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VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF AMLODIPINE, HYDROCHLOROTHIAZIDE AND VALSARTAN IN PHARMACEUTICAL FORMULATION

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Abstract

An accurate, precise and reproducible reverse-phase stability-indicating HPLC method has been developed and validated for the simultaneous estimation of Amlodipine, Hydrochlorothiazide and Valsartan in combined dosage form. Separation was achieved using Phenomenex ODS 5µ, C_{18} column (250×4.6mm) and ACN : Methanol : 1% Triethylamine Buffer (39:26:35) as mobile phase which shows sharp and resolved peak at a flow rate 1.2 mL/min and UV detection at 235 nm. The linearity of the proposed method was investigated in concentration range of 4-20 µg/mL(r = 0.999) for AML, 10-50 µg/mL(r = 0.999) for HTZ and 64-320 µg/mL(r = 0.999) for VAL. The retention time for AML, HTZ and VAL were found to be 2.43, 3.62 and 6.15 respectively. The drugs were subjected to acid degradation, alkali degradation, oxidative degradation and neutral degradation. The mean % estimation and recovery of the drugs was found near to 100 % representing the accuracy of the method. Validation of the proposed method was carried out for its accuracy, precision, specificity and ruggedness according to ICH guidelines. The proposed method can be successfully applied in routine work for the determination of amlodipine, hydrochlorothiazide and valsartan in combined dosage form.

Keywords: Amlodipine, Hydrochlorothiazide, Valsartan, RP-HPLC method.

1. Introduction

Amlodipine besylate (AML) is a drug used in the treatment of hypertension. It acts by inhibiting Ca^{2+} mediated slow channel component of action potential in smooth / cardiac muscle cell. AML chemically[1] is 3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy) methyl]-4- (2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5 pyridinedicarboxylate monobenzenesulphonate. It is calcium channel blocker and freely soluble in methanol. Hydrochlorothiazide (HTZ)

is a drug used in the treatment of hypertension. It blocks the reabsorption of Na^+ in the distal convoluted tubules by inhibiting the luminal membrane bound Na^+ / Cl^- cotransport system. HTZ[2] chemically 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide 1,1 dioxide. It is a diuretic and highly soluble in methanol. Valsartan is a drug used in the treatment of hypertension. It reduces blood pressure by depressing the activity of the sympathetic nervous system. VAL chemically[3] is N-[p-(o-1H-Tetrazol-5-ylphenyl) benzyl]-N- valeryl-L valine. It is angiotension-II receptor antagonist and freely soluble in methanol and ethanol. Literature survey revealed that the methods reported earlier were only for the analysis of single drug and in combination of drugs. Methods are reported for AML with HTZ[4], Valsartan[5] and HTZ with VAL[6], Bisoprolol fumarate[7], Telmisartan[8] for their determinations in pharmaceutical formulation. This paper presents simple, accurate, reproducible, rapid RP-HPLC method for simultaneous analysis of the three components in tablet formulation.

2. Material and Method

2.1 Instrumentation and Condition

Shimadzu HPLC 1100 series chromatograph equipped with binary pump LC-10 ADvp, SPD-10 UV detector, Rheodyne Manual injector 7725i with 20µL loop and a reversed-phase 5µ Phenomenex ODS C18 column (250 x 4.6 mm) with pore size of 100 ^oA was used for the chromatographic studies and ACN: Methanol: 1% Triethylamine Buffer (39:26:35) as mobile phase which shows sharp and resolved peak at a flow rate 1.2 mL/min and UV detection at 235 nm. Shimadzu AUX220 balance was used for weighing the samples. Double distilled water and whatman filter paper (no.41) were used throughout the experimental work.

2.2 Materials

Multi-component tablet Exforge HCT (AML 10mg, HTZ 25mg and VAL 160mg) manufactured by Novartis AG pharmaceutical Ltd. All chemicals and reagents used were of HPLC grade. All the pure drugs samples supplied by Torrent Pharmaceuticals Ltd., Gujarat and were used without further purification.

