Reversed-Phase High Performance Liquid Chromatographic Method for the Determination of Lansoprazole, Omeprazole and Pantoprazole Sodium Sesquihydrate in Presence of Their Acid-Induced Degradation Products

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Received November 25, 2005; accepted March 16, 2006

A simple sensitive, selective and accurate reversed-phase high performance liquid chromatographic method was developed and validated for the quantitative determination of lansoprazole, omeprazole and pantoprazole sodium sesquishydrate in the presence of their acid-induced degradation products. The three compounds were monitored at 280 nm using Nova-Pak C_{18} column and a mobile phase consisting of 0.05 M potassium dihydrogen phosphate : methanol : acetonitrile (5 : 3 : 2 v/v/v). Linearity ranges were 2—20 μg ml^{-1}, 2—36 μg ml^{-1} and 0.5—20 μg ml^{-1} for lansoprazole, omeprazole and pantoprazole, respectively. The corresponding recoveries were 100.61±0.84%, 100.50±0.80% and 99.78±0.88%. The minimum detection limits were 0.55, 0.54 and 0.03 μg ml^{-1} for lansoprazole, omeprazole and pantoprazole, respectively. The method could be successfully applied to the determination of pure, laboratory prepared mixtures and pharmaceutical dosage forms. The results obtained were compared with the reported methods for lansoprazole and pantoprazole or the official U.S.P method for omeprazole.

Key words lansoprazole; omeprazole; pantoprazole sodium sesquihydrate; RP-HPLC; stability-indicating; pharmaceutical dosage form

The proton-pump inhibitors lansoprazole, omeprazole and pantoprazole inhibit gastric acid by blocking the H^+/K^+-adenosine triphosphatase enzyme system (the proton pump) of the gastric parietal cell.[1)] They are α-pyridylmethylsulfinyl benzimidazoles with different substitutions on the pyridine or the benzimidazole groups, their pharmacological properties are similar.[2)]

Proton pump inhibitors are unstable at a low pH, therefore, the oral dosage forms are supplied as enteric-coated granules encapsulated in a gelatin shell (omeprazole and lansoprazole) or as enteric-coated tablets (pantoprazole). The granules dissolve only at alkaline pH, thus preventing degradation of the drug by acid in the esophagus and stomach.[2)]

The structural formulae are shown in Chart 1.

Different analytical methods are reported in the literature for the assay of lansoprazole (Lan), omeprazole (Ome) and pantoprazole (Pan) in dosage forms and in biological fluids including spectrophotometry,[3—10)] Fluorimetry,[10)] TLC,[10,11)] HPTLC,[12—14)] HPLC,[15—21)] capillary electrophoresis[22)] and polarography.[23)]

The aim of this work was to develop new and validated, simple and reproducible stability indicating RP-HPLC method allowing the estimation of Lan, Ome and Pan in presence of their acid-degradation products.

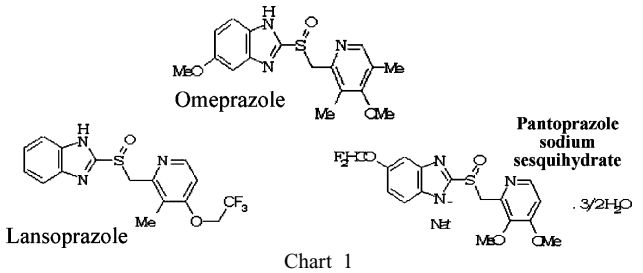

Chart 1

Experimental

Apparatus HPLC system consists of: 600 controller, Waters 486 Tunable absorbance detector, Waters 600 pump, Injector valve with constant 20 μl loop, Waters 746 Data Module, Waters Nova-Pak C_{18} 60 A 4 μm, 3.9×150 mm HPLC cartridge column, Hamilton syringe, Teflon membrane filter, pore size 0.45 μm and 47 mm diameter, Teflon disposable membrane filter, Pore size 0.45 μm for samples, Ultrasonic bath (BANDELIN SONGREX TK 100).

Materials and Chemicals A. Pure Sample:

– Lansoprazole working standard, kindly supplied by Sedico Pharmaceutical Co., 6 October City, Egypt. The purity of the sample was labelled to be 100.98%.

