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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF OMEPRAZOLE & ONDANSETRON IN BULK AND ITS PHARMACEUTICAL FORMULATIONS

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ABSTRACT
A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid Chromatography assay method has been developed for simultaneous estimation of Omeprazole and Ondansetron tablet formulations. The separation was achieved by using column Inertsil ODS-3V (250 x 4.6 mm, 5 µ) in mobile phase consisted of phosphate buffer, methanol and acetonitrile in the ratio of 50:25:25 v/v/v. The flow rate was 1.0 mL/min, column oven temperature 30° C, the injection volume was 10 µL, and detection was performed at 290 nm using a photodiode array detector (PDA), Run time 20 minutes. The retention time of Omeprazole and Ondansetron, was noted to be 8.8 minutes and 11.8 minutes respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

KEYWORDS: Liquid Chromatography; Omeprazole, Ondansetron, combined dosage forms; Simultaneous estimation, Validation.

1.0 INTRODUCTION
Omeprazole, sold under the brand names Prilosec and Losec among others, is a medication used in the treatment of gastroesophageal reflux disease (GERD), peptic ulcer disease, and Zollinger–Ellison syndrome. It is also used to prevent upper gastrointestinal bleeding in people who are at high risk. Omeprazole is a proton-pump inhibitor (PPI) and its effectiveness is similar to other PPIs. It can be taken by mouth or by injection into a vein.

Chemically, it is 5-Methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl) methanesulfinyl]-1H-benzimidazole. Molecular formula is C17H19N3O3S and Molecular weight 345.42 g/mol. The chemical structure of Omeprazole shown in **Figure 1.01.**

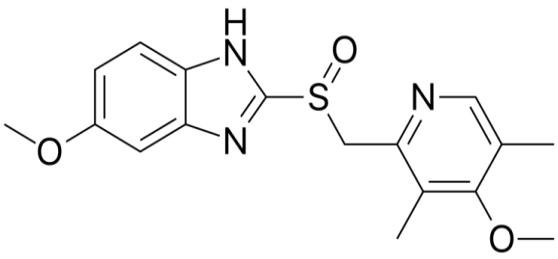

Figure1.01: Chemical structure of Omeprazole.

Ondansetron, sold under the brand name Zofran among others, is a medication used to prevent nausea and vomiting caused by cancer chemotherapy, radiation therapy, or surgery. It is also effective for treating gastroenteritis. It is ineffective for treating vomiting caused by motion sickness. It can be given by mouth or by injection into a muscle or into a vein.

Chemically, it is (*RS*)-9-Methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-2,3-dihydro-1*H*-carbazol-4(9*H*)-one. Molecular formula is $C_{18}H_{19}N_3O$ and Molecular weight 293.370 g/mol. The chemical structure of Ondansetron shown in **Figure: 1.02.**

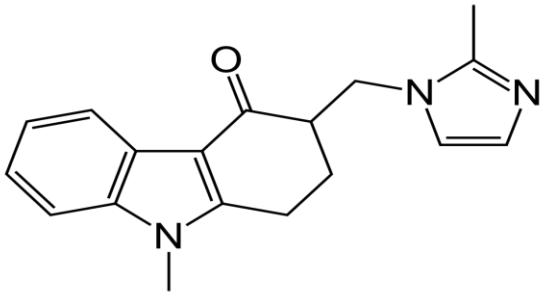

Figure: 1.02 Chemical structure of Ondansetron.

Literature survey reveals that few analytical methods have been reported for the estimation of Omeprazole and Ondansetron in pharmaceutical dosage form including RP-HPLC in formulation[2-3] and in plasma and blood[4-6], HPTLC[7], spectrophotometry[8] are available for the determination of omeprazole and spectrometric[9-10] in formulation, RPHPLC in formulation[11] and blood[12] for determination of Ondansetron. The present work describes a simple, stability indicating HPLC method for the determination of Omeprazole and Ondansetron in bulk and tablet dosage form according to ICH guidelines.[13-14]

2.0 EXPERIMENTAL

2.1. Chemicals and Reagents

Milli-Q Water, Acetonitrile, Methanol (HPLC Grade) and Potassium dihydrogen orthophosphate Sodium phosphate dibasic anhydrous (AR Grade) were obtained from Qualigens Ltd., Mumbai. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard class-A, volumetric glassware.

2.2. Instrumentation and Chromatographic Conditions

Instrumentation

Agilent HPLC model:1260, equipped with open lab software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) were use in the present assay.

