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Validating a Stability Indicating HPLC Method for Kinetic Study of Ondansetron Degradation in Acidic, Basic and Oxidative Conditions.

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ABSTRACT

A high performance liquid chromatographic method was developed for the determination of ondansetron hydrochloride in the presence of its degradation products. Stress degradation studies were performed on ondansetrone hydrochloride bulk powder using 5 M hydrochloric acid, 2 M sodium hydroxide, 10% hydrogen peroxide, heat and light. Chromatographic separation was performed on a Nava-Pak C18 column using a mixture of 20 mM KH_2PO_4 (pH 5.0) and acetonitrile (65:35, v/v) as the mobile phase and UV detection at 284 nm. The developed method was accurate (error <1.5%) and precise (CV <2%) within the linear range of 2-50 μg/ml of ondansetron hydrochloride (r^2 >0.999). The kinetics of degradation of ondansetron hydrochloride in acidic, basic, and oxidative conditions indicated first order profiles with regards to drug concentration in the temperature range of 60-90°C.

Keywords: Ondansetron, HPLC, Degradation, Kinetics, Stability

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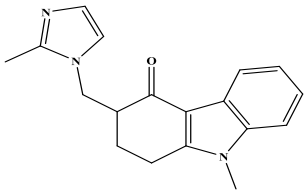
INTRODUCTION

Ondansetron, (±)-9-methyl-3-[(2-methylimidazol-1-yl) methyl]-2,3-dihyro-1H-carbazol-4-one (figure no. 1), is a potent antagonist of 5-hyroxytryptamine (serotonin) type 3 (5-HT_3) receptors. Ondansetron is used effectively for the treatment of nausea and vomiting after surgery, radiotherapy or cancer chemotherapy [1]. High performance liquid chromatography [2-5], LC-MS/MS [6, 7] and enantioselective LC/MS/MS [8, 9] methods have been used for the determination of ondansetron in biological fluids. Few HPLC [10-15] and spctrophotometric methods [16] were also reported for the determination of ondansetron hydrochloride in pharmaceutical dosage forms. The reported method by Mushabbara basha et al. [15] is based on stress degradation of ondansetron hydrochloride. To our best knowledge, there is no research published in the literature in regard to the kinetics of degradation of ondansetron hydrochloride.

This study was undertaken to validate an HPLC method according to the ICH guidelines to be performed for stress degradation of ondansetron hydrochloride in different conditions. An additional goal was to investigate the degradation kinetics of ondansetron hydrochloride in acidic, basic and oxidative conditions.



Ondansetron

Figure 1: Chemical structure of ondansetron

EXPERIMENTAL

Materials

Ondansetron hydrochloride (Batch No: ADEN003127) was from Dr. Reddy's Laboratories Ltd (India) and kindly provided by Exir Pharmaceutical Company (Iran). Methanol (HPLC grade), and acetonitrile (HPLC grade) were purchased from Merck (Darmstadt, Germany). High purity water was prepared by Milli-Q purification system (Millipore, Milford, MA, USA). All other chemicals were of analytical grade and prepared from Merck (Darmstadt, Germany).

Instrumentation

Chromatographic separation was carried out on a Waters HPLC system (Milford, MA, USA) equipped with an isocratic pump (Model 515), an autosampler (Model 710 plus) and a variable UV-vis detector (Modle 480). For data processing a multi channel Chrom&Spec software (version 1.5 x) was used.

The light sources include 100 W Tungsten (visible light) and a low pressure Mercury lamp (UV light). A Melag dry air oven (Germany) was used for heating.

Chromatographic Conditions

A Nova-Pak C_{18} column (4 µm, 150×3.9 mm i.d.) (Waters, MA, USA) was used for chromatographic separation. A mixture of 20 mM NaH_2PO_4 (pH 5.0) and aetonitrile (65:35, v/v) at a flow rate of 1 ml/min was used as the mobile phase. The mobile phase was prepared daily and degassed by filtration through a 0.45 µm Teflon membrane filter (Millipore, Milford, MA, USA) and sonication for 10 min. The UV detection was performed at 284 nm and the injection volume was 20 µl.

