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Research Article

RP-HPLC Method Development and Validation for Estimation of Omeprazole, Pantoprazole Sodium and Ranitidine HCl in Pharmaceutical Dosage Form

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Abstract

A new method was established for simultaneous estimation of Omeprazole Pantoprazole Sodium and Ranitidine Hcl RP-HPLC method. Omeprazole Pantoprazole Sodium was freely soluble in water and alcohol. Ranitidine Hcl was freely soluble in alcohol and sparingly soluble in water. Methanol and potassium dihydrogen ortho phosphate (pH 3) was chosen as the mobile phase. The run time of the HPLC procedure was 5 minutes. The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness robustness, LOD and LOQ. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration range of 10-100 µg /ml. The % recovery of Omeprazole Pantoprazole Sodium and Ranitidine Hcl were found to be in the range of 99.25 % - 98.22 %. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate and Mobile phase composition separately and analysis being performed by different analysts.

Keywords: Omeprazole Pantoprazole Sodium and Ranitidine HCl, RP-HPLC, Methanol.

Article Info

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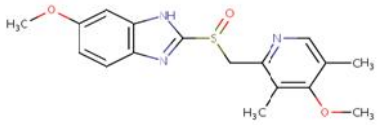
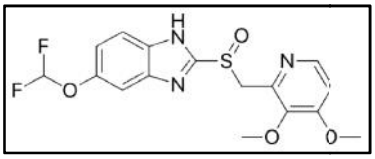
1. Introduction

Omeprazole



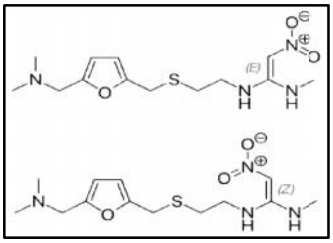
IUPAC Name:
6-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methanesulfinyl]-1H-1,3-benzodiazole
Chemical formula : $C_{17}H_{19}N_3O_3S$
Molecular weight : 345.416
pKa : 1.66
CAS No : 68-35-9
Solubility : Slightly Soluble water
Melting point : 155 °C
Category : Enzyme Inhibitors, Anti-Ulcer Agents, Proton Pump Inhibitors
Mechanism of action: Omeprazole is a proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of the H^+/K^+-ATP ase in the gastric parietal cell. By acting specifically on the proton pump, omeprazole blocks the final step in acid production, thus reducing gastric acidity[1-3].
Generic Name: Omeprazole
Brand Name : Audazol, Ceprandal

Pantoprazole



IUPAC Name:
6-(difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methanesulfinyl]-1H-1,3-benzodiazole
Chemical formula : $C_{16}H_{15}F_2N_3O_4S$
Molecular weight : 383.37
Cas No: : 102625-70-7
Category : Alimentary Tract and Metabolism, Anti-Ulcer Agents, BCRP/ABCG2 Inhibitors, Benzimidazoles.
Mechanism of action : Pantoprazole is a proton pump inhibitor (PPI) that suppresses the final step in gastric acid production by forming a covalent bond to two sites of the (H+,K+)- ATPase enzyme system at the secretory surface of the gastric parietal cell. This effect is dose- related and leads to inhibition of both basal and stimulated gastric acid secretion irrespective of the stimulus[4-5].
Brand Name: Aciban

Ranitidine



Chemical Data
IUPAC Name:
[1-({2-[({5-[(dimethylamino)methyl]furan-2-yl}methyl)sulfanyl]ethyl}amino)-2nitroethenyl](methyl)amine
Chemical formula : $C_{13}H_{22}N_4O_3S$
Molecular weight : 314.40 g/mol
CAS No : 66357-35-5
Category : Anti-Ulcer Agents.
Mechanism of Action :
After a meal, the hormone gastrin, produced by cells in the lining of the stomach, stimulates the release of histamine, which then binds to histamine H2 receptors, leading to the secretion of gastric acid. Ranitidine reduces the secretion of gastric acid by reversible binding to histamine (H2) receptors, which are found on gastric parietal cells. This process leads to the inhibition of histamine binding to this receptor, causing the reduction of gastric acid secretion. The relief of gastric-acid related symptoms can occur as soon as 60 minutes after administration of a single dose, and the effects can last from 4-10 hours, providing fast and effective symptomatic relief[7-9].
Generic name: Zantac
Brand name: Zantac
Aim:
The study aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the simultaneous analysis of Omeprazole, Pantoprazole Sodium and Ranitidine HCL.