– Pantoprazole sodium sesquihydrate, supplied by Egyptian Pharmaceutical and Chemical Co., Ramadan City, Egypt. The purity of the sample was found to be 99.02%.

– Omeprazole supplied by Pharmaceutical Company Alkaloid, Skopje, Macednia The purity of the sample was labeled to be 99.03%.

B. Market Samples:

– Controloc® tablets (BYK) Konstanz, Germany batch no.; 499871. Each tablet was labeled to contain 45.1 mg pantoprazole sodium sesquihydrate equivalent to 40 mg pantoprazole.

– Zollipak capsules (Sedico Pharmaceutical Co.), batch no. 201103, each capsule was labeled to contain of 15 mg of lansoprazole (B.N. 9101) and 30 mg (B.N. 201103).

– Lopral capsules (T_3A, Co.) Egypt, batch no. 010639 labeled to contain 30 mg of lansoprazole.

– Losec® capsules (ASTRA) batch PK. 2670 labeled to contain 20 mg of omeprazole.

– Gastrozole® capsules (AMRIYA PHARAM. IND) batch no. 433109 labeled to contain 20 mg of omeprazole.

– Trio capsules (A1KAN PHARMA), batch no. 010, labeled to contain 20 mg omeprazole.

C. Reagents and Chemicals: All reagents and chemicals used were of analytical grade where as methanol and acetonitrile were of HPLC grade.

Acetonitrile [LAB-SCAN]. Methyl alcohol [LAB-SCAN]. 0.05 M potassium dihydrogen phosphate. 1.0 M potassium hydroxide solution. 0.01 M sodium hydroxide solution. 0.1 M hydrochloric acid solution.

Standard Solutions Stock Solutions of Lan and Ome (0.10 mg ml^{-1}): An accurately weighed 25 mg of each drug was transferred into a separate 25-ml volumetric flask; filtered through 0.45 μm Teflon membrane filter and diluted to volume with methanol.

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Stock Solution of Pan (0.10 mg ml^{-1}): An accurately weighed 25 mg was transferred into 25-ml volumetric flask; dissolved in distilled water, filtered through 0.45 μm Teflon membrane filter. The solutions were further diluted with the same solvent to obtain a final concentration of 0.10 mg ml^{-1}.

Chromatographic Conditions The column used was Nova-Pak C_{18} 60 A 4 μm, 3.9×150 mm HPLC cartridge column. The mobile phase was a mixture of 5 : 3 : 2 (v/v/v) 0.05 M potassium dihydrogen phosphate, methanol and acetonitrile (pH adjusted to 7.0±0.2 with 1.0 M potassium hydroxide) filtered through a 0.45 μm Teflon membrane filter. The flow rate was 1.5 ml/min for Lan and Ome and 0.8 ml/min for Pan. Chart speed: 0.5 AUFS: 0.5. Attenuation: 64 for Ome and Pan, 32 for Lan at ambient temperature. Injection volume was 20 μl. The separation was monitored at a wavelength of 280 nm.

Preparation of the Acid-Degradation Products Twenty milligrams of the drug were transferred into a 50-ml conical flask; 25 ml of 0.1 M hydrochloric acid were added. The solution was set aside at room temperature (25±2 °C) for not less than 1.5 h for Ome and 2.25 h for Lan and Pan, neutralized with 0.1 M sodium hydroxide solution, quantitatively transferred into 100-ml volumetric flask and completed to 100 ml with methanol.

Procedure. Construction of Calibration Curves The stock solutions of the drugs 100 μg ml^{-1} were serially diluted with methanol to get concentration range 2—20 μg ml^{-1}, 2—36 μg ml^{-1} and 0.5—20 μg ml^{-1} for Lan, Ome and Pan, respectively. Three injections of each solution were chromatographed under the chromatographic condition described above, using an injection volume of 20 μl. The peak areas response at t_R=3.34±0.090 (for Lan), t_R=2.1±0.064 min (for Ome) and t_R=4.54±0.049 (for Pan), were plotted against the corresponding concentration.

Laboratory Prepared Mixtures Containing Different Percentages of Degradation Products Aliquots (1.8—0.2 ml) of Lan and Pan (2.7—0.3 ml) of Ome from their standard stock solutions were accurately transferred into three series of 10-ml volumetric flasks. Aliquots (0.1—0.9 ml) from degradation products solution of Lan and Pan (0.15—1.35 ml) from degradation products solution of Ome (0.2 mg ml^{-1}) were added and the flasks were completed to volume with methanol to prepare mixtures containing different ratios 10—90% of degradation products.

