RESEARCH ARTICLE

Analytical Method Development by Using UV-Spectrophotometer for Estimation of Valsartan in Bulk

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ABSTRACT

In order to accurately determine valsartan (API), this work aims to build a straightforward and exact UV spectroscopic approach. To improve sensitivity and accuracy, the procedure entails methodically optimizing experimental parameters, including solvent choice and wavelength selection. The produced stock solutions were tested for absorbance at 249 nm using a UV spectrophotometer. With %RSD values below 2% for both intra- and inter-day, the devised approach was determined to be quite accurate. At each additional concentration, the approach was shown to be accurate, yielding good drug recoveries ranging from 98 to 101%. The process was validated for linearity, specificity, accuracy, precision and limit of detection (LoD) and limit of quantification (LoQ) as per International Council of Harmonization (ICH) criteria. Its dependability for quantitative analysis of valsartan in pharmaceutical formulations was demonstrated. This UV spectroscopic approach offers a cost-effective and efficient alternative for routine quality control in pharmaceutical industries.

Keywords: Valsartan, Angiotensin, Receptor, Hyperaldosteronism, UV spectroscopy, Method validation.

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INTRODUCTION

Adolescents and youngsters 6 years old and up can take valsartan thanks to the drug's approval by the Food and Drug Administration (FDA) to treat hypertension. It has been extensively utilized to treat hypertension since its launch in 1996 because of its effectiveness and the fact that patients tolerate it well.[1]

As a white powder, valsartan is found in nature. While it dissolves almost completely in ethanol and methanol (99.5% solubility), water is an extremely poor solvent. Its chemical name is 2S, and its molecular formula is $C_{24}H_{29}N_5O_3$ (Figure 1). Its molecular weight is 435.52, butanoic acid, which is a ring-shaped molecule, is a ketone molecule.[2]

Novartis was the company that initially created valsartan. To treat hypertension, it is used either singly or in conjunction with other medications.[3,4]

Angiotensin II receptor blockers (ARBs) include valsartan. The renin-angiotensin pathway culminates with angiotensin II, the last messenger. Blood pressure is raised by the effects of vasoconstriction and fluid retention brought about by the binding of angiotensin II to angiotensin1 receptors.[5] Because of this, their action is analogous to that of ACE inhibitors, which prevent ACE from producing angiotensin II.[6] However, some angiotensin II can be produced via non-ACE routes.[7,8] Additionally, ACE inhibitors reduce the breakdown of bradykinin, which may contribute to both the positive and negative effects of this class of medications.[9,10]

Analytical Method Validation

What is method validation?

Laboratory studies validate an analytical method when they guarantee that the method's performance attributes match the needs of the intended analysis application.[11]

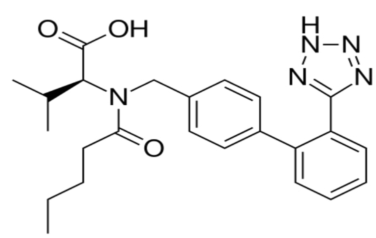

Figure 1: Structure of valsartan

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To ensure that different operators in different labs can reliably and consistently use the same technique to get the same results, validation is an essential step in developing and refining any procedure. What kind of validation programme is required is determined by the specific method and its intended usage.

Importance of method malidation

Regulatory agencies have heavily influenced the literature on method validation.

- Methods that are to be validated are requested by U.S. FDA CGMP (1) in section 211. 65 (e): The company's test processes must be defined and documented with regard to their accuracy, sensitivity, specificity, and reproducibility.
- The ICH has penned an article regarding the verification of analytical methods. The book provides definitions for eight validation characteristics.

Validation Parameters

Precision

The level of settlement between individual test findings when using the method for multiple sampling of a homogeneous sample is the degree to which an analytical procedure is considered precise.

$$\%RSD = SD \times 100/mean$$

It is important to use genuine, homogeneous samples while studying precision. When a homogeneous sample is not possible to collect, alternative methods such as artificially generated samples or sample solutions might be employed for examination. Repeatability, reasonable precision, and reproducibility are the three tiers of accuracy.