2. Methodology

Selection of wavelength
A solution of 10 µg/ml of Omeprazole Pantoprazole Sodium and Ranitidine Hcl were prepared in milli Q water. The resulting solutions were scanned individually on HPLC PDA detector from 190 to 400 nm and also in UV-Visible spectrophotometer[10]. The optimal response for three of them was obtained at 245 nm. Hence the complete method was processed at the wavelength of 245 nm. Overlay spectrum for Omeprazole, Pantoprazole Sodium and Ranitidine HCL.

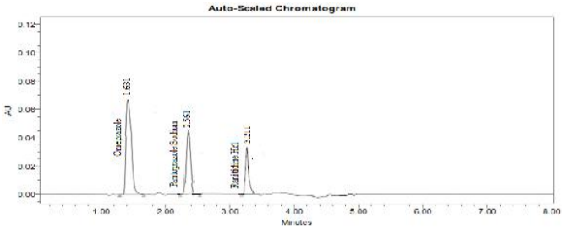


Fig.1: Chromatogram for System suitability injection 1

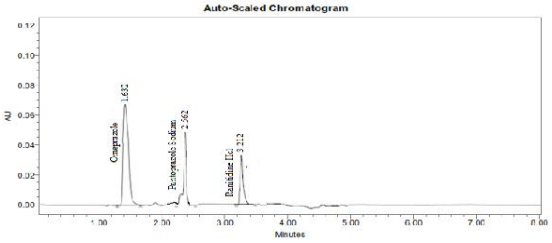


Fig.2: Chromatogram for System suitability injection 2

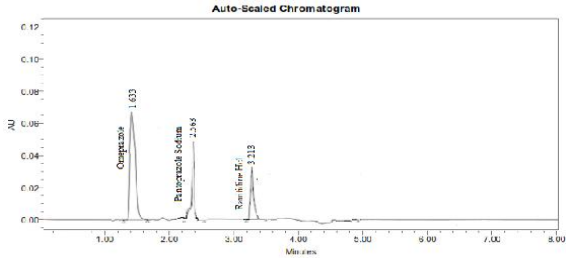


Fig.3: Chromatogram for System suitability injection 3

Table 1: Chromatogram values for System suitability of Omeprazole

Injection	R_t	Peak Area	USP Plate count	USP Tailing
1	1.631	1250763	2489	1.52
2	1.632	1247865	2484	1.52
3	1.633	1255849	2495	1.63
Mean		1251360		
SD		3750.674		
% RSD		0.20728		

Tailing factor Obtained from the standard injection is 1

Theoretical Plates Obtained from the standard injection is 2496

Table 2: Chromatogram values for System suitability of Pantoprazole Sodium

Injection	R_t	Peak Area	USP Plate count	USP Tailing	USP Resolution
1	2.561	740627	2381	1.51	3.04
2	2.562	731161	2245	1.45	3.09
3	2.563	740306	2262	1.45	3.05
Mean		837362.7			
SD		5374.93			
% RSD		0.473408			

Tailing factor Obtained from the standard injection is 1.51

Theoretical Plates Obtained from the standard injection is 2281

Table 3: Chromatogram values for System suitability of Ranitidine Hcl

Injection	R_t	Peak Area	USP Plate count	USP Tailing	USP Resolution
1	3.211	832577	2507	1.24	12.96
2	3.212	834463	2595	1.24	13.25
3	3.213	820415	2566	1.23	13.17
Mean		829167.6			
SD		6598.938			
% RSD		0.617823			

