Drug Development and Industrial Pharmacy, 25(9), 1057–1065 (1999)

COMMUNICATION

Effect of Various Salts on the Stability of Lansoprazole, Omeprazole, and Pantoprazole as Determined by High-Performance Liquid Chromatography

Anthony Ekpe* and Thomas Jacobsen

Bayer Corporation, 36 Columbia Road, P. O. Box 1910, Morristown, NJ 07962-1910

ABSTRACT

A fast and reproducible reverse-phase high-performance liquid chromatography (HPLC) assay method has been developed for the simultaneous quantitation of omeprazole, lansoprazole, and pantoprazole. The three compounds were monitored at 280 nm using Zorbax Eclipse XDB C_8 (5 μm, 150 cm × 4.6 mm i.d.) and a mobile phase consisting of 700:300 phosphate buffer:acetonitrile with the pH adjusted to 7.0 with phosphoric acid. The method was used to study the effect of pH and various salts on the stability of the three compounds. The pH rate profile curve showed that pantoprazole was the most stable compound and lansoprazole the least stable. The stabilities of the compounds in salt solutions were found to be in the following order: phosphate buffer < trisodium citrate < citrate buffer ≤ acetate buffer < citric acid ≤ monosodium citrate ≤ calcium carbonate < sodium bicarbonate < sodium chloride < water. The rate of degradation had a direct relationship with the H^+ and salt concentration.

INTRODUCTION

Omeprazole and several close analogs are potent non-reversible inhibitors of the gastric proton pump H^+/K^+-ATPase (1–3). The enzyme is located in the secretory membrane of the parietal cells and is responsible for gastric acid secretion.

Omeprazole (Fig. 1a), 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole, is a prodrug that is chemically transformed

* To whom correspondence should be addressed. Fax: 973 254 4717. E-mail: Anthony.Ekpe.B@Bayer.com

INTRODUCTION:

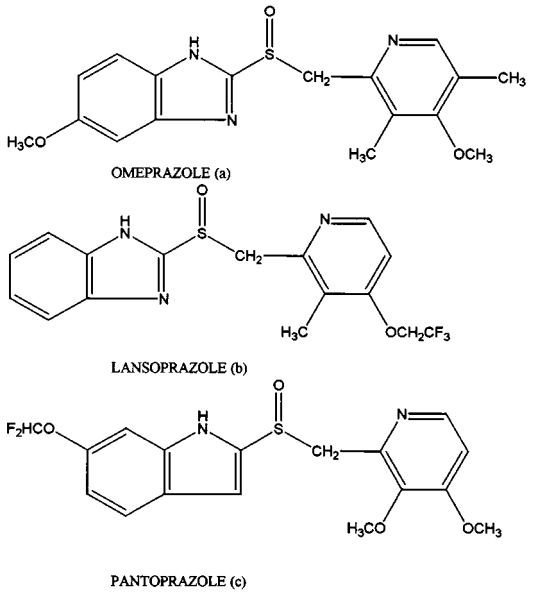

Figure 1. Structural formulas of omeprazole, lansoprazole, and pantoprazole.

within parietal cells into a cyclic sulfenamide. The sulfenamide then reacts with H^+/K^+-ATPase to form a disulfide inhibitor complex (4). Lansoprazole (Fig. 1b), 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl] methyl]sulfinyl]-1H-benzimidazole, is a substituted benzimidazole containing a trifluoroethoxy group with a mechanism of action similar to omeprazole (5). Pantoprazole (Fig. 1c), (5-(difluoromethoxy)-2-[[3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole, is an omeprazole analog with dialkoxy-substituted pyridine. This analog inhibits H^+/K^+-ATPase faster than omeprazole (2).

Many high-performance liquid chromatography (HPLC) methods have been described for the determination of omeprazole (6,7), lansoprazole (8,9), and pantoprazole (10,11) in pharmaceutical dosage forms and in biological fluids. To date, there is no known HPLC method that is capable of resolving omeprazole, lansoprazole, and pantoprazole in the same separation.

