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Stability indicating RP-HPLC method for the determination of Ondansetron (An Anti-emetic agent)

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ABSTRACT

A simple new RP-HPLC method was established for the forced degradation studies of Ondansetron tablets. A mixture of methanol and tetra butyl ammonium hydrogen sulphate was employed with flow rate 0.6 ml/min on Shimadzu HPLC system with Agilent extended C18 column. The regression equation was y = 102382x + 49245 with correlation coefficient 0.9998. Forced degradation studies were conducted by exposing Ondansetron to acidic, alkaline, oxidation, photolytic and thermal stress conditions.

KEY WORDS: Ondansetron, RP-HPLC, validation, stability-indicating.

1. INTRODUCTION

Ondansetron (OND) is a potent, highly selective competitive 5HT3- receptor antagonist that has been introduced to clinical practice as an antiemetic for cancer treatment-induced and anesthesia-related and vomiting (Ye, 2001). Ondansetron is chemically known as 9-methyl-3-[(2-methyl- 1H- imidazol-1-yl) -methyl]-1, 2, 3, 9-tetrahydro- 4H-carbazol-4-one hydrochloride dihydrate with molecular weight 365.86 g/mol (Indian Pharmacopoeia, 2010). It acts both peripherally on vagal nerve terminals and centrally in the chemoreceptor trigger zone of the postrema and specifically vomiting associated with cancer chemotherapy, radiotherapy and anesthesia and surgery (KD. Tripathi, 2010). Ondansetron was determined by different techniques such as Spectrophotometry (Sradhanjali Patra, 2007), (Chennaiah, 2012), (Kalaichelvi, 2012), (Lobhe, 2011), (Chauhan Prakash, 2012), (Asad Raza, 2007), (Lahuerta Zamora, 1996), (Asad Raza, 2007), (Sudhakararao, 2014), (Shirish, 2014), (Kolte Reshma, 2012) and liquid chromatography (Zarna Dedania, 2009), (Mushabbar basha, 2013) in pharmaceutical formulations. A new stability indicating RP-HPLC method has been designed for Ondansetron tablets.

2. MATERIALS AND METHODS

Shimadzu HPLC system with PDA detector and Agilent extended C18 column was used for the study. Reference standards of Ondansetron was obtained from Symed labs pvt. Ltd., (India). Ondansetron is available as tablets and injections with brand names Odep Tab® and Odep Inj®, (Label claim: 4 mg/tablet; Plenus Pharmaceuticals Pvt. Ltd., India).

3.3954 g of Tetra butyl ammonium hydrogen sulphate (TBAHS) was dissolved in HPLC grade water in a 1000 mL volumetric flask and used. TBHS along with methanol (35:65%, v/v) was used as mobile phase (flow rate 0.6 ml/min) (UV detection 305 nm).

Validation: A series of solutions (0.05–200 µg/ml) were prepared from the OND stock solution, injected in to HPLC and peak area was noted. Calibration curve was constructed by plotting concentration on x-axis and peak area values on y-axis (ICH guidelines, 2005). The intra-day precision and the inter-day precision study were performed on different days (50, 100 and 150 µg/ml). Accuracy of the assay method was evaluated from the recovery study. The robustness was established by introducing small changes in the HPLC conditions which included wavelength (303 and 307 nm), percentage of methanol in mobile phase (63 and 67%) and flow rate (0.5 and 0.7 ml/min). Robustness was studied at 50 µg/ml of OND.

Forced degradation studies: These studies were executed to assess the specificity of the method (ICH, 2003). 1 mg/ml of OND was refluxed with 0.1 M HCl for 20 min at 80 ºC and the stressed sample was cooled, neutralized with NaOH and known as acid hydrolysis study. Alkaline hydrolysis study was done with 0.1 N sodium hydroxide at 80 ºC. The stressed sample was cooled, neutralized with HCl. Oxidation degradation was accomplished using 30% H_2O_2 at 80 ºC. Thermal degradation was studied by heating for 3 hours to 80 ºC in a thermostat. Photolytic degradation: Photolytic degradation was achieved by treating the solution with UV light (365 nm) for 3 hours.

Assay of marketed formulations (Tablets): Odep Tab® and Odep Inj® were procured from the local pharmacy store and extracted with mobile phase. The extracted drug solution was diluted after filtration with mobile phase and injected. The peak area of the respective chromatogram was recorded.