Repeatability

The capacity to express accuracy within a short time frame while maintaining identical operating circumstances is known as repeatability.

Intermediate precision

Intermediate precision is used to express variations within laboratories, which can include different days, analyzers, and equipment.

Reproducibility

Reproducibility pertains to the consistency of results obtained across different laboratories, sometimes achieved through joint investigations aimed at establishing standardized methodologies.

Accuracy

Accuracy is a quantitative assessment of how closely the results achieved from a certain method align with the true value. That is, the correspondence between the measured value and the actual value (Figure 2).

Linearity

The term "analytical sensitivity" refers to a technique's outcomes within a specific range; either directly or through a mathematical transformation. Graphing the sensitivity, which represents the response or amount, allows for clear visualization of the linear range (Figure 3).

Range

The method's range refers to the interval between the highest and the lowest levels of analyte that may be accurately and precisely measured based on acceptable levels of precision, accuracy, and linearity.

Ruggedness

Ruggedness refers to the extent to which the results obtained from analyzing the same sample can be reproduced under various typical testing conditions. In other words, it pertains to the consistency of results across different labs and analysts.

Robustness

The effect of operational parameters on analysis results can be investigated by means of robustness testing. Modifying a number of method parameters within a practical range and quantifying their effects allows one to evaluate a method's robustness. These parameters may include pH, flow rate, column temperature, injection volume, detection wavelength, or the composition of the mobile phase if the parameter's effect stays within a predetermined range.

Limit of detection

Under the given experimental parameters, this represents the minimum concentration in a sample that may be identified, although it may not be possible to accurately determine its quantity.

Limit of quantitation

Limit of quantitation (LoQ) refers to the lowest concentration of a substance that can be reliably measured with a specified level of accuracy and precision.

$$LoD = 3.3\sigma / S, \; LoQ = 10\sigma / S$$

Where, S = slope, σ = residual standard deviation.[12]

MATERIALS AND METHODS

It was conducted with a Shimadzu UV 1800 spectrophotometer.

Reagents

The gift sample of valsartan was obtained. The experimentation utilized methanol AR grade as the solvent. A pharmaceutical product was acquired from a nearby drugstore.

Selection of Media

The primary factors to consider when selecting a medium are solubility and stability. In other words, the medicine must possess the ability to dissolve and remain chemically stable in the chosen environment for a enough duration. Valsartan exhibits a high level of solubility in methanol.

Preparation of Standard Stock Solution

A standard solution with a concentration of 1000 μg/mL of valsartan was generated by dissolving 50 mg of pure medication in methanol and diluting it to a volume of 50 mL with methanol.

Selection of λ_{max}

The standard valsartan solution, with a concentration of 10 μg/mL, was analyzed using UV spectroscopy in a 1.0 cm cell. The analysis was performed in the UV region (400–200

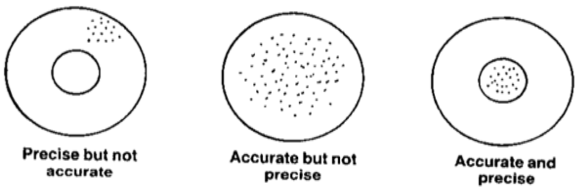

Figure 2: Relation between precision and accuracy

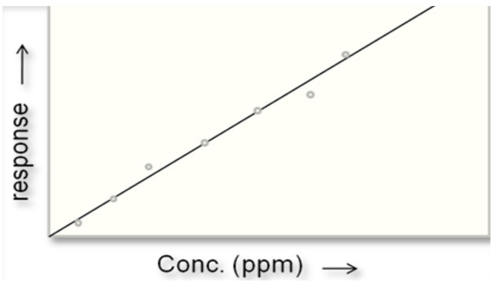

Figure 3: Linearity graph

nm), and the recorded wavelength was 249 nm. A solvent blank was used for comparison.[13]

Preparation of Working Standard Dilutions

A serial dilution was made from stock 2, ranging from 2 to 20 µg/mL. Measurement of absorbance was conducted at the wavelength of maximum absorption, which is 249 nm.

