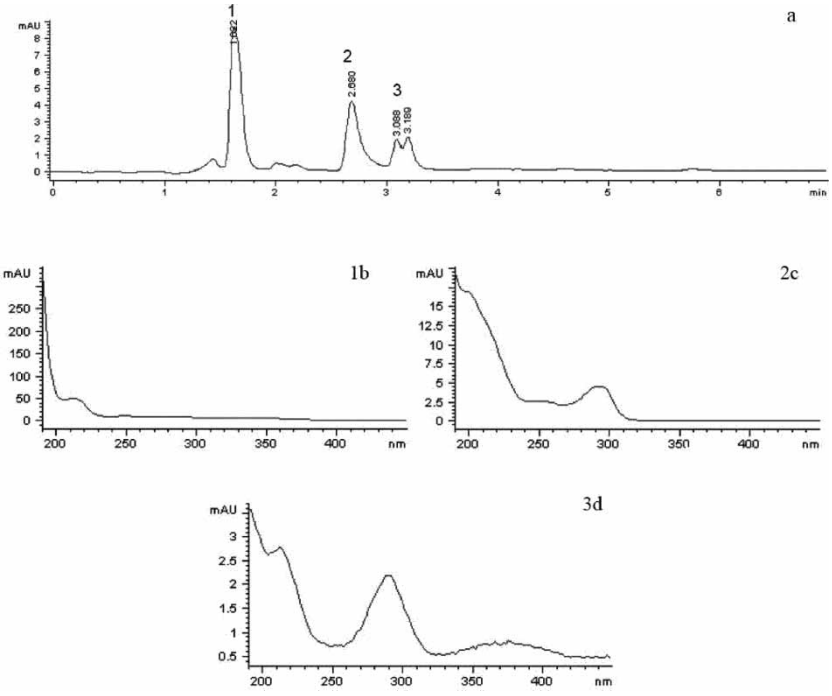

Figure 3. Chromatogram (a) of pantoprazole acid induced degradation products (1, 2, 3) in hydrochloric acid 0.1 M. Ultraviolet spectra of the peaks at 1.6 min (1b), 2.7 min (2c), 3.1 and 3.2 min (3d), respectively. RP-HPLC conditions see Figure 2.

Table 1. Accuracy results (n = 3) for recovery of pantoprazole added to pellets in three concentration levels (2.5; 5.0; 10.0 μg mL^{-1})

Pellet batch	Sample amount (±s.d.)[a] (μg mL^{-1})	Total amount (±s.d.) (μg mL^{-1})	Found (±s.d.) (μg mL^{-1})	%Recovery (%RSD)
P1	5.06 ± 0.12	7.52 ± 0.14	2.46 ± 0.02	98.40 (0.81)
	5.06 ± 0.12	10.20 ± 0.15	5.14 ± 0.03	102.87 (0.59)
	5.06 ± 0.12	15.17 ± 0.03	10.11 ± 0.09	101.07 (0.94)
	4.77 ± 0.15	7.25 ± 0.16	2.48 ± 0.03	99.20 (1.07)
P2	4.77 ± 0.15	9.79 ± 0.12	5.02 ± 0.04	100.47 (0.70)
	4.77 ± 0.15	14.89 ± 0.11	10.12 ± 0.06	101.23 (0.54)
	5.11 ± 0.09	7.52 ± 0.09	2.41 ± 0.03	96.27 (1.20)
P3	5.11 ± 0.09	10.14 ± 0.08	5.02 ± 0.03	100.47 (0.6)
	5.11 ± 0.09	15.18 ± 0.06	10.07 ± 0.06	100.67 (0.06)

[a]s.d., standard deviation.

were greater than 0.999. The relative standard deviation (RSD, 0.57%) calculated for the regression was less than 2% for the common standard curve.

The intra-day and inter-day precisions were evaluated in the pellets. The average pantoprazole percentage ranged from 104.19 ± 2.09 to 106.89 ± 1.96 (% average ± s.d.) with RSD values (0.91–2.00%) not greater than the acceptance limit (2%),[20] thus indicating adequate precision of the method.

Accuracy was expressed as the percent recovery of pantoprazole separately added to the three pellet batches. The results are shown in Table 1. Average recoveries were around 100% and within the usual range of acceptance described for pharmaceutical products (98.0 to 102.0%).[21] The lowest recovery values (96.27%, 98.40%, and 99.20%) occurred for the lower concentrations of all batches, as usually expected. In all cases, RSD values were less than 2%, evidencing the precision of the recovery.

The assay, content uniformity, and dissolution test results of pantoprazole in capsules are shown in Table 2, the assay varying from 93.48% to 105.15% of the labeled amount.

