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# High performance liquid chromatography with PDA detector for combined determination of hydrochlorothiazide, amlodipine and valsartan

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**ABSTRACT**

*A simple, precise, and accurate HPLC method for simultaneous estimation of hydrochlorothiazide, amlodipine and valsartan as the bulk drug and in pharmaceutical dosage forms. Chromatographic separation of the drugs was performed on Zorbax C18 (250 x 4.6 mm; 5 µm particle size) analytical column as the stationary phase. The solvent system consisted of 0.1M $NaH_2PO_4$ and methanol in the ratio of 60:40 (v/v) as mobile phase. Evaluation of the separated drugs was performed using a PDA detector covering the range of 200-400 nm. All the three drugs were resolved with the retention time of 3.624 min, 5.484 min and 14.943 min for hydrochlorothiazide, amlodipine and valsartan, respectively. The method was validated with respect to linearity, sensitivity, precision, accuracy and robustness in accordance with ICH guidelines. The validated method was successfully applied to the commercially available pharmaceutical dosage form, yielding good and reproducible result.*

**Key words:** Hydrochlorothiazide, Amlodipine, Valsartan, HPLC, simultaneous Determination.

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## INTRODUCTION

Hydrochlorothiazide [1,2], chemically known as 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide (Figure 1), is a diuretic belonging to the class of compounds known as benzothiadiazines. It is often used to treat hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis and hypoparathyroidism.

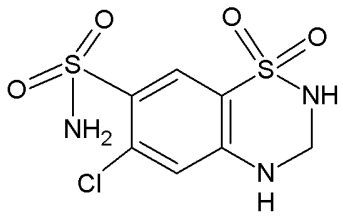


**Figure 1: Structure of hydrochlorothiazide**

Amlodipine [3,4], chemically known as 5-Methyl 3-ethyl 2-(2-aminoethoxy methyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (Figure 2), is a long-acting calcium channel blocker with cardiovascular activity. Amlodipine belongs to the dihydropyridine type third generation calcium channel blocker. It is used as antihypertensive, antiarrhythmic agent and in the treatment of chronic stable angina pectoris and prinzmetal's variant angina.

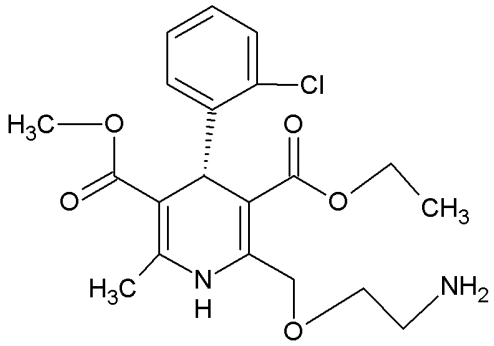


**Figure 2: Structure of amlodipine**

Valsartan [5,6] is an effective and specific competitive antagonist of the angiotensin-II AT1-receptor belonging to the class of compounds known as biphenyltetrazoles and derivatives. Chemically, it is described as N-[*p*-(*o*-1H-tetrazol-5-ylphenyl)benzyl]-N-valeryl-L-valine (Figure 3) and used in treating high blood pressure, heart failure, decreasing the risk of death after a heart attack in patients with left ventricular dysfunction.

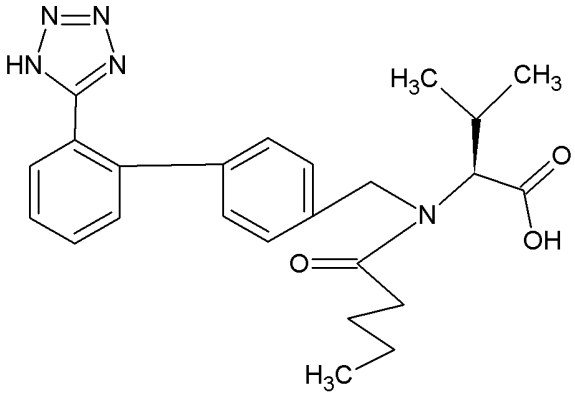


**Figure 3: Structure of valsartan**

The use of multiple antihypertensive agents is often required in the successful treatment of moderate or severe hypertension [7]. For treating hypertension and cardiovascular diseases, hydrochlorothiazide, amlodipine and valsartan are used as combinations in pharmaceutical preparations [8].

