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Efficient Validated HPLC/UV Method for Determination of Valsartan and Atenolol in Dosage Form And *In Vitro* Dissolution Studies

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Abstract: The main purpose of this study was to develop and validate an efficient HPLC/UV method for determination of valsartan and atenolol and to introduce the dissolution profiles of tablets; The resolution of peaks was best achieved with Zorbax C8 (4.6 mm i.d. X 150 mm, 5 µm) column. Samples were chromatographed in a isocratic mode (methanol and 25 mM solution potassium dihydrogen phosphate pH 7.3 (55:45, V/V)), pumped with 1.0 mL/min at 40 °C set temperature of column oven, with UV detector set to 225 nm wavelength; The total chromatographic run time was 6 minutes. The retention time of valsartan is 1.753 min, atenolol – 3.064 min. Linearity was examined and proven at different concentration levels in the range of working concentration of valsartan (0.16-0.96 mg/mL) and atenolol (0.2–1.2 mg/mL). The high value of recoveries obtained for valsartan and atenolol indicates that the proposed method was found to be accurate. In all three dissolution media the releases of valsartan and atenolol are more than 85% in 15 min A rapid, simple, accurate, selective, and sensitive method was developed for the determination of valsartan and atenolol in dosage forms. The method was strictly validated according to the ICH guidelines. Acquired results demonstrate that proposed strategy can be effortlessly and advantageously applied for routine quality control of drugs and *in vitro* dissolution study.

Keywords: Valsartan, Atenolol, HPLC/UV, Dissolution test, Validation.

1. Introduction

Dissolution testing has been used primarily as a quality control (QC) test for solid oral drug products. Indeed, it is the only QC test that provides a measure of the quantitative release rate of the drug from the pharmaceutical product. More recently, the test has been proposed instead of bioequivalence testing. The various pharmacopeias describe four main types of dissolution apparatus; one (basket), two (paddle), three (reciprocating cylinder, and four (flow-through). The three main pharmacopeias (Japanese; JP, US; USP, SPhU, and European; Ph. Eur.) are mostly harmonized, although there are some aspects that are unique to the individual pharmacopeias [1,2]. Apparatus one and two are the most widely used for QC methods, being routinely adopted to evaluate the drug release from several different solid oral formulations such as tablets, capsules, and sachets.

Valsartan (Fig. 1) is chemically described as (2*S*)-3-methyl-2-[pentanoyl-[[4-[2-(2*H*-tetrazol-5-yl)phenyl]phenyl]methyl]amino]butanoic acid. Valsartan is an orally active nonpeptide triazole-derived antagonist of angiotensin (AT) II with antihypertensive properties.

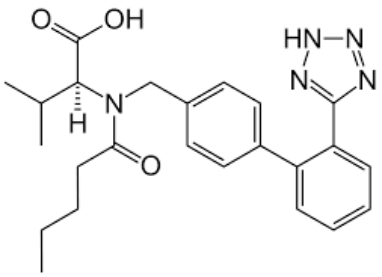

Figure 1. Chemical structure of valsartan.

Atenolol (Fig. 2) is a synthetic isopropylamino-propanol derivative used as an antihypertensive, hypotensive, and antiarrhythmic. Atenolol is chemically known as 2-[4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl]acetamide.

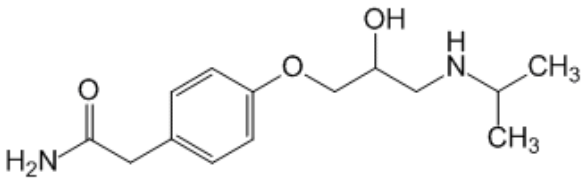

Figure 2. Chemical structure of atenolol.

This unique combination is safe and effective for treating hypertension in older people than using each drug alone [3-10]. In order to elucidate the dissolution profiles valsartan and atenolol, a validated HPLC method is required for the determination of valsartan and atenolol from the tablet in the dissolution matrix. Therefore, this study aimed to develop and validate an efficient HPLC/UV method for the determination of valsartan and atenolol and to introduce the dissolution profiles of tablets. Moreover, this new method could also be used for the routine analysis of valsartan and atenolol in fixed dosage form, provided it is completely validated and rapid. The method was validated according to guidelines and applied for the assay of valsartan and atenolol from their tablets. Also, *in vitro* dissolution of valsartan and atenolol containing tablets were performed to validate the suitability of the proposed method.

2. Materials and Methods

2.1. Chemicals and reagents.

Valsartan was obtained as a gift from Farmak pharmaceuticals (Kyiv, Ukraine), Refik Saydam National Public Health Agency supplied atenolol. The methanol used in experiments was HPLC gradient grade, and potassium dihydrogen phosphate was of Ph. Eur. reagent grade and purchased from Merck Darmstadt, Germany. Analytical Balance Mettler Toledo MPC227, pH–meter Metrohm 827, demineralized water from the TKA Microsystem, with final conductivity less than 0.05µS/cm. IKA orbital shaker KS4000i was used for sample agitation. The nylon and regenerated cellulose RC 0.45um syringe filters were purchased from Agilent Technologies.