3. RESULTS AND DISCUSSION

Method optimization: The stressed samples were initially investigated using TBHS: methanol (50: 50% v/v) with 1.0 ml/min flow rate in which the peak was obtained at Rt 1.27 mins and also the resolution and peak symmetry were not satisfactory. Flow rate was then changed as 0.6ml/min where elution of a sharp peak at 4.11 mins with tailing

was detected. Finally, composition of the mobile phase was modified as 35:65% v/v with flow rate 0.6 ml/min and a symmetrical drug peak was eluted with retention time 2.27 ± 0.03 mins (305 nm). The chromatogram of the standard was shown in Figure 2A.

Method Validation: Validation of the method was finished with system suitability, linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity and robustness studies (ICH, 2005). Ondansetron has shown linearity 0.05–200 µg/ml (Table 1) with y = 102382x + 49245 and correlation coefficient 0.9998 (Figure 3). The method accuracy was proved by the recovery studies. Known quantity of Ondansetron standard (10 µg/ml) was spiked with drug sample solutions and diluted to yield a total concentration 18, 20 and 22 µg/ml (80%, 100% and 120%). The % recovery was 98.85 - 99.04 with % RSD 0.21- 0.93 (<2.0 %) indicating that the method is accurate (Table.3). Precision was studied by assaying three samples of each at three different levels on the same day whereas the inter-day precision was assessed on different days (n=3). Table.2) indicates that the method is precise. Slight changes in parameters affects the chromatographic response in terms of retention time, theoretical plates, tailing factor etc. During the robustness study the chromatographic response was observed at wavelengths; 303 and 307 nm (± 2 nm), ratio of mobile phase (TBHS: methanol); 33:67 and 37:63 (± 2, v/v) and flow rate; 0.5 and 0.7 ml/min (± 0.1 ml/min). The percentage relative standard deviation was less than 2 indicating that this method is robust (Table.3).

Analysis of Ondansetron commercial formulations: The suggested method was applied for Ondansetron in marketed formulations and the recovery was 98.57%-98.34% (Table.4). The chromatograms obtained for the marketed formulations were presented in Figure.2, (C-D).

Forced degradation studies: Stability indicating studies shows the ability to separate Ondansetron peak from its degradants. The characteristic chromatograms obtained during the study were displayed in Figure 4(A-G). A slight decomposition i.e. less than 5 % (Table.4), was observed during all degradations such as acidic (4.16 %), alkaline (3.07 %), thermal (3.82 %), oxidation (2.18 %) and photolysis (3.29 %) (Table.5).

Table.1. Linearity of Ondansetron

Conc. (µg/ml)	*Mean peak area ± SD	RSD (%)	SEM
0.05	10633 ± 12.76	0.12	7.366758495
0.1	20621 ± 68.05	0.33	39.28764659
0.5	106622 ± 298.54	0.28	172.363612
1	204699 ± 225.17	0.11	130.0015367
5	535069 ± 802.60	0.15	463.3833468
10	1070645 ± 3961.39	0.37	2287.10685
20	2127810 ± 5106.74	0.24	2948.380485
50	5107697 ± 5107.70	0.10	2948.930238
80	8503256 ± 15305.86	0.18	8836.842507
100	10225781 ± 22496.72	0.22	12988.48589
150	15370083 ± 26129.14	0.17	15085.66697
200	20490844 ± 116797.81	0.57	67433.2486

*Mean of three replicates

Table.2. Precision and accuracy studies of Ondansetron

Conc. (µg/mL)	Intra-day precision			Inter-day precision		
	*Conc. obtained (µg/mL) ± SD	%RSD	SEM	* Conc. obtained (µg/mL) ± SD	%RSD	SEM
50	49.7997 ± 0.3433	0.69	0.1982	49.82 ± 0.1618	0.32	0.0934
100	99.5374 ± 0.8016	0.81	0.4628	98.82 ± 1.0425	1.05	0.6019
150	148.7097 ± 0.5535	0.37	0.3196	19.87 ± 0.4045	0.95	0.8109
Accuracy						
Spiked conc. (µg/mL)	**Total conc. (µg/mL)**	***Conc. found (µg/mL) ± SD**		**%RSD**	**SEM**	**%Recovery**
8 (80 %)	18	17.79±0.0365		0.21	0.1170	98.85
10 (100 %)	20	19.81±0.0617		0.31	0.1781	99.04
12 (120 %)	22	21.76±0.2020		0.93	0.5302	98.90