Few methods have been reported in the literature for the simultaneous determination of hydrochlorothiazide, amlodipine and valsartan in bulk, pharmaceutical formulations and biological samples. They include: UV-spectrophotometric absorption correction [9], first order derivative UV spectrophotomety [10], Sequential spectrophotometry [11], HPLC [12-18] and HPTLC [17, 18] methods.

The aim of the present investigation is to develop and validate a sensitive, precise and accurate RP-HPLC method for the simultaneous quantification of hydrochlorothiazide, amlodipine and valsartan in bulk and in its combined pharmaceutical formulation.

## MATERIALS AND METHODS

### Apparatus

A Waters 2695 alliance with binary HPLC pump equipped with Waters 2998 PDA detector and Waters Empower2 software is used in the present investigation.

### Mobile phase

The solvents and chemicals used in the preparation of mobile phase were of HPLC grade and analytical grade, respectively. The mobile phase used is 0.1M $NaH_2PO_4$ and methanol in the ratio of 60:40 *v/v*. The mobile phase is filtered before using, through millipore membrane filter and degassed for 15 min by sonication.

### Chromatographic conditions

Zorbax C18 (250 x 4.6 mm; 5 µm particle size) analytical column was used for separation and simultaneous analysis of the hydrochlorothiazide, amlodipine and valsartan. The column temperature was maintained at 30 ± 1°C. The separation was carried out under isocratic elution. The flow rate was maintained 1.0 ml/min. The injection volume was 10 µl. The selected drugs were analyzed using a PDA detector covering the range of 200–400 nm.

### Standard solutions

The standard stock solution was prepared by dissolving 20 mg of amlodipine, 50 mg of hydrochlorothiazide and 640 mg of valsartan in 100 ml mobile phase. Working standard solutions equivalent to 20-60 µg/ml amlodipine, 50-150 µg/ml hydrochloro-thiazide and 640-1920 µg/ml valsartan was prepared from stock solution by appropriately diluting the stock standard solution with the mobile phase.

### Sample Solution

Ten tablets were weighed and crushed to a fine powder. The powder equivalent to 10 mg of amlodipine, 320 mg of valsartan and 25 mg of hydrochlorothiazide was taken in a 100 ml volumetric flask containing 20 ml of mobile phase, sonicate for 20 min and made up to mark with the same solvent. The resultant mixture was filtered through 0.45 µm filter paper. The resultant solution was diluted appropriately with the mobile phase to get a final concentration of 40 µg/ml amlodipine, 1280 µg/ml valsartan and 100 µg/ml hydrochlorothiazide.

## RESULTS AND DISCUSSION

### HPLC parameters optimization

The main aim of this study is to simultaneously analyze amlodipine, hydrochlorothiazide and valsartan with sufficient resolution in reasonable analysis time. To obtain a good chromatographic condition, two different stationary phases were tested by name Hypersil C18 (150 x 4.6mm; 5 µm particle size) and Zorbax C18 (250 x 4.6 mm; 5 µm particle size). Similarly, various mobile phases with isocratic elution were also tested to obtain good chromatographic condition:

1. Water: Methanol (60:40, *v/v*)
2. 0.1M $NaH_2PO_4$: Methanol (60:40, *v/v*)
3. 0.1M $NaH_2PO_4$: Methanol (70:30, *v/v*)
4. 0.1M $NaH_2PO_4$: Methanol (80:20, *v/v*)
5. 0.1M $NaH_2PO_4$: Methanol (60:40, *v/v*)

The peak shape and system suitability parameters of amlodipine, hydrochlorothiazide and valsartan were good with Zorbax C 18 (250 mm × 4.6 mm, 5 µm) column. Hence this analytical column was selected. The good performance and better separation was achieved with the mobile phase combination 0.1M $NaH_2PO_4$ and methanol in the ratio of 60:40 (*v/v*) using Zorbax C 18 (250 mm × 4.6 mm, 5 µm) column. The isocratic elution with a flow rate of 1 ml/min was optimized.

Under the optimized chromatographic conditions, the chromatogram (Figure 4) obtained, demonstrated a good separation of the hydrochlorothiazide (RT= 3.624 min), amlodipine (RT= 5.484 min) and valsartan (RT= 14.943 min) from each other.

