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Stability study of Valsartan in Aqueous Solutions: Effect of different pH, Time and Temperature

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ABSTRACT

Angiotensin II type1 receptor blockers are group of drugs binding to and blocking active site of the receptor Angiotensin II type 1 receptor (AngIItype1 R). According to current good manufacturing practices, all drugs have been tested for their stability before release. In this study we investigated the stability of valsartan in aqueous buffer solutions ($\mu\text{g/ml}$) at various pH in range of pH 2-12 and at various temperatures from 4°C to 37°C with different time ranging from 0-96 h to determine the optimum pH and temperature and time requirements for its stability and eventually performance over various human gastrointestinal pH range. The stability study of valsartan was determined by high performance liquid chromatography (HPLC) and UV-spectrophotometer. Valsartan showed the highest stability after 2 hour sat pH 6.8 with different temperature. While valsartan recovery rate remain stable at pH 12 all over the incubation times and temperatures using HPLC. In contrast, recovery rate of valsartan was much lower at pH 2. In addition, incubation of valsartan in different temperature with different time, a maximum decrease in the valsartan concentrations was noticed. These results were confirmed by using spectrophotometric analysis. Aqueous solution of valsartan ($\mu\text{g/ml}$) adjusted at pH 6.8 and 12 showed the highest maximum stability at room temperature. In contrast pH 2 induced a higher decrease in valsartan concentration with time.

The present study showed that highest recovery rate and stability were achieved at pH 6.8 and 12 in varies temperatures 4, 20 and 40°C with different time. While elevated hydrogen ion concentrations (pH 2) and temperature with different time can significantly lower valsartan concentrations.

In conclusion: The stability study revealed that the neutral and alkaline pH attenuate rate of valsartan degradation suggested. Thus, keeping hydrogen ion concentrations at neutral or alkaline pH over different time and temperature would be a very useful method to overcome the stability problems of valsartan.

KEY WORDS: Valsartan, temperature and Ph

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INTRODUCTION

Valsartan is an active drug that beneficially used in the treatment of cardiovascular diseases as heart failure, myocardial infarction and hypertension through blocking Angiotensin II type 1 receptor. Fleschet al¹ showed that valsartan is tetrazole derivative, orally active drug specifically prevent the interaction of Angiotensin II with its receptor. Pharmacokinetic study of valsartan indicated that it is rapidly absorbed after oral administration and it is highly bound to plasma proteins, therefore its volume of distribution is limited. Valsartan is not subjected to extensive metabolism and the excretion is mainly by non-renal routes. It is used alone or in combination with other antihypertensive drugs for the treatment of mild to moderate hypertension of elderly and pediatric patients. Valsartan monotherapy starting dose (80 mg) can induce the great effect in the treatment of hypertension along with renal impairment. Moreover, the implication of combination therapy succeeded in controlling severe hypertension in patients not responding to other antihypertensive classes especially diuretics with ACE inhibitors or β -blockers. The importance of valsartan in the treatment of blood pressure aggressiveness is not disputed until now. Furthermore, the role of valsartan in slowing down kidney disease progression due to diabetes or elevated blood pressure are very positive.

The physical characters of valsartan showed that it is a white powder, highly soluble in acetonitrile, methanol and ethanol, while slightly soluble in water. Elevating pH from 4 to 6 increasing its solubility in water by a factor of about 1000. Valsartan has melting point of 110°C. Saydamet al² tested the stability of valsartan in storage under dry conditions and reported that valsartan is a highly stable compound. It is a lipophilic drug, however, on increasing the pH it induces the remarkable decrease in its lipophilicity through favoring formation of anionic form. Therefore, intestinal pH along with GI tract can influence rate of absorption of valsartan.

The large variations in the pH of the gastrointestinal tract starting from highly acidic gastric juice with pH 1-2 in the stomach to neutral or slightly alkaline pH 6 or pH 6-7.5 in the duodenum and the jejunum respectively can control rate of activation or de-activation of the drug^{3,4}. Exposure of orally delivered drugs to these different pH values can result in hydrolysis, oxidation or deamidation. Furthermore, the proper pH of the blood maintained by both organs kidneys and lungs. It has been shown that the pharmacokinetic variables of several drugs as bioavailability and volume of distribution were modulated in pulmonary obstructive disease^{5,6,7}, moreover, the alteration of plasma proteins concentration has been reported⁸. The effect of variations in pH on the ionization of the drugs has been reported by Hinderling and Hartmann⁹. They concluded that alteration in pH can induce changes in the fraction of ionized and non-ionized of weak acid or basic drugs which could be responsible for the observed pH dependent interaction with proteins. In alkaline pH, the fraction of

ionized acidic drugs are increased would preferentially bind to albumin, while the fraction of non-ionized basic drugs is elevated which could preferentially bind to both albumin¹⁰ and alpha1 acid glycoprotein AGP¹¹.

Roškar et al.,¹² studied the stability properties of perindopril as angiotensin-converting enzyme inhibitor (ACEI) in different pH solutions (2.0, 6.8 and 12.0) and temperatures (40, 60 and 80 °C) and concluded that the highest stability of perindopril was achieved at pH 2. Furthermore, the detection and identification of the degradation product as hydrolysis and cyclization of perindopril was achieved by HPLC and mass spectroscopy (LC–MS).

Karima¹³ studied the effect of different pH on ciprofloxacin active ingredient on a different mode of drug administration and concluded that there is a clear effect of pH on drug compound or active ingredient ciprofloxacin through both ways qualitative by degradation and quantitative by the reduced level of concentration.