*Mean of three replicates

Table.3. Robustness study of Ondansetron

Parameter	Condition	*Mean peak area	*Mean peak area ± SD (% RSD)	*Assay (%)
Flow rate (± 0.1 ml/min)	0.5	4895485	4953769 ± 59695.33 (1.2)	100.05
	0.6	4951039		
	0.7	5014782		

Detection wavelength (± 2 nm)	303	4912197	4915125±34542.72 (0.7)	99.27
	305	4951039		
	307	4882140		
TBHS: methanol (± 2 %, v/v)	33:67	4857498	4928888 ± 63292.09 (1.28)	99.55
	35:65	4951039		
	37:63	4978128		

*Mean of three replicates

Table.4. Analysis of Ondansetron in commercial formulations

Formulation	Labelled claim (mg)	Amount found* (mg)	Recovery* (%)
Brand I	4.0	3.97	99.37
Brand II	4.0	3.95	98.93

* Mean of three replicates

Table.5. Forced degradation studies of Ondansetron

Stress Conditions	*Mean peak area	*Drug recovered (%)	*Drug decomposed (%)	Theoretical plates	Tailing factor
Standard Drug	5107691	100	-	7222.409	1.437
Acidic degradation	4895172	95.84	4.16	7575.085	1.443
Alkaline degradation	4951039	96.93	3.07	7736.154	1.477
Oxidative degradation	4996137	97.82	2.18	7882.941	1.491
Thermal degradation	4912505	96.18	3.82	7643.248	1.423
Photolytic degradation	4939545	96.71	3.29	8022.939	1.478

*Mean of three replicates

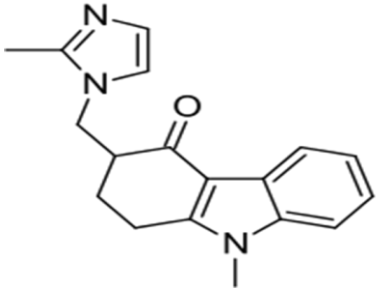

Figure.1. Chemical structure of Ondansetron (OND)

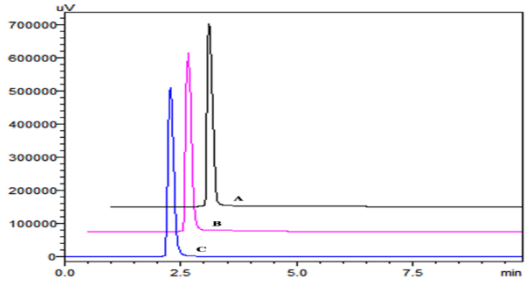

Figure.2. Typical chromatograms of Ondansetron (50 µg/ml) [A], ODEP injection® [B] and ODEP tablet® [C]

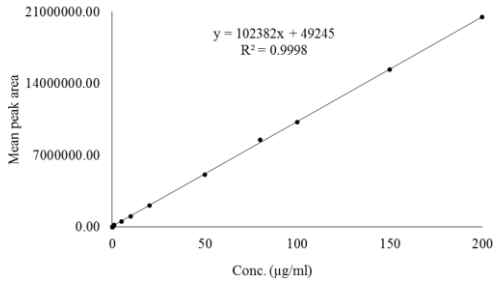

Figure.3. Calibration curve of Ondansetron

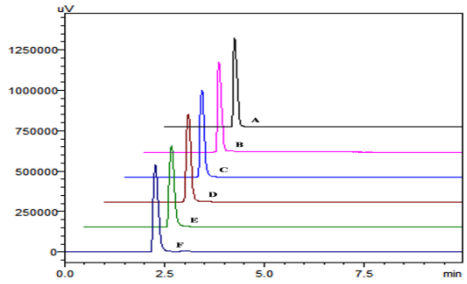

Figure.4. Typical chromatograms of Ondansetron (50 µg/ml) [A], acidic [B], alkaline[C], oxidative [D], thermal [E] and photolytic [F] degradations

4. CONCLUSION

The proposed stability-indicating RP-HPLC method was quite simple and economical for the estimation of Ondansetron in tablet dosage forms. Ondansetron is resistant towards all forced degradation conditions with the established optimized chromatographic conditions.