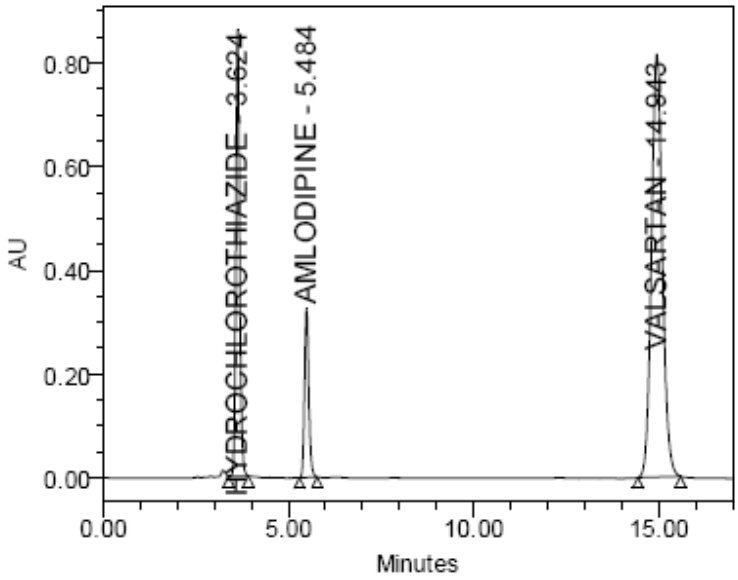


**Figure 4: Chromatogram of hydrochlorothiazide, amlodipine and valsartan**

**Method validation**

The optimized RP-HPLC method for simultaneous assay of amlodipine, hydrochlorothiazide and valsartan was validated according to ICH guidelines [19] with respect to system suitability, linearity, sensitivity, accuracy, precision and robustness.

**System suitability**

Prior to analysis, the chromatographic system must satisfy system suitability test requirements. System suitability test was assessed from five replicate injections of the standard solution containing 40, 100 and 1280 µg/ml amlodipine, hydrochlorothiazide and valsartan, respectively. All the three peaks were well resolved and the precision of injections for all the peaks were acceptable. The percent relative standard deviation of the amlodipine, hydrochlorothiazide and valsartan peaks area responses were determined to be less than 1. The USP tailing factor and USP plate count were also calculated. The results of system suitability in comparison with the required limits are shown in Table 1 and are found to be within the accepted limits.

**Table 1: System suitability of the method**

| Parameters | Results | | | Recommended limits |
|---|---|---|---|---|
| | Valsartan | Hydrochlorothiazide | Amlodipine | |
| **Retention time** | 14.998 | 3.604 | 5.470 | - |
| **Peak area** | 16743876 (%RSD – 0.8) | 6102248 (%RSD – 0.6) | 2520410 (%RSD – 0.6) | RSD ≤1 |
| **USP resolution** | 25.03 | - | 9.41 | > 1.5 |
| **USP plate count** | 12134 | 5986 | 12004 | > 2000 |
| **USP tailing factor** | 1.09 | 1.18 | 1.22 | ≤ 2 |

**Linearity and range**

The linearity of the HPLC method was determined, for the simultaneous assay of hydrochlorothiazide, amlodipine and valsartan, by analyzing five different concentrations of each drug. The calibration curve was plotted by area under the peak responses of the three drugs against their corresponding concentrations. Calibration curves were linear over the concentration range of 50-150 µg/ml for hydrochlorothiazide, 20-60 µg/ml for amlodipine and 640-1920 µg/ml for valsartan. The linearity parameters such as regression equations and regression coefficients are given in Figures 5,6 and 7. The results show a good correlation between the peak areas of the three drugs and their corresponding concentrations.