Dionex Ultimate 3000 UHPLC system controlled by Chromeleon version 6,80, composed of quaternary LPG pump ultimate 3000, autosampler ultimate 3000, ultimate 3000

column compartment, four-channel UV-Vis detector ultimate 3000 RS. Shimadzu Nexera XR UPLC system with LPG Quaternary Pump LC-20AD with degasser DGU-20A5R, Autosempler SIL-20AC, PDA detector M20-A, Column Oven and Controller CBM-20A controlled by Lab Solutions version 5,97. The used column Zorbax C8 (4.6 mm i.d. X 150 mm, 5 µm), purchased from Sigma-Aldrich Supelco.

In vitro dissolution of twelve tablets containing valsartan and atenolol was performed using buffer solutions (pH 1.2; 4.5; 6.8) as the dissolution media at 50 rpm using a USP Apparatus II. The dissolution study was carried out in a 900 mL volume of buffer solution at 37 °C (± 0.5) using the paddle method. One mL of sample was withdrawn and replaced with fresh dissolution medium at the time intervals of 5, 15, 30, 45 minutes. The concentrations of valsartan and atenolol in samples were determined by the proposed HPLC method.

2.2. Chromatographic conditions.

The optimum mobile phase composition was composed of methanol and 25 mM solution potassium dihydrogen phosphate pH 7.3 (55:45, V/V), pumped with 1.0 mL/min at 40 °C set temperature of column oven, with UV detector set to 225 nm wavelength. Analyses performed on column Zorbax C8 (4.6 mm i.d. X 150 mm, 5 µm).

2.3. Sample preparation.

Twelve tablets of each preparation were studied to obtain statistically significant results. The tablets with declared contents of 80 mg valsartan and 100 mg of atenolol were purchased from a local drug store, pharmacy. The tablets were put in 100 mL measuring flasks and dissolved in 50 mL 50% v/v methanol, ultrasound crushed and treated for 2 minutes, and shake 15 min with an orbital shaker. After that, measuring flasks were filled to mark for 100 mL, the final concentrations were 1mg/mL for atenolol and 0.8mg/mL for valsartan. Samples were filtered with RC 0.45um syringe filters and injected.

3. Results and Discussion

The HPLC method was developed to provide specific procedures for the rapid quality control analysis for the dissolution test. This method provides the sensitivity of the technique and allows to separate API with the impurities and components of the placebo [10-22].

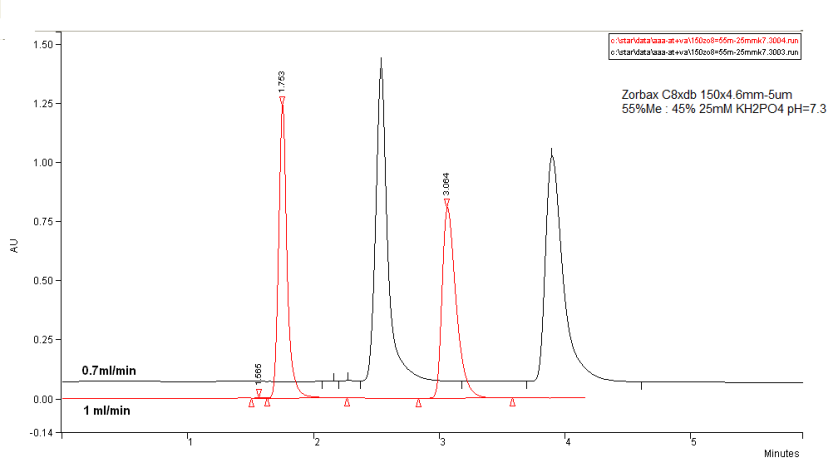

Figure 3. Chromatogram obtained using the final established, optimized, and validated chromatographic method, Dionex Ultimate 3000 UHPLC system with flow rate of 1.0 mL/min and 0.7 mL/min.

To find the appropriate HPLC conditions for the separation of the examined drugs, various columns, isocratic and gradient mobile phase systems were tried, and successful attempts were performed using Zorbax C8 (4.6 mm i.d. X 150 mm, 5 μm) and mobile phase composed of methanol and 25 mM solution potassium dihydrogen phosphate pH 7.3 (55:45, V/V), at a flow rate of 1.0 mL/min and 0.7 mL/min with λ_{max} at 225 nm. Fig. 3 shows a typical chromatogram.

The retention time of valsartan is 1.753 min, atenolol – 3.064 min. Chromatograms were obtained with satisfactory retention factors and perfect peak symmetry of both analyte peaks (tailing factors according to USP of around 1.2 - 1.4), with resolution better than required (R > 7).

The method was validated according to the ICH guideline for the validation of analytical procedures Q2((Q1A (R2) 2003, Q2A 1994, Q2B 1996).

3.1. Specificity.

Commonly used tablet excipients did not interfere with this method. It shows that the method is specific. Furthermore, the well-shaped peaks also indicate the specificity of the method. The specificity results are tabulated in Table 1.

Table 1. Specificity study.

