Research Article

HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE, HYDROCHLOROTHIAZIDE AND VALSARTAN IN BULK AND TABLET DOSAGE FORM

Bodduluri Anil Kumar[1*] and Pankaj Kumar Sharma[2]

[1]Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur-313024, Jaipur, Rajasthan, India.
[2]School of Pharmaceutical Sciences, Jaipur National University, Jaipur-302025, Rajasthan, India.

ABSTRACT
A new reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for simultaneous estimation of Amlodipine, Hydrochlorothiazide and Valsartan in pharmaceutical dosage forms. Chromatography is carried out at 30°C ± 0.5°C on Inertsil ODS (250mm x 4.6 mm, 5μ. particle size) with a mobile phase composed of Phosphate Buffer and Acetonitrile taken in gradient at a flow rate of 1.0 mL/min and the detection was carried out using a PDA detector at 270nm. Validation parameters such as system suitability, linearity, precision, specificity, accuracy and robustness are studied as reported in the International Conference on Harmonization guidelines. The retention times for Amlodipine, Hydrochlorothiazide and Valsartan are 2.4 min, 3.8 min and 7.5 min. The linearity range for Amlodipine, Hydrochlorothiazide and Valsartan are 2.5 - 15μg/mL, 6.25 - 37.5μg/mL and 80 - 480μg/mL The percentage recoveries of Amlodipine, Hydrochlorothiazide and Valsartan are 100.53%, 100.29% and 100.01%. The RSD for assay samples of tablets is always less than 2%.

Keywords: RP-HPLC, Amlodipine, HCTZ, Valsartan, Simultaneous estimation.

INTRODUCTION
Amlodipine is (*RS*)-3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. Amlodipine is a medication used to lower blood pressure and prevent chest pain. It belongs to a group of medications known as dihydropyridine-type calcium   channel blockers. By widening of blood vessels it lowers blood pressure. In angina, amlodipine increases blood flow to the heart muscle to relieve pain due to angina. Amlodipine is used in the management of hypertension and coronary artery disease.
Hydrochlorothiazide is 6-chloro-1, 1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide.   Hydro   chlorothiazide (abbreviated HCTZ, HCT, or HZT), is a diuretic medication often used to treat high blood pressure and swelling due to fluid buildup. Other uses include diabetes insipidus, renal tubular acidosis, and to decrease the risk of kidney stones in those with high calcium level in the urine. It is in the thiazide medication class and acts by decreasing the kidneys' ability to retain water.
Valsartan is (*S*)-3-methyl-2- (*N*-{[2'- (2*H*-1,2,3,4-tetrazol-5-yl)   biphenyl-4-yl]   methyl} pentan amido) butanoic acid. Valsartan (trade name Diovan) is an angiotensin II receptor antagonist (commonly called an ARB, or angiotensin receptor blocker), that is selective

for the type I (AT1) angiotensin receptor. Valsartan is used to treat high blood pressure, congestive heart failure, and to reduce death for people with left ventricular dysfunction after having had a heart attack. Valsartan blocks the actions of angiotensin II, which include constricting blood vessels and activating aldosterone, to reduce blood pressure[1-3].
Various UV & HPLC assay methods are also reported in the literature for the estimation of Amlodipine, hydrochlorothiazide and Valsartan individually and in-combination with