Many studies investigated the structure-activity relationship of different blockers of angiotensin II type 1 receptor suggested the structural requirement needed for a potent blocker for angiotensin II type 1 receptor. Despite the importance of this drug as antihypertensive, little is known about its stability over various time under different temperature and pH. Up to date and to our knowledge, there has been a single study investigating the physicochemical properties of valsartan showing the effect of different pH on its solubility along with quantitation of valsartan and its fragmentation products by HPLC¹⁴. No recent report focused on the stability of valsartan as a blocker for angiotensin II type 1 receptor in different pH under different time and temperature. Therefore, the objective of the present study will elucidate the stability of valsartan, in an aqueous solution of different pH (2.0, 6.8 and 12.0) with different incubation time ranging from 0 to 96 h and temperature from 4 to 37°C.

MATERIALS AND METHODS

Analytically pure valsartan has been purchased from Sigma Chemical Co. All chemicals and reagents used were of HPLC grade. Sodium hydroxide, hydrochloric acid and acetonitrile were purchased from Aldrich Chemie (Steinheim, Germany). Double distilled water was obtained from a water distillation unit in our laboratory. For the stability study, the preparation of phosphate buffers solutions (PBS) with pH ranging from 2.0, 6.8 and 12.0 was used. Phosphate buffers solutions with different selected pH 2.0, 6.8 or 12.0 was prepared using potassium dihydrogen phosphate (KH₂PO₄) by weighting 136mg of KH₂PO₄ and dissolving (Sigma Chemical Company) in 800ml of distilled water, using phosphoric acid or potassium hydroxide (KOH) for adjusting the pH to 2.0, 6.8 or 12.0 with and we completed to 1000ml using distilled water. All these three volumetric flasks

with different pH were kept in the darkness to exclude the possible degradation effect of light. The studied samples of valsartan was dissolved in minute amount of ethanol and the accurately weighed quantity of valsartan was transferred to 100 ml volumetric flask, which was then dissolved and made up to volume to give 1 µg/ml concentration for HPLC analysis. The calibrated glassware's were used all over the experiment. Valsartan initial concentration was 1 µg/ml as an optimum concentration for studying stability by HPLC and 1 µg/ml by UV-spectrophotometer.

20 µL of sample solutions were injected and analyzed against control samples (lacking of degradation treatment). HPLC chromatogram indicated that there is only one peak response which is due to a single component only.

Experimental analysis:

All analytical works were done on Agilent HPLC 1200 series equipped with flow pump, auto injector, Diode array detector of 1260 series (Agilent, CA, USA) using C18 column (150 mm × 3 mm id, 5 µm particle size) as a stationary phase. A calibrated electronic single pan balance Perkin Elmer AD6 auto balanced and pH Meter of OAKTON, were also used during the analysis.

Preparation of mobile phase and standard stock solution

To stabilize HPLC method for valsartan determination. The mobile phase used for HPLC determination of valsartan was: acetonitrile / water (50/50) with HPLC grade. It was obtained from Aldrich Chemie (Steinheim, Germany); using sodium hydroxide NaOH and hydrochloric acid HCl for adjusting pH. The mobile phase was sonicated 10 minutes¹⁵.

Optimized chromatographic conditions

RP- HPLC analysis was performed by isocratic elution with flow rate of 1 ml/min. The mobile phase containing acetonitrile/water in the ratio of 50:50 (v/v) to obtain well-resolved peak of valsartan ($R_t = 2.86$ min) as shown in figure A. Wavelength of maximum absorption was selected by Photo diode Array detector. The drug shows reasonably good response at 273 nm.

A UV-Visible spectrophotometer (ThermoFisher Scientific) with spectral bandwidth of 1 nm, wavelength accuracy ± 0.1 nm, Model-Evolution 60S (China), software VISIONlite, and a pair of 1 cm matched quartz cells, was used to measure the concentration of valsartan in prepared solutions.

All tools and glassware were washed by ethanol and double washed with distilled water, and dried for 24 hours in 40°C oven. Stock solution of 50 µg/mL of valsartan were prepared and scanned in the UV-spectrophotometer (190-500 nm) for determination of maximum absorption (λ_{max}) in which found to be equal to 270 nm.

Stock solutions were diluted into 25, 10, 5, 2.5 and 1 µg/ml. Then, the absorption (A) of diluted solutions were measured in the UV-spectrophotometer. Finally, a calibration curve was

created by plotting the absorbance (A) versus sample concentration ($\mu\text{g/ml}$), and correlation coefficient was detected as well as shown in figure B¹⁶.

Statistical Analysis: Descriptive statistical analyses were performed on the data for the study sample. Difference between obtained data (Mean \pm SE) for the prepared sample in different

pH at a different time and temperature and the drug at 0 time sample was carried out using one way analysis of variance (ANOVA), followed by tukey-Kramer multiple comparison test as an appropriate post hoc test in the case of presence of significant difference. All statistical analysis were done by the statistical software 20.0 version. P-value less than 0.05 will be taken as a criterion for a statistically significant difference.

RESULTS

RP- HPLC chromatogram using acetonitrile/water as the mobile phase to obtain well-resolved peak of valsartan ($R_t = 2.8$ min) as shown in Figure A. Wavelength of maximum absorption 273 was selected to show reasonably good response by Photo diode Array detector. While figure B show the calibration curve of valsartan using spectrophotometric.

