Original Article

Development and Validation of Stability Indicating Nanodrop 2000c UV-Vis Method for Determination of Valsartan in Pharmaceutical formulations

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Abstract

A nanodrop spectrophotometric method was developed and validated for determination of Valsartan (VAL) in both bulk and tablet dosage form. Using methanol as a solvent, valsartan's maximum absorption was observed at 243 nm. The developed method was found to be linear (R2 = 0.9999) within the concentration range of 10-50 µg/mL. The precision study gives RSD less than 1%. The LOD and LOQ values were 0.34 and 1.14 g/mL, respectively. The accuracy study revealed that Valsartan commercial products had a remarkable recovery rate of more than 98%. The current method was successfully applied for valsartan stability indication study in Arbiten, Lastavin, Tabuvan, Extra, and Diostar products commonly found in pharmacies in Iraq's Kurdistan area. The stability-indicating study was investigated under acidic, basic, oxidative, photolytic, and thermal conditions. The results of all products showed that VAL is unstable in acidic, alkaline, and oxidative conditions under heating at 60 ℃up to 3 hrs. While under photolytic and thermal conditions, the degradation percentage was more than 15% indicating to the instability of VAL in all products tablets in accordance to ICH International Conference on Harmonization guideline of drugs.

Key words: Valsartan, UV Nanodrop 2000c spectrophotometer, Development, Validation, Stability Indicating.

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1. INTRODUCTION

Hypertension is one of the most common causes of chronic adult diseases today, and although it cannot be cured, it may be controlled. The pharmacological treatment for control of hypertension uses a variety of medication therapy, single doses or combinations, beta-blockers, calcium channel blockers, angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor (AT1) antagonist (ARA)(De Matos Jensen et al., 2010). Angiotensin II receptor (AT1) antagonists are a class of antihypertensive medications that, in comparison to other drug classes, are usually regarded as safe, well-tolerated, and having higher compliance and persistence rates.

Valsartan (VAL) is an angiotensin II receptor (AT1) antagonist that has been recommended for the treatment of hypertension, It is a nonpeptide that is orally active and highly selective and has been proven to reduce blood pressure (BP) for 24 hours after taking a single oral dose. (Toth & Dayspring, 2011). Overall, the physiologic effects of valsartan result in lower blood pressure, lower aldosterone levels, reduced cardiac activity, and enhanced salt excretion. Valsartan (VAL) is chemically described as (2S)-3-methyl-2-[N-({4-[2-(2H-1,2,3,4-tetrazol-5-yl) phenyl] phenyl} methyl) pentanamido] butanoic acid. Molecular formula of VAL is $C_{24}H_{29}N_5O_3$, while the molecular weight is 435.5188 g/mol. The structure of VAL is shown in figure 1 (Chittedi Anand Reddy, 2022).

Several techniques have been reported in the literature for the determination of VAL individually and in combination with other drugs including, FTIR spectroscopy (Rahman et al., 2020), HPTLC (Shah et al., 2019), HPLC (Çıkla Yılmaz et al., 2019; Peleshok et al., 2021; Piponski et al., 2020), LC-MS/MS (Annadi et al., 2019) and UV-Spectrophotometry (Abdullah & Rashid, 2021; Sonar et al., 2017; Tsvetkova & Ivanova, 2018)

Stability is an important step in the development of a pharmaceutical product. The goal of stability is to show how a drug's quality changes over time and how it responds to different environmental factors (Qasim & Mohammed, 2021). When developing any pharmaceutical product, the study of drug stability allows for the selection of appropriate formulation and packaging for appropriate storage and shelf-life conditions, which are critical for regulatory documents. Using a nanodrop spectrophotometer, the current study aims to develop and validate a new analytical method for routine analysis and stability-indicating study of VAL in pure form and pharmaceutical tablets.

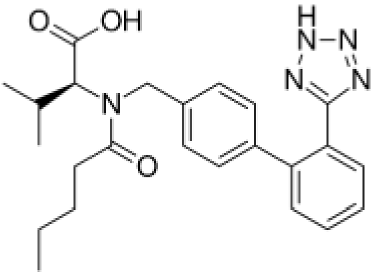

Figure 1. Chemical structure of Valsartan.

2. MATERIALS AND METHODS

2.1 Chemicals

A pharmaceutically active component of pure valsartan (VAL) was obtained from Awa Medical Company (99 % purity) which located in Kurdistan Region of Iraq. Pharmaceutical commercial tablets of VAL (Arbiten, Lastavin, Tabuvan, and Extra Diostar) were purchased randomly from local pharmacies. Analytical grades included methanol, Ethanol, acetonitrile, and water (Merck, Germany) were HPLC grade solvents.

