International Journal of Biomedical And Advance Research

ISSN: 2229-3809 (Online)
Journal DOI:10.7439/ijbar
CODEN:IJBABN

Research Article

HPTLC method for simultaneous determination of amlodipine besylate and enalapril maleate in pharmaceutical formulation

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Abstract

A rapid and simple high performance thin layer chromatography (HPTLC) method with densitometry at λ= 223 nm was developed and validated for simultaneous determination of Amlodipine and Enalapril from pharmaceutical preparation. Separation was performed on aluminum-backed silica gel $60F_{254}$ HPTLC plates as stationary phase and using a mobile phase comprising of Toluene: Isopropanol: Glacial acetic acid (GAA): Methanol (6: 2: 0.6: 0.5 v/v/v/v) and 20 ml of mobile phase was used per chromatography run. After development, plates were observed under UV light. The validated lowest limit of detection was 80ng/spot and 200ng/spot whereas lowest limit of quantification was 150ng/spot and 400ng/spot for Amlodipine and Enalapril respectively. The percentage assay of Amlodipine and Enalapril was found between 99.74 to 100.66% and 99.89 to 100.42% respectively. The described method has the advantage of being rapid and easy. Hence it can be applied for routine quality control analysis of Amlodipine and Enalapril from pharmaceutical preparation and stability studies.

Keywords: Amlodipine, Enalapril, HPTLC, Pharmaceutical formulation

1. Introduction

Amlodipine besylate (I) is a dihydropyridine type (Fig. 1A) long acting calcium channel blocker with slow onset of vasodialatory action[1,2]. The use of this important life saving drug is approved for the treatment of variant and stable angina and hypertension, too[3,4]. As an additional property, Amlodipine inhibits vascular smooth muscle cell growth through interactions with targets other than L-type calcium channels[5] and is more selective for arterial vascular smooth muscle than cardiac tissues. It has been investigated that Amlodipine exhibits ameliorating effects on plasma and myocardial catecholamines with a significant reduction of calcium deposition and may be useful in dilated cardiomyopathy[6]. Enalapril, *N*-[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]-l-proline, belongs to the series of substituted *N*-carboxymethyl dipeptides. Enalapril is a prodrug which is hydrolyzed after absorption forming the active angiotensin converting enzyme (ACE) inhibitor. The active form, enalaprilat (Fig. 1B), is the major metabolite of Enalapril and has been shown to be effective in the treatment of hypertension and congestive heart failure without causing significant side effects. Therefore, Enalapril and enalaprilat are often determined simultaneously in biological fluids[7].

Fig. 1A: Amlodipine

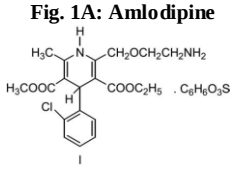
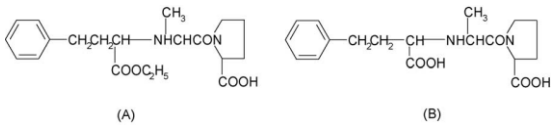


Fig. 1B: Enalapril

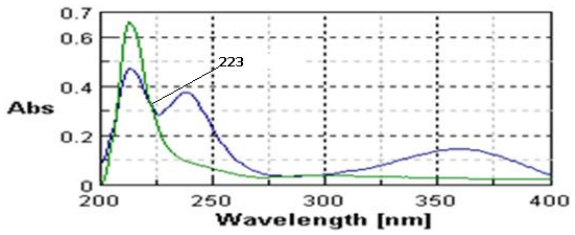


2. Experimental

2.1 Selection of analytical wavelength

From the standard stock solution further dilutions were done using mobile phase and scanned over the range of 200- 400 nm and the spectra were overlain. It was observed that both drugs showed considerable absorbance at 223 nm as shown in Fig.1

Fig. No: 1 Overlain spectra of Amlodipine Besylate and Enalapril Maleate.



2.2 Preparation of standard stock solution

50 mg of each drug AMLO and ENA were weighed separately and dissolved in 20 ml of methanol and then volume was made up to 50 ml so as to get the concentration 1 mg/ml. From each of these solutions 1ml of solution was pipette out and transferred to 10 ml volumetric flasks and volume was made up to the mark using methanol so as to get the concentration 100 µg/ml.

Fig No: 2 Densitogram of Standard Amlodipine Besylate (200ng/spot) and Standard Enalapril Maleate (800ng/spot); peak 1 (R_F: 0.15), peak 2 (R_F: 0.25).

