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# Simultaneous Determination of Amlodipine Besylate and Valsartan in Tablets by High Performance Liquid Chromatography with UV Detection

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## ABSTRACT

A simple, precise, accurate and sensitive high-performance liquid chromatography method with UV detection at 220 nm was developed for the simultaneous determination of Amlodipine and Valsartan drugs in pharmaceutical formulations. The present method presents a narrow range (19.6-78.4 (for Amlodipine) and 32-128 (for Valsartan) µg/ml) of calibration curve and sensitivity. Isocratic separation was employed on a C18 column (250×4.6 mm i.d., 5 µm) at ambient temperature. The mobile phase consisted of water, acetonitrile and glacial acetic acid (300:700:1 by volume). The Drugs under investigation were found to be 93–101% recovery of their label claim in pharmaceutical formulations. The separation was completed within 6 minutes. The calibration curve was linear over the range of 19.6–78.4 µg/mL for Amlodipine and over the range of 32 to 128 µg/mL for Valsartan. The Limits of Quantification (LOQ) were chosen to be the lowest concentrations in the calibration curve (19.6 µg/mL) for Amlodipine and (32 µg/mL) for Valsartan. The Limits of Detection (LOD) were 10 µg/mL and 16 µg/mL for Amlodipine and Valsartan, respectively. Good method precision was demonstrated for the analysis with a coefficient of variation of 0.0049 and 0.002 for amlodipine and valsartan, respectively).

**Keywords**: Amlodipine Besylate, Valsartan, HPLC.

## INTRODUCTION

Amlodipine Besylate (Figure 1a) is a long-acting calcium channel blocker that is used as an anti-hypertensive and in the treatment of angina. Valsartan (Figure 1b) is an angiotensin II receptor antagonist that is indicated for the treatment of high blood pressure and congesive heart failure. Amlodipine and Valsartan relax the blood vessels and increase the supply of blood and oxygen to the heart while reducing its workload.

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With the recent development of an anti-hypertension mixture for their use as heart failure inhibitors, the development of a rapid, accurate, and precise chemical method of simultaneous analysis for Amlodipine and Valsartan was desired. In the past, Amlodipine has been analyzed and/or quantitated by liquid chromatography-mass spectroscopy [1-4], liquid chromatography-ultraviolet spectroscopy[5-8], spectrophotometric determination [9-11], spectrofluorometric determination [12] and others [13-15]. On the other hand, chemical methods that were reported for the determination of Valsartan involve the use of liquid chromatography-mass spectroscopy[16,17], spectrophotometric determination [18,19], electrochemical [20], and liquid chromatography-fluorescence detection [21]. Amlodipine and

Valsartan have been analyzed in plasma samples by Ramadan and coworkers [22]. Stability indicating studies for Amlodipine and Valsartan have been reported by Patel and coworkers [23].

