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Development and Validation of a RP-HPLC Method For Simultaneous Determination of Amlodipine Besylate and Hydrochlorthiazide in Tablet Dosage Form

Sharma Hemandra Kumar *, Vinod Sahu, Rahul Sahu, Neha Sengar, Sneha Kulkerni

Sagar Institute of Research and Technology-Pharmacy,
Ayodhya Bypass Road, Bhopal, M.P.

ABSTRACT

A simple, precise, rapid and reproducible reversed-phase high performance liquid chromatography (RP-HPLC) method is developed for the simultaneous estimation of amlodipine besylate (AML) and hydrochlorthiazide (HCZ) present in multicomponent dosage forms. Chromatography is carried out isocratically at 25°C ± 0.5°C on an Prontosil C-18 column (4.6 x 250mm, 5 particle size) with a mobile phase composed of acetonitrile: methanol: phosphate buffer (pH-3) in the ratio of 48:12:40 v/v/v at a flow rate of 1.2 mL/min. Detection is carried out using a UV-PDA detector at 232 nm. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the International Conference on Harmonization guidelines. The retention times for AML and HCZ are 3.93 ± 0.5 min and 2.61 ± 0.5 min respectively. The linearity range and percentage recoveries for AML and HCZ are 5-25µgml^{-1} for both drugs and 99.05, 98.73% respectively. The correlation coefficients for all components are close to 1. The relative standard deviations for three replicate measurements in three concentrations of samples in tablets are always less than 2%.

KEYWORDS: RP-HPLC, Amlodipine besylate, Hydrochlorthiazide, Simultaneous estimation.

***For correspondence:**

Hemendra Kumar Sharma

Sagar Institute of Research and Technology-Pharmacy,

Ayodhya bypass Road, Bhopal (M.P.) – 462036(India).

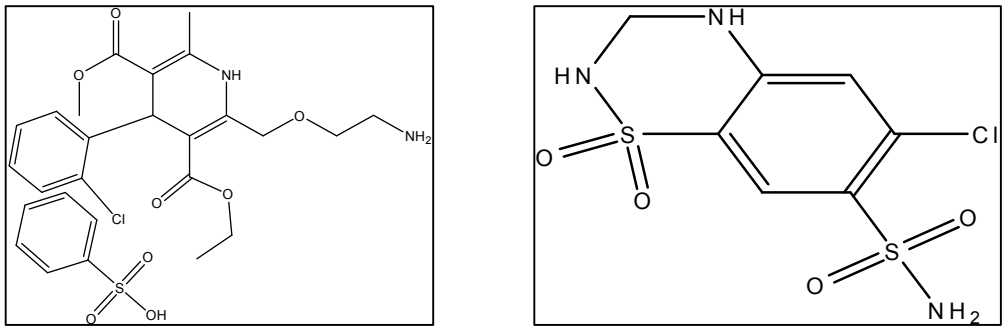
Email: hemendrasharma05@gmail.com

Mobile. No-+917415551032

INTRODUCTION

Amlodipine besylate **(Fig.1A)**[1] is 3-Ethyl 5-methyl (4*RS*)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate is a calcium channel blocker and widely used in the treatment of hyprtension[5].

Hydrochlorthiazide **(Fig.1B)**[1] is 6-chloro-3, 4-dihydro-2*H*-1,2,4-benzothiadiazine-7- Sulphonamide 1, 1-dioxide is diuretic and antihypertensive drug, which inhibits the reabsorption of sodium and calcium at the beginning of distal convoluted tubules[5].



(A) **(B)**

Figure-1: Chemical structures of (A) Amlodipine besylate, (B) Hydrochlorthiazide

Tablet dosage forms containing AML and HCZ in ratio of 5 mg and 12.5 mg are available in market. Literature surveyrevealed that various methods such as UV[9], [10, 15, 16, 19, 20],

HPLC [11, 17], HPTLC[12, 18], LC-MS/MS[13] and UPLC[14] are available in single and combination with other drugs. However, no RP-HPLC method has yet been reported for simultaneous estimation of amlodipine besylate and hydrochlorthiazide in tablet dosage forms. Hence, an attempt has been made to develop and validate in accordance with ICH guidelines [7, 8].