Standard Solutions

Stock standard solution of ondansetron hydrochloride was prepared in acetonitrile to reach a final concentration of 5000 µg**/**ml. Standard calibration solutions (2, 5, 10, 20, 40 and 50 µg/ml) were prepared by appropriate dilution of stock standard solution with mobile phase.

System Suitability

Six replicate injections of ondansetron hydrochloride solution in mobile phase (25 µg/ml) were performed to calculate the system suitability parameters of the HPLC method.

Robustness

To determine the robustness of the proposed method, the influence of varying pH values (5.0± 0.2) of the mobile phase and also perecentage of the organic solvent (35± 2) were studied on the chromatographic parameters.

Stability

The stability of ondansetron hydrochloride stock solutions in acetonitrile kept in refrigerator and also working solutions in the mobile phase, kept at room temperature, was checked by comparison with a freshly prepared solution at the same concentration value.

Linearity

Six series of standard calibration solutions of ondansetron hydrochloride were prepared in mobile phase at six different concentration levels (2, 5, 10, 20, 40 and 50 µg/ml) and injected to the HPLC system. The calibration curves were constructed using the obtained peak areas against ondansetron hydrochloride concentration and statistical analysis was performed.

Precision and Accuracy

Three standard solutions of ondansetron hydrochloride at three different concentration levels (2, 10 and 50 µg/ml) in the calibration range were prepared and injected to the HPLC system to examine the accuracy and precision of the developed method. This procedure was performed in triplicate in one day and three consecutive days to find out the within-day and between-day accuracy and precision.

Ruggedness

To establish the ruggedness of the method, a standard solution of ondansetron hydrochloride was analyzed by two analysts on two different chromatographic systems.

Application of the Method

Twenty Demitron tablets (Tehran Chemie, Pharmaceutical Company, Tehran, Iran, Batch No. 006), containing 4 mg of ondansetron hydrochloride, were accurately weighed and finely pulverized in a mortar. An average weight equivalent to one tablet was transferred to a 100 ml volumetric flask. After addition of 70 ml of the mobile phase, the mixture was sonicated for 15 min. The flask was diluted to volume by mobile phase and centrifuged at 4000 rpm for 10 min. The supernatant was filtered through a 0.45 µm polypropylene syringe filter (Teknokroma, Spain) and injected to the HPLC system after two times dilution.

Relative Recovery

To check the relative recovery of ondansetron hydrochloride, standard addition method was used. Known amounts of a standard solution of ondansetron hydrochloride were added to a sample of tablet powder and the sample was treated according to the assay method. The obtained peak area was compared with a standard solution at the same concentration value to find out the relative recovery.

Stress Degradation

Acidic degradation conditions:

To study the degradation of ondansetron hydrochloride in acidic conditions, 0.5 ml of a standard solution of ondansetron hydrochloride in methanol (5000 µg/ml) was transferred to a 10 ml volumetric flask and after addition of 1 ml methanol and 8.5 ml of 5 M HCl, the resulted solution was kept at 80°C for 30 min. Appropriate amounts of 5 M NaOH were added for neutralization and the solution was diluted with mobile phase to reach the claimed concentration value of 25 µg/ml. After injection to the HPLC system the peak area compared with a standard solution of ondansetron hydrochloride at the same concentration level and the percentage of remained ondansetron hydrochloride was calculated.

Basic degradation conditions:

The same procedure for acidic conditions was performed by using a mixture of 2 M NaOH and methanol (60:40). The solution was kept at 80°C for 30 min and neutralized by hydrochloric acid before dilution and injection to the HPLC system.

Oxidative degradation conditions:

For oxidative conditions, a mixture of 10% H_2O_2 solution and methanol (85:15) was used and the resulting solution was kept at 80°C for 30 min. The solution was diluted with mobile phase to reach a concentration value of 25 µg/ml and injected to the HPLC system.