other drugs[4-6]. According to literature survey there is no official method for the simultaneous estimation of Amlodipine, HCTZ and Valsartan by RP-HPLC in bulk and combined tablet dosage form. Hence, an attempt has been made to develop new method for simultaneous estimation and validation of Amlodipine, HCTZ and Valsartan in bulk and tablet formulation in accordance with the ICH guidelines[7-11].
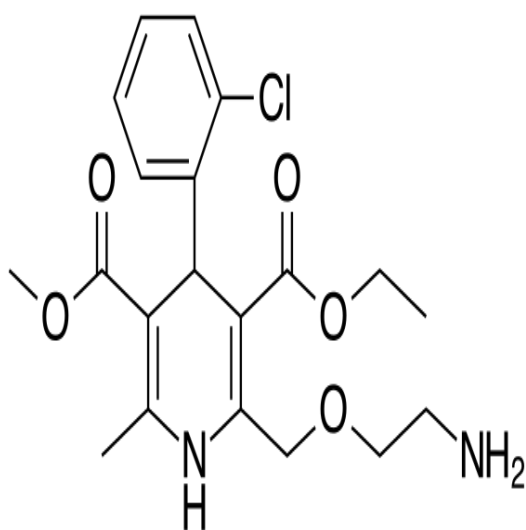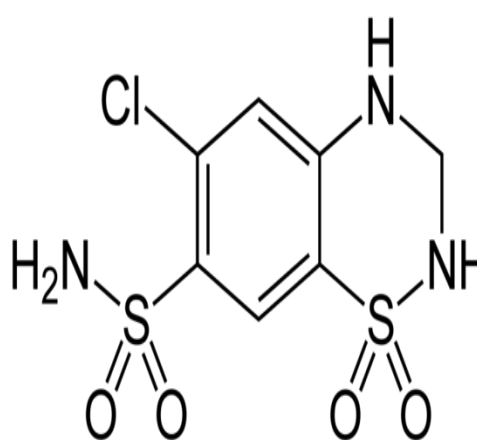

Fig. 1(A): Structure of Amlodipine
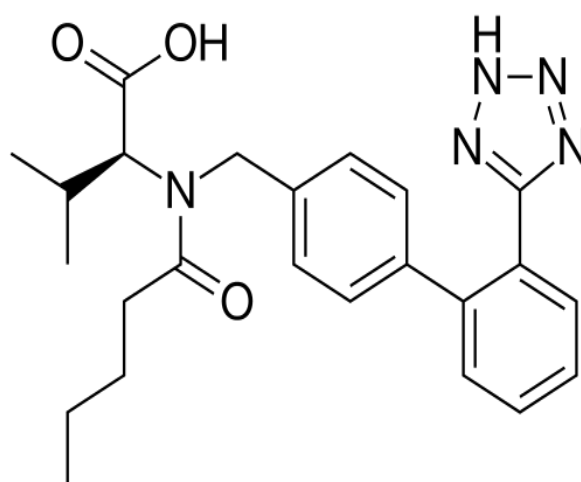

Fig. 1(B): Structure of HCTZ

Fig. 1(C): Structure of Valsartan

EXPERIMENTAL
Instrumentation
Chromatography was performed with Water's 2695 HPLC system provided with Hamilton Syringe, auto sampler and 2996 Photodiode array detector. All HPLC systems were equipped with a column compartment with temperature control and an online degasser. Data acquisition, analysis and reporting were performed by Empower2 software.

Reagents and chemicals
Pharmaceutically pure sample of Amlodipine, HCTZ and Valsartan were obtained from Spectrum Pharma Research Solutions, Hyderabad as gift samples along with their analytical reports. Acetonitrile and Methanol of HPLC grade was obtained from Merck chemical division, Mumbai. Commercial tablets were procured from the local drug market.

Chromatographic condition
Chromatography is carried out at 30°C ± 0.5°C on an Inertsil ODS (250mm x 4.6 mm, 5µ. particle size) with a mobile phase composed of Buffer and Acetonitrile taken in Gradient at a flow rate of 1.0 mL/min. Although the Amlodipine, HCTZ and Valsartan have different λ max, but considering the chromatographic parameters, 270 nm was selected as the detection wavelength for PDA detector.

Preparation of Standard Stock Solution
Accurately Weighed and transferred 5mg of Amlodipine, 12.5mg of HCTZ and 160 mg of Valsartan working Standards into a 50 ml, clean dry volumetric flask, add diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution, 1 ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluents.

Preparation of Working Standard Solutions
Aliquots of 0.25, 0.5, 0.75, 1.0, 1.25 & 1.5 ml were pipetted out from the stock solution and transferred into a 10 ml volumetric flask and volume was made up to 10 ml with diluent. This gives the solutions of 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 µg/ml for Amlodipine and 6.25, 12.5, 18.75, 25.0, 31.25, 37.50 µg/ml for HCTZ and 80, 160, 240, 320, 400, 480 µg/ml Valsartan, respectively.