Tailing factor Obtained from the standard injection is 1.25

Theoretical Plates Obtained from the standard injection is 2594

3. Results and Discussion

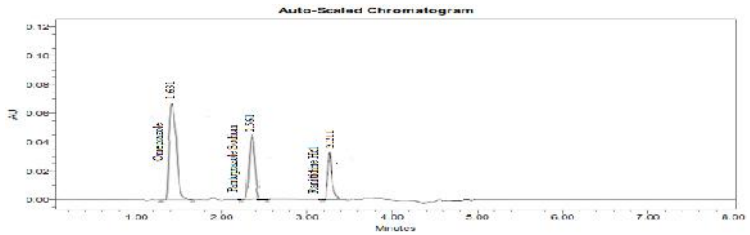


Fig.4: Chromatogram for System suitability injection 1

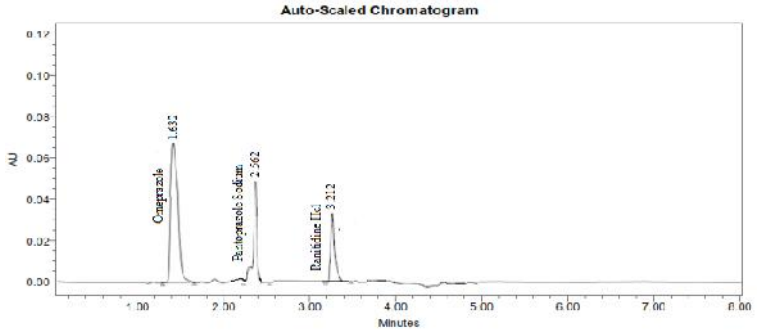


Fig.5: Chromatogram for System suitability injection 2

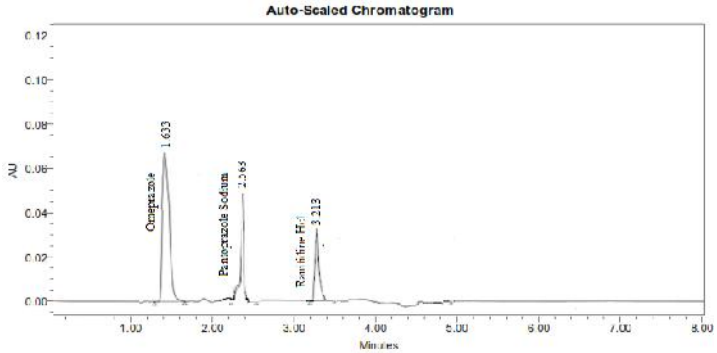


Fig.6: Chromatogram for System suitability injection 3

Table 4: Chromatogram values for System suitability of Omeprazole

Injection	R_t	Peak Area	USP Plate count	USP Tailing
1	1.631	1250763	2489	1.52
2	1.632	1247865	2484	1.52
3	1.633	1255849	2495	1.63
Mean		1251360		
SD		3750.674		
% RSD		0.20728		

Discussion:

1). Tailing factor Obtained from the standard injection is 1.

2). Theoretical Plates Obtained from the standard injection is 2496

Assay Results: (Omeprazole)

1250595 10 0.3 100 10 99.9 1754.5

---------- x ---------- x --------- x -------- x ------- x --------- x100 x --------- =99.84%

1251360 10 10 1754.5 0.1 100 300

Table 5: Chromatogram values for System suitability of Pantoprazole Sodium

Injection	R_t	Peak Area	USP Plate count	USP Tailing	USP Resolution
1	2.561	740627	2381	1.51	3.04
2	2.562	731161	2245	1.45	3.09
3	2.563	740306	2262	1.45	3.05
Mean		837362.7			
SD		5374.93			
% RSD		0.473408			

1) Tailing factor Obtained from the standard injection is 1.51

2) Theoretical Plates Obtained from the standard injection is 2281

Assay Results :(Pantoprazole Sodium)

928829 10 0.3 100 10 99.8 1754.5
---------- x -------- x--------- x ----------x------- x -------- x --------- x100 =98.88%
937364 10 10 1754.5 0.1 100 300

Table 6: Chromatogram values for System suitability of Ranitidine Hcl

Injection	R_t	Peak Area	USP Plate count	USP Tailing	USP Resolution
1	3.211	832577	2507	1.24	12.96
2	3.212	834463	2595	1.24	13.25
3	3.213	820415	2566	1.23	13.17
Mean		829167.6			
SD		6598.938			
% RSD		0.617823			

1) Tailing factor Obtained from the standard injection is 1.25
2) Theoretical Plates Obtained from the standard injection is 2594

Assay Results: (Metformin)

924264 20 0.3 100 10 99.6 1754.5
---------- x -------- x--------- x ----------x------- x -------- x --------- x100 =99.08%
929167 10 10 1754.5 0.1 100 600

Table 7: Assay Results for Omeprazole Pantoprazole Sodium and Ranitidine Hcl

S/no	Name	%Purity
1	Omeprazole	99.84
2	Pantoprazole Sodium	98.88
3	Ranitidine Hcl	99.08

Table 8: Linearity results for Omeprazole

S.No	Linearity Level	Concentration (µg/ml)	Area
1	I	5	226221
2	II	10	478750
3	III	15	721447
4	IV	20	970162
5	V	25	1196060
Correlation Coefficient			0.99916

Table 9: Linearity results for Pantoprazole Sodium

S.No	Linearity Level	Concentration(µg/ml)	Area
1	I	2.5	339286
2	II	5	667774
3	III	7.5	986474
4	IV	10	1339994
5	V	12.5	1639065
Correlation Coefficient			0.99932