Heat and light degradation conditions:

Bulk powder of ondansetron hydrochloride (50 mg) was spread in thin layer in a watch glass and directly exposed to light or heat. Portions of the powder was taken and dissolved in the mobile phase to reach a claimed concentration of 25 µg/ml after 5 days exposure. The resulting solutions were injected to the HPLC system. The peak area was compared with a standard solution at the same concentration level and the percentage of remained ondansetron hydrochloride was calculated.

Samples at the concentration level of 500 µg/ml in water-methanol (85:15) in Pyrex flasks were also used for heat and light degradation. The samples were kept in heat or light for 5 days and injected to the HPLC system after dilution to a concentration level of 25 µg/ml.

Degradation kinetics of ondansetron hydrochloride under different conditions

To study the degradation kinetics of ondansetron hydrochloride in acidic, basic and oxidative conditions, a stock standard solution of 500 µg/ml was used. The samples were prepared according to the degradation conditions and placed in a dry air oven at three different temperatures (60, 70 and 80°C). At appropriate time intervals, 0.5 ml of the samples were extracted and transfermed into a 10 ml volumetric flask and made up to volume by the mobile phase after neutralization. 20 µl of the resulting solutions were injected to the HPLC system. The concentration of remained ondansetron hydrochloride was calculated and a semi-logarithmic plot of the percent remained drug versus time was constructed and degradation rate constants, K_{obs}, were calculated.

RESULTS AND DISCUSSION

Chromatographic Conditions

A Nova-Pak C_{18} stationary phase with varying compositions of phosphate buffer and acetonitrile as the mobile phase was used for chromatographic separation. The composition and pH value of the mobile phase were changed to optimize the separation conditions. By using

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a mixture of 20 mM KH_2PO_4 (pH 5.0) and acetonitrile (65:35), acceptable peak symmetry and retention time were observed. Good chromatographic separation of ondansetron hydrochloride and degradation products in stress conditions and also tablet excipients was achieved. Representative chromatograms are shown in figure no. 2.