Calibration Curve of Valsartan

Created a solution with dilutions of 2 to 10 µg/mL. The measurement of absorbance was conducted at the wavelength of maximum absorption, which is 249 nm. Next, the calibration curve was constructed by graphing the concentration versus absorbance (Figure 4).

Analytical Method Validation of the Proposed Method

The suggested approach was authenticated as per requirements provided by the International Council for Harmonisation (ICH) and the United States Pharmacopoeia (USP-39).[14,15]

Linearity range

The linearity of the drug's reaction was confirmed within a concentrated range of 2 to 20 µg/mL. Calibration graphs were generated by plotting the absorbance data against the concentration data and were analyzed using linear regression.[16]

Precision

The method's accuracy was established through research on intraday and interday fluctuation. During the intraday experiments, measurements of both the standard and sample solutions were conducted three times each day. Interday research involved measuring standard and sample solutions over a period of three consecutive days and then calculating the percentage relative standard deviation (RSD).

Accuracy (Recovery test)

The method's accuracy was assessed by creating solutions with varying concentrations of 80, 100, and 120%. The marketed product was held constant at 10 mg, while the amount of pure drug was adjusted to 8, 10, and 12 mg at the respective concentrations. The solutions were made in three identical sets and the precision was measured as the percentage of recovered material.

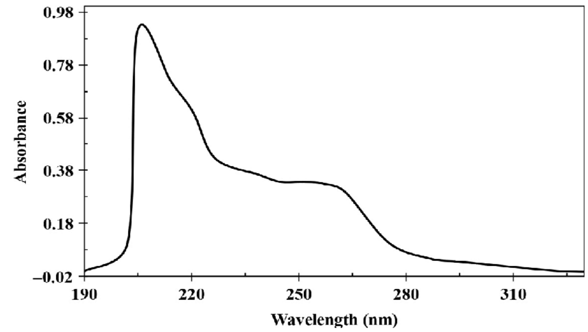

Figure 4: UV spectra of Valsartan

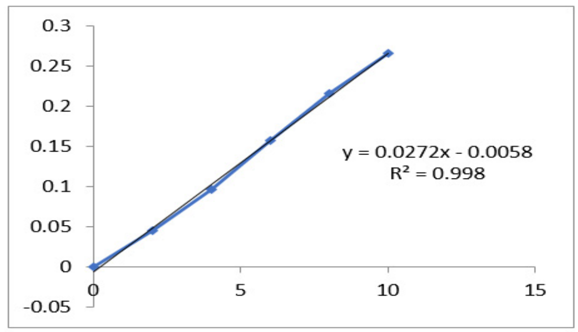

Figure 5: Calibration curve of valsartan

Ruggedness

Ruggedness of the method was assessed by analyzing absorbance data obtained with 2 separate analyzers. Based on the recorded results, it was established that the method is robust. The outcome was denoted with %RSD.

Robustness

It was assessed by conducting the analysis at two distinct temperatures: room temperature 25 and 17ºC. The pertinent absorbance was quantified and stated as a %RSD.

Assay of Valsartan Tablets

Weighing and pulverizing 20 tablets of valsartan were performed. Exactly 50 mg of valsartan powder was carefully

Table 1: Calibration curve of valsartan

Con. (µg/mL)	*Absorbance (nm)*
0	0
2	0.0454
4	0.0965
6	0.1576
8	0.2161
10	0.2664

measured and put into a 50 mL volumetric flask. The 10 mL of methanol was poured into it, followed by an additional 20 mL of solvent. Following 15 minutes of sonication, the mixture underwent filtration using Whatman filter paper (No. 41) and the volume was adjusted using the solvent. The additional dilution resulted in an ultimate concentration of 10 µg/mL. The quantity of valsartan was determined using the calibration curve.[17,18]

RESULTS AND DISCUSSION

Analytical Method Development Using UV Spectrophotometer

The standard dilution of valsartan was prepared with a concentration of 10 μgmL^{-1}. A wavelength of 249 nm was chosen for quantitative analysis.

