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RP-HPLC Method for Simultaneous Estimation of Amlodipine Besylate and Hydrochlorothiazide in Combined Dosage Forms

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ABSTRACT

A reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the simultaneous estimation of Amlodipine Besylate and Hydrochlorothiazide in combine dosage form. Amlodipine Besylate (AML) is a long acting calcium channel blocker and in the treatment of CVS disorder. Hydrochlorothiazide (HCT) is a diuretic and antihypertensive. The mobile phase used was a combination of Water: Methanol (70:30). The detection of the combined dosage form was carried out at 245nm and a flow rate employd was 0.5ml/min. The retention time for Amlodipine Besylate and Hydrochlorothiazide was found to be 6.95 and 2.65 min respectively. Linearity was obtained in the concentration range of 6 to 18µg/ml of Amlodipine Besylate and 6 to 18µg/ml of Hydrochlorothiazide with a correlation coefficient of 0.997 and 0.9974. Detector consists of photodiode array detector; the reversed phase column used was RP-C_{18} (5 µm size, 250mm, 4.6mm i.d.) at ambient temperature. The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. Thus the proposed method is precise, selective and rapid for simultaneous estimation of Amlodipine Besylate and Hydrochlorothiazide in routine analysis.

Key Words: Simultaneous Estimation, Amlodipine Besylate, Hydrochlorothiazide, HPLC.

INTRODUCTION

Amlodipine Besylate (AML) is a long acting calcium channel blocker. It is used in CVS disorder. Chemically it is (R.S.)-2 – [(2-aminoethoxy) methyl] 4-o-chlorophenyl)-14, dihydro-6-methyl-3, 5-pyridinedicarboxylate, monobenzenesulphonate (British Pharmacopoeia, 2005). HPLC (George et al., 2004), HPTLC (Gohil et al., 2005) and UV (Mahadik et al., 2006) methods have been reported for the estimation of AML in dosage forms and in human plasma. Hydrochlorothiazide (HCT) is a diuretic and antihypertensive. It is the 3, 4-dihyro derivative of chlorothiazide. It is chemically 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7 sulphonamide 1,1-dioxide (British Pharmacopoeia, 2005). Spectrophotometric method, HPTLC, and HPLC (George et al., 2004), have been reported for the estimation of HCT alone as well as in combination (Suhagia et al., 2005). The review of the literature revealed that no RP-HPLC method has so far been reported for the combination of Amlodipine Besylate and Hydrochlorothiazide. So an attempt has been made to develop a simple, precise, accurate reverse phase high performance liquid chromatographic method for the simultaneous estimation of Amlodipine Besylate and Hydrochlorothiazide in combined tablet dosage forms.

MATERIALS AND METHODS

Instrumentation

The present work was carried out on isocratic high pressure liquid chromatography liquid chromatography, LC system used consist of pump (Jasco, Japan) with universal loop injector (Rheodyne) of injection capacity 20 µl. Detector consists of photoiode array detector; the reversed phase column used was RP-C_{18} (5 µm size, 250mm, 4.6mm i.d.) at ambient temperature.

AMLONG-H containing 5 mg/tab of Amlodipine Besylate and 12.5 mg/tab Hydrochlorothiazide from local market (Micro Laboratories Ltd, Banglore, India).

Reagents and chemicals
Pure drug samples of AML and HCT were obtained as gift sample from Micro Laboratories Ltd., Banglore, India. Methanol HPLC grade, HPLC grade water, Ortho Phosphoric acid GR grade obtained form E-Merck, Mumbai, India.

Experimental Conditions
The HPLC system was operated isocratically at flow rate of 0.5ml/min at 25℃ ± 0.5° C for 15 min. The mobile phase found to be most suitable for analysis was Water: Methanol 70:30% v/v, 0.5% triethylamine, pH adjusted to 3.0 with O-phosphoric acid, detection was carried out at 245nm.

Preparation of Standard solution
Standard stock solution of 1000µg/ml of each AML and HCT were prepared by dissolving 10 mg of each drug in mobile phase.

Table 1: System Suitability Test Parameters.

System Suitability Parameters	Proposed Method	
	AML	HCT
Retention Times (R_T) min	6.90	2.62
Theoretical Plates (N)	856.75	2343.76
Tailing Factor (A_S)	2.22	2.08
Resolution (R_S)	-	1.49

Sub stock solution was prepared from stock solution by diluting each standard stock solution (1ml) up to 10ml to get 100µg/ml of each drug. The normal concentrations in range 6-18µg/ml were prepared fore calibration. All solutions were stored at room temperature. Each standard solution (20 µl) was injected into the column after filtration using 0.2 micron membrane filter.