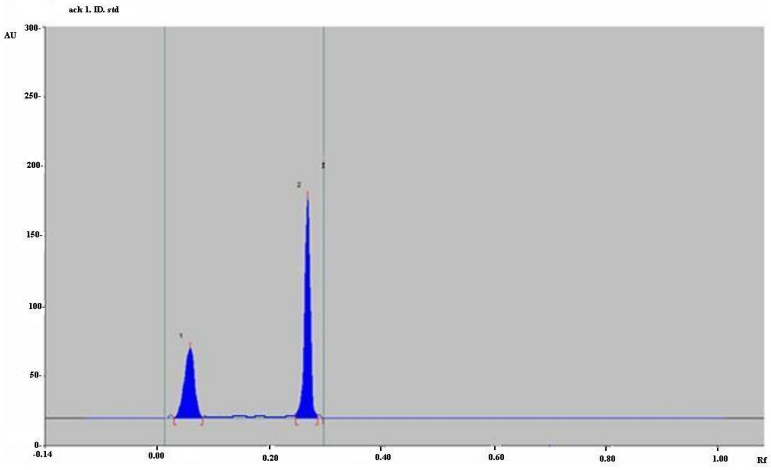


Fig No: 3 Densitogram of Standard Amlodipine Besylate

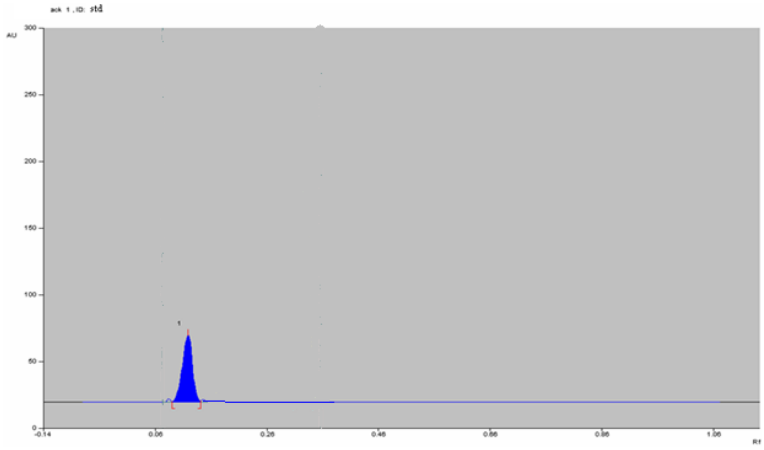
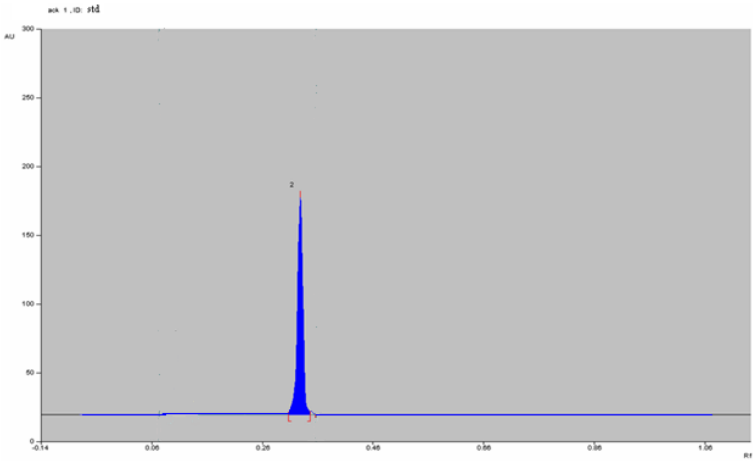


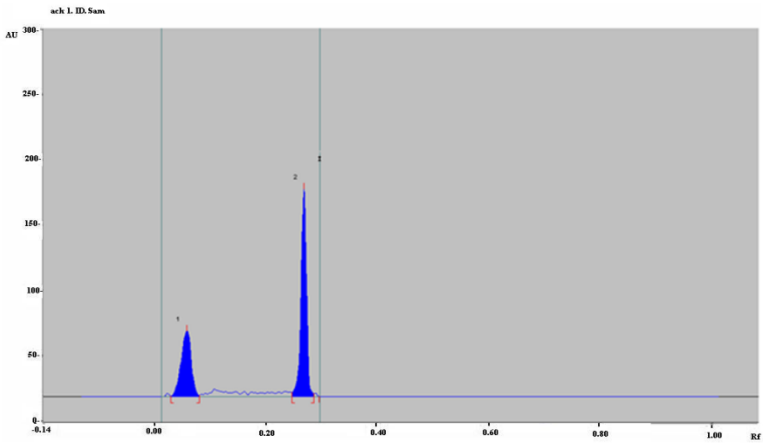
Fig No: 4 Densitogram of Standard Enalapril Maleate



2.3 Sample Preparation

Quantity of tablet powder equivalent to 5 mg of Amlodipine Besylate and 5 mg of Enalapril Maleate was weighed and transferred to a 50 ml volumetric flask containing about 30 ml of mobile phase, ultrasonicated for 5 min, filtered, filter was washed with methanol, washing transfers to flask and volume was made up to the mark with the mobile phase to get sample stock solution. The sample solution was then filtered using 0.45 µ nylon filter.