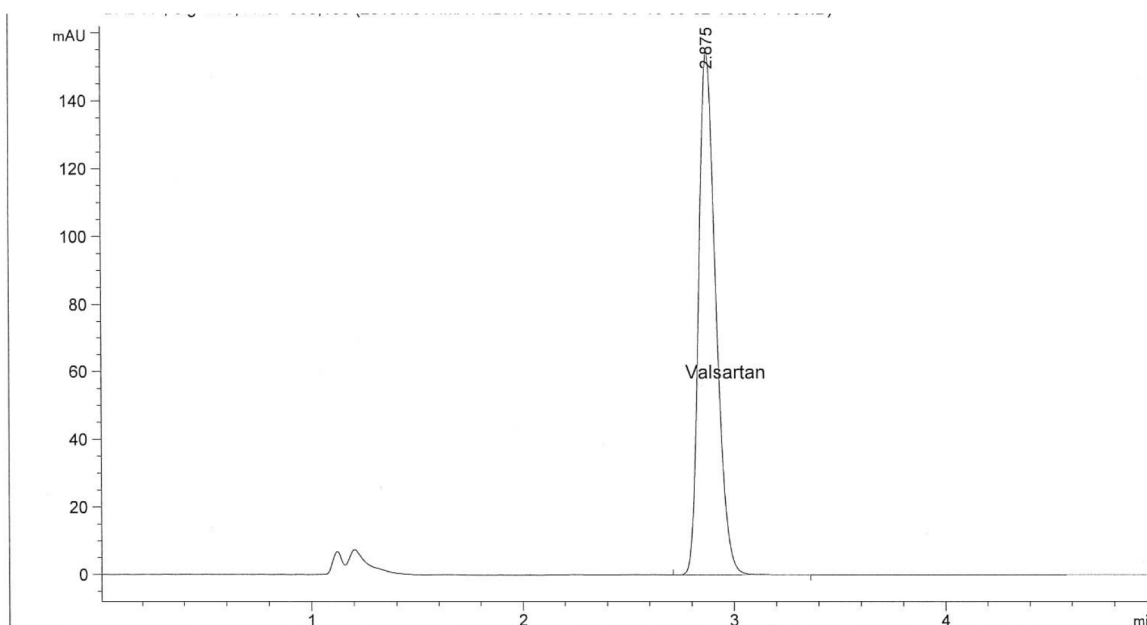


Figure A. The chromatogram of standard valsartan

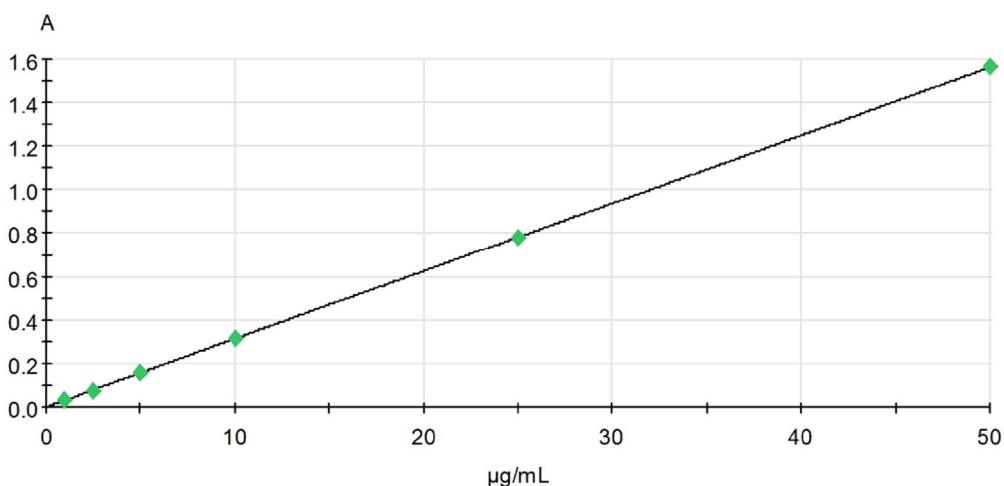
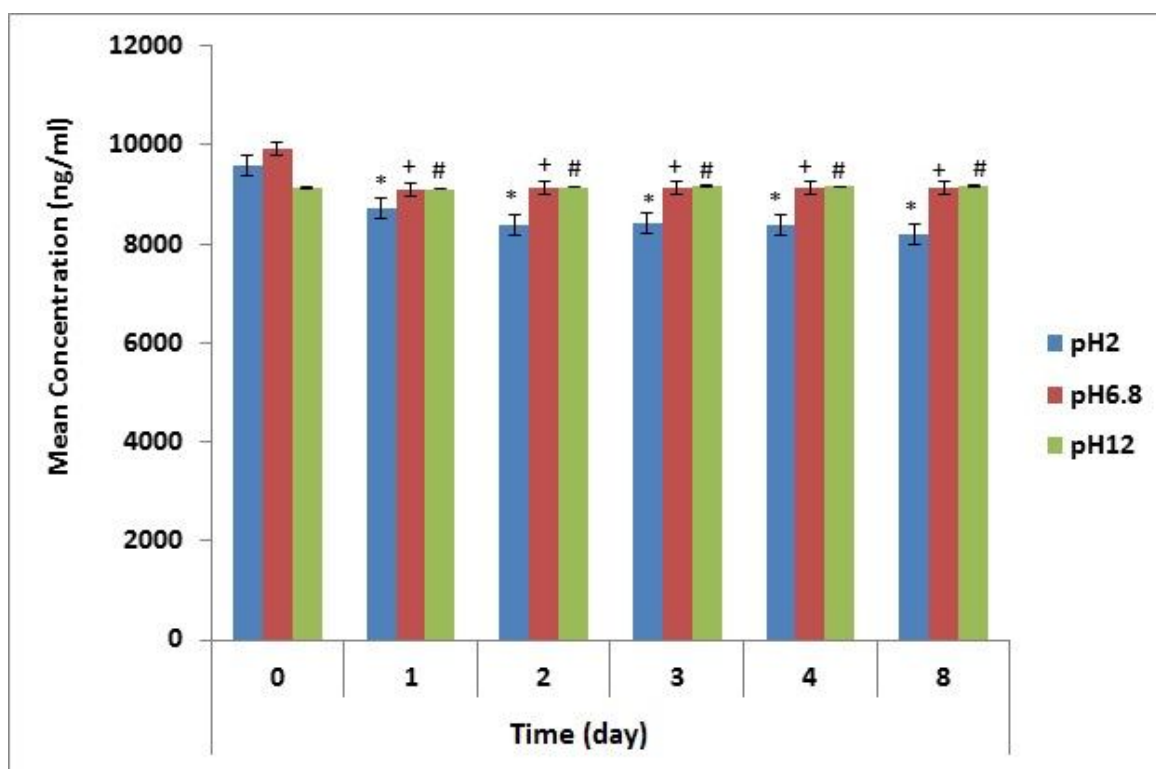