Preparation of buffer

Accurately weighed and transferred 2.54g of sodium phosphate dibasic anhydrous and 0.272g of potassium dihydrogen phosphate into a beaker containing 1000ml of water sonicated to dissolved for 5 minutes. Filtered through 0.45µ nylon membrane filter.

Preparation of mobile phase

Mixed 500ml of phosphate buffer and 250 ml of Acetonitrile and 250 ml of methanol in the ratio of 50:25:25v/v/v.

Preparation of Diluent

Mobile phase used as diluent.

Preparation of standard solution

Weighed accurately 20mg of Omeprazole standard and 4 mg of Ondansetron standard is transferred into 100ml volumetric flask, 50ml of diluent was added and sonicated for 5 minutes to dissolve it and make up to the volume with diluent and mixed well.

Preparation of sample solution

Twenty tablets were weighed and crushed to fine powder. Powder equivalent to 10 mg of Omeprazole and 4mg Ondansetron was accurately weighed and transferred in to 100 mL volumetric flask, added 50 ml of diluent sonicated to dissolved for 30 minutes with intermediate shaking and make up to the volume with diluent and mixed well. The sample solution filtered through 0.45µm PVDF filter.

Chromatographic conditions

Inertsil ODS-3V (250 x 4.6 mm, 5µ) Column was used for analysis at 30°C column temperature. The mobile phase was pumped through the column at a flow rate of 1.0mL/min. The sample injection volume was 10 µL. The photodiode array detector was set to a wavelength of 290nm for the detection and Chromatographic runtime was 20 minutes.

3.0 RESULTS AND DISCUSSION

Method development

To develop a suitable and robust LC method for the determination of Omeprazole and Ondansetron, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Intersil ODS-3V, (250 × 4.6 mm, 5 µ) with the following mobile phase. Phosphate buffer : Acetonitrile (50:50 v/v). Detector wavelength 290 nm, column temperature 30^{o} C, Injection volume 10 µL and Flow rate 1.0 ml/min used. Due to poor separation of peaks proceed to next trail. Hence, another trial was made with change in mobile phase composition.

For next trial mobile phase composition was changed to Phosphate buffer : Acetonitrile (45:55 v/v). from Phosphate buffer : Acetonitrile (50:50 v/v) remaining chromatographic conditions are same. Due to poor separation of peaks proceed to next trail. So, another trial was made with change in mobile phase composition introduced methanol.

For next trial mobile phase composition introduced methanol in the ratio of (50:25:25 v/v/v) remaining chromatographic conditions are same. Peak shape was satisfactory in both standard and sample preparations. Retention time of Omeprazole and Ondansetron, were found to be 8.8 and 11.8 min acceptable. The chromatogram of Omeprazole and Ondansetron standard using the proposed method is shown in **Figure: 1.03** System suitability results of the method are presented in **Table: 1.01.**

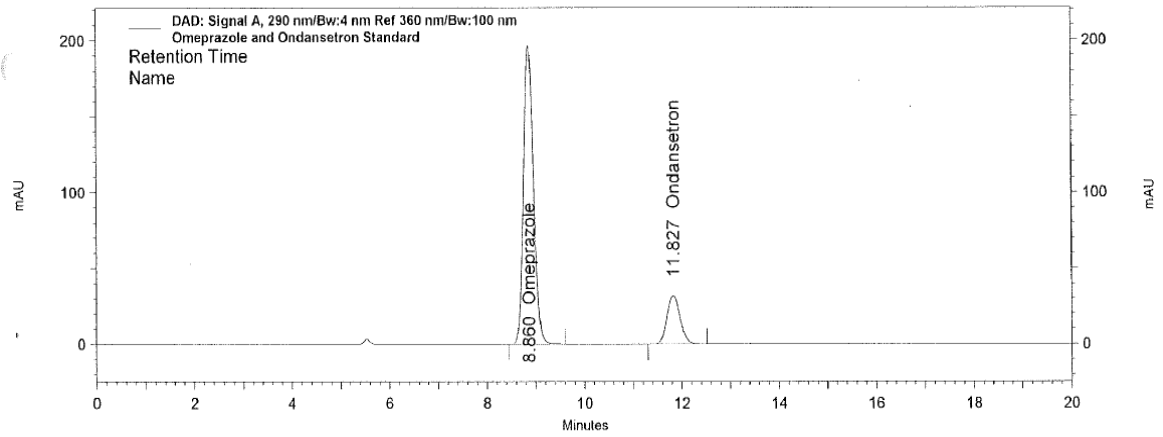

Figure: 1.03 A typical standard chromatogram of Omeprazole and Ondansetron.

4.0 METHOD VALIDATION

The developed RP-LC method extensively validated for assay of Omeprazole and Ondansetron using the following Parameters.