Table 10: Chromatogram Values for Accuracy of Omeprazole

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.12%

		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.68%
		15	14.86	99.0%	
		15	14.82	99.79%	

Table 11: Chromatogram Values for Accuracy of Pantoprazole Sodium

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.32%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.88%
		15	14.86	99.0%	
		15	14.99	99.79%	

Table 12: Chromatogram Values for Accuracy of Ranitidine Hcl

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	10	9.8	98%	100%
		10	10.2	102%	
		10	10	100%	
2	100 %	20	19.8	99%	100%
		20	20.2	101%	
		20	20	100%	
3	150 %	30	29.6	98.66%	99.35%
		30	30	100%	
		30	29.8	99.33%	

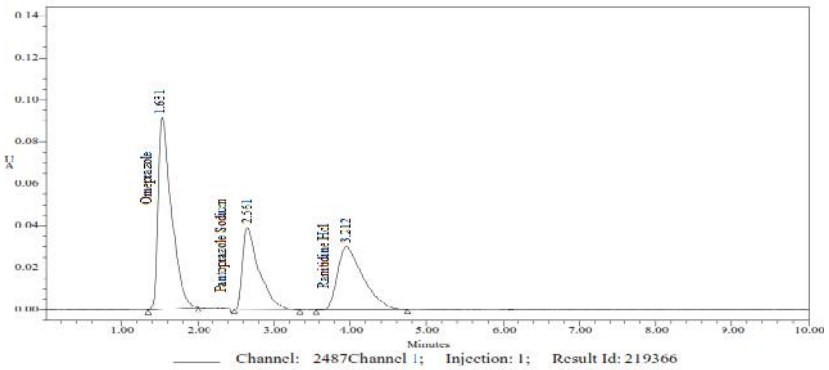


Fig 7: Sample Chromatograms for Repeatability

Table 13: Sample Chromatogram values for Repeatability of Omeprazole

Injection No	Peak Area	R_t
1	1248257	1.631
2	1247578	1.632
3	1245272	1.633
4	1245264	1.634
5	1248573	1.635
Avg	1246487	

SD	2865.61	
% RSD	0.23783	

Table 14: Sample Chromatogram values for Repeatability of Pantoprazole Sodium

Injection No	Peak Area	R_t
1	935136	2.561
2	929455	2.562
3	930458	2.563
4	934387	2.564
5	924058	2.565
Avg	927858.7	
SD	5875.15	
% RSD	0.5231	

Table 15: Sample Chromatogram values for Repeatability of Ranitidine Hcl

Injection No	Peak Area	R_t
1	954857	3.212
2	937616	3.213
3	950692	3.214
4	940253	3.215
5	927055	3.216
Avg	935424.3	
SD	6301.561	
% RSD	0.562	

Table 16: Sample Chromatogram values for intermediate Precision Omeprazole

Injection No	Peak Area	R_t
1	1231405	1.636
2	1232198	1.637
3	1234007	1.638
4	1278576	1.639
5	1272405	1.635
Mean	1243419	
SD	3051.05	
% RSD	0.2572	

Table 17: Sample Chromatogram values for intermediate Precision Pantoprazole Sodium

Injection No	Peak Area	R_t
1	912413	2.564
2	912065	2.565
3	908632	2.566
4	915871	2.565
5	914028	2.564
Mean	915201.3	
SD	2722.875	
% RSD	0.277	

Table 18: Sample Chromatogram values for intermediate Precision Ranitidine Hcl

Injection No	Peak Area	R_t
1	915928	3.217
2	902376	3.217

3	914265	3.218
4	907419	3.217
5	913497	3.215
Mean	912697.3	
SD	2482.867	
% RSD	0.2675	

Acceptance Criteria: The % RSD for the areas and Rt's of five standard injections results should not be more than 2%.

Specificity

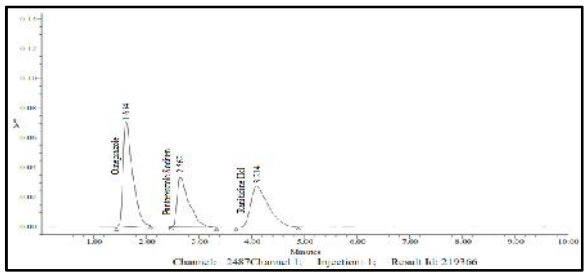


Fig 8: Standard Chromatogram for Omeprazole Pantoprazole Sodium and Ranitidine Hcl Identification