The purpose of this study was to (*a*) develop an HPLC method capable of quantitating omeprazole, lansoprazole, and pantoprazole simultaneously, (*b*) determine the effect of various salts and pH on the stability of the three proton pump inhibitors.

EXPERIMENTAL

Instrumentation

The liquid chromatograph used for the study was from Perkin Elmer and consisted of a series 200 IC pump, series 200 autosampler equipped with a 150-µl loop, series 235C diode array detector with series 600 link, and Laserjet 5 printer (Perkin Elmer, Norwalk, CT).

Chromatographic Conditions

The column used was a reverse-phase Zorbax Eclipse XDB C_8, 150 cm × 4.6 mm i.d., 5 µ particle size (DuPont Company, Rockland Technologies, Inc., Chadds Ford, PA). The mobile phase was a mixture of 700:300 phosphate buffer and acetonitrile (pH adjusted to 7.0 with phosphoric acid) filtered through a 0.45-µm nylon filter membrane. The phosphate buffer was prepared by dissolving 136 mg of sodium phosphate monobasic and 839 mg of sodium phosphate dibasic in 1000 ml of purified water. The flow rate was 2.0 ml/min with the column temperature at ambient conditions. Injection volume was 20 µl. The separation was monitored at a wavelength of 280 nm.

Chemicals and Reagents

All the chemicals and reagents were either USP-NF or ACS grade and were used without further purification. Omeprazole, lansoprazole, and pantoprazole (Cadila Healthcare Limited, Gujarat, India) were used as received. Citric acid, acetonitrile, methanol, acetic acid, phosphoric acid, and sodium phosphate dibasic were purchased from J. T. Baker (Phillipsburg, NJ). Trisodium citrate, sodium bicarbonate, sodium chloride, sodium acetate, and sodium phosphate monobasic were purchased from E. M. Science (Gibbstown, NJ). Monosodium citrate was supplied by Roche (Roche, Belgium). Calcium carbonate was purchased from Specialty Chemicals (Bethlehem, PA).

Standard Preparation

The stock solution of omeprazole, lansoprazole, and pantoprazole was prepared by weighing about 250 mg of each compound into a 100-ml volumetric flask; the compounds were dissolved and diluted to volume with methanol. The stock solution was further diluted to obtain a final concentration of 50 µg/ml (2 ml diluted to 100

Table 1

Assay Results of Omeprazole, Lansoprazole, and Pantoprazole Solutions at pH 4.0

	Percentage Remaining at Time (min)														
	Omeprazole					Pantoprazole					Lansoprazole				
Condition	0	30	60	120	180	0	30	60	120	180	0	30	60	120	180
Water	103	102	103	102	102	102	101	102	101	101	102	101	101	101	100
NaCl (0.025 M)	103	104	104	104	102	101	103	103	103	102	102	103	102	101	100
NaCl (0.25 M)	102	100	99	96	93	101	101	101	100	98	101	98	97	93	91
Citric acid (0.025 M)	91	17	3	—	—	100	60	35	—	—	87	14	9	—	—
Citric acid (0.25 M)	84	8	1	—	—	93	36	16	—	—	77	3	—	—	—
Trisodium citrate (0.025 M)	94	21	6	—	—	98	54	36	19	19	91	23	17	17	16
Trisodium citrate (0.25 M)	102	9	2	—	—	94	35	13	—	—	79	18	18	—	—
Monosodium citrate (0.025 M)	92	26	8	—	—	96	58	43	—	—	88	24	16	—	—
Monosodium citrate (0.25 M)	88	13	3	—	—	93	41	3	—	—	82	16	14	—	—
Sodium bicarbonate (0.025 M)	87	18	19	—	—	97	57	30	—	4	84	13	15	—	—
Calcium carbonate (0.025 M)	74	13	2	—	—	92	50	30	5	2	71	10	15	—	—

ml) with water, and the pH was adjusted to about 10 with 0.1 N NaOH.

