



## RP-HPLC Method Development and Validation for Simultaneous Estimation of Amlodipine besylate, Valsartan and Hydrochlorothiazide in Tablet Dosage Form

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Received on: 15-06-2010; Revised on: 18-08-2010; Accepted on: 13-09-2010

### ABSTRACT

A simple, specific, accurate and precise Reverse Phase High Performance Liquid Chromatographic method was developed for simultaneous estimation of Amlodipine Besylate (AB), Valsartan (VAT) and Hydrochlorothiazide (HTZ) in tablet dosage form on RP C-18 Column (Hypersil 250\*4.6 mm) using Acetonitrile: Mixed Phosphate buffer (6.8 pH) (55:45) (buffer was prepared by mixing the equal proportions of 0.01M Potassium dihydrogenphosphate and 0.001M Dipotassium hydrogenphosphate. pH was adjusted with Orthophosphoric acid) as mobile phase. The flow rate was 1.0 ml/min and effluent was monitored at 237nm. The retention time for VAT, HTZ and AB was found to be as 2.28, 2.99 and 4.57 respectively. Proposed method was validated for Precision, Accuracy, Linearity range, Robustness and Ruggedness.

**Key words:** Amlodipine Besylate, Valsartan, Hydrochlorothiazide, Reverse Phase High Performance Liquid Chromatography

### INTRODUCTION

Amlodipine Besylate, (RS)-3-ethyl-5-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate benzene sulfonate is a long-acting calcium channel blocker (dihydropyridine class) used as an anti-hypertensive and in the treatment of angina<sup>(1,3)</sup>. Valsartan, N-(1-oxopentyl)-N-[[2'-(1H-tetrazol-5-yl) [1,1'-biphenyl]-4-yl] methyl]-L-valine is a specific angiotensin II receptor blocker acting on the AT<sub>1</sub> receptor subtype used as an anti-hypertensive<sup>(2,3)</sup>. Hydrochlorothiazide, 2H-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-3, 4-dihydro-, 1, 1-dioxide is a thiazide diuretic used to treat high blood pressure<sup>(2,3)</sup>.

Literature survey revealed HPLC<sup>(4-7)</sup>, LCMS<sup>(8,9)</sup>, HPTLC<sup>(10,11)</sup>, and Simultaneous UV Spectrophotometric Methods<sup>(12,13)</sup> have been reported for the estimation of AB alone or in combination with other drugs. Methods such as HPLC<sup>(14)</sup>, TLC-Densitometry<sup>(15)</sup>, HPTLC<sup>(16)</sup> and LCMS<sup>(17)</sup> methods have been reported for estimation of VAT alone or in combination with other agents. For HTZ HPLC<sup>(18)</sup>, UV Spectrophotometric<sup>(19)</sup>, and LCMS<sup>(20)</sup> methods have been reported. The analytical method for simultaneous estimation of AB, VAT and HTZ in pharmaceutical dosage form has not been reported. The present paper describes the simple, specific, accurate and precise Reverse Phase High Performance Liquid Chromatographic for Simultaneous Estimation of AB, VAT and HTZ in tablet dosage form.

### MATERIALS AND METHODS

#### Instrument

High Performance Liquid Chromatographic system (Shimadzu) equipped with two LC 20AT liquid pumps, Rheodyne Injector (2E 7725, 20 µl loop), SPD 20A UV/Vis detector and Spinchrome software, Glass Van Hypodermic injecting syringe, an ODS Hypersil C-18 RP column (25 cm\* 4.6 mm ID).

#### Reagents

Acetonitrile of HPLC grade (Merck), Potassium dihydrogenphosphate, Dipotassium hydrogenphosphate and Orthophosphoric acid of reagent grade (Merck), Double Distilled Water.

#### Chromatographic condition

The mobile phase containing Acetonitrile: Mixed Phosphate buffer pH 6.8) (55:45), (buffer was prepared by mixing the equal proportions of 0.01M Potassium dihydrogenphosphate and 0.001M Dipotassium hydrogenphosphate, the

pH was adjusted to 6.8 by using Orthophosphoric acid) was found to resolve AB, VAT and HTZ. The mobile phase was filtered through 0.45-µm-membrane filter and the ultrasonicated for 30 min. the flow rate was set at 1.0 ml/min. All three drugs showed good absorbance at 237 nm, which was selected as wavelength for further analysis all determinations were performed at ambient column temperature.