Calibration Curve of Valsartan

The stock solutions were analyzed for absorbance at a wavelength of 249 nm using a UV spectrophotometer (Table 1, Figure 5).

UV Method Validation

The created method was determined to be exact since %RSD values for intra-day and inter-day were both below 2%. The substance was recovered with high precision (98–101%) at every concentration tested, demonstrating the accuracy of the procedure. LoD and LoQ were determined to be at a sub-microgram level, demonstrating high sensitivity of approach. The validation parameters are reported in Table 2. The approach was determined to be both robust and tough (%RSD <2%).

Table 2: Validation parameters

Parameter	*Result*
	249 nm
Range	2–20 µg/mL
Equation	y = 0.028x - 0.014
R^2	0.999
Standard Deviation	0.00284
LoD (µg/mL)	0.3347
LoQ (µg/mL)	1.01428

Linearity

The linearity of the suggested method was assessed using ten different concentrations ranging from 2 to 20 µg/mL. In the statistical analysis, the correlation coefficient, intercept, and slope were calculated. The graph showed a straight line with a linear equation of y = 0.028x - 0.014 and a R^2 of 0.999. The concentration range used for this analysis was from 2 to 20 µg/mL (Table 3 and Figure 6). Therefore, it may be inferred that the suggested approach exhibits linearity within the designated concentration range.

Precision

The accuracy of the approach was assessed by intra-day and inter-day studies (Table 4 and Table 5).

A %RSD range being less than 2% indicates that the developed method exhibited excellent precision.

Accuracy (Recovery Test)

Recovery trials assessed the accuracy of the approach. Recovery was conducted at three different levels, specifically 80, 100, and 120% of the normal concentration of valsartan. The recovery values pertaining to valsartan were summarized in Table 6.

Ruggedness Study

The results obtained after performing a ruggedness study are reported in Table 7.

Robustness Study

The efficacy of the established process was assessed by varying temperatures at 17 and 25°C with a deviation of ± 5 (Table 8).

The assay was performed and results are expressed in Table 9.[19,20]

Table 3: Linearity

Concentration	*A1*	*A2*	*A3*	*Mean*	*SD*	*%RSD*
2	0.0454	0.0379	0.0376	0.0403	0.0036	8.9528
4	0.0965	0.0971	0.0968	0.0978	0.0003	0.3076
6	0.1576	0.1602	0.1634	0.1604	0.0012	0.8135
8	0.2161	0.2169	0.2145	0.2159	0.0013	0.5862
10	0.2664	0.2702	0.2735	0.2700	0.0036	1.3157
12	0.3150	0.3200	0.3250	0.3200	0.0050	1.5625
14	0.3840	0.3960	0.3902	0.3900	0.0060	1.5387
16	0.4420	0.4350	0.4465	0.4400	0.0058	1.3170
18	0.4941	0.4950	0.4945	0.4945	0.0005	0.0911
20	0.5562	0.5584	0.5575	0.5573	0.0011	0.1984

Table 4: Intraday absorbance of precision study

Concentration	*Morning*	*Afternoon*	*Evening*	*Mean*	*SD*	*%RSD*
4	0.0980	0.0985	0.0993	0.0986	0.0005	0.5425
4	0.103	0.1035	0.1041	0.1035	0.0004	0.4346
4	0.1171	0.119	0.1197	0.1186	0.0010	0.9258
8	0.216	0.217	0.215	0.216	0.0008	0.3777
8	0.229	0.228	0.23	0.229	0.0008	0.3563
8	0.2308	0.2316	0.2324	0.2316	0.0006	0.2819
16	0.442	0.43445	0.4355	0.4377	0.0033	0.7602
16	0.4377	0.4384	0.4406	0.4388	0.0012	0.2889
16	0.4510	04518	0.4524	0.4517	0.0005	0.1268