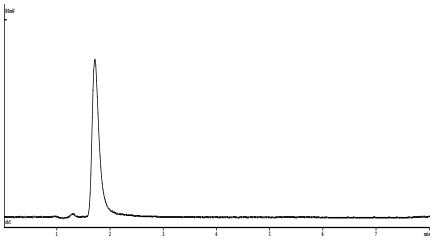

Figure 2a

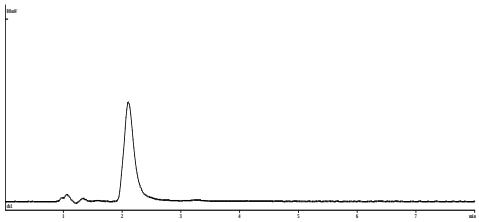

Figure 2b

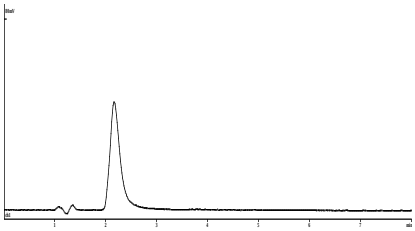

Figure 2c

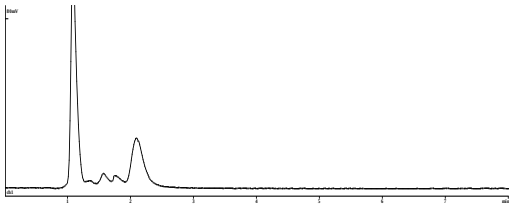

Figure 2d

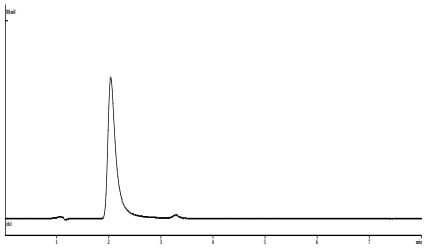

Figure 2e

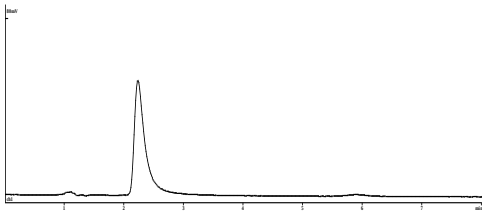

Figure 2f

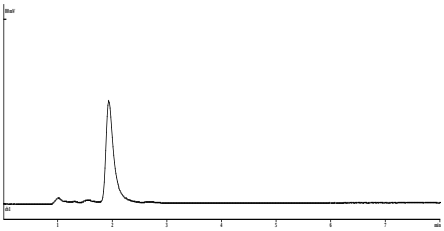

Figure 2g

Figure 2: Typical chromatograms obtained from stability studies of ondansetron. (a) ondansetron hydrochloride standard solution (25 µg/ml); (b) ondansetron hydrochloride solution in 5 M HCl at 80ºC after 30 min; (c) ondansetron hydrochloride solution in 2 M NaOH at 80ºC after 30 min; (d) ondansetron hydrochloride solution in 10 % H_2O_2 at 80ºC after 30 min. (e) ondansetron hydrochloride solution after 5 days at 80 °C; (f) ondansetron hydrochloride solution after 5 days exposure to visible light; (g) ondansetron solution after 5 days exposure to UV light.

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The system suitability parameters (such as theoretical plates, peak symmetry, and repeatability of peak area and retention time) were also tested. The results, given in Table no. 1, showed acceptable criteria.

Table 1: System suitability parameters for chromatographic conditions

Parameters	Found	Acceptable limits
USP theoretical plates (n=6)	2200	N>1500
USP tailing factor (n=6)	1.25	T<1.5
Repeatability (t_R) (n=6)	0.54	RSD<1%
Repeatability (peak area)(n=6)	0.91	RSD<1%

t_R: Retention time (min); N: Theoretical plate; T: Tailing factor; RSD: Relative Standard Deviation

Linearity

Six series of ondansetron hydrochloride calibration solutions at the concentration rage of 2-50 µg/ml were prepared and injected to the HPLC system. Calibration curves were linear over the selected concentration range. The statistical analysis of the calibration curves was done and the results are presented in Table no. 2.

Table 2: Statistical data of calibration curves of ondansetron hydrochloride (n=6)

Parameters	Results
Linearity range	2-50 µg/ml
Regression equation	Y= 24.57X-15.72
Standard deviation of slope	0.16
Relative standard deviation of slope (%)	0.64
Standard deviation of intercept	1.74
Correlation coefficient (r^2)	0.9994
LOQ	0.71 µg/ml
LOD	0.23 µg/ml

The LOD and LOQ were estimated using the following equations.

$$LOQ = 10\sigma/s \quad \text{and} \quad LOD = 3.3\sigma/s$$

where σ is the standard deviation of intercept and s is the slope of the calibration graph.

Precision and Accuracy

The within-day and between-day accuracy and precision data obtained from the analysis of three different concentrations of ondansetron hydrochloride are summarized in Table no. 3.

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Table no. 3: Precision and accuracy of the proposed method for determination of ondansetron hydrochloride (Three sets for 3 days)

Added (μg/ml)	Recovered (mean ± SD) (μg/ml)	CV (%)	Error (%)
Within-day (n=3)			
2.00	2.03±0.04	1.97	1.50
10.00	9.91±0.08	0.81	-0.90
50.00	50.21±0.10	0.20	0.42
Between-day (n=9)			
2.00	2.05±0.03	1.46	1.50
10.00	9.97±0.12	1.20	-0.30
50.00	50.12±0.19	0.38	0.24

The results of the analysis of a standard solution of ondansetron hydrochloride applying the proposed HPLC method by two different analysts with two different HPLC systems showed no significant variations (CV<2%), which showed the ruggedness of the method.