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REFERENCES

Asad Raza, Abdul Subhan Ijaz, Atta-ur-Rehman, Khalida Aslam. Application of certain π-acceptors for the spectrophotometric determination of Ondansetron in pharmaceutical formulations. Analytical chemistry-an Indian journal, 6 (2), 2007, 43-47.

Asad Raza, Abdul Subhan Ijaz, Atta-ur-Rehman and Uzma Rasheed. Spectrophotometric Determination of Ondansetron in pharmaceutical bulk and dosage forms, J. Chinese chem. society, 54, 2007, 223-227.

Chauhan Prakash S, Panchal Vihang, Comparative study of various UV spectrophotometric methods for quantitative determination of Ondansetron in bulk and tablet dosage form, Inverti rapid-pharm analysis and quality assurance**,** 2012.

Chennaiah M, Veeraiah T, Venkateshwarlu G, Extractive spectrophotometric methods for determination of Ondansetron in pharmaceutical formulations using acidic triphenyl methane dyes, Int. J. Res. Pharm. Biomed. Sci, 3, 2012, 846-857.

ICH stability testing of new drug substances and products Q1A (R2), International Conference on Harmonization, 2003.

ICH validation of analytical procedures, text and methodology Q2 (R1), International Conference on Harmonization, 2005.

Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, the Indian Pharmacopoeia Commission, Ghaziabad, 3, 2010, 1815.

Kalaichelvi R, Madhava Rao B, Manikanta S, Gopinath G, Usha M, Venkata Ramana D, Srinivasa Rao D, Jayachandran E, UV spectrophotometric method for determination of Ondansetron in pure and its formulation, International Journal of Pharmacy and Pharmaceutical Sciences, 4, 2012, 151-152.

Kolte Reshma, Tambe Vrushali, Vichare Vijaya, Patil Manaswi, Simultaneous spectrophotometric estimation of omeprazole and Ondansetron by first order derivative spectroscopy method in combined dosage form, Asian Journal of Research in Chemistry, 5, 2012, 787-790.

Lahuerta Zamora L, Extractive Spectrophotometric Determination of Ondansetron by Ion-Pair formation with bromocresol green, Analytical Letters, 29, 1996, 785-792.

Lobhe GA, Banerjee SK, Shirkhedkar AA, Surana SJ, Simultaneous spectrophotometric methods for estimation of Ondansetron and Omeprazole in tablets, International Journal of Research in Pharmacy and chemistry, 1, 2011, 475-480.

Mushabbar basha MD, Pravena B, Srinidhi M, Rahaman SKA, Method development and validation of Ondansetron in bulk and pharmaceutical dosage form by stability- indicating RP-HPLC method, International journal of PharmTech Research, 5, 2013, 86-98.

Shirish Patel R, Patel LJ, Yogeshvar Thakkar P, Nimesh Patel D, Derivative spectrophotometric method for simultaneous determination of Ondansetron and omeprazole in combined dosage form, International Journal of chemtech applications, 3, 2014, 1530-1536.

Sradhanjali Patra, Choudhury AA, Kar RK, Barik BB, Spectrophotometric method for Ondansetron, Indian Journal of Pharmaceutical Sciences, 69, 2007, 840-841.

Sudhakararao GV, Sujana K, Pedababu T, Development and validation of UV spectrophotometric method for the estimation of Ondansetron in bulk and pharmaceutical formulation. World Journal of Pharmaceutical Research, 3, 2014, 2677-2683.

Tripathi KD, Essentials of Medical Pharmacology, 6th edition, New Delhi, J P Brothers Medical Publishers Pvt. Ltd, 2010

Ye JH, Ponnudurai R, Schaefer R, Ondansetron: a selective 5-HT (3) receptor antagonist and its applications in CNS-related disorders, CNS Drug Review, 7, 2001, 199–213.

Zarna Dedania, Ronak Dedania, Vaishali Karkhanis, Vidya Sagar G, Meeta Baldania and Sheth N.R, RP-HPLC Method for Simultaneous Estimation of Omeprazole and Ondansetron in Combined Dosage Forms, Asian Journal of Research in Chemistry, 2, 2009, 108-111.