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Development and Validation of RP-HPLC Method for Simultaneous Estimation of Enalapril Maleate and Amlodipine Besylate in Combined Dosage form

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ABSTRACT

A simple, precise and rapid reverse-phase HPLC method has been developed and subsequently validated for the simultaneous estimation of Amlodipine besylate and Enalapril maleate from their combination drug product. The proposed RP-HPLC method utilizes a Phenomenex C_{18}, 5 µm, 250 mm × 4.6 mm i.d. column, at ambient temperature, optimum mobile phase consisted of Methanol: Acetonitrile : Water (40:50:10, v/v/v), effluent flow rate monitored at 1.0 mL min^{-1}, and detection using PDA detector. The described method was linear over the range of 0.5-6.0 µg/ml and 0.5-8.0 µg/ml for Enalapril maleate and Amlodipine besylate, respectively. The mean recovery was found to be 100.06 ± 0.49 % and 99.98 ± 0.63 % for Enalapril maleate and Amlodipine besylate, respectively. The intermediate precision data obtained under different experimental setup, the calculated value of coefficient of variation (CV, %) was found to be less than critical value. The proposed method can be useful in the quality control of bulk manufacturing and pharmaceutical dosage forms.

INTRODUCTION

Amlodipine besylate (AML) is long-acting calcium channel blocker (dihydropyridine) used as an anti-hypertensive and in the treatment of angina while Enalapril maleate (ENA) is Competitive inhibitor of angiotensin- converting enzyme(ACE). Chemically, AML is (RS)-3-ethyl-5-methyl-2-(2-amino-ethoxymethyl) -4 -(2 -chlorophenyl) -1,4- dihydro -6- methyl 3, 5pyridinedicarboxylatebenzenesulfonate(1) while ENA is (S)-1-[N-[1-(ethoxy carbonyl)-3 phenyle propyl]-L- alanyle]- L-proline maleate (Budavri 2006). AML is official in IP (Indian Pharmacopoeia 2007), BP (British Pharmacopoeia 2005), EP (The European Pharmacopoeia 2002) and USP (The United States Pharmacopoeia 2007) while ENA is official in IP (Indian Pharmacopoeia 2007), BP (British Pharmacopoeia 2005) and USP(The United States Pharmacopoeia 2007) but they do not involve simultaneous determination of AML and ENA. Deep survey of literature for AML revealed methods based on Spectrophotometry (Chaudhari *et al*., 2010, Khopde *et al*., 2000), RP-HPLC (Bahrami *et al*., 2004) using fluorescence detection, HPLC-tandem mass spectrometry (Streel *et al*., 2002, Ceccato *et al*., 2002), RP-HPLC using UV detection (Patki *et al*., 1994, Avadhanulu *et al*., 1996), HPLC (Zarapkar *et al*., 1997, Valiyare *et al*., 2005, Kamble *et al*., 2004, Kulkarni *et al*., 2003, Zarapkar *et al*., 2002) in combination with other drugs, Flow injection analysis using UV-detection (Altiokka *et al*., 2002), HPTLC (Pandya *et al*., 1995), stability indicating HPLC (Kamat *et al*., 2005) and stability indicating HPLC (Naidu *et al*., 2005) in

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combination with benazepril hydrochloride have been reported. Similarly survey of literature for ENA revealed methods based on colorimetric and spectrophotometric (Dubey *et al*., 2010, Patil *et al*., 2011, Rahman *et al*., 2008), HPTLC (Kondawar *et al*., 2011) and HPLC(AL-Momani 2011) in combination with Hydrochlorothiazide. This manuscript describes the development and subsequent validation (International Conference on Harmonization 1996) of RP-HPLC method for the simultaneous determination of ENA and AML form their combination drug products. No interference from excepients of tablet formulation was found. The linearity of response, accuracy and intermediate precision of the described method has been validated.. The proposed method was successfully applied for simultaneous determination of AML and ENA in combined dosage forms that are available in market.