Robustness

To evaluate the method robustness, a standard solution of ondansetron hydrochloride (50 μg/ml) was injected to the HPLC system three times using varied pH value (5.0±0.2) and composition of organic solvent (35±2) of the mobile phase. No significant influence was observed on the peak area or peak shape of ondansetron hydrochloride using all different conditions. On the other hand, the retention time of ondansetron hydrochloride was under the influence of the organic phase composition of the mobile phase.

Relative Recovery

The relative recovery of ondansetron hydrochloride was 99.62±1.03 and no interfering peaks were observed from excipients.

Solution Stability

No significant changes were observed on the stock standard solution of ondansetron hydrochloride kept in refrigerator for 1 week (recovery of 99.9±0.9%). Working standard solutions of ondansetron hydrochloride kept in room temperature was also stable for 1 week (recovery of 99.4±0.5%).

Analysis of Pharmaceutical Product

The content of ondansetron hydrochloride in Demitron tablets was determined. The calculated amount was 3.93±0.12 which is very close to the label claimed of the drug.

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Degradation Studies

The ondansetron hydrochloride samples were treated in different stress degradation conditions according to the experimental section and the remained percent of the drug were calculated. Table no. 4 indicates the results of the degradation studies. Under acidic and basic conditions, ondansetron hydrochloride showed about 16% degradation after 30 min at 80ºC. A small peak was observed in the chromatogram at the retention time of 1.3 min in both acidic and basic media (figures no. 2b and 2c). Ondansetron hydrochloride was more sensitive to stress oxidative conditions and about 51% degradation was observed after 30 min exposure to 10% hydrogen peroxide at 80ºC. Two unknown peaks were detected before the drug peak (figure no. 2d).

No significant degradation was observed when the ondansetron hydrochloride bulk powder was subjected to light and heat for 5 days. On the other hand, the ondansetron hydrochloride solutions were relatively unstable and showed 30% and 12% degradation after 5 days exposure to UV light or heat, respectively (Table no. 4). In these conditions, no new peak was observed (figures no. 2e and 2g).

Table 4: The results of the stress degradation tests on ondansetron hydrochloride bulk powder using different conditions

Stress test condition	Solvent	Temperature	Time	% of ondansetron
Acidic	5 M HCl- methanol (85:15)	80ºC	30 min	83.7
Basic	2 M NaOH- methanol (60:40)	80ºC	30 min	83.9
Oxidative	10% H_2O_2- methanol (85:15)	80ºC	30 min	48.7
Photolytic				
UV light	Solid form	Room temperature	5 days	100.3
UV light	Water-methanol (85:15)	Room temperature	5 days	69.2
Visible light	Solid form	Room temperature	5 days	100.1
Visible light	Water-methanol (85:15)	Room temperature	5 days	98.8
Heat	Solid form	80 °C	5 days	99.9
	Water-methanol (85:15)	80 °C	5 days	88.3

Degradation kinetics of ondansetron hydrochloride

The degradation of ondansetron hydrochloride was kinetically followed in 5 M HCl, 2 M NaOH and 10% H_2O_2 solutions at 70, 80 and 90°C. The semi-logarithmic plots of residual percent ondansetron hydrochloride (Ct) versus time showed good linearity according to the followind equation

$$\log C_t = \log C_0 - kt/2.303$$

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where C_t is the remained percent ondansetrone hydrochloride, C_0 is the initial percent of ondansetron hydrochloride (100%), k is the apparent first order rate constant with a negative sign and t is the time. The correlation coefficients (r^2) of the semi-logarithmic plots of remained percent ondansetron hydrochloride concentration over time were more then 0.93 which showed a first order kinetics. The plots are shown in figures no. 3-5. The degradation equation, estimated degradation rate constants and half-lives, obtained from the slopes of the linear semi-logarithmic plots, are demonstrated in Table no. 5.

