IJBPAS, April, 2022, 11(4): 1968-1978

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF OMEPRAZOLE AND ONDANSETRON BY (UHPLC) RP-HPLC IN BULK AND TABLET DOSAGE FORMS

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Received 25th April 2021; Revised 26th May 2021; Accepted 30th July 2021; Available online 1st April 2022

https://doi.org/10.31032/IJBPAS/2022/11.4.6034

ABSTRACT

An accurate, precise and reproducible HPLC method was developed for quantitative estimation of Omeprazole (OPZ) and Ondansetron (ODN) simultaneously in tablet dosage forms. Omeprazole is a proton pump inhibitor and in the treatment of gastro-oesophageal reflux disease (GERD), peptic ulcer and Zollinger-Ellison syndrome. Ondansetron is used as selective 5-HT3 receptor antagonist and used in the management of nausea and vomiting induced by cytotoxic chemotherapy and radio therapy and also post operative nausea and vomiting. Agilent (S. K.) gradient system UV detector and C_{18} column with 100 mm x 4.6 mm i.d. and 5µm particle size. Methanol: water (70:30) 0.1 % OPA was used as the mobile phase for the method. The detection wavelength was 259 nm and flow rate was 0.8 ml/min and temperature 26° C ambien. In the developed method, the retention time of omeprazole and ondansetron were found to be 4.01 min and 6.95 min. The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and

reproducible. So the proposed methods can be used for the routine quality control analysis of omeprazole and ondansetron in bulk drug as well as in formulations.

Keywords: Omeprazole and Ondansetron, method development, validation, simultaneous estimation, HPLC

INTRODUCTION

Omeprazole is chemically 5-methoxy-2-[[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole and it is used as proton pump inhibitor and in the treatment of gastro-oesophageal reflux disease (GERD), peptic ulcer and Zollinger-Ellison syndrome **[1]**. Ondansetron is chemically (±) 1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl) methyl]-4H-carbazol-4-one, monohydrochloride, dehydrate **(Figure 1)**. Ondansetron was developed around 1984 by scientists working at Glaxo's laboratories in London. It is in both the imidazole and carbazole families of heterocyclic compounds. it is used as selective 5-HT3 receptor antagonist and used in the management of nausea and vomiting induced by cytotoxic chemotherapy and radio therapy and also post operative nausea and vomiting. Literature survey reveals that 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 spectro-metric **[9, 10]** in formulation, RP-HPLC in formulation **[11]** and blood **[12]** for determination of ondansetron.

The review of the literature revealed that no (UHPLC) RP-HPLC method has so far been reported for the combination of omeprazole and ondansetron. So an attempt has been made to develop a simple, precise, accurate reverse phase high performance liquid chromatographic method for the simultaneous estimation of omeprazole and ondansetron in combined tablet dosage forms.

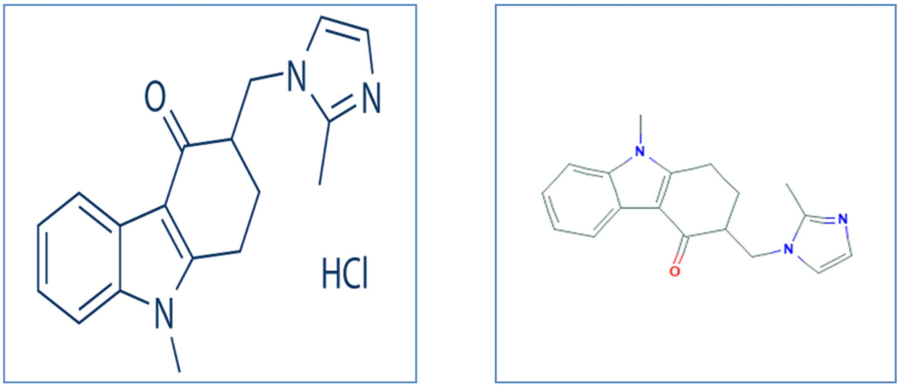

Figure 1: Structure of omeprazole and ondansetron

MATERIALS AND METHODS

Materials and Reagents

The analysis of the drug was carried out on Agilent (S. K.) Gradient System UV Detector. Equipped with Reverse Phase (Grace) C18 column (4.6mm x 100mm; 5µm), a SP930D pump, a 20µl injection loop and UV730D Absorbance detector and running autochro-3000 software. The API of both drugs Omeprazole (OPZ) and Ondansetron (ODN) procured from Donal healthcare. Orthophopsphoric acid (OPA), methanol, acetonitrile, water (HPLC grade Merck Specialties Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai.), 0.45µm filter (Millipore, Bangalore). A combination of Omeprazole (10 mg) and Ondansetron (4 mg) in tablet formulation was procured from local pharmacy **(Donrid - O).**