Figure B. Calibration curve of valsartan solution using spectrophotometric analysis at 270 nm

The effect of different pH on the valsartan stability at room temperature with different incubation time were shown in (table1). It was noticed that the highest recovery rate of valsartan occurred at pH 6.8 after few hours of dissolutions. In contrast, the lowest recovery rate was recorded at pH 12. However, assessment of the recovery rate after different incubation time ranging from 1 to 8 days revealed that there was a significant decrease in recovery rate at all tested pH(Figure 1). Their degradation rate of valsartan was markedly elevated at pH 2 in comparison with pH 6.8 and 12.

Table 1:Effect of different pH and incubation time(days) at room temperature on the stability of valsartan (ng/ml)

pH Level	Time(Days)					
	0	1	2	3	4	8
pH2	9584.88	8717.09	8375.815	8414.59	8372.065	8179.203
pH6.8	9930.697	9110.797	9136.427	9139.95	9123.36	9137.753
pH12	9128.287	9113.11	9143.48	9163.217	9151.65	9171.933



P<0.05 statistically significant between time 0 and with different days

Figure 1: Effect of different pH and incubation time(days) at room temperature on the stability of valsartan (ng/ml)

However, incubation of aqueous solution of valsartan at 4°C in different pH are shown in table 2. It has been shown that the highest recovery rate of valsartan occurred at pH 6.8 after few hours of dissolutions. In contrast the lowest recovery rate was recorded at pH 12. The recovery rate of valsartan were at the same level at pH 6.8 and 12, while much lower at acidic pH (Figure 2).

Table 2: Recovery study of valsartan in different pH and incubation time(days) at 4°C on the stability of valsartan (ng/ml)

pH Level	Time(Days)					
	0	1	2	3	4	8
pH2	9551.547	8708.463	8559.833	8691.327	8739.437	8892.183
pH6.8	9930.697	9004.1	9020.55	9031.927	9036.45	9030.427
pH12	9128.287	9119.863	9148.347	9149.89	9139.807	9184.133

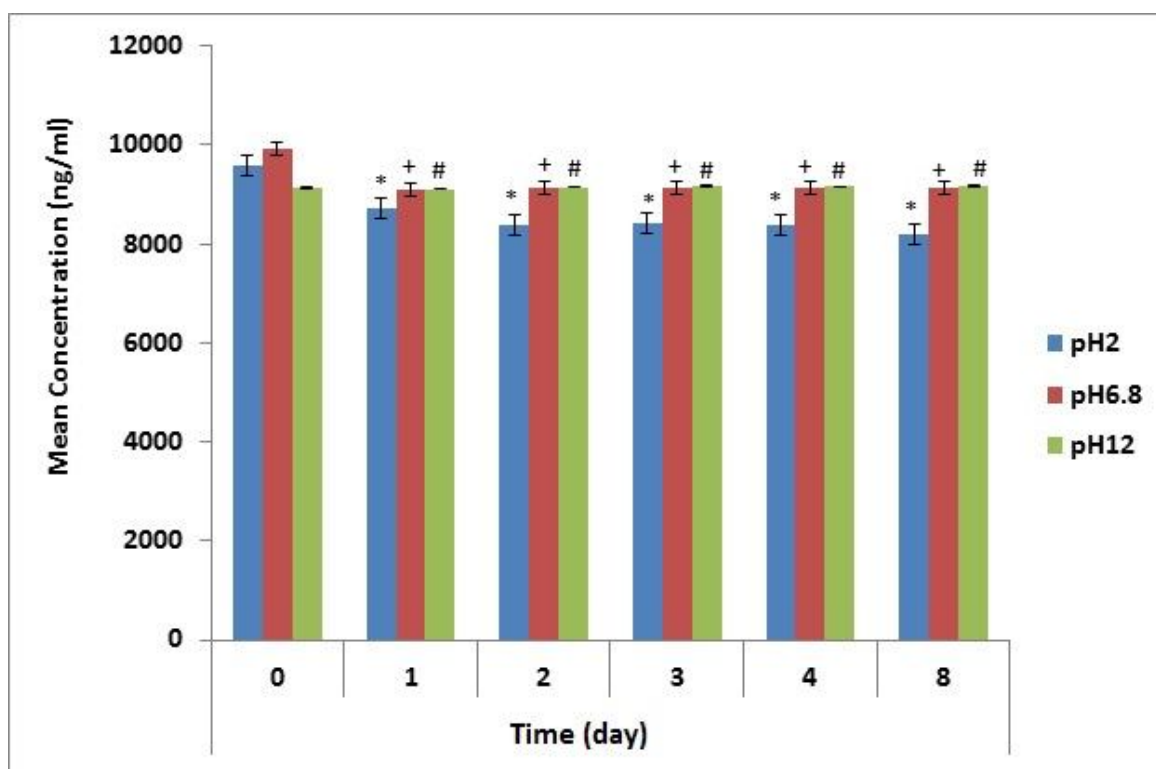


Figure 2: Recovery study of valsartan in different pH and incubation time(days) at 4°C on the stability of valsartan (ng/ml)

Incubation of different aqueous solutions of valsartan at pH ranging from 2 to 12 and different incubation time from 1 to 8 days at 37°C were shown in Table 3. There was a remarkable decrease in valsartan concentration in all tested pH at all time. However, the highest recovery rate was achieved with pH 6.8 (Figure3).