2.2 Apparatus

The nanodrop spectrophotometer instrument (2000C Micro volume A Thermo Scientific) was used for the spectrophotometric analysis. Voyager® was the analytical balance. Elmasonic P (100W, 80 kHz) manufactured the oven, and Lab Tech manufactured the water bath shaker (LVO-2030).

2.3 Preparation of stock and working solutions

To prepare a stock standard solution containing 1000 µg/mL of pure Valsartan (VAL), accurately 0.25 g of the pure drug are weighted and dissolved in methanol as solvent, the solution is transferred to a 250 mL volumetric flask, the total volume was then made up to the mark with methanol solvent, this stock solution is used to prepare working solution with various concentration.

2.4 Method Optimization

The nanodrop 2000c spectrophotometer with in wavelength between 200 and 700 nm is used to determine the maximum wavelength (λ max) of VAL, using a solution of 50 µg/mL concentration, and against methanol solvent as blank, λ max of VAL was discovered to be 243 nm. A preliminary examining for solubility of VAL was also performed testing other solvents including Ethanol, methanol, acetonitrile and water.

2.5 Method Validation

In compliance with the International Conference on Harmonization guideline (ICH), and using nanodrop 2000c spectrophotometer, the spectrophotometric method was validated in terms of system, precision, linearity, limit of detection, limit of quantification, specificity, accuracy, and robustness

2.6 Degradation study

Stability examining of VAL in its formulation was applied under Examine conditions including acidic and alkaline (1N), oxidant (30% (v/v) H_2O_2), photolytic condition (UV, dark and sunlight) and thermal (90 ºC), at various periods. The VAL products Arbiten, Lastavin, Tabuvan, and Extra Diostar, were examined in this study

2.6.1 Blank solution preparation:

The different solution of blank, (including 1N of both hydrochloric acid and sodium hydroxide, and 30% hydrogen peroxide H_2O_2) are prepared by taking 12.5 mL of each one to 25 mL volumetric flask, the total volume then completed with methanol solvent the solution then heated to 60℃. 2 mL of the solution of are taken in separate 10 mL volumetric flask at different time intervals and neutralized with appropriate reagent, and after that, the final volume was completed with methanol, and the absorbance at 243 nm was measured.

2.6.2 Stock solution preparation of formulation:

Twenty tablets (2.5 g) from each commercial product were accurately weighed and powdered (one tablet contains 80 mg of VAL). The powder equivalent to 250 mg of VAL was placed in a 100 mL beaker and 40 mL of methanol was added to the beaker and the solution is sonicated for 20 minutes. The solution was filtered in 250 mL volumetric flask using Whatman filter paper (0.45 µm) and the volume was then filled with methanol to make a stock solution containing 1000 µg/mL of VAL. From this solution of formulation, different concentration of working solutions was prepared.

2.6.3 Acidic& alkaline condition:

For acidic condition, 12.5 mL of the stock solution of drug (1000 µg/mL) was placed into 25 ml volumetric flask. to that, 12.5 mL of hydrochloric acid 1 M was added, the solution then heated to 60℃. 2 mL of the solution was then transferred into another 10 mL volumetric flask at different time, 1hrs and 3 hrs. the solution then had to be neutralizing with a suitable reagent, the volume was completed with methanol solvent to acquire 10 µg/mL of VAL against acid condition. The absorbance was measured against blank at 243 nm. For alkaline condition, the same procedure of acidic condition was followed replacing 1M of hydrochloric acid with 1M of sodium hydroxide.

2.6.4 Oxidation condition:

The procedure listed in above section 2.6.3 was followed replacing 1 M of hydrochloric acid with 30% of hydrogen peroxide.

2.6.5 Photo decay:

Three solutions containing 50 µg/mL of VAL are prepared from stock solution 1000 µg/mL by taking 12.5 mL of the solution into 25 mL volumetric flask the volume was completed by methanol. The three solutions are then exposed to different circumstances including UV, sunlight, and dark. After 2 days and 5 days 2 mL from each solution was transferred into 10 mL of volumetric flask, the final volume was completed with methanol to form solution containing 10µg/mL of VAL. The absorbance of these solutions (against methanol as blank) was measured at 243 nm.