Chromatographic Conditions

Column C18 (100 mm× 4.6mm); particle size packing 5µm ; detection wavelength 259 nm; flow rate 0.8 ml/min; temperature 26 ^{0}C ambient; sample size 20 µl; mobile phase methanol: water (OPA 0.1%) (70:30); run time 15 min.

Preparation of standard stock solution

10 mg of Omeprazole and 4 mg of Ondansetron was weighed accurately and transferred to separate 10 ml volumetric flask dissolved in methanol and diluted to 10ml with the solvent (Methanol**:** Water, 70:30v/v) to give a stock solution of 1000 µgm/ml Omeprazole and 400 µgm/ml Ondansetron **(Table 1 and Figure 2)**.

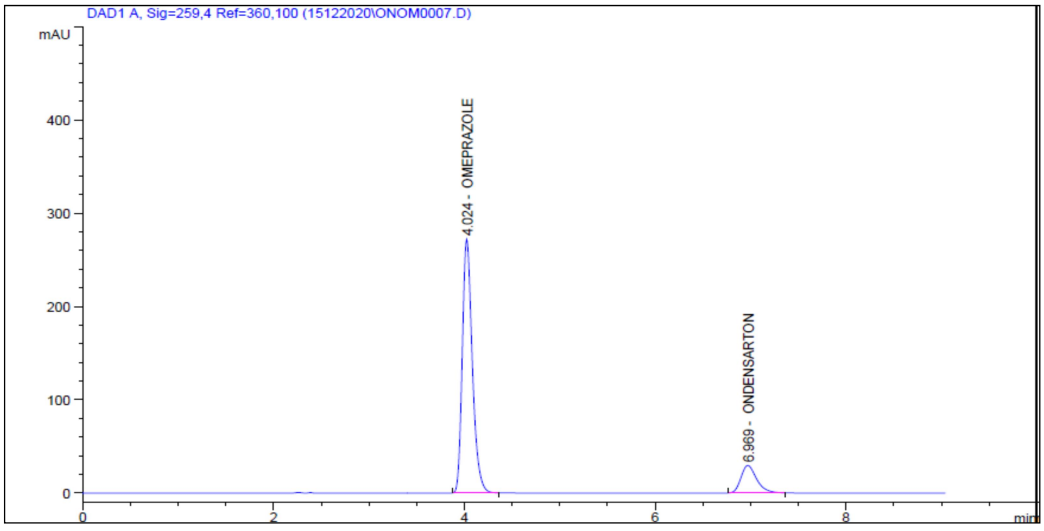

Figure 2: Chromatogram of standard mixture of Omeprazole and Ondansetron

Table 1: Details of chromatogram of standard mixture Omeprazole and Ondansetron

Drug name	R.T	Area	Height	TH.Plates	SYMM	Resolution
OPZ	2.720	7364.42139	1319.52087	6197	0.76	---
ODN	5.868	672.20447	66.34810	8869	0.59	16.22

Method development and validation:

Working standard of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected **[13 - 20]**.

Assay preparation for commercial formulation

For analysis of the tablet dosage form, weigh 20 Omeprazole and Ondansetron combination tablets and calculated the average weight, accurately weigh and transfer the sample equivalent to 41 mg Omeprazole and Ondansetron into 10 ml volumetric flask. Add about 10ml methanol of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm nylon membrane filter. Then volume was made up to the mark with methanol + water (0.1% OPA) (70 + 30% v/v). The simple chromatogram of test Omeprazole and Ondansetron shown in **(Figure 3)**. The amounts of SOFO and LEDI per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet Assay for % Label claim for % RSD Calculated, Result was shown in **(Table 2)**.