Sample Preparation

The stock solution of omeprazole, lansoprazole, and pantoprazole was prepared by weighing about 250 mg of each into a 100-ml volumetric flask; the compounds were dissolved and diluted to volume with methanol. This solution was further diluted to a concentration of 50 μg/ml (2 ml diluted to 100 ml) using the different buffers at various pH values (Tables 1–7). The solutions were stored at room temperature.

System Suitability Test

The system suitability was evaluated by making six replicate injections of standard preparation and recording peak area responses. The system was deemed to be suitable for use if the coefficient of variation was <2.0%,

Table 2

Assay Results of Omeprazole, Lansoprazole, and Pantoprazole Solutions at pH 5.0

	Percentage Remaining at Time (min)														
	Omeprazole					Pantoprazole					Lansoprazole				
Condition	0	30	60	120	180	0	30	60	120	180	0	30	60	120	180
Water	103	102	102	103	102	102	101	101	101	101	101	101	101	100	99
NaCl (0.025 M)	102	103	102	102	102	101	101	101	101	101	101	102	101	101	101
NaCl (0.25 M)	101	103	102	100	101	101	101	100	99	100	104	101	101	99	100
Citric acid (0.025 M)	104	82	56	27	13	104	100	93	27	13	102	75	50	24	12
Citric acid (0.25 M)	96	53	31	10	3	97	82	69	49	35	92	42	20	4	—
Trisodium citrate (0.025 M)	100	69	42	17	6	100	92	84	70	54	100	63	31	14	7
Trisodium citrate (0.25 M)	98	50	28	10	2	99	86	65	43	2	94	43	23	14	8
Monosodium citrate (0.025 M)	102	69	49	25	13	101	91	84	70	59	99	63	43	23	16
Monosodium citrate (0.25 M)	101	55	31	11	7	99	84	72	53	49	95	46	25	14	14
Sodium bicarbonate (0.025 M)	101	72	51	27	9	101	93	85	76	59	98	76	44	25	12
Calcium carbonate (0.025 M)	101	72	51	30	12	103	93	85	68	54	98	66	51	14	8

Table 3

Assay Results of Omeprazole, Lansoprazole, and Pantoprazole Solutions at pH 6.0

	Percentage Remaining at Time (min)														
	Omeprazole					Pantoprazole					Lansoprazole				
Condition	0	30	60	120	180	0	30	60	120	180	0	30	60	120	180
Water	101	102	102	102	102	100	101	101	101	101	100	101	101	100	99
NaCl (0.025 M)	102	103	103	103	103	101	102	102	102	101	102	102	102	101	100
NaCl (0.25 M)	104	103	104	103	103	102	102	102	102	102	102	102	103	102	100
Citric acid (0.025 M)	102	98	95	88	80	101	99	98	97	93	100	100	91	82	74
Citric acid (0.25 M)	100	93	86	73	62	99	98	96	91	86	97	97	81	64	52
Trisodium citrate (0.025 M)	100	94	88	78	69	100	99	97	95	91	98	98	84	73	63
Trisodium citrate (0.25 M)	100	91	82	68	56	100	98	95	91	85	99	99	75	58	46
Monosodium citrate (0.025 M)	101	97	94	86	79	100	100	99	97	94	100	100	91	81	73
Monosodium citrate (0.25 M)	101	93	86	74	63	100	98	95	91	87	99	99	79	65	53
Sodium bicarbonate (0.025 M)	102	99	100	—	84	102	101	101	—	97	101	101	92	—	79
Calcium carbonate (0.025 M)	102	98	96	90	84	102	100	100	100	99	100	100	92	86	80
Acetate buffer (0.05 M)	100	94	91	81	74	101	99	98	95	93	98	91	86	76	68
Phosphate buffer (0.05 M)	99	92	86	77	69	100	98	96	93	90	96	88	82	82	61
Citrate buffer (0.05 M)	101	96	91	84	78	102	100	99	96	94	99	93	87	78	69