Sample preparation
20 tablets were weighed and powdered and it was taken into a 50ml volumetric flask and made up with diluents and labeled as Sample stock solution. Sample stock solution was filtered by HPLC filters.1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluents.

Method validation
System suitability tests
To ensure the resolution and reproducibility of the HPLC system was adequate for the analysis, a system suitability test was established. Data from six injections of 10µl of the working standard solutions of Amlodipine, HCTZ and Valsartan were used for the evaluation of the system suitability parameters like tailing factor, the number of theoretical plates, retention time and resolution factor.

Linearity
The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. By appropriate aliquots of the standard Amlodipine, HCTZ and Valsartan solutions with the mobile phase, six working solutions ranging between 2.5 - 15µg/ml, 6.25 – 37.5µg/ml and 80 - 480µg/ml were prepared. Each experiment linearity point was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of Amlodipine, HCTZ and Valsartan to obtain the calibration curve.

Accuracy
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of Amlodipine, HCTZ and Valsartan to which known amounts of standard Amlodipine, HCTZ and Valsartan corresponding to 50%, 100% and 150% of target concentration were added. The accuracy was expressed as the percentage of analyte recovered by the proposed method.

Precision
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines.

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision. Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc. The repeatability and intermediate precision were determined by analyzing the samples of Amlodipine, HCTZ and Valsartan.

Limit of detection and the limit of quantification
Limit of detection (LOD) and limit of quantification (LOD) of AMLO, HCTZ and VAL were determined by calibration curve method. Solutions of AMLO, HCTZ and VAL were prepared in linearity range and injected (n=3). Average peak areas were plotted against concentration. These were calculated by using following equations. $LOD = (3.3 \times Syx)/b$, $LOQ = (10.0 \times Syx)/b$
Where Syx is residual variance due to regression; b is slope.

Robustness
The robustness of the method was performed by changing the chromatographic conditions. The organic strength was varied by ±5%, column temperature was varied by ±5^{0}c and the flow rate ±0.1mL.

RESULT AND DISCUSSION
Method development
Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol: Water, Acetonitrile and Water as mobile phases, in which these drugs did not show good resolution. The organic portion of mobile phase was changed to optimize the separation of these drugs. The pH of mobile phase was changed to get good plate count, tailing factor and area. At pH: 3.3 both drugs eluted with better separation. Thereafter, phosphate buffer and acetonitrile were taken in gradient with flow rate of 1.0 mL/min was employed. Inertsil ODS 250mm x 4.6 mm, 5μ particle size was used as the stationary phase to improve resolution and the tailing of three peaks was reduced considerably. The detection of these drugs was tried at various wavelengths from 215nm to 280nm. The wavelength at which Amlodipine, HCTZ and Valsartan showed maximum absorption at 270nm was selected as the detection wavelength for PDA detector. The retention times were found to about 2.4 min, 3.8 min and 7.5 min for Amlodipine, HCTZ and Valsartan, respectively. The chromatogram obtained was shown in the figure 2.
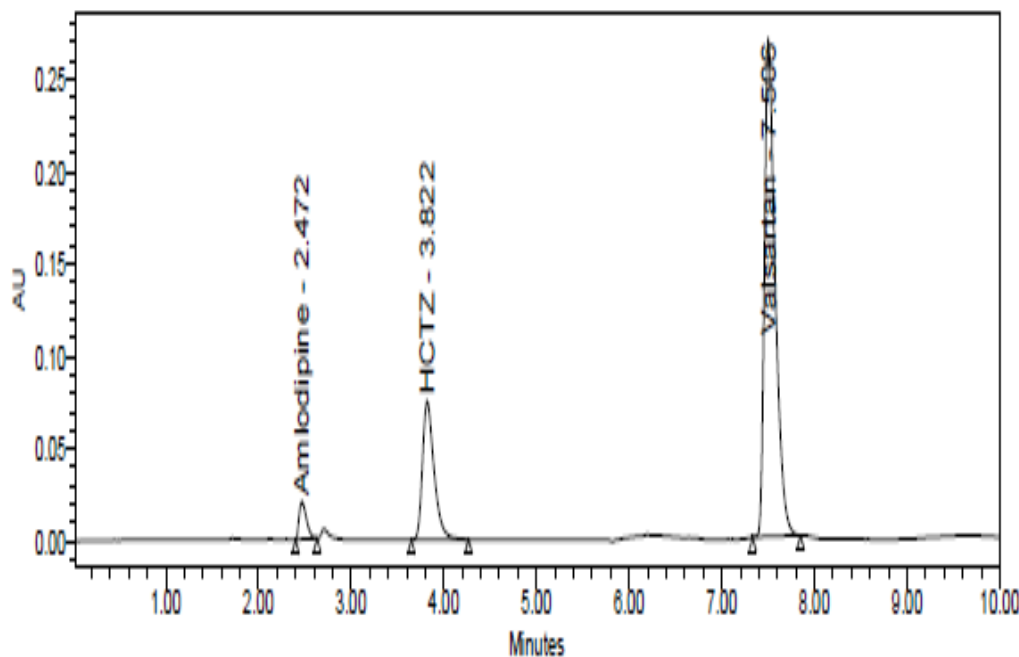