Name of the solution	Retention time (tR) min
mobile phase	No peaks
placebo	No peaks
valsartan 0.8 mg/mL	1.75
atenolol 1.0 mg/mL	3.06

3.2. Linearity.

Calibration curve representing the relation between the concentrations of drugs versus the peak area was constructed. In triplicate run from which the linear regression equation was calculated. Linearity was examined and proven at different concentration levels in the range of working concentration of valsartan (16–96 mg/mL) and atenolol (20–120 mg/mL). For valsartan linearity regression equation y = 2E+045x-12099 and an obtained correlation coefficient of R^2 = 0.9997. For atenolol linearity regression equation y = 2E+012x+ 78985 and an obtained correlation coefficient of R^2 = 0.9995. The results show that a phenomenal relationship between peak area and concentration of the drugs in the calibration curves and indicate high sensitivity of the proposed HPLC method.

Table 2. Intra-day and inter-day precision for the HPLC determination of valsartan

Day	Intra-day precision		Inter-day precision	
	Mean	RSD %	Mean	RSD %
1	98.01	0.389	100.63	0.418
2	101.19	0.543	99.01	0.367
3	100.12	0.481	101.98	0.564

*Each value is represented as a mean±SD of observations (n=5), SD: Standard Deviation, RSD: Relative Standard Deviation

3.3. Accuracy and precision.

Intra-day and inter-day % RSD values lower than 2%, clearly assuring that this method was found to be relatively precise and reproducible (Table 2, 3). Regarding accuracy, a known amount of the standard drug was added to the fixed amount of pre-analyzed sample solution.

The % recovery was calculated by comparing the area before and after the addition of the standard drug. The high value of recoveries obtained for valsartan and atenolol indicates that the proposed method was found to be accurate.

Table 3. Intra-day and inter-day precision for the HPLC determination of atenolol

Day	Intra-day precision		Inter-day precision	
	Mean	RSD %	Mean	RSD %
1	99.24	0.376	100.98	0.397
2	101.98	0.588	99.87	0.241
3	100.33	0.385	100.01	0.588

*Each value is represented as a mean±SD of observations (n=5), SD: Standard Deviation, RSD: Relative Standard Deviation

3.4. Robustness.

The robustness of the developed method was evaluated by small, deliberate changes in method parameters such as flow rate (+10 %) and temperature of column (± 7 %). The % RSD values of robustness, which is less than 2 %, reveal that the proposed method is robust. The results of robustness study results are shown in Table 4, 5.

Table 4. Results of the study of robustness for the HPLC determination of valsartan

Conditions of analysis	Retention time, min
Standard conditions	1.75
flow rate 1.1 mL/min, (+10 %)	1.83
flow rate 0.9 mL/min, (-10 %)	1.91
temperature of column 38° C	1.79
temperature of column 42° C	1.81

Table 5. Results of the study of robustness for the HPLC determination of atenolol

Conditions of analysis	Retention time, min
Standard conditions	3.06
flow rate 1.1 mL/min, (+10 %)	3.17
flow rate 0.9 mL/min, (-10 %)	3.19
temperature of column 28° C	3.18
temperature of column 32° C	3.22

The small changes did not significantly affect the retention time of valsartan and atenolol.

3.5. Dissolution studies.

Dissolution testing is a requirement for all solid oral dosage forms and is used in all phases of development for product release and stability testing. It is a critical analytical test used for detecting physical changes in an active pharmaceutical ingredient (API) and the formulated product. Corresponding data of dissolution studies are shown in Table 6.

Table 6. Dissolution amount for evaluated drugs

Medium	Valsartan			Atenolol		
	% dissolved 15 min	% dissolved 30 min	% dissolved 45 min	% dissolved 15 min	% dissolved 30 min	% dissolved 45 min
pH 1.2	85.34	94.21	97.87	89.18	90.18	92.59
pH 4.5	86.24	95.14	98.23	92.84	93.02	93.98
pH 6.8	90.21	96.63	98.98	88.99	91.98	94.67

Based on the data obtained, it has been found that the equivalence of dissolution profiles for all recommended dissolution media is observed (pH 1.2, 4.5, and 6.8) for the drugs studied. In all three dissolution media, the releases of valsartan and atenolol are more than 85% in 15 min (Table 6).

4. Conclusions

In summary, chromatographic separation achieved isocratically on Zorbax C8 (4.6 mm i.d. X 150 mm, 5 μm) using a mobile phase composed of methanol and 25 mM solution potassium dihydrogen phosphate pH 7.3 (55:45, V/V), pumped with 1.0 mL/min at 40 °C set temperature of column oven, with UV detector set to 225 nm wavelength. Statistical analysis proves that the method is reproducible and selective for the simultaneous estimation of valsartan and atenolol. The developed method was validated as per ICH guidelines in terms of accuracy, precision, linearity, robustness, and specificity. Thus the study aimed at developing and validating the new HPLC method, being simple, accurate, selective, and sensitive and can be applied for the estimation of valsartan and atenolol in pharmaceutical dosage forms and can be used for routine quality control of drugs and *in vitro* dissolution study.

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Conflicts of Interest

The authors declare no conflict of interest.

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