Sample preparation
Twenty tablets were weighed and crushed to fine powder. Powder equivalent to 5 mg of AML and 12.5 mg HCT was accurately weighed and dissolved in mobile phase, sonicated for 10 min and filtered through Whatman filter paper no. 42, finally different concentrations of tablet sample were prepared by serial dilution technique.

Chromatographic condition
Chromatographic separation was achieved by using mobile phase consisted of Water: Methanol 70:30% v/v, 0.5% Triethylamine, pH adjusted to 3.0 with O-phosphoric acid, flowing through RP-C_{18} column flow rate of 0.5ml/min for 15 min. A RP-C_{18} column was used as the separation phase. Detection was carried out using a photodiode array detector at 245nm.

Linearity
To establish the linearity a series of dilutions ranging from 6-18µg/ml for AML and 6-18µg/ml for HCT were prepared separately and calibration graph was plotted between the mean peak area Vs respective concentration and regression equation was derived.

Method Validation
The accuracy, precision and robustness were determined by analyzing a set of laboratory sample (n=5) with each of the five concentrations ranging from 6-18µg/ml for both drugs.

RESULTS AND DISCUSSION

Chromatographic Method
Initially Water: Methanol was tried as mobile phase, in which satisfactory peak was not obtained. Then different ratio of Water: Methanol were tried but resolution was not satisfactory. After adding 0.5% Triethylamine in water and pH of water adjusted to 3 by O-phosphoric acid.

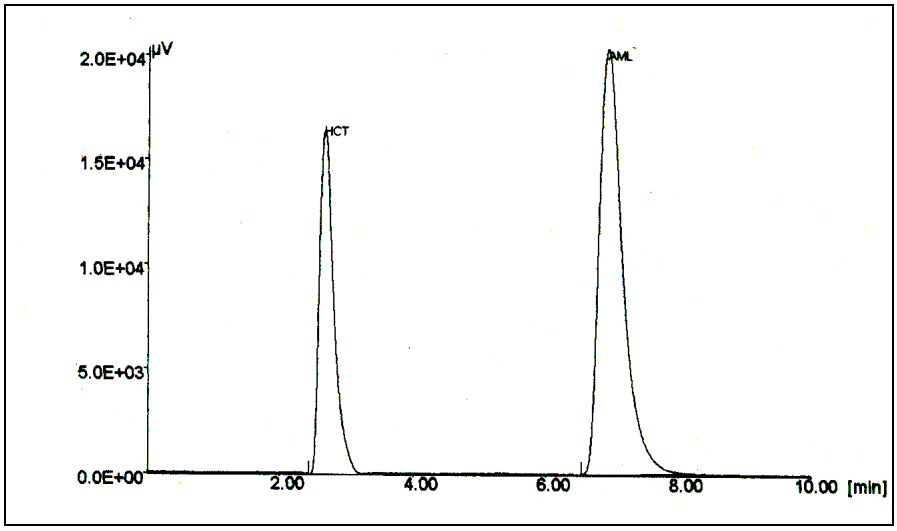

Figure 1: Chromatogram containing 18µg/ml standard Amlodipine Besylate (AML) and Hydrochlorothiazide (HCT) in Water: Methanol (70: 30), 0.5% TEA, pH adjusted to 3 by O-phosphoric acid, Flow rate 0.5ml/min.

Finally the system containing Water: Methanol (70:30) was found to be satisfactory and gave two well resolved peaks for AML and HCT with retention time for AML and HCT 6.90 min and 2.62 min respectively. A representative graph of this is shown in Figure 1.

Table 2: Determination of Accuracy.

Amount of Sample		Amount of drug added		Amount recovered		% Recovery	
AML (µg/ml)	HCT (µg/ml)	AML (µg/ml)	HCT (µg/ml)	AML (µg/ml)	HCT (µg/ml)	AML (%)	HCT (%)
12.5	12.5	0	0	12.47	12.32	-	-
12.5	12.5	6.25	6.25	18.64	18.54	101	99.6
12.5	12.5	12.5	12.5	24.65	25.10	98.7	102.3
12.5	12.5	18.75	18.75	30.97	30.88	99.5	99.0

System Suitability

The system suitability test was applied to a representative chromatogram to check the various parameters like column efficiency, resolution, precision and peak tailing. The result obtained is shown in Table 1. The resolution between AML and HCT was 1.49. The number of theoretical plate for AML and HCT were 856.75 and 2343.76 respectively. All these parameters were evaluated with the background of regulatory requirements which also suggests good chromatographic condition.