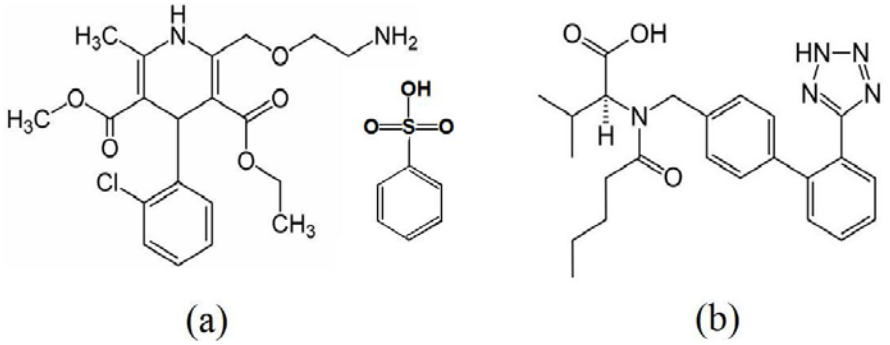



**Figure 1. The chemical structure of a) Amlodipine b) Valsartan.**

Although some of the listed chromatographic methods reported above are precise and accurate, the simultaneous determination of Amlodipine and Valsartan is not investigated very well; only three studies appeared in the literature [24-26]. The previous studies stimulate us to develop a method for analysis of Amlodipine and Valsartan in tablets and extend our work to investigate the Stability indication application. In the present work, optimum stability, selectivity, and sensitivity for the assay of Amlodipine and Valsartan levels have been investigated in bulk drug samples and in combined dosage formulation. The advantage of the present method over others was the application of a narrow range (19.6-78.4 (for Amlodipine) and 32-128 (for Valsartan) µg/ml) of calibration curve and sensitivity. The method has been developed by using reversed-phase High-Performance Liquid Chromatography (HPLC) connected to a C18 (250 mm × 4.6 mm *i.d*., 5 µm) column. The analytical method was validated according to the "Guidance for industry, Bioanalytical Method Validation, FDA" [27-28].

## 2. Experimental:

### 2.1 Materials

Amlodipine and Valsartan were used as received from Merck. Acetonitrile was of chromatographic grade purchased from Merck. Water used in the preparation of the standards and mobile phase was distillated and then deionized in the Albalqa Applied University. Glacial Acetic Acid (GAA) was purchased from Merck.

### 2.2 Instrumentation

A spectra-physics pump SP8810 was used and the injections were performed using a Hamilton 250 µL syringe via a rheodyne 7725 injector connected to a 20 µL loop. The eluent was monitored using a spectra-physics variable wavelength UV-vis detector type spectra-100 at 220 nm. The used column was a C18 (250mm × 4.6mm i.d., 5µm). An ODS guard column was used to protect the analytical column.

### 2.3 Chromatographic Condition

The mobile phase (Water: Acetonitrile: GAA; 300:700:1) is introduced to the chromatographic system via isocratic elution solvents. Before use, the mobile phase was vacuum filtered through a 0.22 µm membrane filter (Lida, Kenosha, WI, USA).

### 2.4 Standard Preparation

Stock solutions of Amlodipine (0.5 mg/mL) and Valsartan (0.8 mg/mL) were prepared in the mobile phase. The solutions were stored at –20 °C and remained stable for at least 1 month. Standard solutions were freshly prepared for each run day. The standard solutions of amlodipine (19.6, 29.4, 39.2, 49.0, 58.8, and 78.4 µg/mL)

and Valsartan (32, 48, 64, 70, 96 and 128 µg/mL) were prepared by dilution of the stock solutions with the mobile phase. Table (1) shows the method for standard solution preparation of Amlodipine Besylate and Valsartan.

**Table (1): Method for preparation of standard solution of Amlodipine Besylate and Valsartan**

| Dilution | | Final Concentration (µg/mL) | |
|---|---|---|---|
| **Amount withdrawn from stock solutin (mL)** | **Dilution Volume (mL)** | **Amlodipine Besylate** | **Valsartan** |
| 2 | 50 | 19.6 | 32 |
| 3 | 50 | 29.4 | 48 |
| 4 | 50 | 39.2 | 64 |
| 5 | 50 | 49.0 | 70 |
| 3 | 25 | 58.8 | 96 |
| 4 | 25 | 78.4 | 128 |

**2.5 Calibration Curve**

Calibrators for Amlodipine analysis were prepared in the concentration range of 19.6-78.4 µg/mL by the dilution of the stock solutions with the mobile phase while the calibrations for Valsartan analysis were prepared in the concentration range of 32–128 µg/mL by the dilution of the stock solutions with the mobile phase.

**2.6 Sample Solution**

The contents of 20 tablets were mixed and accurately weighed. An amount equivalent to one tablet (containing 5 mg amlodipine as amlodipine besylate and 80 mg Valsartan) was transferred to a 100 ml volumetric flask and dissolved with the mobile phase by using ultrasonic bath. The resulting solution was filtered through 0.2 micron nylon membrane filter and was used in the analysis. The sample was then directly injected for amlodipine determination while a dilution step was performed (5 mL of the filtrate with 50 mL mobile phase) for the determination Valsartan.