Table 3: Degradation of valsartan in aqueous solutions of different pH and different incubation time (days) at 37°C on the Stability of valsartan (ng/ml)

pH Level	Time(Days)					
	0	1	2	3	4	8
pH2	9551.547	8804.91	8370.757	8368.51	8332.1	8353.74
pH6.8	9930.697	9272.067	9300.3	9283.793	9289.963	9305.323
pH12	9128.287	9117.693	9221.293	9220.2	9243.387	9260.767

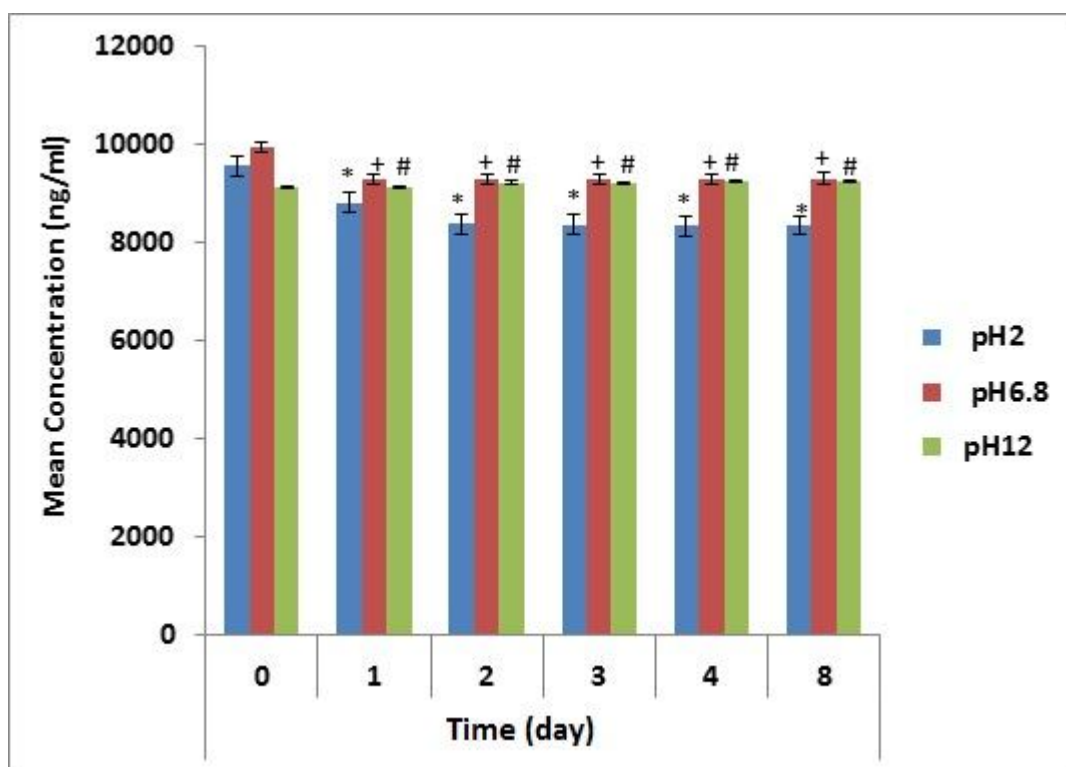


Figure 3: Degradation of valsartan in aqueous solutions of different pH and different incubation time (days) at 37°C on the Stability of valsartan (ng/ml)

Incubation of valsartan at different temperature and time indicated a marked decrease in valsartan concentration were observed and remain constant all over the incubation time (table 4). The results may indicate that neutral and alkaline pH can protect or enhance stability of valsartan (figure4)

Table 4: Residual drug of valsartan versus time and different temperature (ng/ml)

Temperature	Time(Days)					
	0	1	2	3	4	8
Room T.	8971.123	8944.273	8920.65	8859.54	8935.39	8983.213
Refrigerator	8971.123	8968.077	8960.193	8918.527	8918.577	9086.55
37°C	8971.123	9035.38	8902.223	8990.457	9038.133	9036.167