2.6.6 Thermal decay:

Twenty tablets of VAL drugs formulation are powdered by mortal and exposed in an oven at 90 ºC up to 48 hrs. from that 20 mg was dissolved in methanol then transferred into 10 mL Volumetric flask, after sonicating the solution for 20 minutes the final volume was completed with methanol to prepare a solution containing 10 µg/mL of VAL drug. The absorbance of the solution was measured at 243nm against methanol as blank.

3. RESULT AND DISCUSSION

3.1 Method Optimization

The main objective of developing and validating analytical methods is to validate an appropriate technique for analyzing a particular analyte with specified, accurate, and exact findings.

3.1.1 Determination (Scanning) of VAL λmax:

VAL spectrum showed maximum absorption (λmax) at 243 nm (Figure 2). This absorbance λmax was used later for all measurements study.

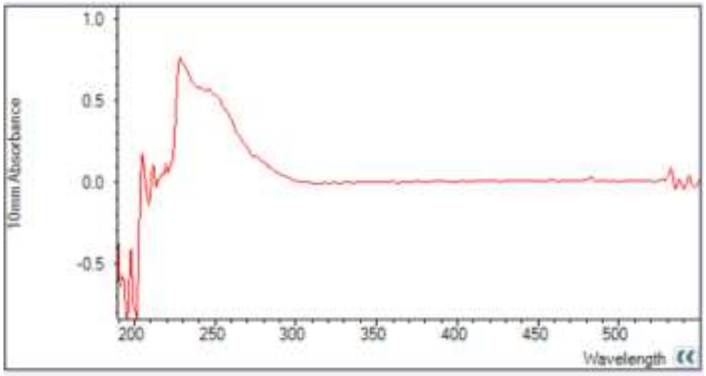

Figure 2: The UV-Visible spectrum of Valsartan 243 nm

3.1.2 Type of solvent

The influence of several solvents, such as methanol, ethanol, water and acetonitrile were investigated. 20 mL of solvents were used to test the solubility of pure VAL, the pure drug was soluble more easily in methanol than in, ethanol, water, and acetonitrile. The absorbance was measured, and it was discovered that pure drug in methanol had the maximum absorbance (0.74) at 243 nm compared in ethanol (0.66), water (0.32) and Acetonitrile (0.534). Therefore, according to the highest Absorbance, methanol was used in this research as a solvent.

3.2 Method Validation

Method validation is a data gathering and analysis procedure that establishes exact proof that an analytical method is capable of providing high-quality results. According to the ICH guidelines developed method was validated for the linearity, precision, accuracy, limit of detection LOD, limit of quantification LOQ, specificity and robustness.

3.2.1 Linearity:

The Linearity of the method was estimated by analysing five concentrations at the concentration range 10-50 µg/mL. The regression equations for VAL were found to be $Y = 0.0238X - 0.007$with correlation coefficients (R2) 0.9999.

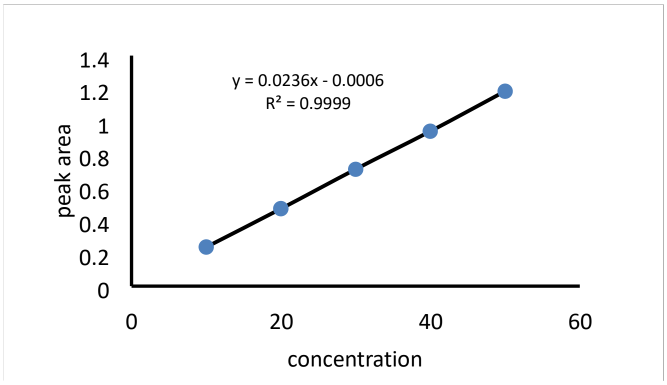

Figure 4: Calibration curve 10-50 µg/mL of VAL

3.2.2 Precision:

The precision was assessed using repeatability, intra-day and inter-day study of 5 µg/mL of VAL solution. As shown in Table 1, the RSD% values of drug are less than 1% indicating to good precision according to ICH standard criteria.