Table 4

Assay Results of Omeprazole, Lansoprazole, and Pantoprazole Solutions at pH 6.0

	Percentage Remaining at Time (Days)														
	Omeprazole					Pantoprazole					Lansoprazole				
Condition	0	1	6	8	12	0	1	6	8	12	0	1	6	8	12
Water	101	93	72	61	25	100	95	78	71	46	100	85	44	34	8
NaCl (0.025 M)	100	91	58	37	1	100	94	72	60	23	100	83	34	17	1
NaCl (0.25 M)	100	91	46	26	0	100	96	68	55	20	100	88	26	12	0
Citric acid (0.025 M)	101	6	1	0	0	100	45	1	0	0	101	3	0	0	0
Citric acid (0.25 M)	100	1	1	0	0	100	22	0	0	0	101	0	0	0	0
Trisodium citrate (0.025 M)	101	3	1	0	0	100	36	0	0	0	100	1	0	0	0
Trisodium citrate (0.25 M)	100	1	2	0	0	101	19	0	0	0	100	0	0	0	0
Monosodium citrate (0.025 M)	100	16	1	0	0	100	60	5	0	0	100	10	0	0	0
Monosodium citrate (0.25 M)	100	1	1	0	0	101	19	0	0	0	100	0	0	0	0
Acetate buffer (0.05 M)[a]	100	13	0			101	58	8			98	7	0		
Phosphate buffer (0.05 M)[b]	101	20	0			102	58	14			99	8	0		
Citrate buffer (0.05 M)[c]	99	9	0			100	50	4			96	4	0		

[a]Acetate buffer was prepared by dissolving 6.81 g of sodium acetate trihydrate in 900 ml of purified water, adjusting the pH to 6.0 with acetic acid, and diluting to 1000 ml with purified water.

[b]Phosphate buffer was prepared by dissolving 6.86 g of sodium phosphate monobasic mono hydrate in 900 ml of purified water, adjusting the pH to 6.0 with phosphoric acid, and diluting to 1000 ml with purified water.

[c]Citrate buffer was prepared by dissolving 14.71 g of trisodium citrate and 0.830 g of citric acid in 1000 ml of purified water. The pH of the buffer was 6.0.

Table 5

Assay Results of Omeprazole, Lansoprazole, and Pantoprazole Solutions at pH 7.0

	Percentage Remaining at Time (Days)														
	Omeprazole					Pantoprazole					Lansoprazole				
Condition	0	1	6	8	12	0	1	6	8	12	0	1	6	8	12
Water	100	95	73	65	27	100	97	80	72	48	101	87	45	35	9
NaCl (0.025 M)	100	93	60	40	12	100	96	73	62	26	100	82	36	21	1
NaCl (0.25 M)	100	91	49	28	4	101	95	70	57	20	100	84	28	14	1
Citric acid (0.025 M)	101	74	16	10	0	101	89	49	40	23	100	64	7	4	0
Citric acid (0.25 M)	102	65	9	4	0	102	85	38	29	15	100	54	3	0	0
Trisodium citrate (0.025 M)	100	71	11	6	0	100	87	44	35	19	101	62	5	2	0
Trisodium citrate (0.25 M)	100	67	11	5	0	100	87	40	31	16	100	58	3	1	0
Monosodium citrate (0.025 M)	100	78	16	9	0	100	88	48	39	23	101	64	7	4	0
Monosodium citrate (0.25 M)	101	72	7	3	0	102	84	35	26	13	100	52	2	0	0

tailing factor was <1.5, resolution between omeprazole and pantoprazole was >3.0, and the resolution between pantoprazole and lansoprazole was >10.0.