#### Preparation of Stock solution and Standard solution

Accurately weighed 10 mg of Amlodipine, 160 mg of Valsartan and 25 mg of Hydrochlorothiazide dissolved in 100 ml of mobile phase (Stock solution). The stock solution was further diluted by using mobile phase to get the concentration of 10 mcg/ml, 160 mcg/ml, and 25 mcg/ml of Amlodipine, Valsartan and Hydrochlorothiazide respectively (standard solution).

#### System suitability

Standard solution was injected five times and Flow rate was maintained at 1.0 ml/min. temperature of column kept ambient and the column effluents were monitored at 237 nm chromatograms were taken and System suitability parameters were computed.

#### Calibration Curve

Calibration curves were prepared by taking appropriate aliquots of AB, VAT and HTZ stock solution in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 10, 20, 30, 40, 50 and 60 mcg/ml of AB, 160, 320, 480, 600, 720, 800 and 960 mcg/ml of VAT and 25, 50, 75, 100, 125 and 150 mcg/ml of HTZ. These solutions (n=6) were injected and chromatogram were taken. Flow rate was maintained at 1.0 ml/min. temperature of column kept ambient and the column effluents were monitored at 237 nm. Calibration curve was constructed by plotting peak area Vs concentration and regression equation was computed. R<sup>2</sup> values of AB, VAT and HTZ were found to be as 0.9996, 0.9995 and 0.9996 respectively.

#### Validation of method

The developed method was validated in terms of linearity, specificity, precision, accuracy, robustness and ruggedness.

#### Sample preparation

A total of 20 tablets were taken and accurately weighed and finely powdered. An amount equivalent to 10 mg of AB, 160 mg of VAT and 25 mg of HTZ (one tablet) was taken into 100 ml volumetric flask and dissolved in 50 ml of mobile phase, ultrasonicated for 15 min and filtered through 0.2 µ filter. Final volume was made up to 100ml. From this solution 5ml was taken and diluted to 10ml in a volumetric flask. The diluted solution was analyzed under optimized chromatographic conditions.

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Fig. No 1 Typical Chromatogram of Blank