2.3 Preparation of stock and standard solution

Mix Stock solution containing AML, HTZ and VAL was prepared in mobile phase having concentration 100µg/ mL AML, 250µg/ mL HTZ and 1600µg/ mL VAL in 50mL volumetric flask. Aliquot of the standard solution was appropriately diluted with the mobile phase to get the concentration of 10µg/mL for AML, 25µg/mL for HTZ and 160µg/mL for VAL respectively.

2.3.1 Preparation of buffer solution

Triethylamine, 10 mL was dissolved in 1000.0 mL of double distilled water and pH was adjusted to 3.0 with orthophosphoric acid.

2.4 Development and validation of HPLC Method

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of amlodipine, hydrochlorthiazide, valsartan in tablet dosage form. The method was validated for the parameters like system suitability, linearity, accuracy, precision, and robustness.

2.4.1 System suitability

The system suitability was assessed by six replicate analysis of amlodipine, hydrochlorthiazide, valsartan at a 100% level to verify the resolution and reproducibility of the chromatographic system adequate for the analysis to be done. This method was evaluated by analyzing the repeatability of retention time, peak area for amlodipine, hydrochlorthiazide, valsartan tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks of amlodipine, hydrochlorthiazide, valsartan.

2.4.2 Linearity and Range

Linearity of the method was determined by constructing calibration curves. Standard solutions of amlodipine, hydrochlorthiazide, valsartan at different concentrations level (80%, 90%, 100%, 110%, and 120%) were used for this purpose. Aliquots of mixed standard stock solution were diluted in range 1.0 mL to 5.0 mL in a series of 25 mL volumetric flasks with mobile phase, volumes were made up to mark with mobile phase to obtain concentration 4-20µg/mL for aml, 10-50µg/mL for HTZ and 64-320µg/mL for Val. Before injection of the solutions, the column was equilibrated for at least 30 min with the mobile phase. The peak areas of the chromatograms were plotted against the concentrations of amlodipine, hydrochlorthiazide, valsartan to obtain the calibration curves. The five concentrations of the standard were subjected to regression analysis to calculate calibration equation and correlation coefficients.

2.4.3 Accuracy

The accuracy is the closeness of agreement between the true value and test result. Accuracy was determined by means of recovery experiments, by addition of active drug to placebo formulations. The accuracy was calculated from the test results as the percentage of the analyte recovered by the assay.

2.4.4. Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) study. Repeatability was determined by performing four repeated analysis of the three standard solutions (90%, 100% and 110% of target concentration) of amlodipine, hydrochlorthiazide, valsartan on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis of previous standard solutions on three different days (inter-day) in the same laboratory. The relative standard deviation (% RSD) was determined in order to assess the precision of the method.

2.4.5 Assay in Marketed Formulation

An accurately weighed quantity of tablet powder equivalent to 5.0 mg of AML (~12.5 mg of HTZ and ~80 mg of VAL) was transferred to 50.0 mL volumetric flask, sonicated for 30 minutes with sufficient quantity of mobile phase and volume was made up to mark with mobile phase. The contents of the flask were filtered through whatman filter paper (no.41). A 1.0 mL portion of the filtrate was further diluted to 25.0 mL volumetric flask with a mobile phase. The sample solution was injected and the chromatogram was recorded. The content of AML, HTZ and VAL were calculated by comparison of the standard area with sample area and results are shown in Table 1.

3. RESULTS AND DISCUSSION

3.1 Selection of Detection Wavelength

The standard solution of each drug prepared (10µg/ mL) and scanned over the range 200- 400 nm. The overlay spectrum recorded is shown in the Fig 1.