Procedure

Six injections of the standard preparation and one injection of sample preparation were chromatographed using an injection volume of 20 µl. The percentages of the compounds remaining were calculated by the following formula:

$$\%\text{remaining} = \frac{R_{\text{sam}}}{R_{\text{std}}} \times 100\%$$

Table 6

Assay Results of Pantoprazole Solutions at pH 7.0

	% Remaining Pantoprazole at pH 7.0				
	Concentration (0.025 M)				
Time (Days)	HOH	NaCl	Citric Acid	Monosodium Citrate	Trisodium Citrate
0	100	100	100	100	100
1	97	96	89	88	87
2	96	94	81	80	78
5	84	80	56	55	51
6	80	73	49	48	44
7	77	69	45	44	40
8	72	62	40	39	35
12	48	26	23	23	19

Table 7

Assay Results of Lansoprazole Solutions at pH 7.0

	% Remaining Lansoprazole at pH 7.0				
	Concentration (0.025 M)				
Time (Days)	HOH	NaCl	Citric Acid	Monosodium Citrate	Trisodium Citrate
0	101	100	100	100	101
1	87	82	64	64	62
2	83	80	44	43	36
5	54	47	11	11	8
6	45	36	7	7	5
7	42	30	5	5	3
8	35	21	4	4	2
12	9	1	0	0	0

where R_{sam} = peak area response of sample preparation, and R_{std} = average peak area response of standard preparation.

RESULTS AND DISCUSSION

The HPLC method developed for the separation of omeprazole, pantoprazole, and lansoprazole appeared to be stability indicating since there was complete resolution of all the degradation products from the drug components (Figs. 2–6). The peak purity of the drug substances was assessed using a Perkin Elmer diode array detector. System suitability was evaluated by making six replicate injections of standard preparation and recording peak re-

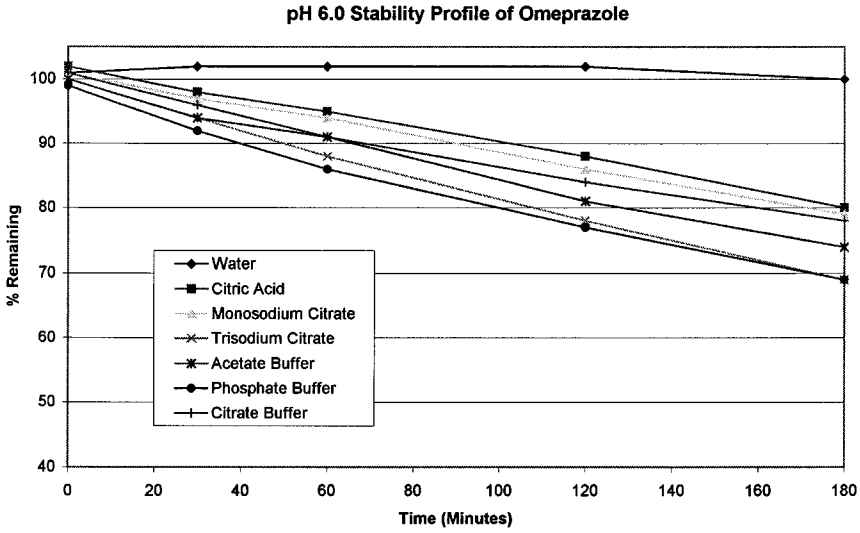

Figure 2. Stability profile of omeprazole at pH 6.0. The concentration of citric acid, monosodium citrate, and trisodium citrate was 0.025 M, and the concentration of the buffers was 0.05 M.