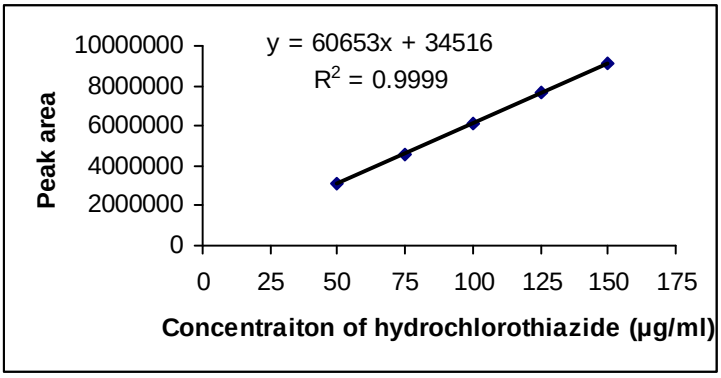


**Figure 5: Linearity curve of hydrochlorothiazide**

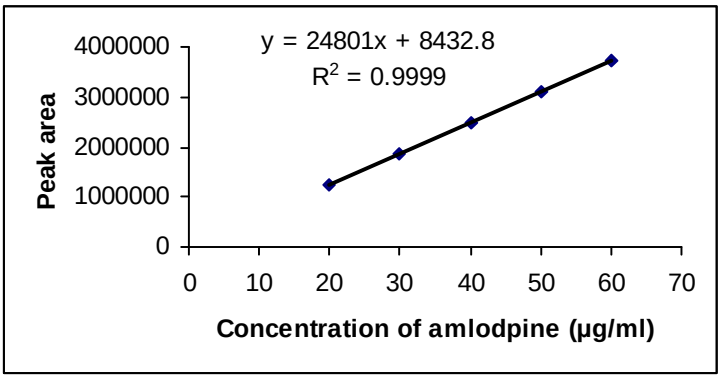


**Figure 6: Linearity curve of amlodipine**

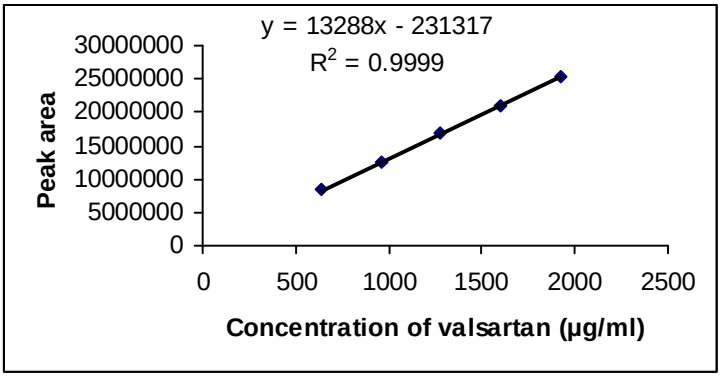


**Figure 7: Linearity curve of valsartan**

**Sensitivity**

The sensitivity of the method was assessed by calculating limit of detection (LOD) and limit of quantification (LOQ). LOD was found to be 0.205 µg/ml, 0.218 µg/ml and 2.796 µg/ml for hydrochlorothiazide, amlodipine and valsartan, respectively (signal to noise ratio of 3:1). LOQ was found to be 0.682 µg/ml, 0.728 µg/ml and 9.320 µg/ml for hydrochlorothiazide, amlodipine and valsartan, respectively (signal to noise ratio of 10:1). The low values of LOD and LOQ demonstrate sufficient sensitivity of the HPLC method.

**Precision**

Precision was determined by injecting six standard solutions of amlodipine, hydrochlorothiazide and valsartan with 40,100 and 1280 µg/ml concentration, respectively. The peak areas were determined. Relative standard deviation of amlodipine, hydrochlorothiazide and valsartan peaks was then calculated to represent precision. The results are summarized in Table 2. The low % RSD values indicated that the method is precise.

**Table 2: Precision of the method**

| Hydrochlorothiazide (100 µg/ml) | | Amlodipine (40 µg/ml) | | Valsartan (1280 µg/ml) | |
|---|---|---|---|---|---|
| **Peak area** | **%RSD** | **Peak area** | **%RSD** | **Peak area** | **%RSD** |
| 6119365 | 0.45 | 2524024 | 0.18 | 16893463 | 0.39 |
| 6131421 | | 2523176 | | 16798656 | |
| 6131602 | | 2531704 | | 16885621 | |
| 6188208 | | 2522874 | | 16944569 | |
| 6123035 | | 2519700 | | 16788941 | |
| 6110598 | | 2518995 | | 16926989 | |

## Accuracy

Accuracy of the method was evaluated by recovery studies at three concentration (50%, 100%, and 150%) levels by standard addition method. The mean percentage recoveries obtained were in the range of 99-100% for all the three drugs (Table 3). The good % recovery values showed the method to be highly accurate.