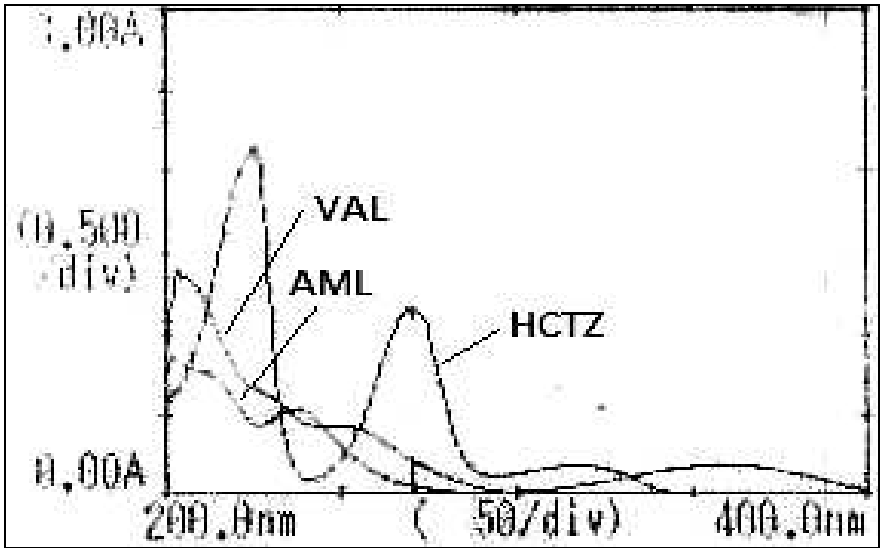

Fig 1: Overlain spectra of Amlodipine, Hydrochlorothiazide & Valsartan.

3.2 HPLC method development and optimization

The optimized chromatographic condition mentioned below was kept constant throughout the experimentation and mobile phase was allowed to equilibrate with stationary phase which was indicated by a steady line.

Column - Phenomenex ODS 5 µ C18 column (250 X 4.6mm)

Detection Wavelength - 235 nm

Flow rate - 1.2 mL/min

Temperature: Ambient - (28-30^0 C)

pH - 3.0

Mobile Phase - ACN : METH : Buffer :: 39 :26 :35

A 20 µL solution of above mix standard was injected through manual injector and chromatogram was recorded using mobile phase containing Acetonitrile, Methanol and Buffer (39: 26: 35). Amlodipine, Hydrochlorothiazide and Valsartan were resolved properly with sharp peak and showing reasonable retention time in the above selected mobile phase. A standard chromatogram for all three drugs so recorded in shown in fig 2.

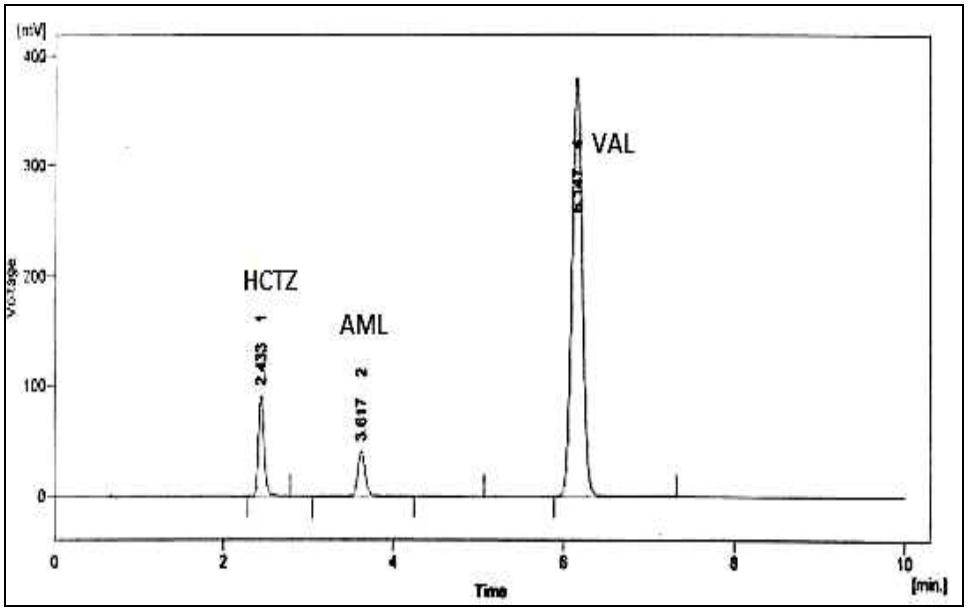

Fig 2: Chromatogram for AML, HTZ and VAL

3.3 Study of system suitability parameters

After equilibration of column with mobile phase, seven replicate injections of 20 µL solution of mix standard solution was injected through the manual injector and the chromatograms were recorded and the system suitability parameter were noted and values are shown in Table 1.