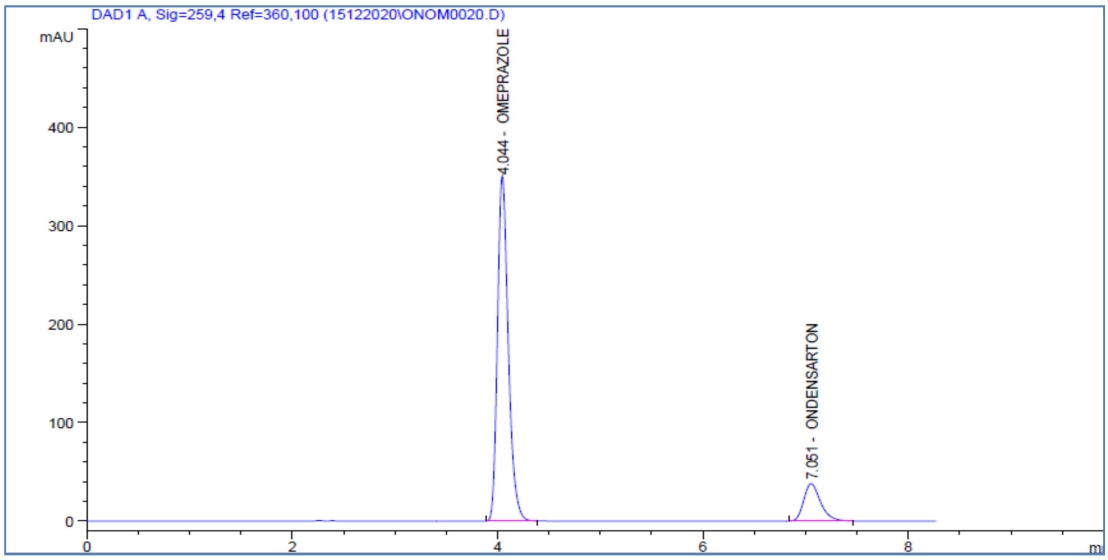

Figure 3: Chromatogram of Omeprazole and Ondansetron tablet formulation

Table 2: Analysis of marketed formulation

Assay	Drug	Amt. Found	%Label Claim	SD	%RSD
Rp-HPLC Method	OPZ	50.14	99.89	0.06	0.06
	ODN	50.14	99.33	0.03	0.03

RESULTS

Linearity and Range:

From Omeprazole and Ondansetron standard stock solution, different working standard solutions (10-50µg/ml) were prepared in mobile phase. Likewise from Omeprazole and Ondansetron standard stock solution different working standard solution (4-20 µg/ml) Were prepared in mobile phase. 20 µl of sample solution was injected into the chromatographic System using fixed volume loop injector. Chromatograms were recorded. The respective linear equation for Omeprazole was y = 62.931 x + 40.126 and Ondansetron equation y = 26.939 x + 4.2302 where x is the concentration and y is area of peak. The correlation coefficient was 0.999 and 0.999. The calibration curve of Omeprazole and Ondansetron. The area for each concentration were recorded **(Table 3, 4)**. The Calibration curves are shown in **(Figure 4, 5)**.

Accuracy:

Recovery studies were performed to validate the accuracy of developed method. To pre-analysed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) **(Figure 6, 7 and 8)** was added and then its recovery was analyzed **(Table 5)**. Statistical validation of recovery studies shown in **(Table 6)**.

System suitability parameters:

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Omeprazole and Ondansetron system suitability parameters were studied. The result shown in below **(Figure 9 and Table 7)**.

Precision:

The method was established by analyzing various replicates standards of Omeprazole and Ondansetron. All the solution were analyzed thrice in order to record any intra-day and interday variation in the result. The result obtained for interday and intraday variation are shown in the **(Table 8)**.

Robustness:

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate on retention time and tailing factor of drug peak was studied. The results indicate that less variability in retention time and tailing factor were observed **(Table 9)**.