MATERIAL AND METHOD

Instruments and Apparatus

RP-HPLC instrument (Shimadzu, LC-2010$C_{HT,}$ Japan,) equipped with a UV-Visible detector and a photodiode array detector, auto sampler, Phenomenex (Torrance, CA) C_{18} column (250 mm × 4.6 mm id, 5 µm particle size) and LC-solution software, Analytical Balance (CP224S, Sartorius, Germany), Ultrasonic Cleaner (Frontline FS 4, Mumbai, India), Corning volumetric flasks, pipettes of borosilicate glass were used in the study, and Water Purification System (Millipore Bioscience Division Pvt.Ltd, India) was used during study.

Chemicals and Reagents

Kindly gifted reference standards of AML and ENA (Zydus cadila Healthcare Ltd, Moraiya, Ahmedabad, India), were used without further purification. Tablet preparations containing 5 mg AML and 5 mg ENA were purchase from Local pharmacy. HPLC grade methanol (Merck Ltd, Mumbai, India) and acetonitrile (Finar Chemicals Ltd.,Mumbai, India) were used. The water for RP-HPLC was prepared by triple glass distillation and filtered through a nylon 0.45 µm – 47 mm membrane filter. Nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India) and Whatman filter paper no. 41 (Whatman International Ltd., England) was used for the study.

Preparation of solutions

Standard stock solution of AML (100 µg/ml)

An accurately weighed quantity of about 5 mg AML was transferred into 50 ml volumetric flask. About 25 ml of methanol was added and sonicated for 10 min. The solution was made upto volume with methanol to obtained final solution of 100 µg/ml.

Standard stock solution of ENA (100 µg/ml)

An accurately weighed quantity of about 5 mg ENA was transferred into 50 ml volumetric flask. About 25 ml of methanol was added and sonicated for 10 min. The solution was made upto volume with methanol to obtained final solution of 100 µg/ml.

Mixed standard stock solution of AML & ENA

Accurately weighed AML (5 mg) and ENA (5mg) were transferred to a 50 ml volumetric flask, dissolved and diluted up to the mark with methanol to get final concentration of 100 µg/ml for both drugs.

Sample Solution

To determine the content of ENA and AML in tablets; twenty tablets were weighed and average weight was determined. The accurately weighed powder equivalent to 5 mg a each of ENA and AML were transferred in a 50 ml volumetric flask and methanol (30 ml) was added. The solution was sonicated for 15 min. The flask was allowed to stand for 5 min at room temperature, and the volume was diluted up to the mark with methanol to achieve the sample stock solution for ENA and AML having 100 µg/ml concentration. The solution was filtered through 0.45µm, 47mm membrane filter. An aliquot (1ml) was transferred to a 10 ml volumetric flask, and diluted up to the mark with methanol used for HPLC to obtain working sample solution for ENA (10 µg/ml) and AML (10µg/ml). An aliquot (0.5 ml) of the working test solution was transferred to a 10 ml volumetric flask, and diluted up to the mark with mobile phase to obtain the sample solution for ENA (0.5 µg/ml) and AML (0.5 µg/ml).

Chromatographic Conditions

The proposed RP-HPLC method utilizes a Phenomenex C_{18}, 5 µm, 250 mm × 4.6 mm i.d. column, at ambient temperature, optimum mobile phase consisted of Methanol: Acetonitrile: Water (40:50:10, v/v/v), injection volume 20 µl, effluent flow rate monitored at 1.0 ml/min, and detection using PDA detector.

Preparation of Calibration curve

Aliquots (0.5, 1.0, 2.0, 4.0, 6.0, 8.0 ml) of mixed working standard solution (equivalent to 0.5, 1, 2, 4, 6µg/ml, for ENA and 0.5, 1, 2, 4, 6, 8 µg/ml for AML) were transferred in a series of 10 ml volumetric flasks, and the volume was made up to the mark with methanol. An aliquot (20 µl) of each solution was injected under the operating chromatographic conditions as described above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentrations, and the regression equations were calculated. Each response was average of three determinations.