4.1 Specificity

Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution **Figure: .1.04** showed no peaks at the retention time of Omeprazole and Ondansetron peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Omeprazole and Ondansetron in tablets. Similarly chromatogram of placebo solution **Figure: 1.05** showed no peaks at the retention time of Omeprazole and Ondansetron peak. This indicates that the placebo used in sample preparation do not interfere in estimation of Omeprazole and Ondansetron in Omeprazole and Ondansetron tablets.

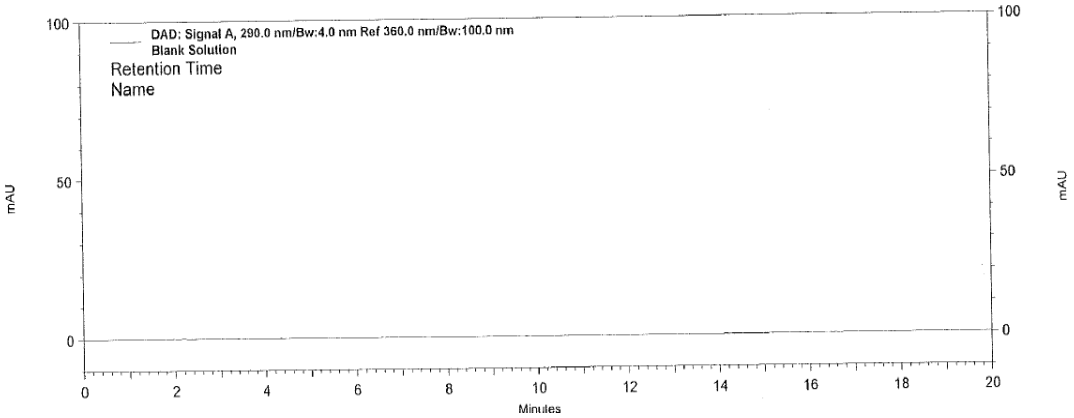

Figure 1.04: Typical chromatogram of Blank.

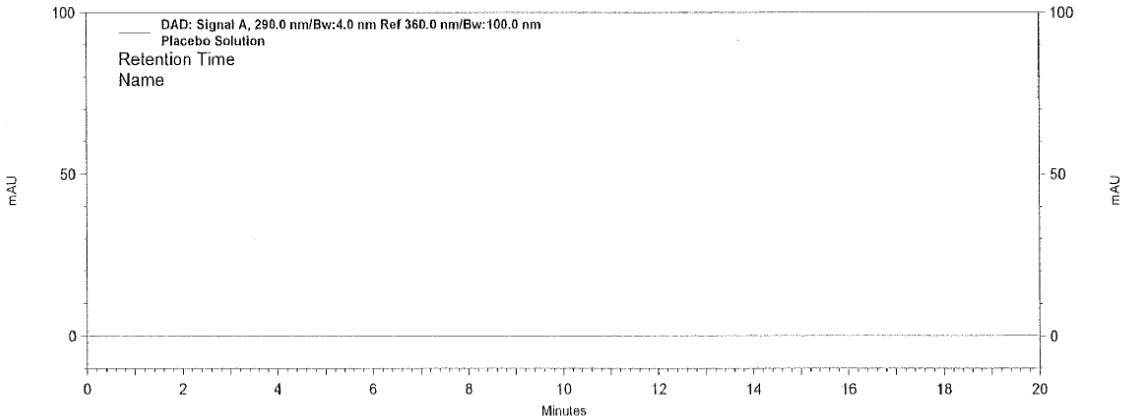

Figure 1.05: Typical chromatogram of Placebo.

Table 1.01: System suitability results.

Parameters	Omeprazole	Ondansetron
Resolution	8.59	
Retention time (min)	8.86	11.83
No. of Theoretical plates	5079	6400
Tailing factor	1.28	1.19

4.2 Precision

The method precision study for six sample preparations in marketed samples showed a RSD of 1.02% for Omeprazole. Similarly the method precision study for six sample preparations in marketed samples showed a RSD of 0.79% for Ondansetron.

Table 1.02: Method Precision results of Omeprazole and Ondansetron.

S.No	Number Samples	% Assay of Omeprazole	% Assay of Ondansetron
1	Method precison-1	103.6	102.8
2	Method precison-2	105.2	101.8
3	Method precison-3	104.0	103.8
4	Method precison-4	103.0	103.0
5	Method precison-5	102.2	103.7
6	Method precison-6	103.0	102.1
Mean		**103.5**	**102.9**
SD		**1.0516**	**0.8174**
% RSD		**1.02**	**0.79**

4.3 Accuracy

A series of solutions were prepared by spiking the placebo and API in the range of about 50% to 150% of test concentration in triplicate and injected into HPLC system and analyzed as per the test method. The results are reported in % mean recovery.