Fig. 2: Representative chromatogram of Amlodipine, HCTZ and Valsartan

Method Validation
System suitability
System suitability parameters such as number of theoretical plates, area and peak tailing were determined. RSD of AMLO, HCTZ and VAL areas were found to be 0.67,0.4 and 0.6, respectively.

Linearity
AMLO, HCTZ and VAL showed a linearity of response between 2.5 - 15µg/ml, 6.25 – 37.5µg/ml and 80 - 480µg/ml (Figure 3A, 3B, 3C) and the linearity were represented by a linear regression equation.
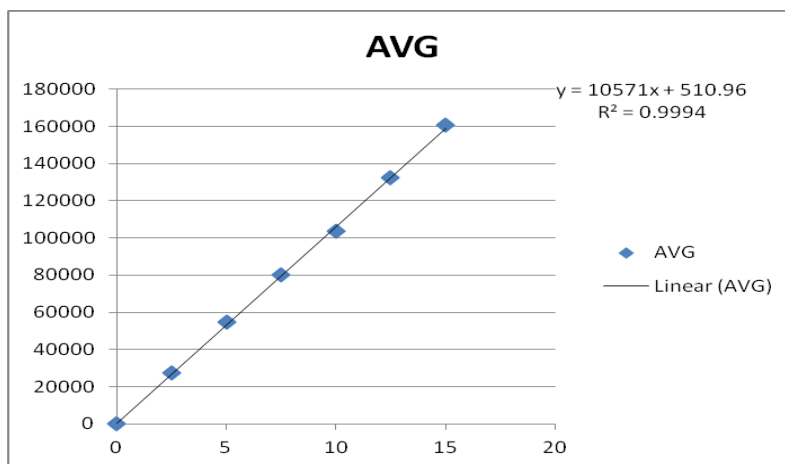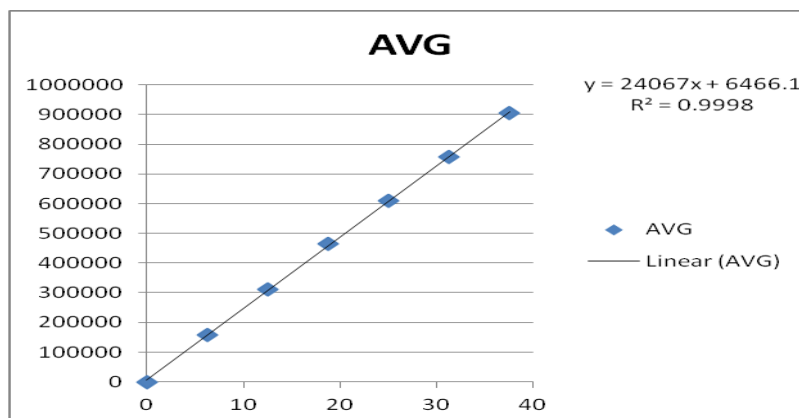