3.4 Study of Linearity

The graphs of concentration of drug vs. area under curve were plotted for both the drugs. The correlation coefficient was found to be (*r=0.999*) for AML, (*r=0.999*) for HTZ and (*r=0.999*) for VAL respectively.

3.5 Linearity and Range

Accurately weighed quantities of tablet content equivalent to about 80, 90, 100, 110 and 120% of label claim of AML were taken and dilutions were made as described under assay. The chromatograms of the resulting solutions were recorded. The plot of AUC Vs Percent label claim was found to be linear with correlation coefficient of 0.998 for AML, 0.999 for HTZ and 0.999 for VAL.

From all the observation, it was concluded that the optimised chromatographic conditions gave well resolved and sharp peaks of AML, HTZ and VAL with retention times 2.43, 3.62 and 6.15 respectively. It was observed that the proposed method can be easily applied to marketed formulation and the statistical parameter viz. S.D., CV is in the acceptable range for quantitative determination of AML, HTZ and VAL.

3.6 Accuracy

To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analysed sample and contents were reanalysed by proposed method and the mean % recovery were found to be 100.92, 99.47 and 99.06 for AML, HTZ and VAL respectively, shown in Table 1.

Table 1: Observations of System Suitability Parameter.

Parameter	**Values**		
	HTZ	**AML**	**VAL**
Mobile phase	[Acetonitrile, Methanol and Triethylamine Buffer (39:26:35)]		
Retention time:	2.43	3.62	6.15
Asymmetry:	1.15	1.31	1.04
Resolution:	-----	8.4	10.9
Efficiency (tl/m):	33275	26778	47095

3.7 Precision and Intermediate precision

Precision and Intermediate precision (Intraday and Interday) shows the % Label claim values within limits (% R.S.D. not more than 2). The method was found to be précised. The ruggedness studies were carried out using

different analyst variation. The results of intermediate precision and ruggedness parameter are shown in Table 2.

Table 2: Intermediate precision and Ruggedness study.

Parameters	Mean % label claim ±S.D.		
	AML	HTZ	VAL
Different Analyst (n=3)	100.41 ±1.184	99.43±1.370	100.59±1.059
Intraday Variation (n=3)	99.40±0.977	100.56 ±0.633	98.90±0.336
Inter day Variation (n=3)	103.81±3.134	100.71±2.673	98.02±1.928

3.8 Stability studies

Sample solution: Accurately weighed quantities of tablet content equivalent to about 5.0 mg AML (~12.5 mg of HTZ and ~80.0 mg VAL) were transferred to 50.0 mL volumetric flask. These entire samples were stored for 24 hrs under following different conditions.

At 50^0C after addition of 20.0 mL of 0.1N NaOH (Alkali), 0.1N HCl (Acid), 3% H_2O_2 (Oxide) and DDW (Distilled Water)

After 24 hrs, shaken for 20 min with sufficient quantity of mobile phase and volume was made up to the mark. The contents of the flask were filtered through whatman filter paper (no. 41). A 1.0 mL portion of the filtrate was further diluted to 10.0 mL with a mobile phase. Sample solution were injected separately, after equilibration of stationary phase, the chromatograms were recorded. The chromatograms were recorded. The amount estimated for AML, HTZ and VAL under different stress conditions like acids, base, oxide and water were found to be different when compared with untreated sample (result obtained by proposed method under the normal condition) shown in Table 3. Hence, we can say that all the three drugs had undergone degradation.

Table 3: Forced degradation study.