Table 5: Interday precision absorbance of precision study

Conc. (µg/mL)				*Statistical result*		
	Day 1	*Day 2*	*Day 3*	*mean*	*SD*	*%RSD*
4	0.1021	0.1031	0.1008	0.102	0.0011533	1.13064
4	0.1038	0.1035	0.1005	0.1026	0.0018248	1.77858
4	0.1071	0.1077	0.1086	0.1078	0.000755	0.70035
8	0.2061	0.2002	0.2009	0.2024	0.0032234	1.59256
8	0.2029	0.2006	0.2012	0.20156	0.001193	0.591881
8	0.2108	0.211	0.2158	0.21253	0.0028308	1.33192
16	0.4028	0.4065	0.4144	0.4079	0.0059254	1.452652
16	0.4077	0.4079	0.4108	0.4088	0.0017349	0.42439
16	0.4101	0.4118	0.4175	0.41313	0.003876	0.938194

Table 6: Accuracy study of valsartan

S. No.	*Fix conc. Taken (ppm)*	*Conc. added (ppm)*	*%added*	*%recovery*	*Average recovery (%)*	*SD*	*%RSD*
1	10	8	80	79.25			
2	10	8	80	80.15	79.81	0.004864	0.60950974
3	10	8	80	80.02			
4	10	10	100	100.32			
5	10	10	100	99.56	99.86	0.004025	0.40309834
6	10	10	100	99.71			
7	10	12	120	119.52		0.009584	
8	10	12	120	118.35	119.37		0.80292703
9	10	12	120	120.25			

Table 7: Ruggedness study of valsartan

ANALYST 1			*ANALYST 2*		
Conc. (µg/mL)	*Absorbance*	*Statistical analysis*	*Conc. (µg/mL)*	*Absorbance*	*Statistical analysis*
10	0.2664	Mean = 0.2625 SD= 0.002693 RSD= 1.0259%	10	0.2658	Mean = 0.2653 SD = 0.001868 RSD = 0.704167%
10	0.2741		10	0.2668	
10	0.2598		10	0.2664	
10	0.2612		10	0.2654	
10	0.2688		10	0.2621	

Table 8: Robustness study of valsartan

At room temperature (at 25°C)			*At Temperature 17°C*		
Conc. (µg/mL)	*Absorbance*	*Statistical analysis*	*Conc. (µg/mL)*	*Absorbance*	*Statistical analysis*
12	0.3150	Mean = 0.31792 SD = 0.002475 RSD = 0.7785s%	12	0.3245	Mean = 0.32306 SD = 0.002512 RSD = 0.7774%
12	0.3205		12	0.3215	
12	0.3182		12	0.3261	
12	0.3158		12	0.3197	
12	0.3201		12	0.3235	

Table 9: Assay of valsartan tablets

Sample	*Label claim (mg)*	*Amount (mg)*	*% amount found*
DIVION tablet	10	9.85	98.5
	10	9.91	99.1
	10	10.08	100.8

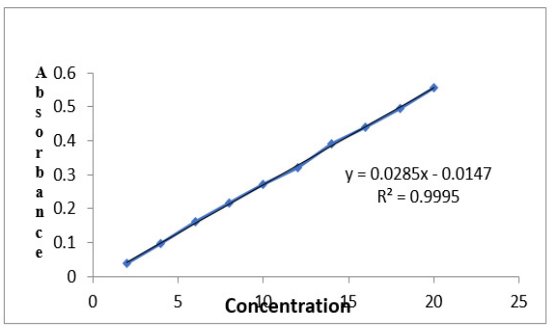

Figure 6 : Linearity graph

CONCLUSION

An analytical approach utilizing a UV-spectrophotometer was developed and validated to accurately and precisely estimate the amount of valsartan in bulk. The method is simple, selective, quick, and adheres to the guidelines set by USP-39 and ICH. A readily accessible solvent and procedure were employed for the experimental study. Therefore, it can be concluded that the implemented analytical approach is also economically efficient. Based on the aforementioned results and discussion, the method developed for quantifying valsartan in large quantities can be employed for regular analysis. The valsartan solution is stable for a duration of 24 hours under typical environmental conditions. Furthermore, the new technique is also effectively utilized for valsartan in solid formulations. Brands that were advertised successfully met the standards set by the pharmacopeia.

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