Table 5: Degradation equation, apparent rate constant (k) and half-life ($t_{1/2}$) for ondansetron hydrochloride in 5 M HCl, 2 M NaOH and 10% H_2O_2

Temperature (°C)	Equation	r^2 value	k(min^{-1})	$t_{1/2}$ (min)
5 M Hydrochloric acid				
70	y = -0.0010x + 2.010	0.939	0.0023	301.3
80	y = -0.0016x + 2.005	0.981	0.0037	187.3
90	y = -0.0027x + 2.021	0.964	0.0062	11.8
2 M Sodium hydroxide				
70	y = -0.0015x + 1.998	0.992	0.0035	198.0
80	y = -0.0018x + 2.017	0.943	0.0041	169.0
90	y = -0.0028x + 2.016	0.962	0.0064	188.3
10% hydrogen peroxide				
70	y = -0.0041x + 1.947	0.992	0.0094	73.7
80	y = -0.0065x + 2.017	0.944	0.0150	46.2
90	y = -0.0073x + 2.016	0.934	0.0168	41.3

Based on the Arrhenius relationship in the temperature range of 70-90°C, the following equation was used to calculate the activation energy for the degradation process of ondansetron hydrochloride in different conditions.

$$k = Ae^{-Eact/RT}$$

where k is the apparent first order rate constant. A is the pre-exponential factor, E_{act} is the activation energy and T is the temperature. The calculated activation energies for the acidic, basic and oxidative conditions were 51.3, 31.1, and 30.2 KJ/mole, respectively.

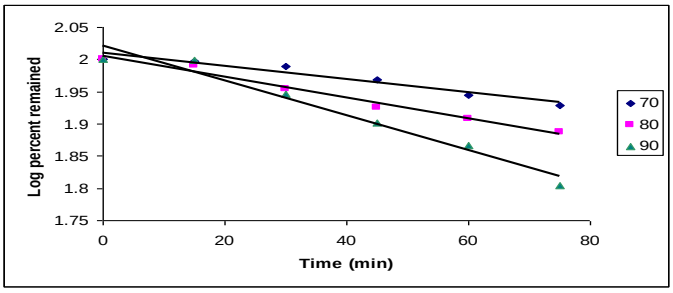

Figure 3: First order plots for the degradation of ondansetron hydrochloride in 5 M HCl at various temperatures using the proposed HPLC method.

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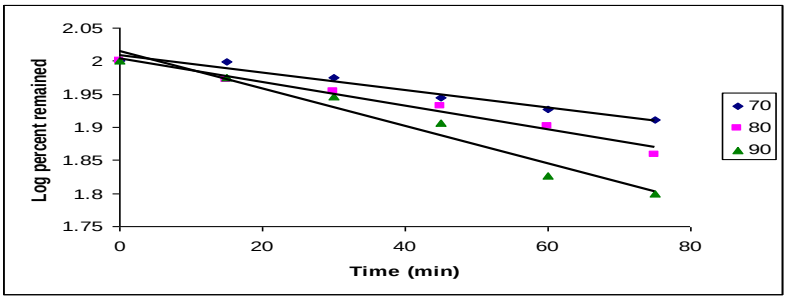

Figure 4: First order plots for the degradation of ondansetron hydrochloride in 2 M NaOH at various temperatures using the proposed HPLC method.

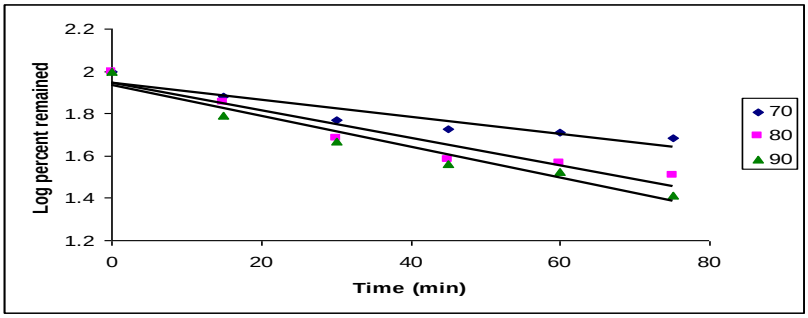

Figure 5: First order plots for the degradation of ondansetron hydrochloride in 10% H_2O_2 at various temperatures using the proposed HPLC method.

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