Analysis of ENA and AML in combined dosage form:-

The response of the sample solution was measured under the chromatographic conditions mentioned above for the quantitation of ENA and AML. The amounts of ENA and AML present in sample solution were determined by applying values of the peak area to the regression equations of the calibration graph.

RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. The mobile phase consisting of

Methanol: Acetontrile: Water, (40:50:10, v/v/v) at a flow rate of 1 ml/min, was found to be satisfactory to obtain good peak symmetry, better reproducibility and repeatability for ENA and AML. The retention times were found to about 2.269 and 5.074 min for ENA and AML, respectively. Complete resolution of the peaks with clear baseline was obtained (Figure 1). Peak purity of drugs was confirmed by comparing the spectra of standard and sample solutions. System suitability test parameters for ENA and AML for the proposed method are reported in Table 1.

Table. 1: System suitability parameters of chromatogram for ENA and AML.

Parameters	RP-HPLC method	
	ENA ± % RSD	AML ± % RSD
Retention time, min	2.269±0.15	5.074±0.17
Tailing factor	1.167±0.65	1.392±0.54
Theoretical plates	2010±1.16	2312±1.38
Resolution	2.14 ±1.23	

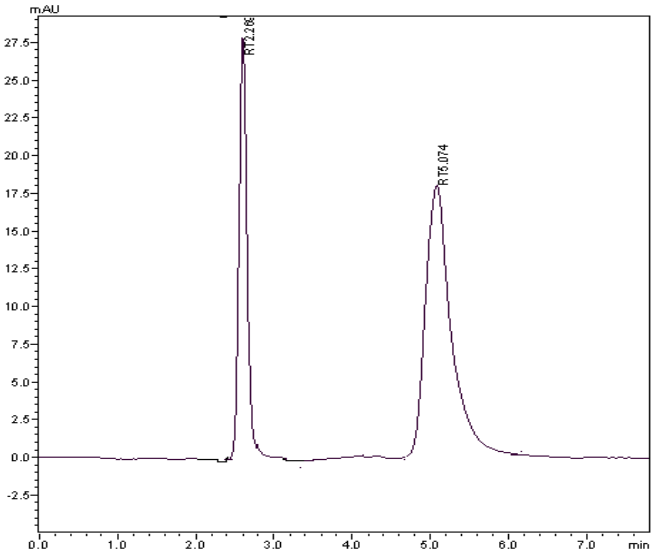

Fig. 1: Chromatogram of standard solution of ENA and AML.

Linear correlation was obtained between peak area versus concentrations of ENA and AML in the concentration ranges of 0.5-6 µg/ml and 0.5-8 µg/ml, respectively. The regression analysis data are depicted in Table 2. The specificity of the method was ascertained by analyzing standard drugs and sample of ENA and AML. The peak purity of standard ENA and AML were 0.999 and 1.000 respectively while for sample ENA and AML peak purity were 0.999 and 1.000, respectively. The results obtained suggested that proposed method was specific for the simultaneous estimation of ENA and AML. The peak purity graphs for ENA and AML for standard as well as for sample solution are given in Figure 2. The accuracy of the method was determined by calculating recovery of ENA and AML by the standard addition method. Known amounts of standard solutions of ENA and AML (1.5, 3, 4.5 µg/ml) were added to pre-quantified sample solutions of ENA and AML (3 + 3 µg/ml). The amounts of ENA and AML were estimated by applying obtained values to the regression equation of the calibration curve.

Table. 2: Regression Analysis Data and Summary of Validation Parameter for the proposed Method.