Sr. No.	Condition	A.U.C. (mV)			% Label Claim		
		AML	HTZ	VAL	AML	HTZ	VAL
1	Alkali	17.36	489.16	4272.77	6.19	110.02	104.56
2	Acid	88.11	432.79	3332.24	31.89	98.72	82.70
3	Oxide	198.95	426.24	3730.35	70.85	95.68	91.11
4	Water	239.1	462.28	4105.87	86.04	104.85	101.33

The study of chromatogram reveals that in basic condition HTZ shows two peaks, AML shows four peaks and VAL shows two peaks. In acidic condition HTZ shows two peaks, AML shows four peaks and VAL shows single peak. In oxidizing condition HTZ shows two peaks, AML shows two peaks and VAL shows two peaks. In neutral treatment HTZ shows single peak, AML shows two peaks and VAL shows single peak (Fig 3A-D). The retention time and percent area of degraded peaks in various conditions are shown in Table 4.

Table 4: Precent area of degraded peaks in forced degradation study.

Sr. No.	Condition	% Area of peaks					
	Retention time	2.640	3.127	3.170	3.710	4.983	5.600
1	Alkali	0.2	0.2	-	-	-	0.1
2	Acid	-	-	0.1	0.2	-	-
3	Oxide	-	-	-	-	0.1	-

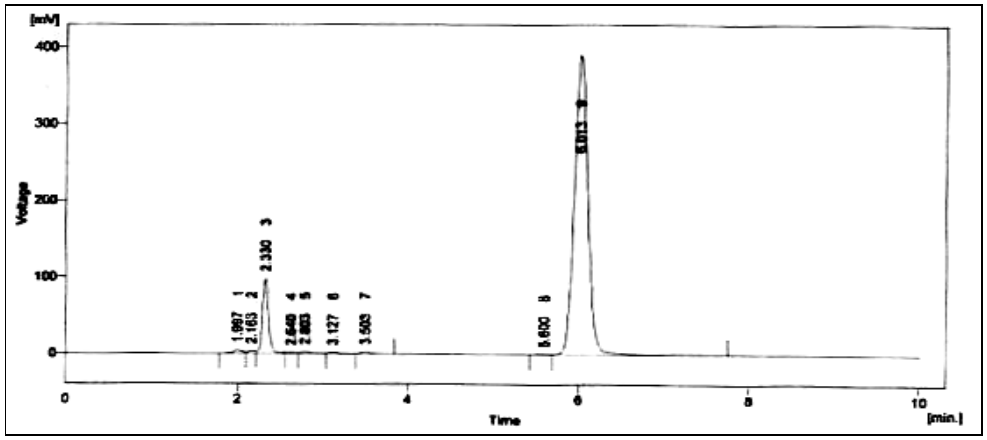

Fig 3A: Chromatogram of powder formulation (0.1 N NaOH)

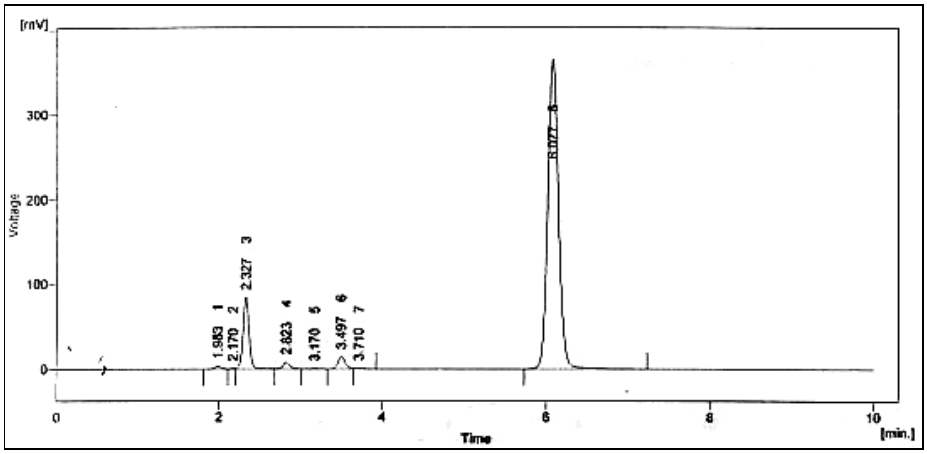

Fig 3B: Chromatogram of powder formulation (0.1 N HCl)