Table 1.03: Results for accuracy studies of Omeprazole.

Recovery sample	Amount added(ppm)	Amount found(ppm)	% recovery	Mean of recovery	% RSD
50%- 1	100.30	100.65	100.3	**99.8**	**1.08**
50%- 2	100.50	99.10	98.6		
50%- 3	100.10	100.73	100.6		
100%-1	200.20	202.02	100.9	**100.2**	**0.58**
100%-2	200.60	200.51	100.0		
100%-3	200.40	200.04	99.8		
150%-1	300.10	302.15	100.7	**100.4**	**0.59**
150%-2	299.74	298.89	99.7		
150%-3	299.80	302.29	100.8		

Table 1.04: Results for accuracy studies of Ondansetron.

Recovery sample	Amount added(ppm)	Amount found(ppm)	% recovery	Mean of recovery	% RSD
50%- 1	20.3	20.11	99.0	**99.8**	**0.85**
50%- 2	20.5	20.87	99.8		
50%- 3	20.9	21.26	100.7		
100%-1	40.6	40.40	99.4	**99.9**	**1.27**
100%-2	40.2	39.78	98.9		
100%-3	40.0	40.54	101.3		
150%-1	60.2	60.79	101.0	**100.3**	**0.81**
150%-2	60.6	60.22	99.4		
150%-3	59.8	60.19	100.6		

The mean recovery for Omeprazole 50%, 100% and 150% levels are respectively 99.8, 100.2 and 100.4 respectively. The mean recovery for Ondansetron 50%, 100% and 150% levels are respectively 99.8, 99.9 and 100.3 respectively. The recovery result indicates that the test method has an acceptance level of accuracy.

4.4 Linearity of detector response

The standard curve was obtained in the concentration range of 100-300 µg/ml for Omeprazole and 20-60 µg/ml for Ondansetron. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in **Figure: 1.06** for Omeprazole and **Figure: 1.07** for Ondansetron to demonstrate the linearity of the proposed method. From the data obtained which given in **Table: 1.05** for Omeprazole and **Table: 1.06** for Ondansetron the method was found to be linear within the proposed range.

Table 1.05: Linearity of omeprazole.

Linearity level	Concentration(ppm)	Mean response
50% Level	103.9	1966272814
75% Level	155.84	291778021
100% Level	207.79	395715146
125% Level	259.74	495418192
150% Level	311.69	590087506
Slope		19,08,195.80
Intercept		26,53,486.20
%y intercept		-0.67
Correlation coefficient		0.99988

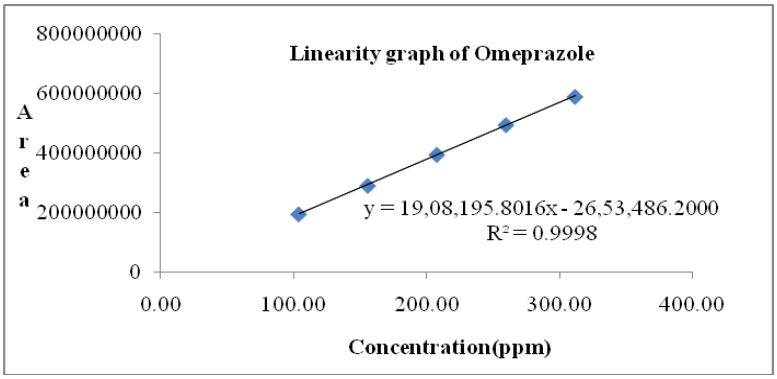

Figure 1.06: Calibration plot of Omeprazole.

Table 1.06: Linearity of Ondansetron.

Linearity level	Concentration(ppm)	Mean response
50% Level	20.42	34789164
75% Level	30.63	52208654
100% Level	40.84	69065965
125% Level	51.05	86792930
150% Level	61.25	104019250
Slope		1695018.5914
Intercept		157413.4000
%y intercept		0.23
Correlation coefficient		1.0000