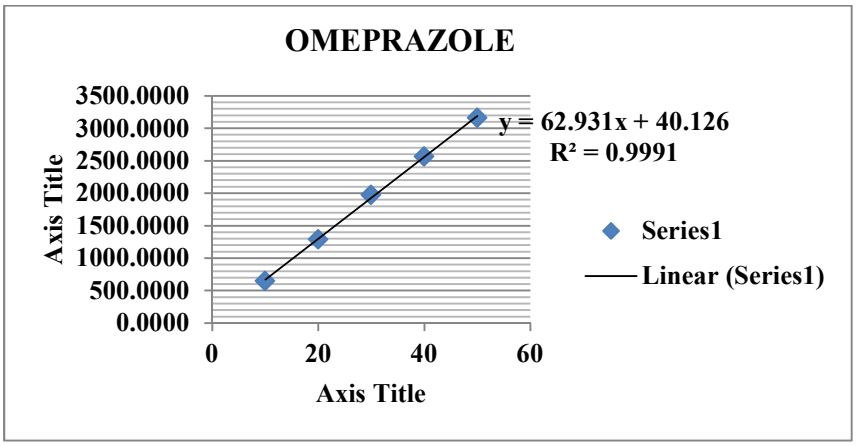

Figure 4: Calibration curve of Omeprazole

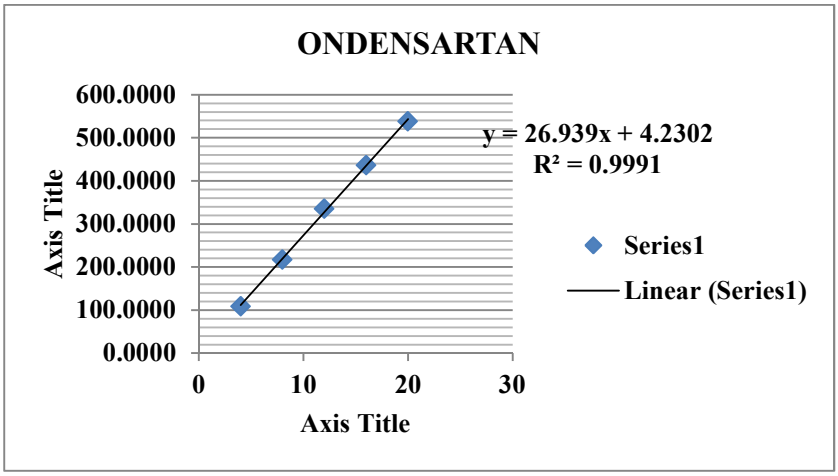

Figure 5: Calibration curve of Ondansetron

Table 3: Linearity data for Omeprazole

Method	Conc. µg/ml	Peak area(µV.sec) 1	Peak area(µV.sec) 2	Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
UHPLC Method	10	648.6549	651.1769	0.1123	0.1123	0.1123
	20	1288.5513	1292.5655	0.1123	0.1123	0.1123
	30	1972.5950	1977.7552	0.1123	0.1123	0.1123
	40	2563.0397	2568.5949	0.1123	0.1123	0.1123
	50	3161.3559	3156.3413	0.1123	0.1123	0.1123
	Equation		y =62.931 x + 40.126			
	R^2		0.999			

Table 4: Linearity data for Ondansetron

Method	Conc. µg/ml	Peak area(µV.sec) 1	Peak area(µV.sec) 2	Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
UHPLC Method	4	108.4747	109.1448	108.8098	0.4738	0.4355
	8	218.0994	217.7225	217.9110	0.2665	0.1223
	12	336.6392	335.4468	336.0430	0.8432	0.2509
	16	435.8655	436.8359	436.3507	0.6862	0.1573
	20	538.2570	538.4765	538.3668	0.1552	0.0288
	Equation		y = 26.939 X + 4.2302			
	R^2		0.999			