**Table 3: Accuracy of the method**

| Drug | Spiked Level | µg/ml added | µg/ml found | % Recovery | % Mean |
|---|---|---|---|---|---|
| **Hydrochlorothiazide** | 50% | 50.000 | 49.27 | 99 | 99 |
| | 50% | 50.000 | 49.75 | 100 | |
| | 50% | 50.000 | 49.71 | 99 | |
| | 100% | 100.000 | 100.62 | 101 | 100 |
| | 100% | 100.000 | 99.23 | 99 | |
| | 100% | 100.000 | 99.19 | 99 | |
| | 150% | 150.000 | 149.23 | 99 | 99 |
| | 150% | 150.000 | 149.06 | 99 | |
| | 150% | 150.000 | 149.02 | 99 | |
| **Amlodipine** | 50% | 20.000 | 19.99 | 100 | 100 |
| | 50% | 20.000 | 19.90 | 100 | |
| | 50% | 20.000 | 20.02 | 100 | |
| | 100% | 40.000 | 39.68 | 99 | 99 |
| | 100% | 40.000 | 39.82 | 100 | |
| | 100% | 40.000 | 39.79 | 99 | |
| | 150% | 60.000 | 59.42 | 99 | 99 |
| | 150% | 60.000 | 59.71 | 100 | |
| | 150% | 60.000 | 59.51 | 99 | |
| **Valsartan** | 50% | 640.000 | 634.07 | 99 | 99 |
| | 50% | 640.000 | 632.84 | 99 | |
| | 50% | 640.000 | 633.46 | 99 | |
| | 100% | 1280.000 | 1285.84 | 100 | 100 |
| | 100% | 1280.000 | 1274.53 | 100 | |
| | 100% | 1280.000 | 1276.57 | 100 | |
| | 150% | 1920.000 | 1924.90 | 100 | 99 |
| | 150% | 1920.000 | 1902.23 | 99 | |
| | **150%** | 1920.000 | 1900.73 | 99 | |

## Robustness

In order to show the robustness of the method, system suitability parameters were evaluated by slightly varying flow rate and column temperature. The parameters used to define robustness were retention time, USP tailing factor and USP plate count. The results showed (Table 4) that slight variations in method parameters had a negligible effect on the analysis.

**Table 4: Robustness of the method**

| Drug | Parameter | Retention time | Peak area | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|
| **Hydrochlorothiazide** | Flow 1 | 3.207 | 5483310 | 6151 | 1.14 |
| | Flow 2 | 3.084 | 5383307 | 5518 | 1.12 |
| | Temperature 1 | 3.901 | 5471602 | 5190 | 1.21 |
| | Temperature 2 | 3.085 | 5387381 | 5492 | 1.12 |
| **Amlodipine** | Flow 1 | 4.912 | 2218934 | 11062 | 1.18 |
| | Flow 2 | 4.688 | 2128941 | 10477 | 1.17 |
| | Temperature 1 | 4.791 | 2186220 | 10120 | 1.19 |
| | Temperature 2 | 4.696 | 2136225 | 10460 | 1.18 |
| **Valsartan** | Flow 1 | 12.990 | 15024181 | 11301 | 1.11 |
| | Flow 2 | 12.926 | 14023082 | 10809 | 1.09 |
| | Temperature 1 | 12.980 | 15012174 | 10124 | 1.10 |
| | Temperature 2 | 12.968 | 14092473 | 10609 | 1.09 |

## CONCLUSION

A RP-HPLC method has been reported for simultaneous estimation amlodipine, hydrochlorothiazide and valsartan. The proposed method gives good resolution of the above said drugs. The validation of developed method was done as per ICH guidelines and proved that method to be simple, sensitive, precise, accurate and robust. The validated method was successfully applied to the determination of commercially available pharmaceutical dosage form. Hence, the method can be used for the routine quality control analysis of pharmaceutical dosage forms containing amlodipine, hydrochlorothiazide and valsartan.

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