Linearity

AML and HCT showed a linearity of response between 6-18µg/ml. This linearity was represented by a linear regression equation as follows.

Y_{AML} = 26238 x – 24316 (r^2 = 0.9970)

Y_{HCT} = 11728 x – 28364 (r^2 = 0.9974)

Accuracy and Precision

The recovery experiment was carried out by spiking the already analyzed sample of the tablets with their different known concentration of standard AML and HCT. The result is summarized in Table 2. The percent recovery for AML ranges from 98.7 to 101% and HCT ranges from 99 to 102.3%. The summary of other validation parameter is shown in Table 3.

Table 3: Summary of Validation Parameters of RP-HPLC.

Parameters	AML	HCT
%Recovery	98.7-101	99-102.3
Repeatability (RSD, n=5)	0.026-0.17	0.12-0.24
Precision (CV)		
Intra-day (n=3)	0.017-0.1	0.12-0.16
Inter-day (n=3)	0.020-0.17	0.25-0.31
Specificity	specific	specific
Solvent suitability	99.7-101.2	99.3-102.5
Limit of Detection (µg/ml)	0.014	0.079
Limit of Quantitation (µg/ml)	0.046	0.26

Assay

The content of AML and HCT found in the tablets by the proposed method are shown in Table 4 and chromatograph is shown in Figure 2. The low RSD indicates that the method is precise and accurate.

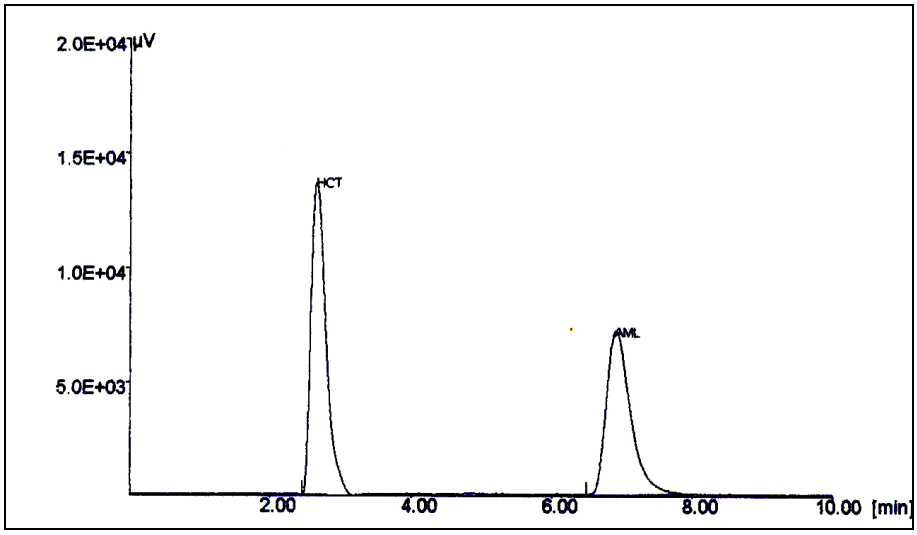

Figure 2: Chromatogram containing tablet formulation of standard Amlodipine Besylate (AML) and Hydrochlorothiazide (HCT) in Water: Methanol (70: 30), 0.5% TEA, pH adjusted to 3 by O-phosphoric acid, Flow rate 0.5ml/min, retention time 2.62 min for HCT and 6.90 min for AML.

Table: 4 Assay Results of Marketed formulation.

Formulation	Actual concentration (µg/ml)		% AML	% HCT
	AML	HCT		
Tablet	5	12.5	100.23±1.3	99.98±1.3

CONCLUSION

The proposed RP-HPLC method allows for accurate, precise and reliable measurement of AML and HCT simultaneously in combined dosage form. The developed RP-HPLC method was found to be simple, rapid, selective, accurate and precise for the concurrent estimation of drugs in respective two-component tablet dosage form of AML and HCT. The method was evaluated in a mass of facets, such as best correlation, robustness, accuracy, reproducibility and precision. The RSD for all parameters was found to be less than one, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of AML and HCT in pharmaceutical preparation.

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