Table 2: Evaluation of precision study

No. of Sample	**Intra-day**			**Interday**			**Repeatability**		
	5	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**
1	0.113	0.23	0.345	0.113	0.227	0.344	0.113	0.23	0.344
2	0.114	0.23	0.344	0.112	0.229	0.343	0.113	0.229	0.345
3	0.114	0.229	0.344	0.114	0.228	0.344	0.114	0.23	0.345
Mean (%)	**0.11**	**0.23**	**0.34**	**0.11**	**0.23**	**0.34**	**0.11**	**0.23**	**0.34**
SD	**0.0006**	**0.0006**	**0.0006**	**0.0010**	**0.0010**	**0.0006**	**0.0006**	**0.0006**	**0.0006**
% RSD	**0.51**	**0.25**	**0.17**	**0.88**	**0.44**	**0.17**	**0.51**	**0.25**	**0.17**

3.2.3 LOD & LOQ:

Limit of detection (LOD) limit of quantification (LOQ) can be calculated from the following formulas:

$$3.3 * \sigma / S = LOD \quad (1)$$

$$10 * \sigma / S = LOQ \quad (2)$$

Where σ = standard deviation of response, S = slope of the calibration curve, Table 2, shows LOD and LOQ values at minimum levels of VAL.

Table 2: LOD and LOQ Values for VAL.

Parameter	Value
Linearity Range (µg/mL)	10-50
Regression equation	y = 0.0238x - 0.007
R^2	0.9997
Slope	0.0238
Intercept	- 0.007
SD	0.0037
LOD µg/mL	0.4418
LOQ µg/mL	1.4728

3.2.4 Accuracy and Recovery: The validity and accuracy of the proposed method were assessed by recovery study testing three replicates sample at three concentration level 50%, 100% and 150% of pure drug. The recovery results were shown in table 3. According to standard criteria of ICH which indicating that the method was accuracy.

Table 3: Evaluation of accuracy study of pure VAL drug.

Level of Recovery (%)	**Label Claimed Amount add (mg)**	**Amount of Pure Drug Abs**	**conc. Obtained**	**% Recovery**	**Statistical Analysis**		
					Mean (%)	**SD**	**RSD %**
sample 1	5	0.117	4.92	98.32	98.60	0.49	0.49
	5	0.118	4.96	99.16			
	5	0.117	4.92	98.32			
sample 2	10	0.237	9.96	99.58	99.86	0.24	0.24
	10	0.238	10.00	100.00			

	10	0.238	10.00	100.00			
sample 3	15	0.355	14.92	99.44	99.63	0.32	0.32
	15	0.355	14.92	99.44			
	15	0.357	15.00	100.00			

3.2.5 Specificity: By adding (starch, lactose, and magnesium) to the VAL standard solution and analyzing the percent of recovery across three replications, it was discovered that the specificity of the current approach was 100.05% with an RSD% of 0.25. The outcome demonstrated that excipients did not interfere with the analysis of standard VAL.

3.2.6 Robustness: Study was done on how the absorbance varied when the wavelength changed from 241 to 245. Different wavelengths of the VAL standard solution (5 µg/mL) were examined. It was discovered that the RSD% for the absorbance measurement was less than 1.6%.

Table 4: Robustness study of VAL at different wavelength range.

No of Sample	**Absorbance at 241 nm**	**Absorbance at 242 nm**	**Absorbance at 243 nm**	**Absorbance at 244**	**Absorbance at 245 nm**
1	0.115	0.117	0.118	0.117	0.115
2	0.117	0.114	0.118	0.118	0.116
3	0.117	0.118	0.117	0.116	0.116
4	0.118	0.119	0.118	0.117	0.117
5	0.115	0.117	0.119	0.118	0.118
Mean	0.1164	0.1170	0.1180	0.1172	0.1164
SD	0.0013	0.0019	0.0007	0.0008	0.0011
% RSD	1.1526	1.5990	0.5992	0.7139	0.9795

3.2.7 Essay of drug products:

To ensure the accuracy of pure sample, the recovery and RSD% experiments were performed using a known concentration of the commercial products of VAL The percentage recovered was depicted in Table 5.

Table 5: Recovery and RSD% of VAL products.

Tablet formulation	**Conc. Added**	**Conc. Founded**	**Recovery %**	**RSD%**
pure valsartan	80µg/ml	80.01	100.01	0.03
Arbiten	80µg/ml	79.95	99.95	0.053
Lastavin	80µg/ml	79.97	99.96	0.08
Tabuvan	80µg/ml	79.98	99.98	0.1
Extra	80µg/ml	79.95	99.95	0.09
Diostar	80µg/ml	79.95	99.95	0.1

3.3 Forced degradation studies The active component may degrade under experimental settings such as acid, alkaline, oxidative, thermal, and light. Forced degradation trials help identify this potential. For a stability-indicating investigation of VAL in five locally available products under different circumstances, the following procedure was used:

3.3.1 Acid & Alkaline degradation: The VAL products (Arbiten, Lastavin, Tabuvan, and Extra Diostar) were subjected to 1N HCl and 1N NaOH individually at 60°C for 3 hours as part of a stress degradation research in both acidic and basic conditions. As stated in Table 3, it was discovered that VAL in all products began to deteriorate at room temperature before heating. When the temperature was raised to 60°C, the products degraded more quickly, taking 1 to 3 hours. It can be concluded that the longer the drugs were kept under acidic and basic with heating conditions, the more degradation can occur.