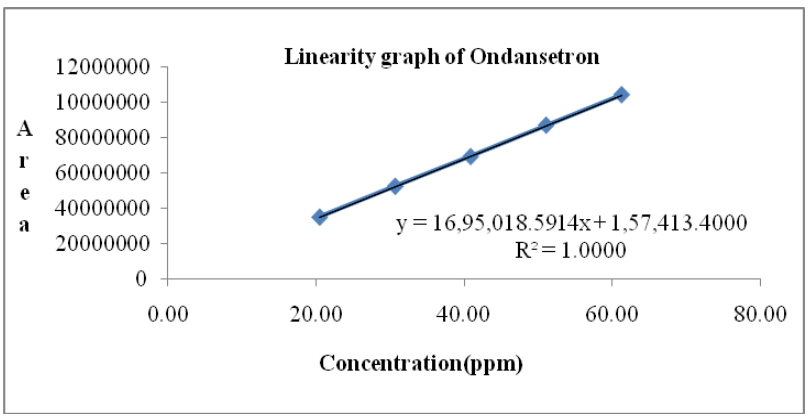

Figure 1.07: Calibration plot of Ondansetron.

4.5 Robustness

Robustness of test method was demonstrated by preparing all system suitability solutions as per test method and chromatographed same into the HPLC system. Carrying out system suitability under normal condition and each of the altered conditions mentioned below. The robustness was evaluated by report the system suitability parameters as per test method.

Table 1.07: Robustness results of Omeprazole.

Parameter	Altered condition	Theoretical Plates	Tailing factor	%RSD
As such condition		5592	1.29	0.98
Flow variation	Low flow (0.9 mL/min)	5896	1.28	1.64
	High flow (1.1 mL/min)	5491	1.29	1.24
Temperature variation (25°C)	Low Temperature 25°C	5891	1.29	1.65
	High Temperature 35°C	5534	1.28	1.24

Table 1.08: Robustness results of Ondansetron.

Parameter	Altered condition	Theoretical Plates	Tailing factor	%RSD
As such condition		7158	1.18	1.12
Flow variation	Low flow (0.9 mL/min)	6958	1.19	1.27
	High flow (1.1 mL/min)	6365	1.18	1.87
Temperature variation (25°C)	Low Temperature 25°C	6358	1.19	1.52
	High Temperature 35°C	7059	1.17	1.41

4.6 Solution stability of analytical solutions

Standard and sample solutions were kept for about 24 hrs at room temperature in transparent bottles in auto sampler and in refrigerator 2-8°C. The stability of standard and sample solutions was determined by comparison of "old" prepared standard solutions with freshly prepared standard solutions.

Table 1.09: Results for solution stability of Omeprazole standard.

Time Interval	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA
24hrs	1.02	1.01

Table 1.10: Results for solution stability of Ondansetron standard.

Time Interval	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA
24hrs	1.01	1.00

Table 1.11: Results for solution stability of Omeprazole sample.

Condition	Bench Top (RT)		Refrigerator (2-8°C)	
Time Interval	%Assay	% of Assay difference	%Assay	% of Assay difference
Initial	103.5	NA	103.5	NA
24hrs	103.9	0.4	103.6	0.1

Table 1.12: Results for solution stability of Ondansetron sample.

Condition	Bench Top (RT)		Refrigerator (2-8°C)	
Time Interval	%Assay	% of Assay difference	%Assay	% of Assay difference
Initial	102.9	NA	102.9	NA
24hrs	103.1	0.2	102.8	-0.1

5.0 CONCLUSION

An RP-HPLC method for simultaneous estimation of Omeprazole and Ondansetron was developed and validated as per ICH guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible.

The accuracy studies were shown as % recovery for Omeprazole and Ondansetron 50%, 100% and 150% level. The limit of % recovered shown is in the range of 98 and 102% and the results obtained were found to be within the limits. Hence the method was found to be accurate.

The method precision studies were shown as % RSD for Omeprazole and Ondansetron less than 2.0%. The limit of % RSD shown is in the below 2.0% and the results

obtained were found to be within the limits. Hence the method was found to be precise.

Linearity was observed over a concentration range of 100-300µg/ml for Omeprazole and 20-60µg/mL for Ondansetron. The solution stability of standard and samples are stable upto 24hrs on bench top and refrigerator (2-8°C).

The method has been successfully applied for the analysis of marketed tablets. It can be used for the routine analysis of formulations containing any one of the above drugs or their combinations without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations. Therefore, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing that are typically associated when different formulations and their individual drug substances are analyzed. We have developed a fast, simple and reliable analytical method for determination of Omeprazole and Ondansetron in pharmaceutical preparation using RP-LC. As there is no interference of blank and placebo at the retention time of Omeprazole and Ondansetron. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and precision. It allows reliably the analysis of Omeprazole and Ondansetron in bulk, its different pharmaceutical dosage forms.

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Conflict of interests

The authors claim that there is no conflict of interest.

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