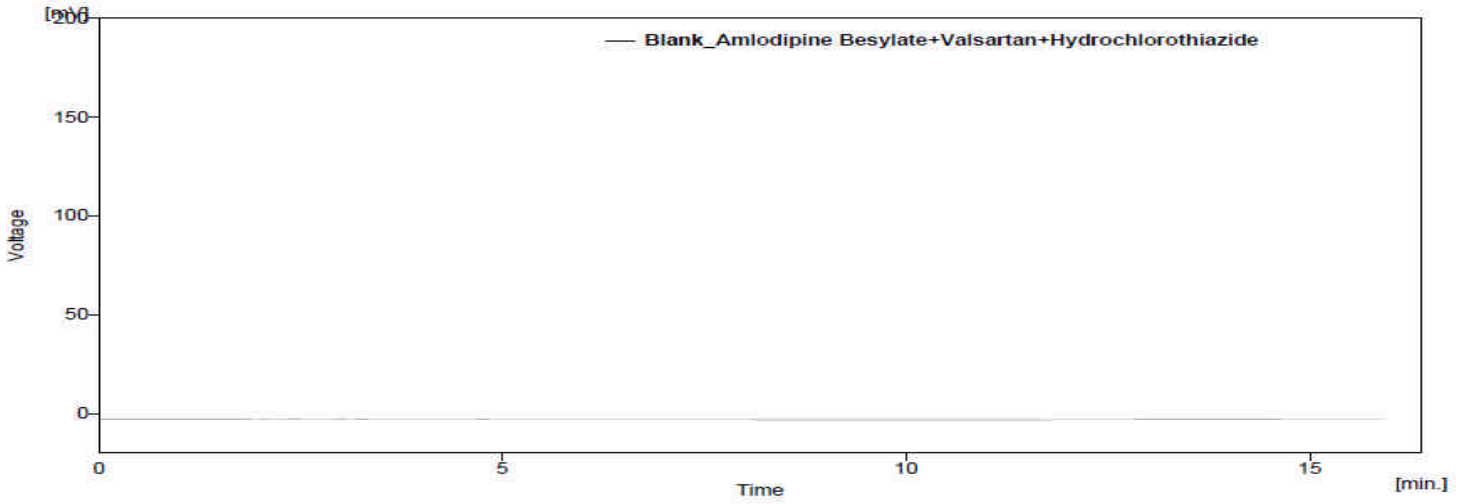


Fig. No 2 Typical Chromatogram of Standard AB, VAT and HTZ

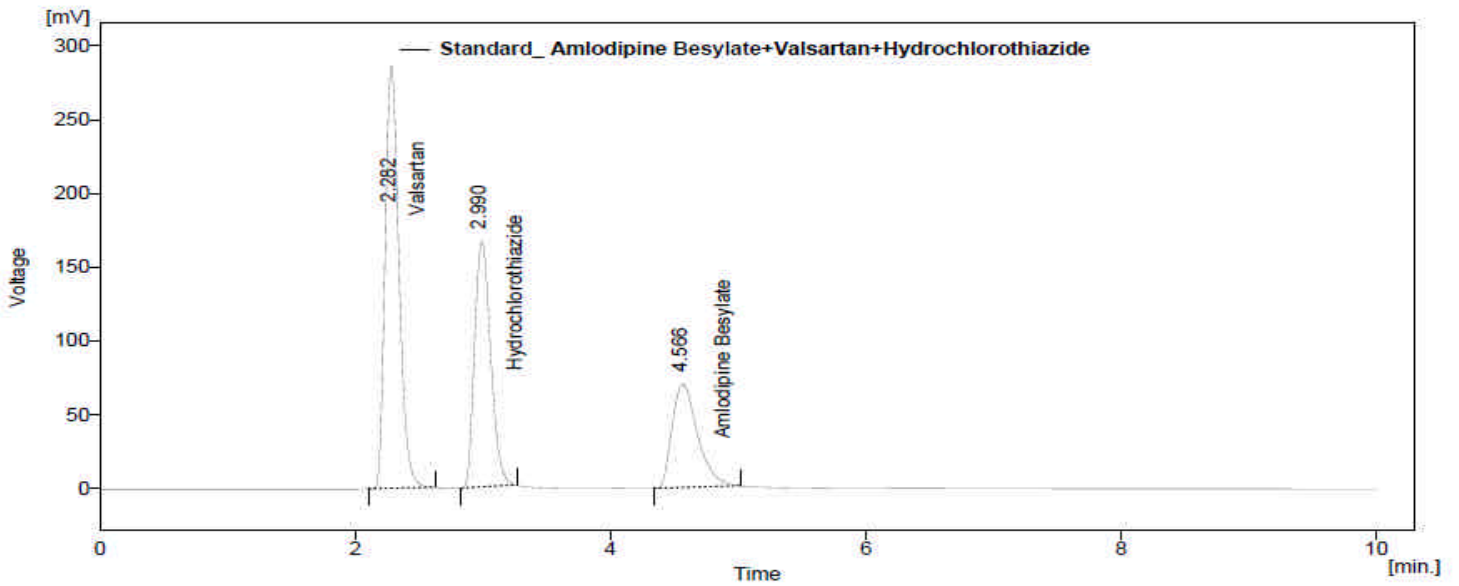


Fig. No 3 Typical Chromatogram of Sample AB, VAT and HTZ

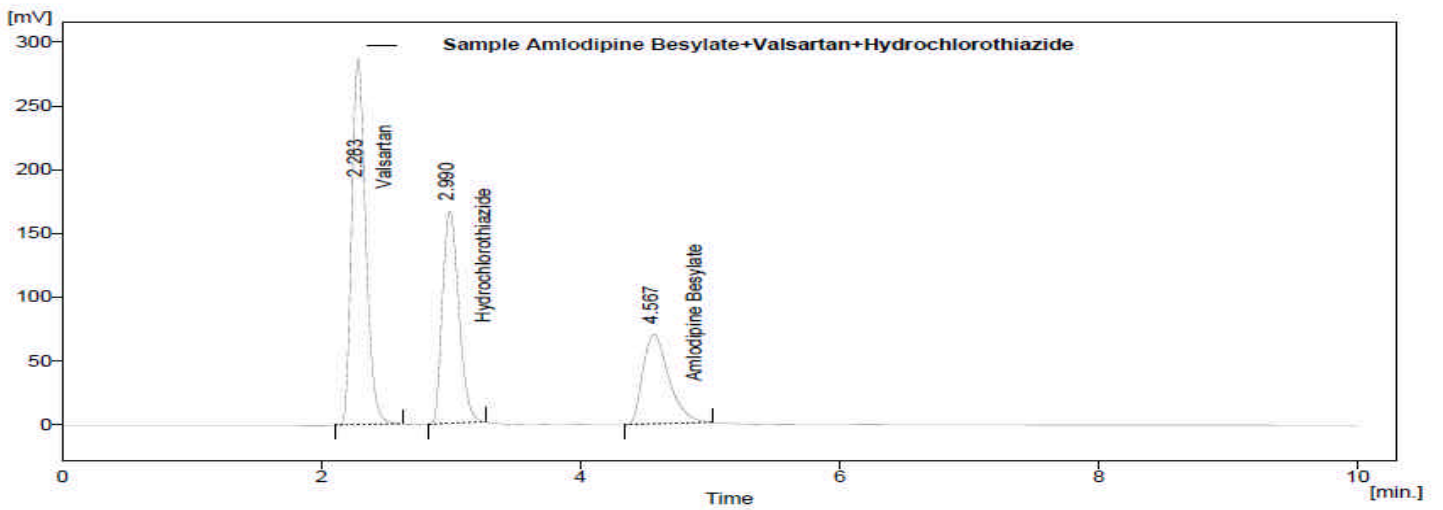


Fig. No 4 Typical Chromatogram of Placebo AB, VAT and HTZ

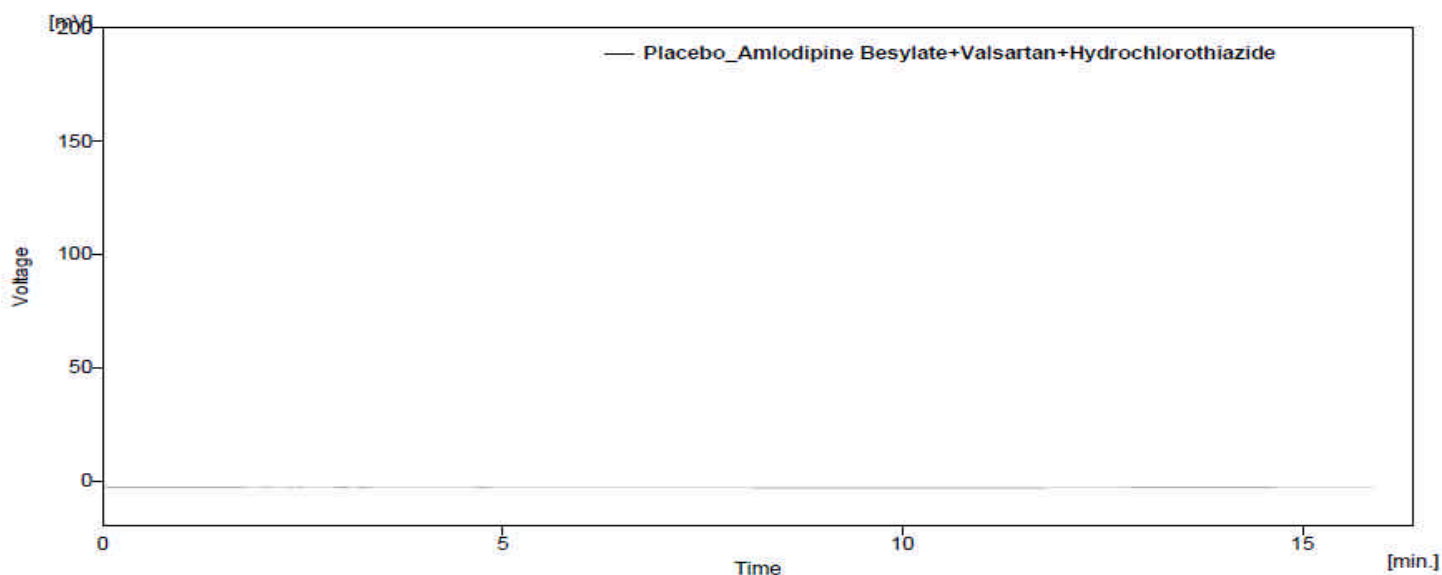


Table No 1 results of System Suitability tests

Parameter	Valsartan (VAT)	Hydrochlorothiazide (HTZ)	Amlodipine Besylate (AB)
% RSD of Retention time	0.071	0.076	0.036
% RSD of Peak Area	0.081	0.101	0.166
Tailing factor	1.389	1.399	1.710
Theoretical Plates	3125	3820	3450
Resolution	-	3.190	5.200

\* Average of 5 readings. \*RSD-Relative Standard Deviation

Table No 2 results of Precision and Intermediate Precision

Amlodipine Besylate (AB)		Valsartan (VAT)		Hydrochlorothiazide (HTZ)	
Precision %RSD	Intermediate Precision %RSD	Precision %RSD	Intermediate Precision %RSD	Precision %RSD	Intermediate Precision %RSD
0.275	0.337	0.200	0.184	0.263	0.232

\* Average of 6 readings.

Table No 3 results of Accuracy (Recovery Studies)

	Amlodipine Besylate (AB) Mean % Recovery	Valsartan (VAT) Mean % Recovery	Hydrochlorothiazide (HTZ) Mean % Recovery
80%	99.21	99.63	99.75
100%	100.09	100.09	100.57
120%	99.86	100.00	99.57

\* Average of 3 readings at each concentration level.

## RESULT AND DISCUSSIONS

To develop a simple, specific, accurate and precise Reverse Phase High Performance Liquid Chromatographic method for simultaneous estimation of AB, VAT, and HTZ, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. System suitability tests were carried as per ICH guidelines and parameters are summarized in table 1.