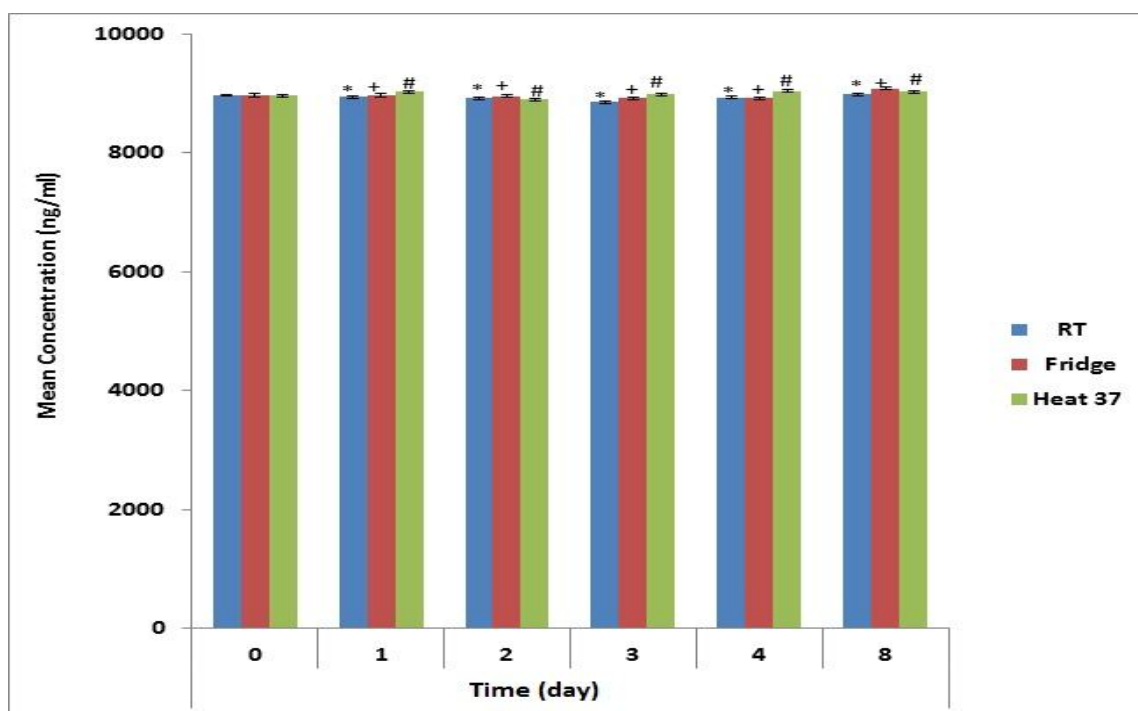


Figure 4: Residual drug of valsartan versus time and different temperature (ng/ml)

Using UV-spectrophotometer as method of analysis, table 5 shows that there was no change in valsartan concentration dissolved in solutions adjusted at pH 6.8 and 12. While there was a decrease in the recovery rate of valsartan incubated in acid solutions in room temperature over different time (figure 5A and B). In contrast, incubation at 37 °C recovery rate of valsartan at pH 2 and 6.8 showed a significant decrease while the concentration of valsartan incubated at 12 remain constant (figure 6A,B)

Table 5: Comparison between different pH and different days at room temperature on the Stability of valsartan using UV Spectrophotometer (µg/ml).

pH Level	Time(day)				
	0	1	2	3	4
pH2	0.201	0.191	0.191	0.184	0.179
pH6.8	0.200	0.199	0.200	0.200	0.200
pH12	0.199	0.200	0.200	0.201	0.200

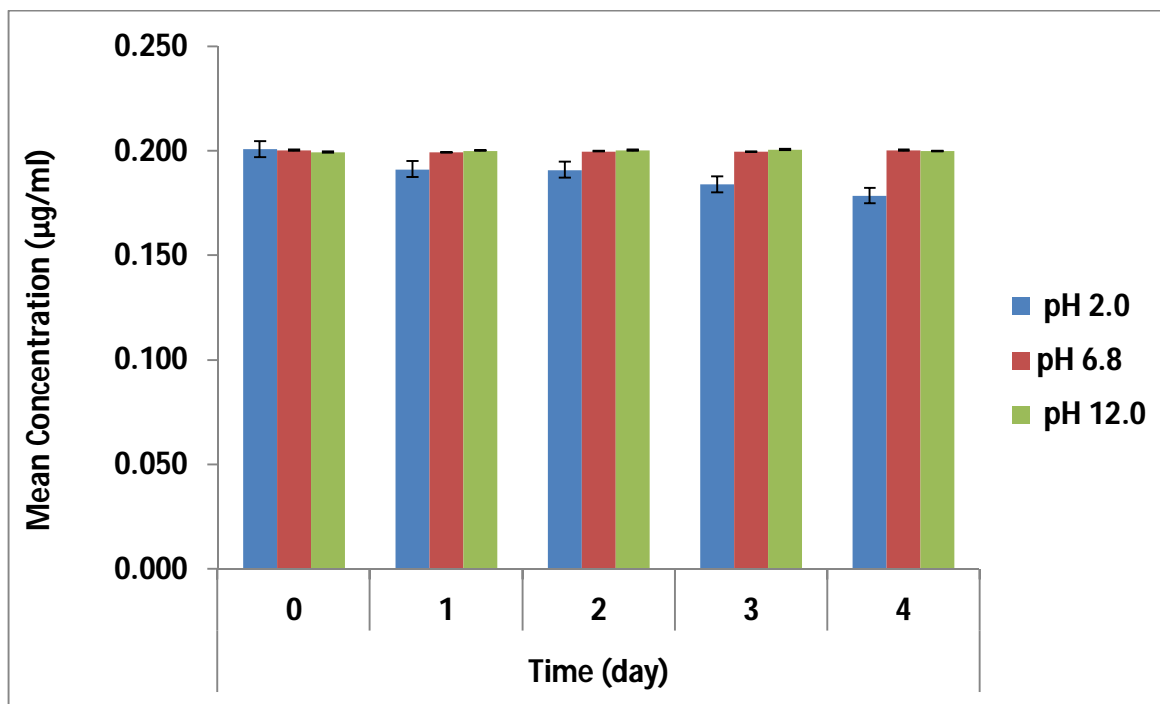


Figure 5 A: Comparison between different pH and different days at room temperature on the Stability of valsartan using UV Spectrophotometer (µg/ml).