**2.7 Stability Studies**

Stability solutions were prepared by dissolving 100 mg of amlodipine and 160 mg of Valsartan in 200 ml mobile phase (each solution was prepared separately) then 5 mL of each solution was diluted to 50 mL before measurements.

**3. Results and Discussion**

**3.1 Method Development**

C8 and C18 columns were tested for the separation of amlodipine and Valsartan. It has been found that C8 column shows no adequate resolution, while C18 shows a good resolution of these analytes; therefore it was employed during this work. Regarding the mobile phase, filtered and degassed mixtures of acetonitrile and water for HPLC (different volume fractions) were tested. The best combination was (Water: Acetonitrile: GAA; 300:700:1). Different flow rates (0.5, 0.7, and 1.2 mL/min) were tested and an optimum of 0.7 ml/min has been chosen in the study (Figure 3). UV detection was performed at 220 nm, and injection volume was 20 µL. Figure 2 shows chromatograms of amlodipine Besylate and Valsartan.

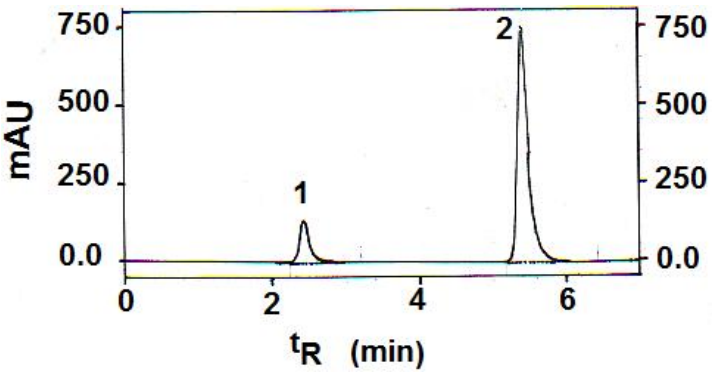
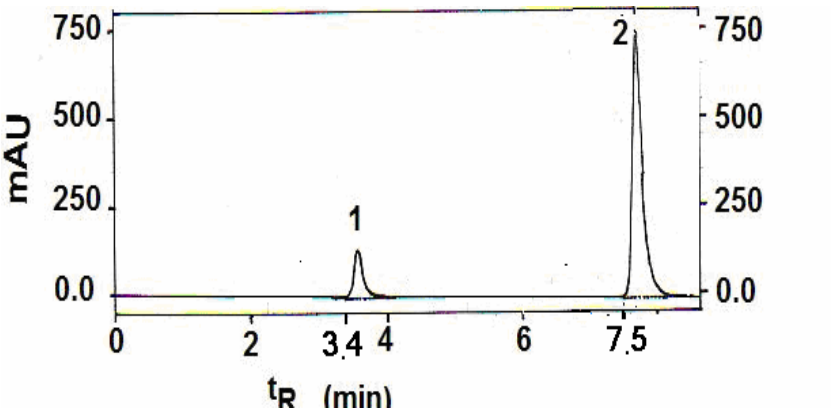
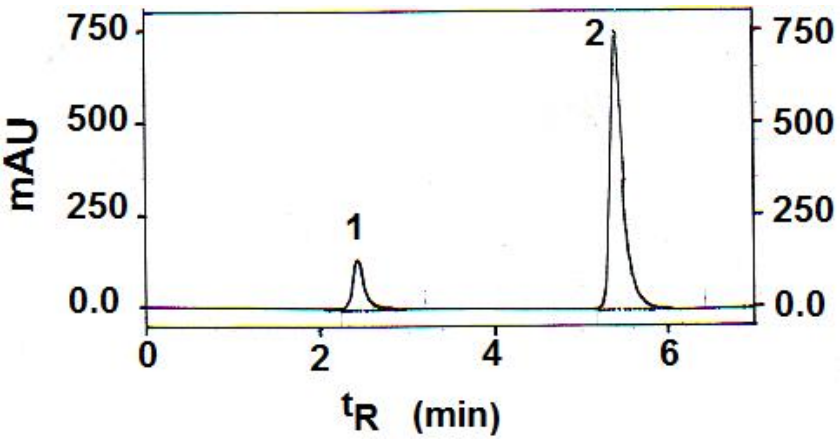