Application of the Proposed Method for the Determination of Lansoprazole, Omeprazole and Pantoprazole in Their Pharmaceutical Formulations 1. For Lansoprazole and Omeprazole: Quantity of the mixed content of 10 capsules equivalent to 30 mg of Lan (20 mg of Ome) was transferred into a conical flask, 1.0 ml of 0.01 M sodium hydroxide was added set aside for 10 min. Eighty milliliters of methanol were added stirred mechanically for 1 h and filtered through 0.45 μm Teflon membrane filter. The filtrate was quantitatively transferred into 100-ml volumetric flask and completed to volume with methanol. One milliliter of the prepared solution of Lan was transferred into 25-ml volumetric flask (10-ml volumetric flask for Ome) and completed to volume with methanol. Triplicate 20 μl injections were made and chromatographed. The average peak areas were calculated.

2. For Pantoprazole: The colored coats of ten tablets were removed with methanol, air dried and powdered. A quantity of the powdered tablets equivalent to 45 mg of Pan were accurately weighed and transferred quantitatively into a conical flask, 1.0 ml of 0.01 M sodium hydroxide was added set aside for 10 min. Eighty milliliters of distilled water was added, stirred mechanically for 1 h and filtered through 0.45 μm Teflon membrane filter. The solution was quantitatively transferred into 100-ml volumetric flask and completed to 100 ml with water. Five milliliters of the filtrate was diluted into 100 ml with water. Two milliliters of the prepared solution was diluted to 5 ml with methanol. Triplicate 20 ml injectionswere made and chromatographed. The average peak areas were calculated.

The concentration of each drug was calculated from the corresponding regression equation.

Results and Discussion

Safety and efficacy of Pharmaceuticals are two fundamental issues of importance in drug therapy. The impurities in drugs often possess unwanted pharmacological or toxicological effects by which any benefit from their administration may be outweighed. Therefore, it is quite obvious that the products intended for human consumption must be characterized as completely as possible. Among the quality and safety of a drug is generally assured by monitoring and controlling the impurities effectively. Thus, the analytical activities concerning impurities in drugs are among the most important issues in modern pharmaceutical analysis. This has become evident by the recent publications of this topic.[24)]

A stability indicating method is an analytical procedure that is capable of discriminating between the major active pharmaceutical ingredients (intact) from many degradation products formed under defined storage conditions.

High performance liquid Chromatography (HPLC) is probably the most powerful and versatile tool for quantitative determination of many individual components in a mixture in one single procedure.[25)] It has many applications in the field of pharmaceuticals which include; stability and pharmacokinetic studies, separation of impurities, enantiomeric purity and drug monitoring in biological fluid.[26,27)]

In this work HPLC was chosen as stability indicating method for the determination of the proton pump inhibitors Lan, Ome and Pan in presence of their acid-induced degradation products.

The rate of degradation of these drugs had a direct relationship with the H^+, as the pH value increased, the rate of degradation decreased.[28)] Pan is a relatively more acid stable compound,[2)] the highest stability can be achieved at pH higher than 5.5.[29)]

The instability of these drugs in acid medium was studied. Complete degradation was affected after 2.25 h (for Lan and Pan) and 1.5 h (for Ome). The disappearance of the intact peak at its retention time by the proposed HPLC method and its spot at the specified R_F by the proposed TLC method confirmed complete degradation. Various mobile phases were tried in attempts to separate each drug from its degradation products. Acidic mobile phases were not suitable since these drugs are acid liable. The small particle size bonded phase of Nova-Pak C_{18} 60A 4 μm, 3.9×150 mm columns offers high resolution, more efficient and fast chromatogram. The mobile phase consisting of methanol–acetonitrile–0.05 M potassium dihydrogen phosphate (3 : 2 : 5 v/v/v) adjusted to pH 7±0.2 gave adequate separation of the drug from its degradation products without affecting the stability of these drugs.