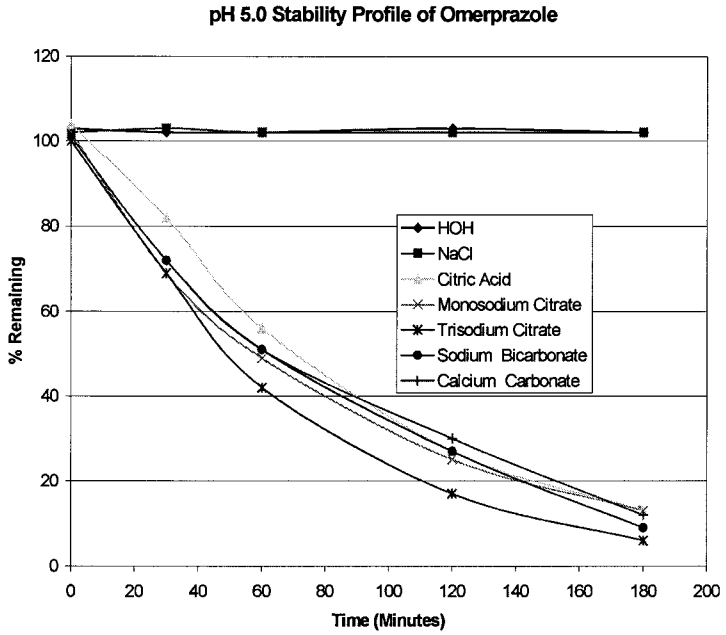

Figure 3. Stability profile of omeprazole at pH 5.0. The concentration of all the salt solutions was 0.025 M.

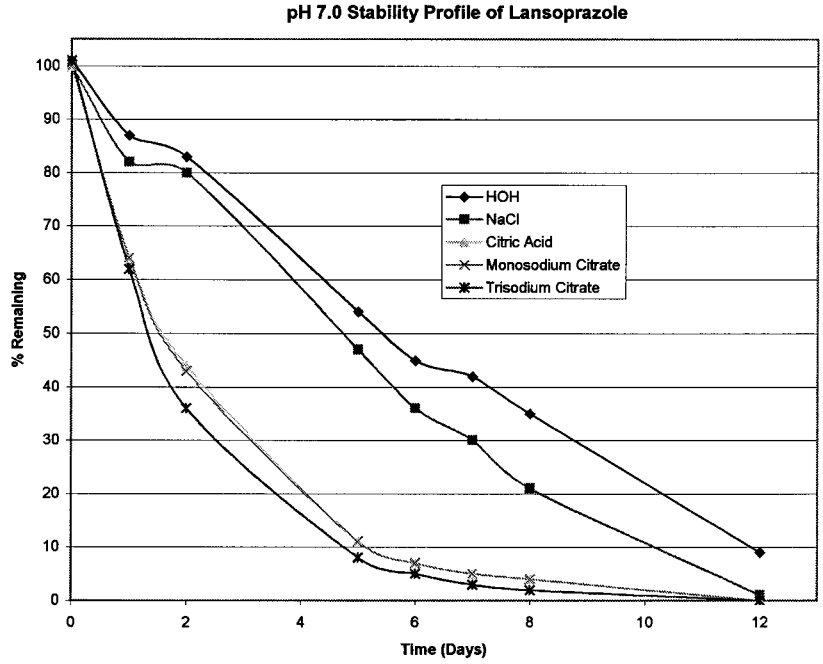

Figure 4. Stability profile of lansoprazole at pH 7.0. The concentration of the salts was 0.025 M.