**Figure 2. HPLC Chromatogram of amlodipine (1) and Valsartan (2). Retention times of Amlodipine and Valsartan are (2.4 and 5.4 min, respectively); concentrations of Amlodipine and Valsartan are equal to 80 and 49 µL respectively**

**a**




**b**



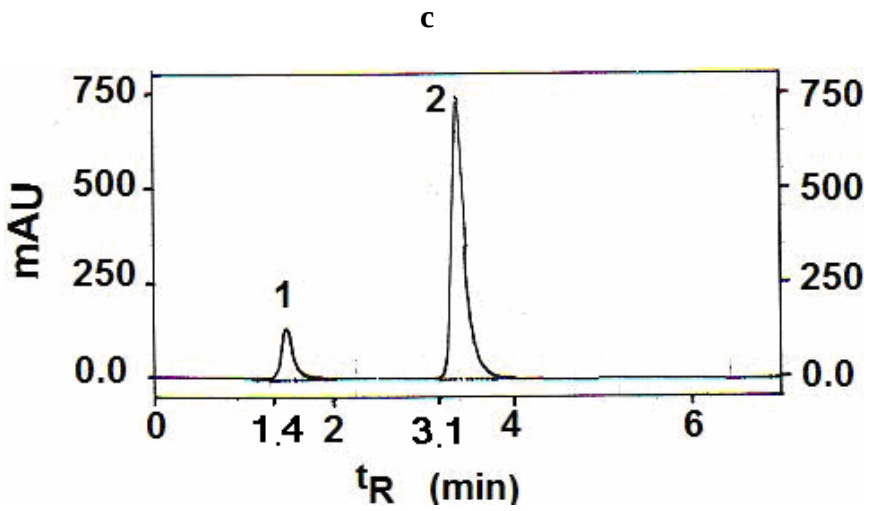



**Figure 3. Flow Rate Chromatograms: (a) 0.5 ml/min, (b) 0.7ml/min, (c) 1.2 ml/min**

### 3.2. Linearity

Standard solutions covering the range between 50-150% of the nominal standard concentration (0.1mg/mL) were prepared by diluting specific volume of the stock standard to get several concentrations (0.05, 0.075, 0.1, 0.125, 0.15 mg/mL). The peak area was recorded and plotted versus standard concentrations. Results have shown that the method is linear over the specified range with $r^2$ of 0.9980 and 0.9992 for Amlodipine and Valsartan, respectively. These findings demonstrate linearity of this method over the specified range (Table 2).

**Table 2: Linearity, precision, repeatability, stability indication data for the analysis of Amlodipine besylate and Valsartan**

| | | **Amlodipine** | **Valsartan** |
|---|---|---|---|
| General information | Retention time (min) | 2.40 | 5.40 |
| | Assymetry | 1.60 | 1.50 |
| | %Recovery±SD | 99.6±0.5 | 99.4±0.4 |
| | Resolution | 8 | 8 |
| Linearity | Range (mg/100 mL) | 1.96 - 7.84 | 3.2 - 12.8 |
| | $R^2$ | 0.998 | 0.999 |
| | Slope | 250275 | 978168 |
| | Detection limit (mg/100 mL) | 0.002 | 0.3 |
| | Quantitation limit (mg/100 mL) | 0.006 | 0.9 |
| | RSD% of AVER R.F | 1.70 | 1.90 |
| Precission | RSD% of 50% | 1.98 | 0.95 |
| | RSD% of 100% | 0.49 | 0.20 |
| | RSD% of 150% | 0.23 | 0.17 |
| Repetability of assay% | RSD% $1^{ST}$ Analyst | 1.40 | 0.83 |
| | RSD% $1^{2D}$ Analyst | 1.20 | 0.50 |