EXPERIMENTAL

INSTRUMENTATION

Liquid chromatographic system from Young Lin 9100 comprising of manual injector, YL 9111 quaternary pump for constant flow and constant pressure delivery and Photodiode array detector YL

9160 detector connected to software YL clarity for controlling the instrumentation as well as processing the data generated was used.

REAGENTS AND CHEMICALS

Pharmaceutically pure sample of amlodipine besylate was obtained from Sun pharmaceuticals, Silvasa(GJ and hydrochlorthiazide was obtained from Matrix laboratory Mumbai as gift samples along with there analytical reports. Methanol AR grade was obtained from Merck chemical division, Mumbai and Commercial tablet of amlodipine besylate (5mg) and Hydrochlorthiazide (12.5mg) were procured from the local drug market.

CHROMATOGRAPHIC CONDITION

The isocratic mobile phase consisted of acetonitrile: methanol: phosphate buffer (pH-3) in the ratio of 48:12:40 v/v/v at a flow rate of 1.2 ml min^{-1}. An Prontosil C-18 column (4.6 x 250mm, 5 particle size) was used as the stationary phase. Although the AML and HCZ have different λmax viz 238 and 271nm respectively, but considering the chromatographic parameter, sensitivity and selectivity of method for both drugs, 232 nm was selected as the detection wavelength for UV-PDA detector.

STANDARD SOLUTION PREPARATION

STANDARD STOCK SOLUTION

Standard stock solutions of 1000 µg ml^{-1} of AML and HCZ were prepared in mobile phase respectively.

WORKING STANDARD SOLUTION

Working standard solutions were prepared by taking dilutions ranging from 5-25 µg/ml for AML and HCZ respectively.

SAMPLE PREPARATION

Twenty tablets of amlodipine and hydrochlorthiazide combination were weighed and crushed to fine powder. Powder equivalent to 5mg amlodipine besylate was weighed and dissolved in 100 ml mobile phase, sonicated for 10 min and filtered through whatmann filter paper No. 42, finally different concentrations of tablet sample were prepared by serial dilution technique.

RESULTS AND DISCUSSION

CHROMATOGRAPHY

Initially reverse phase LC separation was tried to develop using methanol and water (60:40) as mobile phase, in which amlodipine gave tailing of 2.6 although hydrochlorthiazide responded properly, and the resolution was also poor. The organic content of mobile phase was also investigated to optimize the separation of both drugs. To improve the tailing factor, the pH of mobile phase becomes important factor. At pH 3 the signal to noise ratio for AML is less and RT was also 3.92 mins. Thereafter, buffer (pH-3) in the ratio of 48:12:40 v/v/v was selected to improve resolution and the tailing for the two peaks were reduced considerably and brought close to 1. To analyze both drugs detection were tried at various wavelengths from 225nm to 290nm. Initially 232 was selected, considering the λmax of both drugs (λmax of amlodipine 238nm and λmax of hydrochlorthiazide 271 nm) chromatogram (Fig.2).

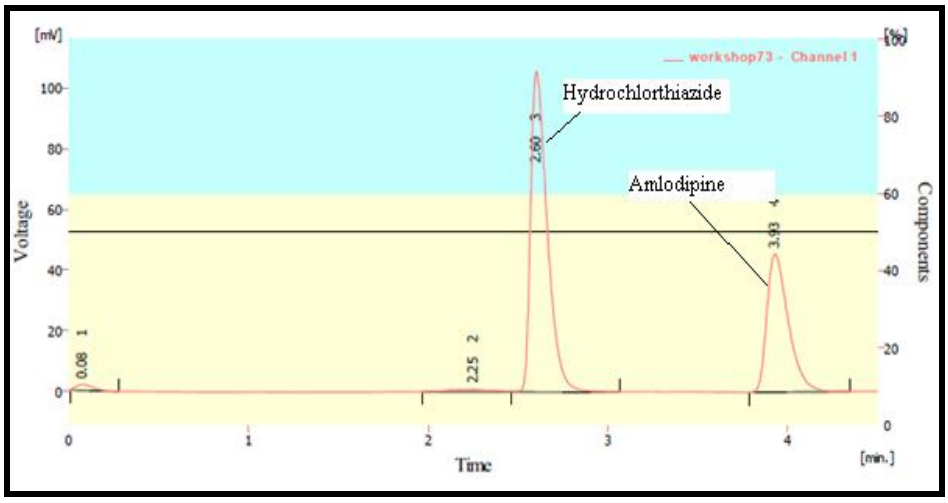


Figure 2: Representative chromatogram of amlodipine and hydrochlortiazide

SYSTEM SUITABILITY

System suitability parameters such as number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in Table No.1. The number of theoretical plates for AML and HCZ were 5123 and 4285 respectively.