Fig No: 5 Densitogram of sample Amlodipine Besylate (200ng/spot) and Enalapril Maleate (800ng/spot); peak 1 (R_F: 0.15), peak 2 (R_F: 0.25).



2.4 Method validation

2.4.1 Linearity

Stock standard solution was prepared by dissolving 10 mg of Enalapril Maleate and Amlodipine Besylate in 10 ml methanol (1000 µg/ml) separately. The standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration 0.1 mg/ml. From each stock solution Enalapril Maleate and Amlodipine Besylate was separately spotted on the TLC plate to obtain final concentration 800-5600ng/spot and 200-1400ng/spot of Enalapril Maleate and Amlodipine Besylate respectively. Each concentration was spotted 3 times on the TLC plate. The plate was developed on previously described mobile phase. Peak areas were plotted against corresponding concentrations to obtain the calibration graphs. Results are shown in table 1 and 2 & see fig no. 6 & 7.

Table 1: Linearity of Enalapril Maleate (n=6)

Std. Conc. (ng /ml)	800	1600	2400	3200	4000	4800	5600
Replicates	Peak Area						
1	1695	2338	2805	3129	3464	3805	4236
2	1628	2356	2865	3136	3469	3810	4240
3	1635	2338	2830	3127	3465	3825	4241
4	1640	2390	2840	3129	3460	3800	4238
5	1685	2364	2830	3145	3462	3815	4232
6	1680	2336	2864	3136	3464	3815	4236
Mean	1694	23380.2	2810.4	3128.9	3464	3805.4	4236
SD	7.099	13.982	28.181	39.073	59.368	62.407	39.601
% RSD	1.811	1.786	1.803	1.634	1.886	1.596	0.849

Regression equation: y = 458.2X +1647 ; r = 0.99832

Table 2: Linearity of Amlodipine Besylate (n=6)

Std. Conc. (ng/ml)	200	400	600	800	1000	1200	1400
Replicates	Peak Area						
1	682	1178	1660	2115	2521	2920	3449
2	679	1182	1666	2120	2528	2929	3442
3	645	1170	1652	2122	2530	2936	3439
4	683	1190	1680	2115	2540	2989	3445
5	690	1188	1662	2119	2528	2910	3442
6	682	1169	1630	2118	2534	2956	3438
Mean	682.4	1178.1	1660	2116.8	2521	2920.1	3449.7
SD	7.222	14.302	14.800	12.998	15.600	13.623	11.409
% RSD	1.881	1.882	0.969	0.569	0.507	0.360	0.250

Regression equation: y = 2.22X +309.5 ; r = 0.99920

Fig no: 6 Linearity of Amlodipine Besylate

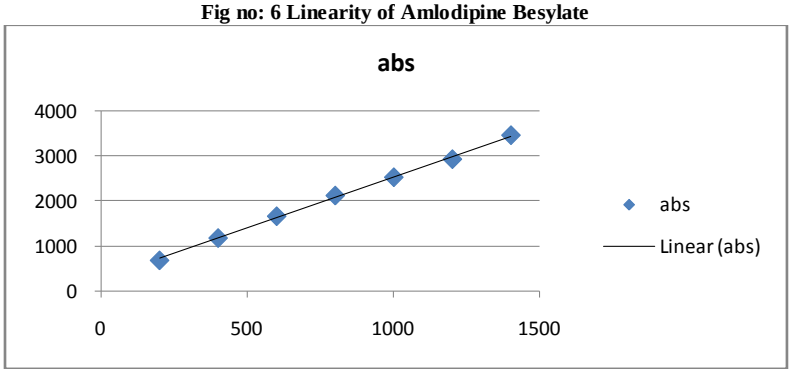
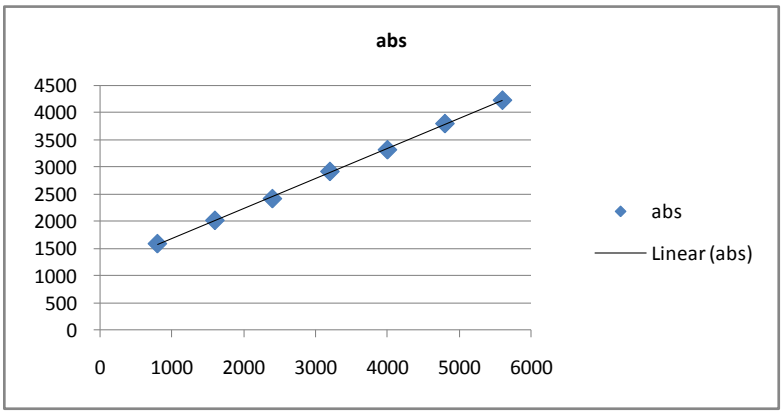


Fig no: 7 Linearity of Enalapril Maleate



2.4.2 Accuracy

Chromatogram was developed and the peak areas were noted. At each levels of the amount, three determinations were performed. The proposed methods when used for extraction and subsequent estimation of Amlodipine Besylate and Enalapril Maleate from pharmaceutical dosage form after spiking with additional drug afforded recovery of 98 – 102 % and mean recovery for Drugs from the marketed formulation are listed in (Table 3 & 4).