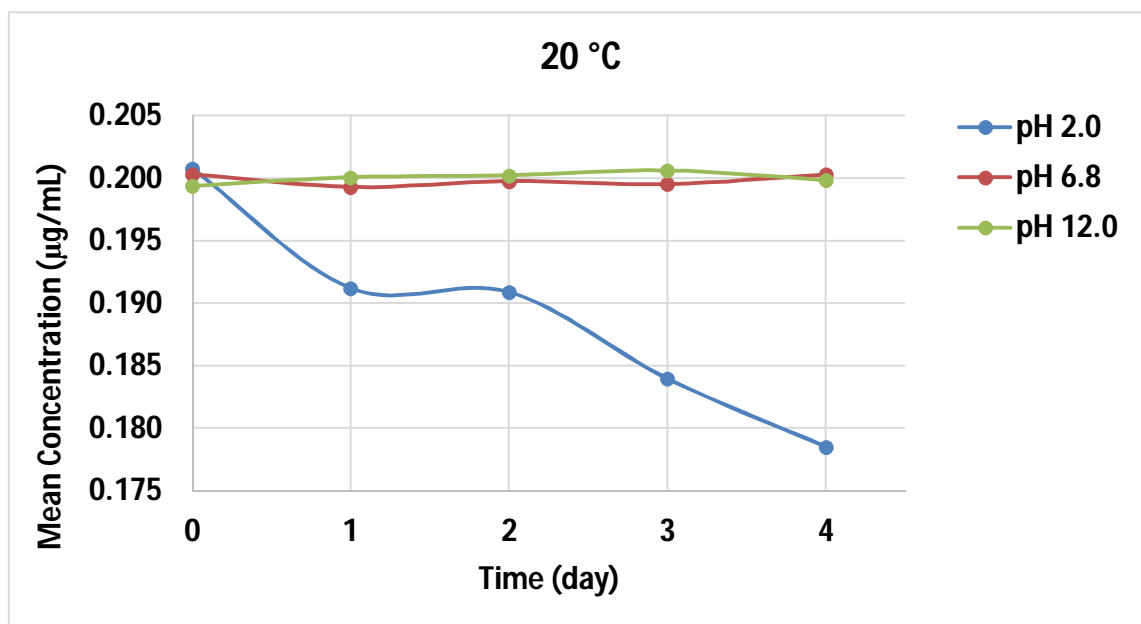


Figure 5 B: Comparison between different pH and different days at room temperature on the Stability of valsartan using UV Spectrophotometer (µg/ml).

Table6: Recovery study of valsartan using different pH and different days at 37°C on the Stability of valsartan using UV Spectrophotometer(µg/ml)

pH Level	Time(day)				
	0	1	2	3	4
pH2	0.201	0.172	0.172	0.166	0.161
pH6.8	0.200	0.180	0.180	0.180	0.181
pH12	0.199	0.196	0.196	0.197	0.196

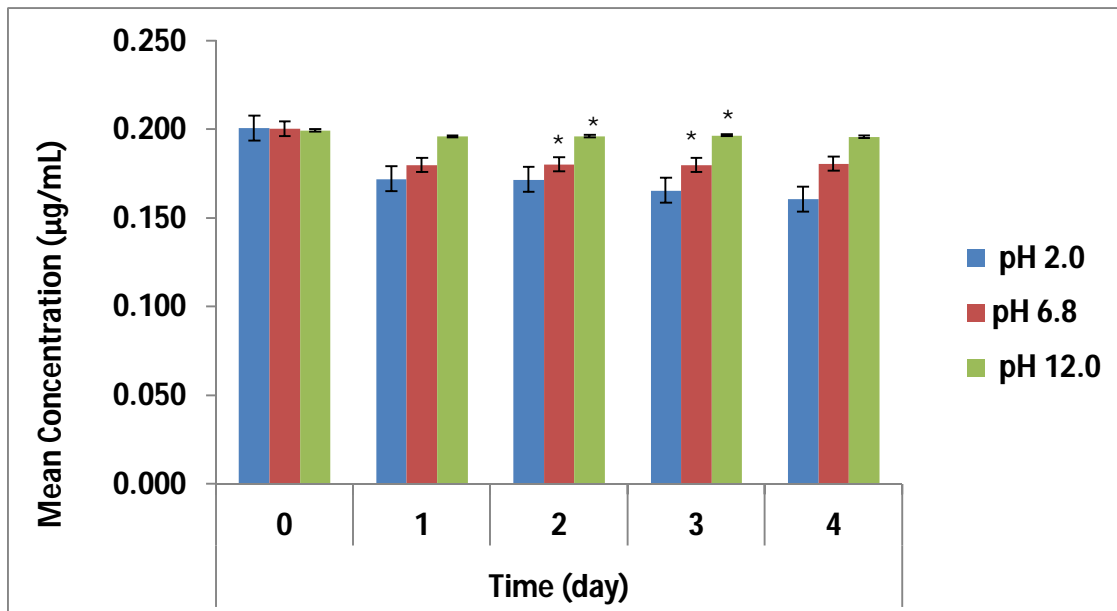


Figure 6 A: Recovery study of valsartan using different pH and different days at 37°C on the Stability of valsartan using UV Spectrophotometer (µg/ml).

