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SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF AMLODIPINE BESYLATE AND HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORM

Patel Gaurav *, Patel Sanjay [1], Mehta Rajendra [2], Patel Mandev
Nootan Pharmacy College, Visnagar - 384 315, Gujarat, India.
[1]Shri B.M. Shah College of Pharmaceutical Education & Research, Modasa , Gujarat, India.
[2]A. R. College of Pharmacy, Vallabh Vidyanagar - 388 120, Anand, Gujarat, India.

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ABSTRACT

A method for simultaneous estimation of Amlodipine besylate and Hydrochlorothiazide in tablet dosage form has been described. The method is based on UV-Spectrophotometric determination of two drugs, using simultaneous equation method. It involves absorbance measurement at 236.17 nm (ëmax of Amlodipine besylate) and 268.58 nm (ëmax of Hydrochlorothiazide) in methanol. Linearity was obtained in the range of 6-33 µg/ml for Amlodipine besylate and 3-33 µg/ml for Hydrochlorothiazide. The method allows rapid analysis of binary pharmaceutical formulation with accuracy. Limit of Quantification and Limit of Detection for Amlodipine besylate at 236.17 nm was found to be 2.1 and 0.6, respectively and for Hydrochlorothiazide 0.9 and 0.28, respectively. Limit of Quantification and Limit of Detection for Amlodipine besylate at 268.58 nm was found to be 1.0 and 0.31, respectively and for Hydrochlorothiazide 0.15 and 0.045, respectively. Results of analysis for this method were validated statistically and were found satisfactory.

Keywords: *Amlodipine Besylate (AML); Hydrochlorothiazide (HCT); UV- Spectrophotometry*

INTRODUCTION

Amlodipine Besylate (AML) is a long acting calcium channel blocker. It is used in CVS disorder. Chemically it is 3-ethyl 5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulphonate[1]. HPLC, HPTLC and UV methods have been reported for the estimation of AML in dosage forms and in human plasma[2,3,4]. Hydrochlorothiazide (HCT) is a diuretic and antihypertensive .It is the 3,4-dihyro derivative of chlorothiazide. It is chemically 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide1,1-dioxide[5]. HPLC, HPTLC, and spectrophotometric methods have been reported for the estimation of HCT alone as well as in combinations[6,7,8]. The extensive literature survey revealed that no UV spectrophotometric method is reported for the simultaneous estimation of both the drugs in tablet formulation. Therefore, it was thought worthwhile to develop simple, precise, accurate method to estimate AML and HCT simultaneously by Spectrophotometric method.

EXPERIMENTAL

Materials and Method

Pure drug samples of AML and HCT were kindly gifted by Micro Laboratories ltd, Banglore-India. Methanol analytical reagent grade (E-Merck, Mumbai, India) was used as solvent in this work. Marketed formulations used (Amlong-H, Micro Laboratories Ltd. Banglore, India) was procured from local market. UV-Visible double beam Spectrophotometer, model: Lambda19 with a pair of 10 mm Quartz cells was used.

UV- Visible Spectrophotometric Method

Standard stock solutions of 100 ìg/mL were prepared by dissolving 10 mg of each in 100 mL of methanol. From these stock solutions, working standard solutions having concentration 9 ìg/mL each were prepared by appropriate dilutions. They were scanned in the wavelength range of 400"200 nm and the overlain spectrum was obtained (Fig 1). Two wavelengths 236.17 nm (ëmax of Amlodipine besylate) and 268.58 nm (ëmax of Hydrochlorothiazide) were selected for the formation of simultaneous equation. The calibration curves were found to be linear in the concentration

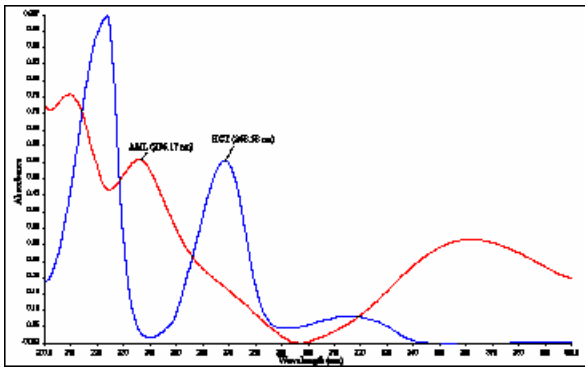

***Fig. 1:** Overlain UV Spectrum of AML and HCT (each 9 □g/mL) in methanol*

***Correspondence :** Gauravpatel1981@yahoo.com

range of 6-33 ìg.mL^{-1} for Amlodipine besylate and 3-33 ìg.mL^{-1} for Hydrochlorothiazide. The calibration curves were plotted at 236.17 and 268.58 nm. The absorptivities (A1%, 1 cm) of both the drugs at both the wavelengths were determined. These calculated values were the mean of five independent determinations. Two simultaneous equations were formed using these absorptivity coefficient values.