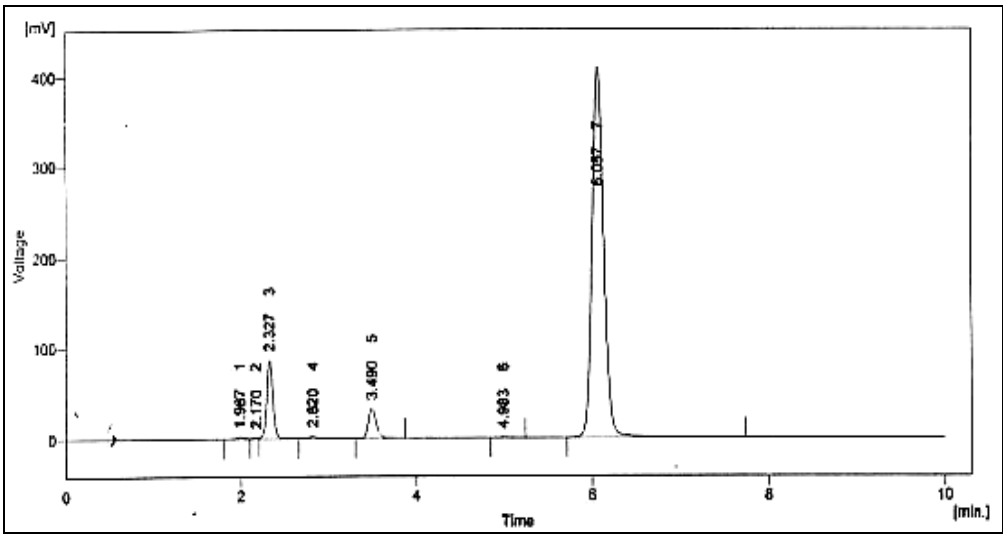

Fig 3C: Chromatogram of powder formulation (3% H_2O_2)

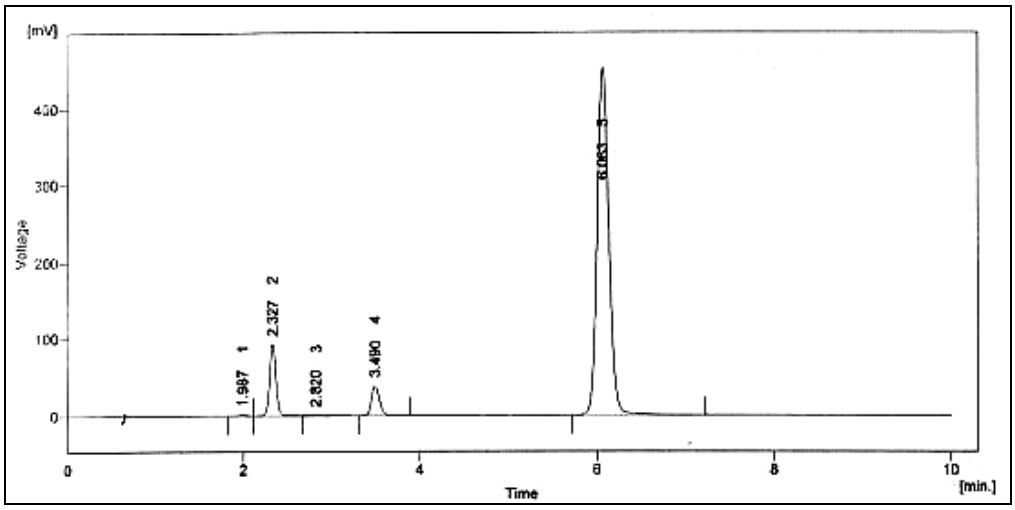

Fig 3D: Chromatogram of powder formulation (DDW)

4. Conclusion

The results obtained by the proposed method for determination of AML, HTZ and VAL are reliable, accurate and precise. The values of standard deviation were found satisfactory and the recovery studies were close to 100%. The method does not require prior separation of one drug from other. Hence it can be employed for routine quality control analysis of AML, HTZ and VAL in combined dosage form.

5. Acknowledgements

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