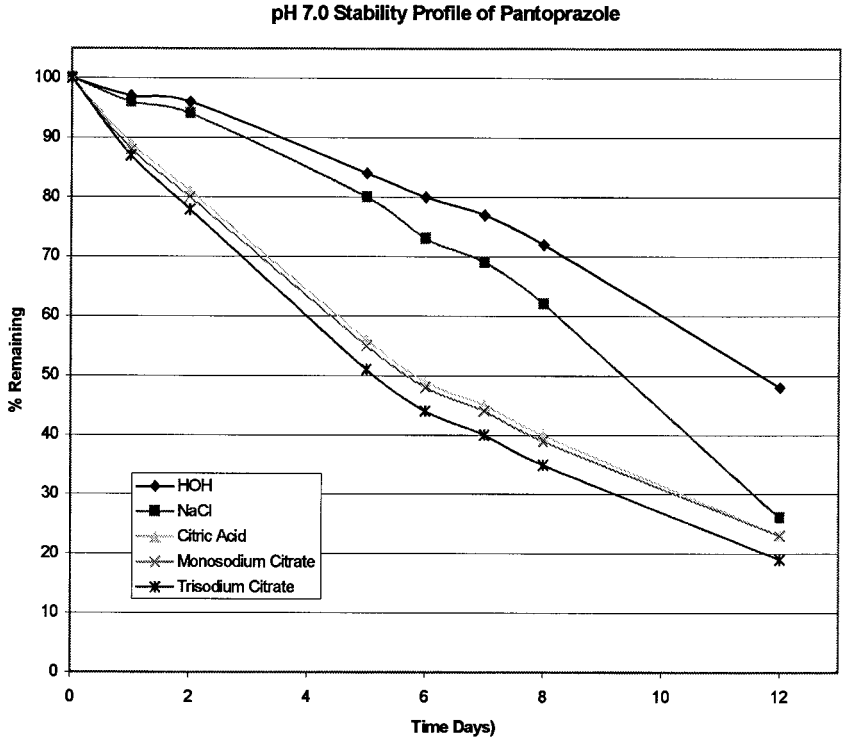

Figure 5. Stability profile of pantoprazole at pH 7.0. The concentration of the salts was 0.025 M.

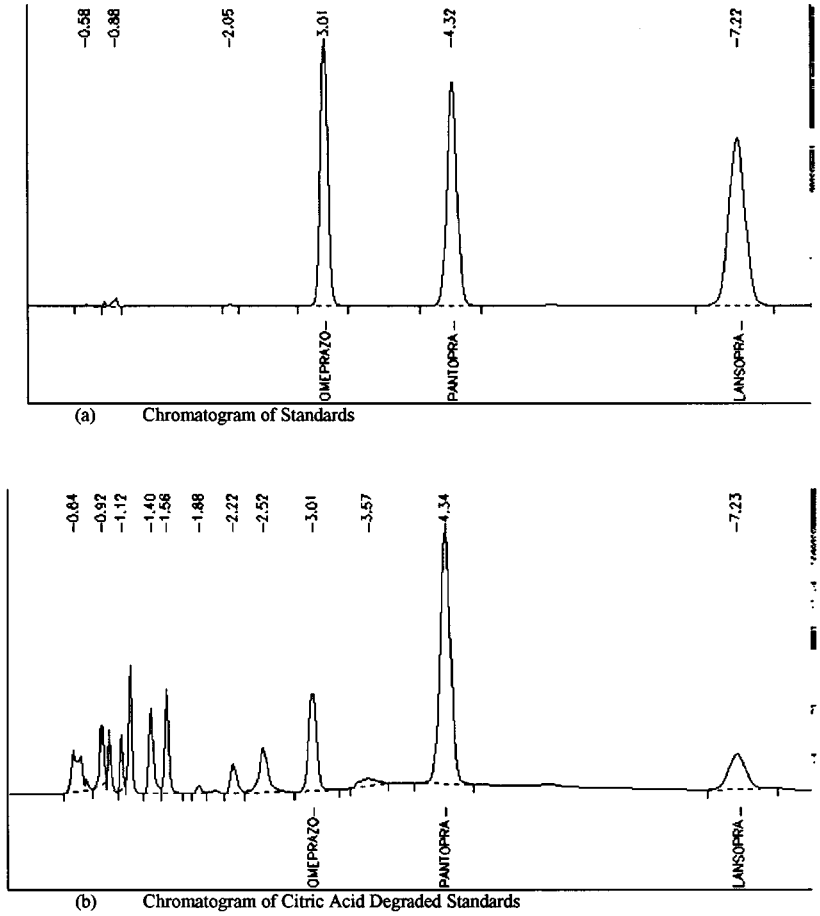

Figure 6. (a) Chromatogram of standards; (b) chromatogram of standards degraded by citric acid.

sponses. The chromatograms were checked for various parameters such as coefficient of variation, column efficiency, resolution, and peak tailing. The results of these parameters are illustrated in Table 8.

The pH of the standard solution was adjusted to about 10 with 0.1 N sodium hydroxide in order to minimize degradation of the drug substances, and it was stored at 4°C. Due to the instability of the drug substances at low pH values, the mobile phase was adjusted to a neutral pH of 7.0.