Table 1 : System suitability

S.No.	Parameters	AML	HCZ
1.	No. of Theoretical plates	5123	4285
2.	HETP	0.035	0.054
3.	Tailing factor	1.58	1.72

LINEARITY

AML and HCZ showed a linearity of response between 5-25µg ml^{-1} and the linearity was represented by a linear regression equation as follows.

Y (AML)=65.22 conc. + 1.91 (r^2=0.9999)

Y (HCZ)= 95.42conc. + 3.07 (r^2=0.9997)

ACCURACY

Recovery studies were performed to validate the accuracy of developed method. To preanalyzed sample solution, a definite concentration of standard drug was added and recovery was studied. These results are summarized in Table No. 2.

Table 2 : Results of Recovery Experiments

Serial. No.	Conc. of drug in preanalyzed samples (µg/ml)		Std. drug sol Added (µg/ml)		Recoverd* amount (µg/ml)		%Recovered	
	AML	HCZ	AML	HCZ	AML	HCZ	AML	HCZ
1	5	5	5	5	4.88	4.85	97.7	97
2	10	10	10	10	9.99	9.92	99.9	99.2
3	15	15	15	15	14.8	14.8	98.9	98.8
						Mean	98.8	98.3
						SD	1.10	1.17
						%RSD	1.11	1.19

*Mean of three reading

PRECISION

Repeatability

Five dilutions in five replicates were analyzed in same day for repeatability and results were found within acceptable limits (RSD ‹ 2) as shown in Table 3.

Intermediate Precision

Five dilutions in five replicates were analyzed on two different days and by two analysts for day to day and analyst to analyst variation and results were found within acceptable limits (RSD ‹ 2) as shown in Table No. 3.

Table 3 : Results of Precision

Serial No.	Validation Parameter	% Mean*		S.D.		% R.S.D.	
		AML	HCZ	AML	HCZ	AML	HCZ
1	Repeatability	98.88	98.67	0.09	0.04	0.46	0.46
2	Intermediate precision						
	Day to Day	98.6	98.57	0.06	0.05	0.53	0.59
3	Intermediate precision						
	Analyst to Analyst	98.26	98.34	0.04	0.08	0.51	0.72

* Mean of 25 determinations (5 replicates at 5 concentration level)

ROBUSTNESS

As per ICH norms, small, but deliberate variations, by altering the pH or concentration of the mobile phase were made to check the method's capacity to remain unaffected. The change was made in the ratio of mobile phase, instead of ACN: Methanol: Phosphate buffer (pH-3) (48:12:40 % V/V/V), ACN: Methanol: Phosphate buffer (pH-3) (55: 10:35% V/V/V) was used as a Mobile Phase. Results of analysis were summarized in Table No. 4.

Table 4 : Results of Robustness

Serial No.	Validation Parameter	% Mean*		S.D.		% R.S.D.	
		AML	HCZ	AML	HCZ	AML	HCZ
1	Robustness	98.34	98.65	0.07	0.06	0.76	0.85

* Mean of six determinations

STABILITY OF SAMPLE SOLUTION

The sample solution injected after 12 hr did not show any appreciable change. Results are shown in Table No. 5.

Table 5 : Stability data of AML and HCZ

AUC ±%RSD		
Hours	AML (15µg/ml)	HCZ (15µg/ml)
0	972±1.11	1436±0.92
6	955±1.15	1442±1.03
12	961±1.17	1424±1.11

TABLET ANALYSIS

Content of AML and HCZ found in the tablets by the proposed method are shown in Table No. 6. The low values of R.S.D. indicate that the method is precise and accurate.

Table 6 : Results of the HPLC analysis for tablets

Serial No.	Drug	Mean*	SD	% CoV	SEσ.
1.	AML	98.03	1.30	1.33	1.13
2.	HCZ	99.02	0.66	0.67	0.57

* Mean of fifteen determinations (3 replicates at 5 concentration level)

CONCLUSIONS

R-HPLC method was developed and validated for simultaneous estimation of AML and HCZ in tablet dosage form. Proposed method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in industries.

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