The use of flow rate of 1.5 ml/min for Lan and Ome or 0.8 ml/min for Pan was lead to good separation for the three drugs from their degradation products. It was found that the optimum ratio of water to methanol for preparation of a standard solution of Pan was 2 : 3 v/v. The average retention times±S.D. for Lan, Ome and Pan were found to be 3.34±0.090, 2.10±0.064 and 4.54±0.049 min, respectively for ten replicates. The robustness was assessed by investigating the dependence of the system to minor change in the mobile phase and pH; little changes in the mobile phase composition and pH did not produce major changes in t_R of the cited drugs and their degradations, demonstrating the robustness of the method.

The selectivity of the HPLC method was illustrated in their chromatograms, where complete separation of Lan, Ome and Pan from their degradation products were noticed with sharp peaks and clear baseline separation. Typical chromatograms are shown in Figs. 1—3.

Linear correlation was obtained between the peak areas and the corresponding concentrations in the range of 2—20 μg ml^{-1}, 2—36 μg ml^{-1} and 0.5—20 μg ml^{-1} for Lan, Ome and Pan, respectively. The regression equations were

computed (Table 1).

The accuracy of the proposed method was checked by analyzing different concentration of the drugs in pure powdered form. The recovery was found to be 100.61%, 100.50% and 99.78% for Lan, Ome and Pan, respectively; indicating good accuracy (Table 1). The results were statistically compared to those obtained by the reported methods for Lan and Pan[30,31] or the official method for Ome.[32] Table 2 shows that the calculated t and F values are less than the tabulated ones. Therefore no significant difference was observed regarding both accuracy and precision at 95% confidence level.

The specificity of the proposed method was studied by analyzing laboratory prepared mixtures of the drug and its degradation products. The results shown in Table 3 indicate that the accuracy of the proposed method is not affected by the presence of up to 90% of degradation products.

Also, the proposed HPLC method was successfully applied to the determination of these drugs in their formulations (tablets and capsules), the standard addition technique was applied and the recoveries of the three drugs were calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug (Table 4). The results obtained were compared with the reported[30,31] and the official methods.[32] Satisfactory results were obtained and in a good agreement with the labeled amount (Table 5). Expired

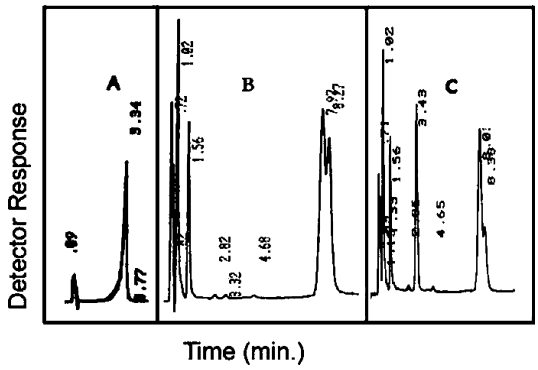

Fig. 1. HPLC Chromatogram

(A) Lansoprazole 4 μg ml^{-1}, retention time: 3.34±0.090. (B) Degraded lansoprazole 14 μg ml^{-1}. (C) Synthetic mixture of 2.8 μg ml^{-1} intact lansoprazole and 16.8 μg ml^{-1} degradation products.

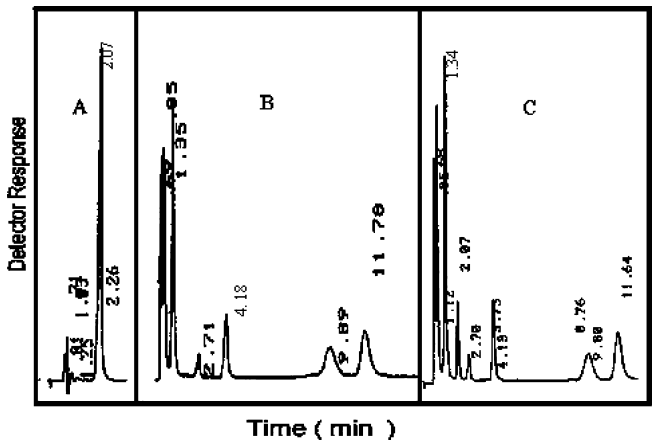

Fig. 2. HPLC Chromatogram

(A) Omeprazole 14 μg ml^{-1}, retention time: 2.10±0.64. (B) Degraded omeprazole 15 μg ml^{-1}. (C) Synthetic mixture of intact omeprazole 3 μg ml^{-1} and 27 μg ml^{-1} degradation products.