Fig. 3(A): Calibration Curve for Amlodipine
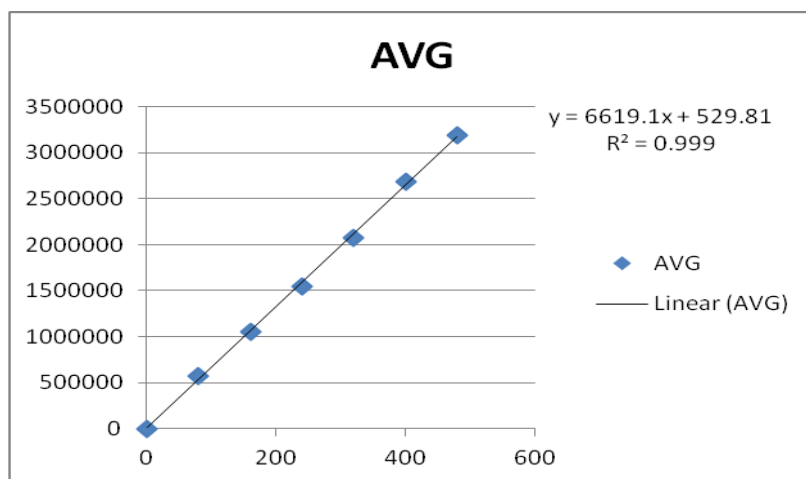

Fig. 3(B): Calibration Curve for HCTZ

Fig. 3(C): Calibration Curve for Valsartan

Accuracy

To Preanalysed sample solution, different concentrations of standard drug were added and recovery was studied and found with in the limits.

Precision
Repeatability

Six replicates in same concentration were analyzed in same day for repeatability and results were found to be within acceptable limits (RSD <2) as shown in table 1**.**

Intermediate Precision

Six replicates in same concentration were analyzed on two different days for day to day variation and results were found to be within acceptable limits (RSD<2) as shown in table 1.

Table 1: Precision of AMLO, HCTZ and VAL

	Repeatability (%Assay)			Day to Day (%Assay)		
	AMLO	HCTZ	VAL	AMLO	HCTZ	VAL
%Mean	100.46	99.90	100.96	100.40	100.60	100.32
SD	1.9	0.9	0.7	0.95	0.41	0.74
%RSD	1.9	0.9	0.7	0.94	0.41	0.73

Robustness

This was carried out by changing mobile phase by ±5%, column temperature ±5^{0}c and the flow rate ±0.1mL. There were no significant changes in the chromatography pattern when the above modifications were made in the experimental conditions, showing that the method is robust.

Stability of sample solution

The sample solution injected after 24 hr did not show any appreciable change.

LOD and LOQ

LOD and LOQ of AMLO, HCTZ and VAL were determined by calibration curve method and the results were shown in the Table 2.

Table 2: LOD and LOQ of AMLO, HCTZ and VAL

	LOD (µg/ml)	LOQ (µg/ml)
AMLO	0.09	0.28
HCTZ	0.35	1.08
VAL	0.19	1.57

Tablet Analysis

Content of AMLO, HCTZ and VAL found in the tablets by the proposed method were 100.3-100.60%.

CONCLUSION

RP-HPLC method was developed and validated for simultaneous estimation of Amlodipine, HCTZ and Valsartan in tablet dosage form. The regression value was found to be 0.999 for Amlodipine, HCTZ and Valsartan, which shows the response, is linear from 2.5 - 15µg/mL, 6.25 – 37.5µg/mL and 80-480µg/mL respectively. Selectivity experiment showed that there is no interference or overlapping of the peaks either due to excipients or diluents with the main peak of Amlodipine, HCTZ and Valsartan. The percentage RSD for precision is <2 which confirms that method is sufficiently precise and the total run time required for the method is only 10 minutes for eluting Amlodipine, HCTZ and Valsartan. So, this method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing three drugs in industries.

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