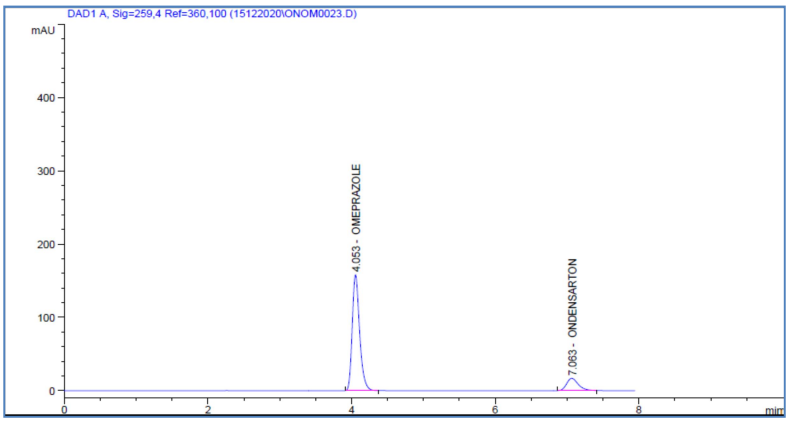

Figure 6: Chromatogram of Accuracy 80%

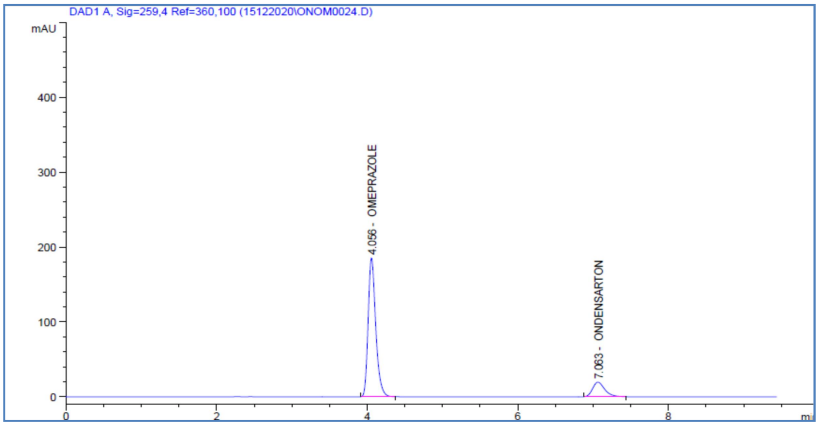

Figure 7: Chromatogram of accuracy 100%

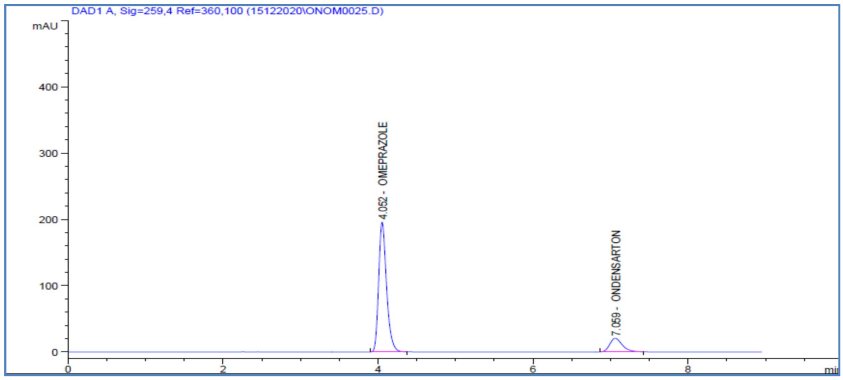

Figure 8: Chromatogram of accuracy 120%

Table 5: Result of Recovery data for Omeprazole and Ondansetron

Method	Drug	Level (%)	Amt. taken (µg/ml)	Amt. Added (µg/ml)	Absorbance Mean*±S.D.	Amt. recovered Mean ±S.D.	% Recovery Mean *± S.D.
RP-HPLC Method	OPZ	80%	10	8	17.85±0.28	8.00±0.08	99.99±1.01
		100%	10	10	20.01±0.06	10.01±0.06	100.15±0.61
		120%	10	12	21.98±0.02	11.98±0.02	99.81±0.21
	OND	80%	5	4	9.02±0.04	4.02±0.04	100.47±0.76
		100%	5	5	9.96±0.001	4.96±0.001	99.72±0.02
		120%	5	6	11.02±0.07	6.02±0.07	101.58±1.14

***mean of each 3 reading for RP-HPLC method**

Table 6: Statistical validation of Recovery studies Omeprazole and Ondansetron

Method	Level of Recovery (%)	Drug	% RSD	S. D.*	Mean % Recovery
Rp-HPLC Method	80%	OPZ	1.01	1.01	99.99
		OND	0.75	0.75	100.47
	100%	OPZ	OPZ	0.61	100.15
		OND	OND	0.02	99.22
	120%	OPZ	OPZ	0.21	99.81
		OND	OND	1.14	101.58