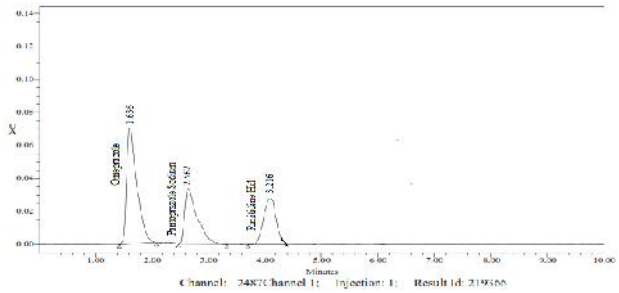


Fig 9. Chromatogram for flow rate of 0.8 ml/min

Table 10: Robustness results for Omeprazole (flow rate)

S.No	Drug	Flow Rate ml/min		
		0.8ml/min	1.0ml/min	1.2ml /min
1	Omeprazole	1.636	1.635	1.635
USP Plate count		2512	2495	2488
USP Tailing		1.65	1.63	1.67

Table 11: Robstness results for Pantoprazole Sodium (flow rate)

S.No	Drug	Flow Rate ml/min		
		0.8 ml/min	1.0ml/min	1.2m l/min
1	Pantoprazole Sodium	2.562	2.561	2.561
USP Plate count		2178	2467	2287
USP Tailing		1.46	1.47	1.47

Table 12: Robstness results for Ranitidine Hcl (flow rate)

S.No	Drug	Flow Rate ml/min		
		0.8ml/min	1.0ml/min	1.2m l/min
1	Ranitidine Hcl	3.216	3.215	3.215
USP Plate count		2347	2546	2087
USP Tailing		1.25	1.26	1.26

Table 13: Roubstness results for Omeprazole

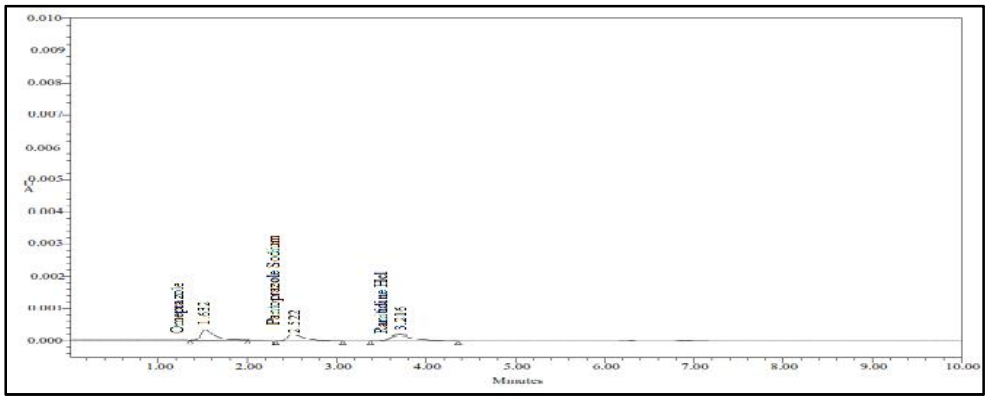
S.No	Drug	Mobile phase		
		Less organic	Normal	More organic
1	Omeprazole	1.634	1.635	1.633
USP Plate count		2511	2397	2595
USP Tailing		1.44	1.63	1.65

Table 14: Roubstness results for Pantoprazole Sodium

S.No	Drug	Mobile phase		
		Less organic	Normal	More organic
1	Pantoprazole Sodium	2.562	2.561	2.561
USP Plate count		2434	2263	2522
USP Tailing		1.34	1.47	1.6

Table no: Roubstness results for Ranitidine Hcl

S.No	Drug	Mobile phase		
		Less organic	Normal	More organic
1	Ranitidine Hcl	3.214	3.213	3.215
USP Plate count		2483	2545	2135
USP Tailing		1.23	1.25	1.32



Limit of detection (LOD)

4. Conclusion

For establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation step. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. Omeprazole & Pantoprazole Sodium was freely soluble in Methanol. Metformin was freely soluble in sparingly soluble in water[11-13]. Methanol and potassium dihydrogen ortho phosphate (pH 3) was chosen as the mobile phase. The run time of the HPLC procedure was 5 minutes. The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness robustness, LOD and LOQ. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration range of 10-100 µg/ml. The % recovery of Omeprazole Pantoprazole Sodium and Metformin were found to be in the range of 99.25% - 98.22 %. As there was no interference due to excipients and mobile phase, the method was found to be specific. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate and Mobile phase composition separately and analysis being performed by different analysts. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Omeprazole Pantoprazole Sodium and Metformin in Bulk drug and Pharmaceutical formulation.

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