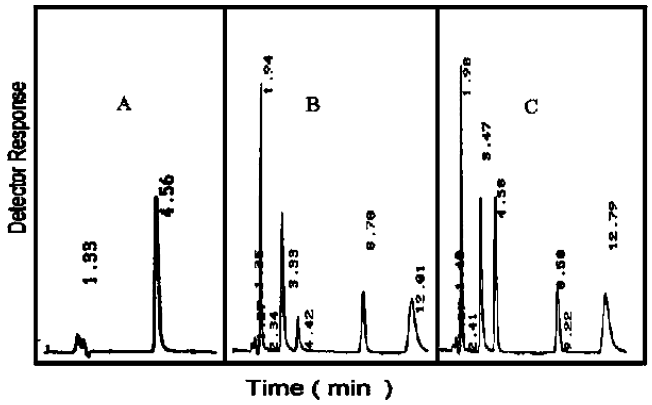

Fig. 3. HPLC Chromatogram

(A) Pantoprazole sodium sesquihydrate 2 μg ml^{-1}, retention time: 4.54±0.049. (B) Degraded pantoprazole sodium 18 μg ml^{-1}. (C) Synthetic mixture of intact pantoprazole sodium 2 μg ml^{-1} and 18 μg ml^{-1} degradation products.

Table 1. Results of Assay Validation Obtained by Applying the Proposed HPLC Method for the Determination of Lansoprazole, Omeprazole and Pantoprazole

Parameters	Lansoprazole	Omeprazole	Pantoprazole
	Linearity range (μg ml^{-1})		
	2—20	2—36	0.5—20
Accuracy			
Mean±RSD	100.61±0.83	100.50±0.80	99.78±0.88
Selectivity			
Mean±RSD	100.00±0.78	99.51±1.04	99.85±1.24
Regression			
Slope	54939.06	40002.93	84280.20
S.E. of slope	263.80	223.43	464.08
Intercept	5172.83	5568.09	3090.48
S.E. of intercept	2989.22	5268.48	4696.76
Correlation coefficient	0.9999	0.9999	0.9999
S.E. of estimation	5278.65	8824.91	9723.89

$Y=a+bC$, where C is the concentration of the drug in μg ml^{-1} and Y is the peak area response.

Table 2. Statistical Analysis of the Results Obtained by the Proposed Method and the Reported Methods for the Analysis of Lansoprazole, Omeprazole and Pantoprazole Sodium Sesquihydrate in Pure Form

Values	Lansoprazole HPLC	Reported method[30]	Omeprazole HPLC	Official method[32]	Pantoprazole HPLC	Reported method[31]
Mean	100.61	100.98	100.50	99.02	99.78	99.03
S.D.	0.84	0.55	0.80	1.00	0.88	0.59
n	5	3	5	3	5	3
F	2.33			1.56	2.22	
Students' t	0.75			2.18	1.44	

The theoretical t=2.447, F=19.300 values at p=(0.05).

Table 3. Results of Analysis of Laboratory Prepared Mixtures Containing Different Percentage of Lansoprazole, Omeprazole and Pantoprazole Sodium Sesquihydrate and Their Acid Degradates (Deg) by the Proposed HPLC Method

Mix. No.	Lansoprazole				Omeprazole				Pantoprazole			
	Lan ($\mu g\,ml^{-1}$)	(Deg) ($\mu g\,ml^{-1}$)	Deg (%)	Recovery (%)	Ome ($\mu g\,ml^{-1}$)	(Deg) ($\mu g\,ml^{-1}$)	Deg (%)	Recovery (%)	Pan ($\mu g\,ml^{-1}$)	(Deg) ($\mu g\,ml^{-1}$)	Deg (%)	Recovery (%)
1	18	2	10	99.92	27	3	10	101.02	18	2	10	101.49
2	14	6	30	100.17	21	9	30	100.04	14	6	30	100.47
3	10	10	50	100.62	15	15	50	98.41	10	10	50	99.07
4	6	14	70	100.59	9	21	70	99.22	8	12	60	98.29
5	2	18	90	98.71	3	27	90	98.87	2	18	90	99.92
Mean				100.00				99.51				99.85
S.D.				0.78				1.03				1.24
RSD				0.78				1.04				1.24