| | | **Amlodipine** | **Valsartan** |
|---|---|---|---|
| General information | Retention time (min) | 2.40 | 5.40 |
| | Assymetry | 1.60 | 1.50 |
| | %Recovery±SD | 99.6±0.5 | 99.4±0.4 |
| | Resolution | 8 | 8 |
| Retention time | RSD% $1^{2D}$ Analyst | 0 | 0 |
| Accuracy | %Recovery of 50% | 100.80 | 100.50 |
| | %Recovery of 100% | 101.30 | 101.60 |
| | %Recovery of 150% | 97.50 | 98.20 |
| | RSD% of 50% | 0.74 | 0.89 |
| | RSD% of 100% | 0.65 | 0.70 |
| | RSD% of 150% | 2 | 2 |
| Stability indicating | 0.1M HCl 90 ºC, 24 Hours | 57.80 | 54.20 |
| | 0.1M NaOH 90 ºC, 24 Hours | 59.1 | 54 |
| | 10.0% $H_2O_2$ 90 ºC, 2 Hours | 73 | 76 |
| Robustness | | | |
| Wavelength | 215 nm | 101.3, R.T 2.5 min | 102.2, R.T 5.4 min |
| | 225 nm | 98.2, R.T 2.5 min | 101.6, R.T 5.4 min |
| Flow rate | 1.2 mL/min | 101.9, R.T 1.4 min | 102.4, R.T 3.1 min |
| | 0.5 mL/min | 98.5, R.T 3.4 min | 99.4, R.T 7.5 min |
| Mobile phase ratio | | | |
| Water:Acetonitrile:GAA | 450:550:1 | 100.4, R.T 2.5 min | 99.6, R.T 7.6 min |
| | 250:750:1 | 100.5, R.T 2.5 min | 100.8, R.T 4.6 min |
| | 300:700:2 | 100.4, R.T 2.5 min | 99.3, R.T 4.5 min |
| | 300:700:0.5 | 98.2, R.T 2.5 min | 99.2, R.T 5.7 min |

**3.3 Degradation Studies**

Stress testing of the drug substance can help identify the likely degradation products, the stability and specificity of the analytical procedure.

**3.3.1 Stress Degradation by Hydrolysis under Acidic Condition (Using 1M HCl)**

Stress degradation by hydrolysis under acidic conditions was employed. Hydrochloric acid (1.0 M, 45 mL) was added to 5 ml of amlodipine and Valsartan Stock solutions (concentrations of 500 mg/mL and 800 mg/100mL, respectively) to get final concentrations of 50 and 80 mg/100mL, respectively. This solution was allowed to stand for 24 hrs at 90 ºC in a closed container, then 5 mL diluted upto 50 mL with the mobile phase. Considerable losses were observed when exposed to harm acidic conditions. The relative recovery of amlodipine and Valsartan was found to be 57.80% and 54.20% (Table 2).

**3.3.2 Stress Degradation by Hydrolysis under Alkaline Condition (Using 1M NaOH)**

Stress degradation by hydrolysis under alkaline conditions was employed. Sodium hydroxide (1.0 M, 45 mL) was added to 5 ml of amlodipine and Valsartan Stock solutions (concentrations of 500 mg/mL and 800 mg/100mL, respectively) to get final concentrations of 50 and 80 mg/100mL, respectively. This solution was allowed to stand for 24 hrs 90 ºC in a closed container,

then 5 mL diluted upto 50 mL with the mobile phase. Considerable losses were observed when exposed to harm basic conditions. The relative recovery of amlodipine and Valsartan was found to be 73.00% and 76.00% (Table 2).