A1= 660 X Cx + 70 X Cy ——— (1)

A2= 66 X Cx + 662 X Cy ——— (2)

Where Cx and Cy are concentrations of AML and HCT, respectively.

Method Validation

The proposed method for estimation of Amlodipine Besylate and Hydrochlorothiazide was validated for linearity, range, stability, accuracy, precision (repeatability and intermediate precision). As per ICH guideline[9] for linearity, series of dilutions were prepared and response ratios were determined. The range was determined by preparing a series of dilutions from 80 % to 120 % of test concentration in five replicate. By recovery studies in which known amounts of standard drug were added to the previously analyzed tablet sample and mixture were analyzed by the proposed method, accuracy of methods was determined. Precision was studied for repeatability and intermediate precision (days, and analysts).

Procedure for Analysis of Tablet Formulation

Twenty tablets were weighed accurately. The average weight was determined and then ground to a fine powder. A quantity equivalent to 5 mg of AML and 12.5 mg of HCT was taken in 10 ml volumetric flask. The contents were ultrasonicated for 10 min with methanol (60 ml), made to volume (100 ml) and filtered through Whatmann filter paper No.42. The solution was further diluted with methanol to give concentrations of within the Beer's Law range. Absorbance of these solutions was measured at 236.17 nm and 268.58 nm as A1 and A2 respectively and concentrations of these two drugs in the sample were calculated using equation (1) and equations (2). Results of the analysis of the tablet formulations are reported in Table 1.

Table 1: *Validation Data of Amlodipine besylate and Hydrochlorothiazide*

Parameters	At 236.17 nm		At 268.58 nm	
	AML	HCT	AML	HCT
Beers Law Limit (µg/ml)	6-33	3-33	6-33	3-33
Regression Coefficient (r²)	0.9977	0.9973	0.9932	0.9977
Slope	0.056	0.007	0.0066	0.066
Intercept	0.0538	-0.0262	0.1159	-0.0327
Precision*				
Intraday (n=3)	0.5603	0.3389	0.3865	0.6276
Interday (n=3)	0.5866	0.4536	0.3889	0.6545
Accuracy (%) ± RSD	97.7 ± 1.94	98.8 ± 1.23	98.6 ± 0.45	101.5 ± 0.23
LOD (µg/ml)	0.6	0.28	0.31	0.045
LOQ (µg/ml)	2.1	0.9	1.0	0.15
Repeatability (n=5)	0.059-0.96	0.09-0.97	0.09-0.97	0.09-0.13
% Recovery	96.5 -98.2	97.63-101.19	95.88-98.87	97.2 – 98.6

RESULTS AND DISCUSSION

Absorbance was determined at both the wavelengths. AML obeyed linearity in the concentration range of 6-33 ìg.mL^{-1}, HCT in the concentration range of 3-33 ìg.mL^{-1} and the correlation coefficient (r^{2}sub) was <1 in both the case. The absorptivity was then calculated and along with absorbance, these values were submitted in the equations 1 and 2 to obtain concentration of drugs. The experiment was repeated five times in a day for intra-day and on five different days for inter-day precision. The method was found to be precise. The reproducibility of the method was determined by using methanol from three different manufacturers for the preparation of stock solution of standard drugs. The accuracy of the method was determined by performing recovery studies by standard addition method in which preanalyzed samples were taken and standard drug was added at five different levels. The % recovery lies in the range of 96.5 to 98.2 for AML and 96.7 to 98.6 for HCT. The method was successfully used to estimate the amounts Amlodipine besylate and Hydrochlorothiazide in marketed tablet formulation containing Amlodipine besylate 5 mg and hydrochlorothiazide 12.5 mg. By using this analytical method the amount of AML was found 5.029 mg and HCT was found 12.48 mg both are 100.58 % and 99.87 % w/w label claim, respectively. Percentage RSD was found to be less than 2 % (Table 1).

CONCLUSIONS

Amlodipine besylate and Hydrochlorothiazide are available in combined tablet dosage form for the treatment of hypertension. No single UV spectrophotometric method was reported for the estimation of the two drugs in combination. UV spectrophotometric method is reported for their simultaneous estimations. The method was validated as per ICH guidelines, the average recoveries being 97.4 % for both the compounds. The proposed method can be employed for the routine estimation of Amlodipine besylate and Hydrochlorothiazide in both bulk and combined dosage form.

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