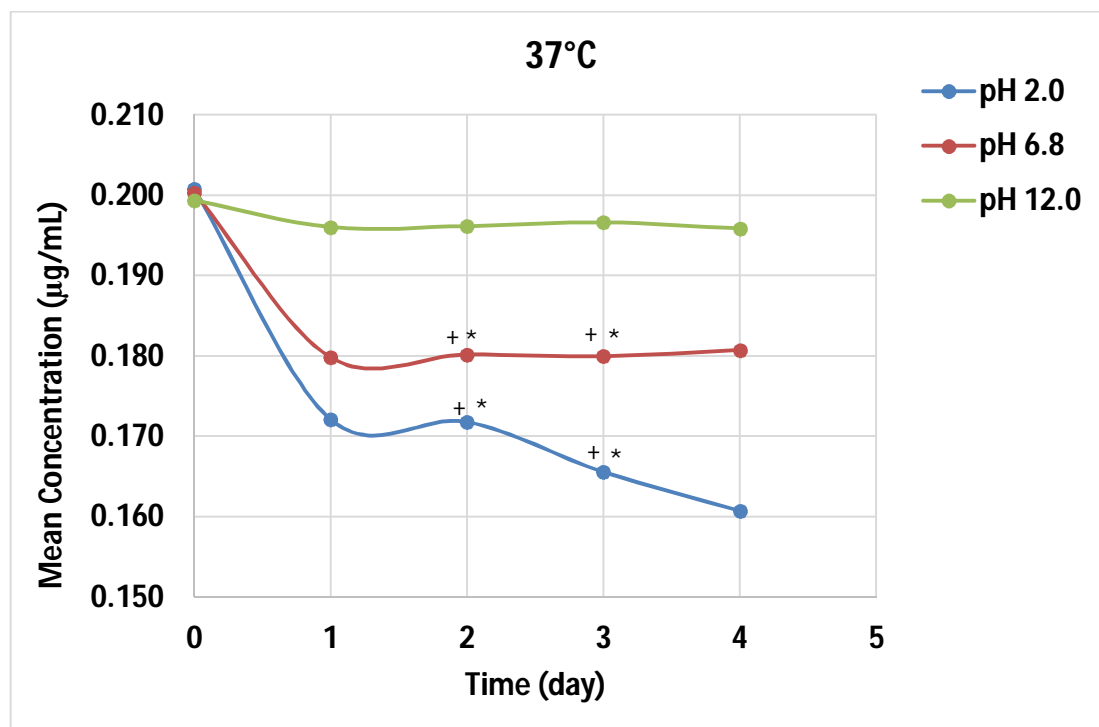


Figure 6 B Recovery study of valsartan using different pH and different days at 37°C on the Stability of valsartan using UV Spectrophotometer (µg/ml).

DISCUSSION

The implication of certain physicochemical studies in the stability performance of the medicinal agents has proved to be of considerable advantage in the development of stable dosage forms. There for stability study of an active substance or medicinal can provide evidence on how the quality of drug product varies with time. It provides information about potential degradation products and mechanism of degradation, as well as an interaction between the drug and excipients in the drug product¹⁷. It is controlled by a variety of environmental factors such as temperature, light, humidity and pH.

The present study was undertaken to assess valsartan stability to the prevailing physical factors like incubation time, temperature and pH. The stability study was carried out by storing valsartan at three different temperatures *i.e.*, 4, 24 and 37 °C and different pH *i.e.*, 2, 6.8 and 12. Drug contents were determined at 0, 24, 48, 72, 96 and 192 h intervals by high performance liquid chromatography and UV-Spectrophotometer that was developed in our laboratory. The percentage of drug remaining was plotted against time for each temperature and pH.

When valsartan was incubated for different times at 0, 24, 48,72 and 96 hours intervals, with different temperatures at 4, 23, and 37 °C it was found that the mean percentage residual drug was 89.7, 89.35 and 89.95% respectively using HPLC method of analysis.

Investigating the effect of different selected pH on stability of valsartan, we dissolved valsartan in different solutions with pH 2, 6.8 and 12. Data obtained from HPLC analysis suggested that incubation of valsartan at pH 2 with different temperatures 4, 20 and 37°C at different time can induce a much lower decrease in valsartan concentration. It was found that percentage residual drug were 88.56, 86.06 and 86.29% respectively. These results may indicate that when valsartan was incubating at pH 2 for different times at 0, 24, 48,72 and 96 h intervals, with different temperature at 4, 23, and 37 °C significantly decrease stability of valsartan as compared with aqueous solutions without pH adjustment. It verifies that at high hydrogen ion concentrations, hydrolysis takes place which results in a significant decrease in valsartan concentrations. Our results are in parallel with Patro et al.,¹⁸ who showed that recovery percentage of valsartan in acidic media was 93.55% while was 101% in alkaline pH after 2 hours exposure. Moreover, Bhatia and Kokil¹⁹ reported validation of valsartan and its degradation products by HPLC and showed that degradation product appeared at a relative retention time of 0.4 min after exposure to acid (acid hydrolysis). In contrast decrease, hydrogen ion concentrations, pH 6.8 and pH 12 can keep valsartan concentration high. On comparing the effect of pH 6.8 and pH 12 on the stability of valsartan with different temperature at 4, 23, and 37 °C using HPLC method of analysis. It was found that the mean of valsartan recovery rate was higher at pH 6.8(91.75, 92.62, and 93.96%) at 4, 24 and 37 °C while at pH 12 were 91.44, 91.44

and 91.98% respectively. These results were confirmed by using spectrophotometer analysis. Aqueous solutions of valsartan ($\mu\text{g/ml}$) were adjusted to pH (2.0, 6.8 and 12) using acid or alkali and were incubated in room temperature for different times (day 0 to day 4), valsartan stability was higher at pH 6.8 and 12. While valsartan concentration showed a higher degradation rate at pH 2. It indicates that not only temperature and time accelerates the rate of degradation but acidic pH also increases the rate of valsartan degradation.

CONCLUSION

The present results suggested that keeping hydrogen ion concentrations at neutral or alkaline pH over different time and temperature would be a very useful method to overcome the stability problems of valsartan

DECLARATION OF CONFLICTING INTEREST

The authors of this manuscript declare that they have no conflicts of interest to disclose.

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