**3.3.3 Oxidative Degradation (using 30% $H_2O_2$ in water)**

Oxidative degradation on amlodipine and Valsartan solutions was investigated by employing 10% of Hydrogen peroxide. The oxidant was added to 5 ml of amlodipine and Valsartan Stock solutions (500 mg/mL and 800 mg/100 mL, respectively) to get final concentrations of 50 and 80 mg/100mL. This solution was allowed to stand for 24 hrs 90 ºC in a closed container, then 5 mL diluted upto 50 mL with the mobile phase. Considerable losses were observed when exposed to harm oxidative conditions. The relative recovery of amlodipine and Valsartan was found to be 59.10% and 54.00% (Table 2).

**3.4 Precision and Accuracy**

Precision of the method for amlodipine and Valsartan analysis were demonstrated by analyzing 6 replicates of three working concentrations namely, (2.04, 5.0, 7.67 mg/ml for Amlodipine and 31.7, 81.3, 128.4 for Valsartan) and calculating the RSD for the peak responses (Area). Results have shown that the RSD for these 9 replicates is 0.82%, 0.76%, and 0.6% for Amlodipine and 1.98%, 0.50%, and 0.23% for Valsartan (Table 2). Good method/system precision was demonstrated for analysis. Accuracy of the method was determined by comparing the means of the measured concentrations (back calculation) of the quality controls with their nominal concentrations. Accuracy results were found to be consistent, precise, and reproducible (Table 2).

**4. Conclusions**

A rapid and simple HPLC method for the simultaneous analysis of amlodipine and Valsartan was developed and validated. The chromatographic elution step was undertaken in a short time with high resolution. In addition, the method is suitable for tablet formulations and can be tested in other biomedical applications and can be used in the routine analysis with the applied calibration range. The calibration curves were linear over a narrow concentration range. The method is accurate, analytical recoveries were high. Amlodipine and Valsartan samples are found to exhibit low resistance when exposed to harm acidic and basic conditions with significant changes of concentrations.

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# التحديد المتزامن للأمالوديبين باسلات و الفالزارتان في الأقراص باستخدام الفصل عالي الكفاءة الموصول بمحساس فوق بنفسجي

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## ملخـص

لقـد تم تطوير طريقه سهلة ودقيقة وحساسة لتحليل الأمالوديبين باسلات والفالزارتان بشكل متزامن باستخدام الفصل عالي الكفاءة موصولاً بمـحساس فوق بنفسجي على طول موجه 220 نانومتراً وذلك في الاقراص الدوائية .
إن الطريقة الحاليـة لها خـط معايـرة ضيق لكلا الدوائيـن .
لقـد تم استخدام عمود من نوع كربون 18 بطول 25 سم ونصف قطر داخلي 4.6 مليمتر .
إن الشكل المتحرك في النظـام هـو ماء مع اسيتو نيترايل وحامض الاسيتك بنسبـة 300 إلى 700 إلى 1.
لقد كانت نسبة الاسترجاع 93 الى 101% وقد اكتملت كل عمليـة تحليل في ست دقائق .
لقد كان منحنى المعايـرة للاملوديبين من 19.6 الى 78.4 مايكروجرام لكل مليلتر بينما كان منحني المعايـرة للفالزارتان من 32 إلى 128 مايكروجرام لكل مليلتر واحد .
لقد كان الحد الادنى للقياس 19.6 مايكروجرام لكل مللتر للاملوديبين بينما كان الحد الادنى للقياس للفالزارتان هو 32 مايكرو جرام لكل مليلتر وقد كان الحد الادنى لتحديد ومشاهدة الاملوديبين للفالزارتان على الجهاز هـو 10 و16 مايكروجرام لكل مليلتر على التوالي .
لقد كانت دقة الطريقة هي 0.0049 و 0.002 ك(معامل التغير) على التوالي لكل من الاملوديبين للفالزارتان.

**الكلمات الدالة**: الاملوديبين ، الفالزارتان ، جهاز الفصل عالي الكفاءة.

تاريخ استلام البحث 2010/3/9 وتاريخ قبوله للنشر2010/8/8.