The dissolution test conditions were adapted from the monographs of delayed release lansoprazole and omeprazole capsules[5] and omeprazole capsules gastro-resistant granules,[7] since there is no official available

Table 2. Results of pantoprazole assay, content uniformity and dissolution test in capsules obtained from five different compounding pharmacies

	Average labelled amount (%RSD)			
Capsule batch	Assay[a] (n = 5)	Content uniformity[a] (n = 10)	Dissolution test[a,b] (n = 6 or n = 12[d])	
C1[c]	100.03 (1.29)	99.27 (1.74)	77.92 (6.65)[d]	81.19 (6.58)[e]
		96.56–102.31[c]	69.00–84.57[c]	80.50–89.54[c]
C2	105.15 (1.00)	109.64 (2.96)	96.98 (5.70)	
		102.78 –113.38[c]	91.35–103.11[c]	
C3	102.13 (1.89)	104.21 (2.16)	96.67 (2.08) 93.91–98.81[c]	
		99.14–107.88[c]		
C4[d]	93.48 (1.51)	94.94 (3.95)	87.70 (2.83)[d]	88.25 (4.08)[e]
		89.43–102.09[c]	84.13–90.33[c]	83.58–95.79[c]
C5	97.90 (1.69)	95.89 (3.30)	90.31 (3.35)	
		89.82–101.24[c]	88.25–96.05[c]	

[a]For RP-HPLC conditions see Figure 2.

[b]Baskets, 100 rpm, 60 min, 37ºC. For other conditions, see experimental section.

[c]Lowest and greatest values.

[d]Samples to be re-submitted to re-test because either one or more units released below Q + 5% (80 + 5%).

[e]Re-test results.

monograph for pantoprazole capsules containing gastro-resistant pellets. The procedure followed the United States Pharmacopeia general method for delayed release dosage forms <724>, except that the apparatus and speed control were baskets and 100 rpm, instead of paddles and 75 rpm, respectively. Baskets were used since they do not require the use of sinkers and because they all can be lifted at once. This favors the rapid passage from acid to buffer stage, so that capsule dissolution can practically be assessed at the same time. Empty capsules were tested in the same manner to verify interferences and evaluated by the validated RP-HPLC method.

It is worthy to mention that pantoprazole in the dissolution medium first had to be diluted in a mixture of acetonitrile-water (50:50), otherwise the drug would precipitate in a higher acetonitrile concentration, as in the utilized mobile phase ratio (75:25).

Capsules from all batches (Table 2) passed the dissolution test using six units. The requirement is that each unit should yield dissolution greater than Q (80%) + 5%. Batches C1 and C4 did not satisfy the requirement and had to be retested with six additional units. The new criteria after the retest is that the dissolution average should be greater than (Q) 80% and no units should be less than 65% (Q-15%) of the labeled amount. No peak elution was observed in the acid stage, while in the buffered stage the pantoprazole peak eluted between 1.9 and 2.2 min. None of the capsule batches showed

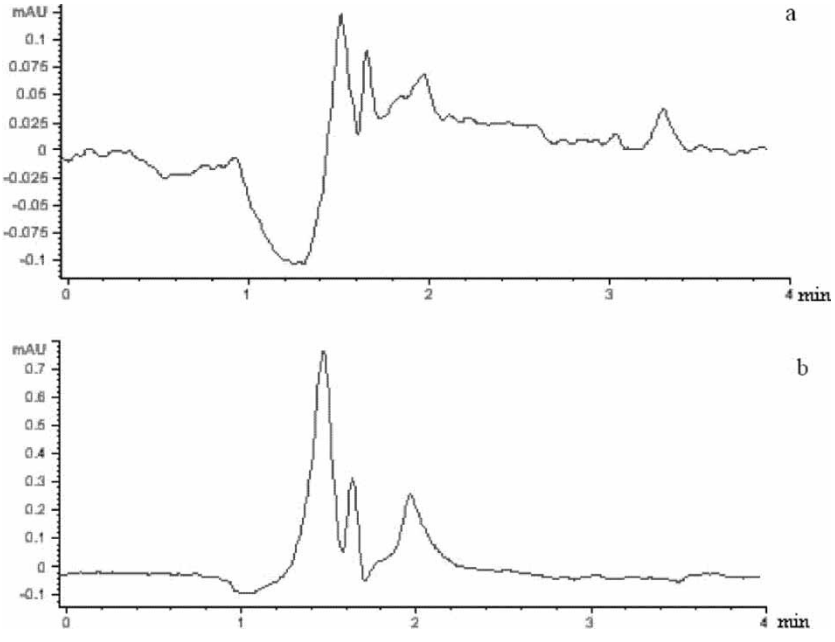

Figure 4. Typical chromatogram of empty capsules after dissolution test acid (a) and buffered (b) stages. RP-HPLC conditions see Figure 2.

signs of disintegration in the acid stage (colorless medium) and all of them totally disintegrated at the buffered stage (yellowish colored medium), except for C1 batch, which presented capsule residues at the end of the first stage of the test.

Figure 4 shows a typical chromatogram of empty capsules after the dissolution test, acid (a) and buffered (b) stages. As it can be seen, only peaks of very low intensity were eluted around 2 min in both stages, thus, not yielding potential shell interference (below 0.5% relative to the expected pantoprazole area).

CONCLUSION

An isocratic and fast HPLC method was developed and validated to determine pantoprazole in capsules containing gastro-resistant pellets. The method employed a mobile phase composed of acetonitrile and water (75:25) with a total elution time of less than 4 min. The method showed linearity, precision, accuracy, and selectivity regarding pantoprazole acid degradation products and pellets' excipients. This rapid method is of great feasibility since it can be used to determine pantoprazole in the quality control routine for assay, content uniformity, as well as for dissolution test of pantoprazole capsules.

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