Table 6: Acid & base hydrolysis/ effect on the VAL products

Stress Condition/Conc.	**Duration**	**Degradation %**					
		Valsartan Standard	**Arbiten drug**	**Lastavin drug**	**Tabuvan drug**	**Extra drug**	**Diostar drug**
Acidic / 1M	**1hr**	9.43	9.20	9.43	8.96	9.43	10.13
Acidic / 1M	**3hrs**	13.17	12.23	11.76	12.70	13.17	11.06
Basic / 1M	**1hr**	10.60	9.43	10.60	9.90	9.66	10.83
Basic / 1M	**3hrs**	15.73	14.10	13.17	13.87	12.46	12.23

3.3.2 Oxidation degradation: The maximum degradation for VAL was seen in this investigation under oxidative conditions using 30% H_2O_2. At room temperature, the VAL in all products began to degrade. Heating to 60 ℃, the degradation percentage is raised with time increasing from 1 to 3 hrs as shown in results of Table 7.

Table 7: Oxidative effect on the VAL products

Duration	**% Degradation**					
	Valsartan standard	**ARBITEN drug**	**Lastavin drug**	**Tabuvan drug**	**Extra drug**	**Diostar drug**
1 h	16.20	16.43	16.20	18.07	17.93	16.43
3 h	18.53	20.63	19.00	18.77	19.47	18.77

3.3.3 Thermal degradation: The thermal degradation was investigated exposing VAL products at solid state to 90 ºC for 48 hrs in an oven. The loss percentages for Arbiten, Lastavin, Tabuvan, Extra, and Diostar were determined to be close to 20.17, 23.44, 21.10, 18.53, 17.60, and 19.47, respectively. These results of the five products indicate that VAL are unstable in the formulation in accordance to the ICH guideline.

3.3.4 Photolytic degradation: In research on stress degradation in photolytic conditions, VAL products (Arbiten, Lastavin, Tabuvan, Extra, and Diostar) were exposed to light, UV, and darkness at various times during the day. According to the ICH recommendation that less than 15% is not measured as degradation because some of this degradation is related to the drug formulation and preparation, all products show a minimal amount of degradation (less than 15%) up to 2 days and 5 days, and as a result, the VAL was found to be stable under photolytic conditions. Results are displayed in Table 8.

Table 8: Photolytic effect on the valsartan products.

Stress condition/ state	**Duration (days)**	**% Degradation**					
		Valsartan standard	**ARBITEN drug**	**Lastavin drug**	**Tabuvan drug**	**Extra drug**	**Diostar drug**
UV	**2 days**	7.10	11.76	9.43	7.80	8.50	8.50
Sun		4.76	5.93	4.76	5.00	5.46	6.16
Dark		1.49	2.43	1.49	1.26	0.33	2.66
UV	**5 days**	10.43	12.83	11.5	10.77	12.16	11.15
Sun		6.58	6.46	6.86	7.92	9.46	7.23
Dark		1.83	3.13	2.76	3.34	1.37	3.05

4. CONCLUSION

Nanodrop spectrophotometer method was developed and validated for determination of valasartan in tablets dosage formulation. The development approach was discovered to be quick, linear, accurate, specific, sensitive, robust, and economical. The current methodology was effectively used to analyze the forced degradation of valsartane in five different products (Arbiten, Lastavin, Tabuvan, Extra, and Diostar) under conditions that included acidic, basic, oxidative, photolytic, and thermal degradation. In accordance to International Conference on Harmonization guideline ICH guideline of drugs, the VAL products (Arbiten, Lastavin, Tabuvan, Extra, and Diostar) was found to be unstable in acidic, basic, and oxidative conditions under heating at 60°C showing high degradation percentage more than 15%. The results revealed that VAL degraded the most under oxidative conditions using 30% H2O2 with all products. The passage of time is the most important factor affecting the degradation of VAL. Different stress factors raise the percentage of degradation, but the effect of time remains stronger.

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