*Denotes average of three determinations for RP-HPLC method

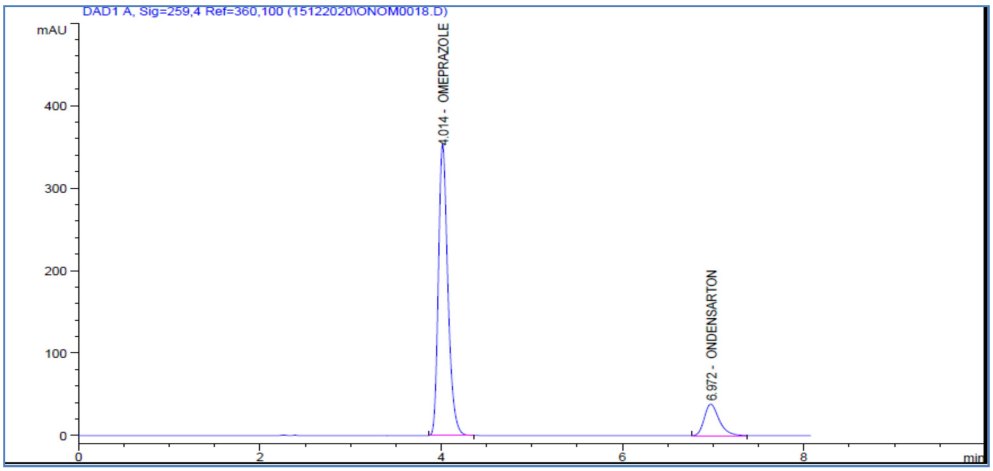

Figure 9: Chromatogram of System suitability studies

Table 7: Repeatability studies on RP-HPLC for Omeprazole and Ondansetron

Method	Concentration of OPZ and ODN (mg/ml)	Peak area	Amount found (mg)	% Amount found
HPLC OPZ Method	20	945.06	19.99	99.99
	20	944.25		
		Mean	944.66	
		SD	0.57	
		%RSD	0.06	
HPLC ODN Method	10	730.14	10.01	100.17
	10	728.29		
		Mean	729.22	
		SD	1.31	
		%RSD	0.18	

Table 8: Result of Intraday and Inter day precision studies on RP-HPLC method for Omeprazole and Ondansetron

Method	Drug	Conc. (µg/ml)	Interday Precision		Intraday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
Rp-HPLC Method	OPZ	10	462.24±1.43	101.76	460.97±0.78	101.50
		30	1431.44±1.62	99.70	1430.78±0.67	99.65
		50	2427.94±1.48	100.44	2429.47±1.56	100.46
	ODN	5	356.57±0.93	100.83	355.93±0.52	100.87
		15	1092.03±0.22	99.00	1090.18±1.39	98.84
		30	1848.22±2.98	99.24	1851.03±0.75	94.96

*Mean of each 3 reading for RP-HPLC method

Table 9: Result of Robustness study of Omeprazole and Ondansetron

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	% RSD	Amount of detected (mean ±SD)	% RSD
		For OPZ		For ODN	
Chromatogram of flow change 0.9 ml	8+20	1296.26±1.61	0.12	1689.44±1.16	0.07
Chromatogram of flow change 1.1 ml	8+20	969.58±1.39	0.14	1260.09±2.11	0.17
Chromatogram of comp change wavelength change 248 nm	8+20	1247.9±1.50	0.12	1674.5±1.24	0.07
Chromatogram of comp change wavelength change 250 nm	8+20	975.56±1.45	0.15	1233.80±2.49	0.20
Chromatogram of mobile phase change 74+26 ml	8+20	1673.9±0.64	0.47	1447.2±0.41	0.03
Chromatogram of mobile phase change 76+24 ml	8+20	1108.46±2.51	0.23	1448.21±2.84	00.20

DISCUSSION

The proposed methods for simultaneous estimation of OPZ and ODN in tablet dosage forms were found to be simple, accurate, economical and rapid. The method was validated as per the ICH Q2 (R1) guidelines. Standard calibration yielded correlation coefficient (r^2) 0.999 & 0.999 for OPZ and ODN respectively at all the selected wavelengths and the values were average of three readings. The values of % RSD are within the prescribed limit of 2 %, showing high precision of methods and recovery was close to 101% for both the drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully for simultaneous estimation of OPZ and ODN in formulations.

CONCLUSION

The developed HPLC methods were found to be more accurate, precise and reproducible. The analysis of tablets containing two drugs gave the satisfactory results. The statistical parameter of these methods showed good results. The recovery studies revealed excellent accuracy and high precision of the method. The methods were found to be simple & time saving. All proposed methods could be applied for routine analysis in quality control laboratories.

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