### Method Validation <sup>(21, 22)</sup>

The proposed RP-HPLC method was validated as per ICH guidelines.

### Specificity

The peak purity of AB, VAT and HTZ were assessed by comparing the retention time of standard AB, VAT and HTZ and sample, good correlation was obtained between the retention time of standard and sample. Placebo and blank were injected and there were no peaks. There are no interferences hence method is specific.

### Linearity

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for AB, VAT and HTZ were found to be as 10-60mcg/ml, 160-960mcg/ml and 25-150mcg/ml respectively. The regression equation for AB, VAT and HTZ were found to be as  $y = 20.705x - 2.9447$ ,  $y = 2.8049x + 17.243$  and  $y = 11.807 + 115.43$  with correlation coefficient ( $R^2$ ) 0.9996, .9995 and 0.9996 respectively.

### Precision

Precision was evaluated by carrying out six independent sample preparations of a single lot of formulation. The sample preparation was carried out in same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% that proves method is precise.

### Accuracy (Recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplet by standard addition method at 80%, 100% and 120% concentration levels. Known amounts of standard AB, VAT and HTZ were added to the pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies are shown in table 3.

### Robustness of the method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in optimized method parameters were done. The effect of change in flow rate, change in pH and change in column temperature retention time and tailing factor were studied. The method was found to be unaffected by small changes like +/- 0.2 in flow rate, +/- 0.1 change in pH, +/-5°C in column temperature.

### Intermediate Precision (Ruggedness)

Different analyst carried out precision studies in a similar manner carried out by first analyst. Percentage relative standard deviation (%RSD) was found to be less than 2% that proves method is rugged.

## CONCLUSION

The proposed method is simple, specific, accurate and precise and hence can be used in routine for simultaneous estimation of AB, VAT and HTZ in tablet dosage. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The %RSD for all parameters was found to be less than one, which indicates the validity of the method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of AB, VAT and HTZ in tablet dosage form.

#### ACKNOWLEDGEMENTS

The authors are thankful to Mr. K. Chandra Shekar, Managing Director of Chandra Labs, Balanagar, Hyderabad for providing necessary facilities and Mr. Mahesh Naidu for his support during this work. We are also thankful to Dr. G.S Kumar for his moral support and guidance.

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**Source of support: Nil, Conflict of interest: None Declared**