Parameters	RP-HPLC method	
	ENA	AML
Concentration range (µg/ml)	0.5-6	0.5-8
Slope	143599	13342
Intercept	15627	30911
Correlation coefficient	0.9989	0.9983
LOD(µg/ml)	0.04	0.05
LOQ(µg/ml)	0.4	0.4
% recovery (Accuracy, n = 6)	100.67 ± 0.56	99.98 ± 0.64
Repeatability (% RSD, n = 6)	0.0006	0.004
Interday (*n* = 6)	0.47-0.98	0.53-1.07
Intraday (*n* = 6)	0.34-0.82	0.65-1.19

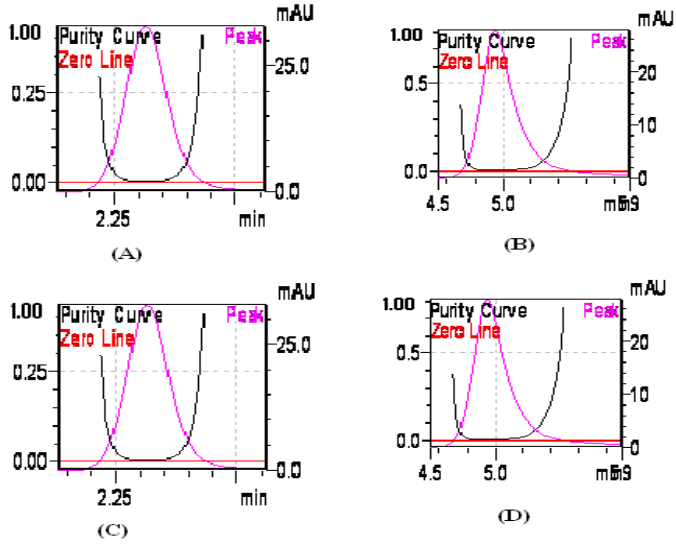

Fig. 2: Peak Purity of (A) ENA in Standard Solution (B) Peak Purity of AML in Standard Solution (C) Peak Purity of ENA in Sample Solution (D) Peak Purity of AML in Sample Solution **.**

The recoveries obtained were 100.06 ± 0.49 % and 99.98 ± 0.63 % for ENA and AML, respectively. The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 3. The limit of detection (LOD) and limit of quantification (LOQ) were determined by visual methods as suggested in ICH guidelines, were found to be 0.04 µg/ml and 0.05 µg/ml, respectively and LOQ values for ENA and AML were found to be 0.4 µg/ml. The low RSD value for repeatability of method as well as within a day and day to day variation suggested that method was found to be precise in the range of measurement (Table 2). The method was applied for the analysis of three marketed formulations containing ENA 5 mg and AML 5 mg per tablet. The results of analysis of tablet formulations are shown in Table 4. All of them meet pharmacopoeial requirement of ETV and AML.

Table. 3: Recovery Data for the proposed Method.

Amount of sample taken (µg/ml)		Amount of standard Added (%)		Total Amount of standard Added (µg/ml)		Amount of standard Recovered (µg/ml)		% Recovery ± S.D.	
ENA	AML	ENA	AML	ENA	AML	ENA	AML	ENA	AML
3	3	50	50	1.5	1.5	3.01	2.99	100.4 ± 0.51	99.98 ± 0.65
3	3	100	100	3.0	3.0	3.02	2.99	100.9 ± 0.49	99.91 ± 0.63
3	3	150	150	4.5	4.5	3.01	2.99	100.5 ± 0.47	99.95 ± 0.62

Table. 4: Analysis of Marketed Formulation of ENA and AML by Proposed Method (n = 6).

Formulation	Amount of drug taken (mg)		Amount of drug found (mg)		% Amount found (n=3) ± SD	
	AML	ENA	AML	ENA	AML	ENA
Tablet 1	5	5	5.03	4.98	100.6 ± 0.23	99.6 ± 0.54
Tablet 2	5	5	4.94	5.07	98.8 ± 1.01	101.4 ± 1.52
Tablet 3	5	5	5.10	4.98	102.0 ± 1.46	99.6 ± 1.21

CONCLUSION

In this proposed method the linearity was observed in the concentration range of 0.5-6 µg/ml and 0.5-8 µg/ml with co-efficient of correlation, (r^2) = 0.9989 and (r^2) = 0.9983 for ENA and AML, respectively. The result of the analysis of pharmaceutical formulation by the proposed method was highly reproducible and reliable and it was in good agreement with the label claim of the drug. The method can be used for the routine analysis of the ENA and AML in combined dosage form without any interference of excipients.

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