The stability of omeprazole, pantoprazole, and lansoprazole was examined at pH 4.0 (Table 1), 5.0 (Table 2),

Table 8

System Suitability Parameters

Number	Parameter	Omeprazole	Pantoprazole	Lansoprazole
1	Theoretical plates	7205	7998	8795
2	Resolution factor	—	7.78	11.38
3	Tailing factor	1.03	1.01	0.99
4	Coefficient of variation	0.20	0.20	0.20

6.0 (Tables 3 and 4), and 7.0 (Tables 5–7). Water (pH adjusted with 0.1 N HCl) was used to compare the effect of various salt solutions and buffers.

Pantoprazole was more stable than omeprazole and lansoprazole in all the solutions. All the compounds were more stable in acidified water than in diluted sodium chloride (0.025 M). A higher sodium salt concentration increased the rate of degradation of all the compounds. The pH rate profile curves of omeprazole (Fig. 3), pantoprazole (Table 6, Fig. 5), and lansoprazole (Table 7, Fig. 4) showed that the degradation followed a first-order reaction profile. As the pH value increased, the rate of degradation decreased.

The kinetic effects of citric acid, monosodium citrate, trisodium citrate, sodium bicarbonate, calcium carbonate, acetate buffer, phosphate buffer, and citrate buffer was significantly different from that of sodium chloride (Figs. 2 and 3). The rate of degradation of omeprazole, lansoprazole, and pantoprazole was accelerated in the following order: phosphate buffer $>$ trisodium citrate $>$ acetate buffer $\geq$ citrate buffer $>$ citric acid $>$ monosodium citrate $\geq$ calcium carbonate $>$ sodium bicarbonate (Fig. 2, Table 3). The degradation kinetics of the compounds in all the salt solutions except sodium chloride appeared to be a second-order reaction (Fig. 3). A brown precipitate was observed in all salt solutions over time at the lower pH levels (pH $<$ 5.0). The buffer solutions used in this study did not improve the stability of any of the drug substances.

CONCLUSION

The proposed method is fast and precise for the separation and quantitation of omeprazole, lansoprazole, and pantoprazole. This method can also be used to demonstrate the effect of various pH levels and salts with respect to the stability of the three compounds. The pH rate profile curves showed that pantoprazole was the most stable compound and lansoprazole was the least stable compound. The stabilities of the compounds in salt solutions were found to be in the following order: phosphate buffer $<$ trisodium citrate $<$ citrate buffer $\leq$ acetate buffer $<$ citric acid $\leq$ monosodium citrate $\leq$ calcium carbonate $<$ sodium bicarbonate $<$ sodium chloride $<$ water. The rate of degradation had a direct relationship with the H^+ and salt concentration.

REFERENCES

1. W. Beil and K.-Fr. Sewing, Gastroenterology, 88, 1320 (1985).
2. W. Beil, U. Staar, and K.-Fr. Sewing, Eur. J. Pharmacol., 218, 265 (1992).
3. H. Nagaya, H. Satoh, and Y. Maki, J. Pharmacol. Exp. Ther., 252, 1289 (1990).
4. D. J. Keeling, K. J. Milliner, S. K. Tingley, R. J. Ive, and A. H. Underwood, Biochem. Pharmacol., 34, 2967 (1985).
5. H. Nagayam, H. Satoh, and Y. Maki, J. Pharmacol. Exp. Ther., 252, 1289 (1989).
   U.S. Pharmacopeial Convention, *U.S. Pharmacopeia*, *Suppl. 7*, 23rd rev., Author, Rockville, MD, 1995, pp. 3914–3915.
7. M. Matthew, V. D. Gupta, and E. Baily, Drug Dev. Ind. Pharm., 21, 965 (1995).
8. S. N. Meyyanathan, J. R. Aravinda, and B. Suresh, Indian Drugs, 34, 403 (1997).
9. N. Badwe, G. C. Kandpal, and S. T. Hathiari, East. Pharm., 39, 127 (1996).
10. M. Tanaka and Y. Hideki, Anal. Chem., 68, 1513 (1996).
11. R. Huber, W. Mueller, M. C. Banke, S. J. Rogers, P. C. Norwood, and E. Doyle, J. Chromatogr., 529, 389 (1990).