Table 4. Results of the Application of the Standard Addition for the Determination of Lansoprazole, Omeprazole and Pantoprazole Sodium Sesquihydrate by the Proposed HPLC Method and Reported Methods

Preparations	HPLC[a] $X\pm$S.D.
1. Zollipak capsules B.N. 0201103	100.32±0.81
2. Lopral capsules B.N. 010639	100.40±0.86
3. Zollipak capsules B.N. 9101	99.54±0.37
4. Losec® capsules PK 2670	100.19±0.44
5. Gastrozole® capsules B.N. 433109	99.66±0.24
6. Trio capsules B.N. 010	100.38±0.73
7. Controloc® tablets B.N. 499871	100.58±1.00

a) *X*: Mean of five different experiments. S.D.: Standard deviation.

Table 5. Comparison between the Results Obtained by Applying the Proposed HPLC Method and Reported Methods for the Determination of Lansoprazole, Omeprazole and Pantoprazole Sodium Sesquihydrate in Their Pharmaceutical Formulations (Some are Expired Batches)

Preparation	HPLC method[a] Recovery % ±S.D.	Comparison method[b] Recovery % ±S.D.
1. Zollipak capsules[23] B.N. 201103	100.80±0.82	100.71±0.68
2. Lopral capsules[23] B.N. 010639	100.79±0.93	101.17±0.67
5. Gastrozole® capsules[25] B.N. 433109	100.40±1.35	101.98±0.12
7. Controloc® tables[24] B.N. 499871	101.55±1.18	100.21±0.68
3. Zollipak capsules[23] B.N. 9101[c]	77.39±1.80	78.45±0.54
4. Losec® capsules[25] PK 2670[c]	94.16±1.02	94.87±1.20
6. Trio capsules[26] B.N. 010[c]	83.34±0.80	85.27±0.82

a) Mean of five determinations. *b*) Mean of three determinations; reported for Lansoprazple and Pantoprazole[30,31] and official[32] for Omeprazole. *c*) Expired batches.

Table 6. Intra- and Inter-day Accuracy and Precision Data

Conc. added $\mu g\,ml^{-1}$	*n*	Lansoprazole		Omeprazole		Pantoprazole sodium sesquihydrate	
		Found mean conc. ±S.D. ($\mu g\,ml^{-1}$)	RSD	Found mean conc. ±S.D. ($\mu g\,ml^{-1}$)	RSD	Found mean conc. ±S.D. ($\mu g\,ml^{-1}$)	RSD
Intra-day							
4	3	99.16±0.82	0.83	99.64±1.28	1.28	100.65±1.11	1.10
8	3	99.98±1.35	1.35	101.57±0.37	0.36	100.59±1.60	1.59
20[a]	3	99.26±1.21	1.22	98.32±0.39	0.4	99.26±1.12	1.13
Inter-day							
4	3	100.03±1.12	1.12	100.73±1.42	1.41	100.30±0.66	0.66
8	3	101.28±1.13	1.12	101.54±0.77	0.76	100.62±0.87	0.86
20[a]	3	100.75±1.42	1.41	98.13±0.69	0.70	99.04±0.34	0.34

a) In case of omeprazole 28 $\mu g\,ml^{-1}$ and in case of lansoprazole 16 $\mu g\,ml^{-1}$.

batches of Zollipak 15 mg capasules, Trio capsules and Losec capsules stored under normal conditions were analyzed by the proposed HPLC method (Table 5). The method was validated by evaluation of the intra and interday precision. The assay validation[32] was performed and the results obtained are shown in Table 6.

Conclusion

The goal of this work was achieved by separating and quantitating the proton-pump inhibitors lansoprazole, omeprazole and pantoprazole sodium sesquihdrate in pharmaceutical preparations (tablets and capsules). The proposed reversed-phase, isocratic HPLC method on Waters Nova-Pak C_{18} column have been developed and validated, the concomitant quantitation provides significant decrease in sample preparation, instrument run time, solvent and drug waste over the separation methods of analysis. Moreover, the method could separate the intact drugs in presence of more than 7 main degradation products; indicating system suitability and efficient separation. The method is also more specific and selective than the manufacturer spectrophotometric method for determination of omeprazole in Trio capsules [$E_{1\,cm}^{1\%}$ at 302.2 nm in methanol[33]] or acid base titration for the determination of omeprazole raw material.[34]

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