Table 3: Recovery Studies of Enalapril Maleate

Enalapril Maleate	**Densitometric peak area**		
	Level of Recovery		
	50 %	**100 %**	**150 %**
	800ng/spot	**1600ng/spot**	**2400ng/spot**
Replicate 1	1690	2338	2805
Replicate 2	1687	2336	2793
Replicate 3	1707	2361	2844
Mean	1694.67	2345	2814
SD	10.78	13.89	26.66
% RSD	0.63	0.59	0.94
Mean conc. found (µg/ml)	801.3	1598.14	2410.23
Mean % Recovery	100.17	99.89	100.42

Table 4: Recovery Studies of Amlodipine Besylate

Amlodipine Besylate	**Densitometric peak area**		
	Level of Recovery		
	50 %	**100 %**	**150 %**
	200ng/spot	**400ng/spot**	**600ng/spot**
Replicate 1	680	1178	1660
Replicate 2	671	1186	1631
Replicate 3	674	1190	1632
Mean	675	1184.66	1641
SD	4.58	6.11	16.46
% RSD	0.67	0.51	1.003
Mean conc. found (µg/ml)	201.32	401.21	598.47
Mean % Recovery	100.66	100.31	99.74

2.4.3 Precision

The intra-day precision of the developed TLC method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each on same day. The inter-day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days. The result obtained for intraday and Inter day variations are shown for Amlodipine Besylate and Enalapril Maleate in Table 5 and 6, respectively.

Table 5: Intraday and Inter day precision of Enalapril Maleate (n=3).

Enalapril Maleate	**Measured concentration (µg/ml), % R.S.D**	
Conc. (ng/spot)	**Intra day**	**Inter day**
2400	2400.07, 0.65	2421,0.97
3200	3211,1.33	3221,1.45
4000	4025,1.28	4111,1.37

Table 6: Intraday and Interday precision of Amlodipine Besylate (n=3).

Amlodipine Besylate	**Measured concentration (µg/ml), % R.S.D**	
Conc. (ng/spot)	**Intra day**	**Inter day**
600	623.01,0.83	601.03,1.68
800	811,1.35	816.12,1.42
1000	1012.01,1.22	1121.23,1.45

2.4.4 LOD and LOQ

The LOD and LOQ were found to be 200ng/spot and 400ng/spot respectively for Enalaprile Maleate and 80ng/spot and 150ng/spot respectively for Amlodipine Besylate.

3. Result and Discussion

Literature survey revealed that no one HPTLC method reported so far for the estimation of Amlodipine Besylate and Enalapril Maleate in pharmaceutical dosage forms.

Hence, it was proposed to attempt the development of HPTLC analytical methods for estimation of Amlodipine Besylate and Enalapril Maleate in pharmaceutical formulations. A simple, accurate and rapid HPTLC method hence, the project was undertaken to develop precise, accurate, reliable, rapid, simple and specific method for estimation of Amlodipine Besylate and Enalapril well resolved by the relevant ICH guidelines and other current regulatory guidelines. The chromatographic techniques exploited for the study were HPTLC as they are more selective and specific.

Standard stock solution of Amlodipine Besylate and Enalapril Maleate (1 mg/ml) as well as working standard of 100µg/ml was prepared in methanol because methanol is a volatile solvent. Various pure solvents of varying polarity viz. methanol, toluene, ethyl acetate, etc. and their mixtures in different proportions were tried as mobile phase for development of chromatogram which was capable of resolving the products. The mobile phase which was found to be most suitable was Toluene: Isopropanol: Glacial acetic acid (GAA): Methanol (6: 2: 0.6: 0.5 v/v/v/v) as it gave the satisfactory baseline resolution of all components (API) with reasonably acceptable R_f values for quantitation purpose. The retention factor (R_f) values observed were 0.15 and 0.23 for the Amlodipine Besylate and Enalapril Maleate. The chamber saturation period of 10 min was found to be suitable as higher saturation period has resulted in band broadening. The λ_{max} of Amlodipine Besylate and Enalapril Maleate, 223 nm.

The developed method was then validated for parameters like limit of detection & limit of quantitation, precision, accuracy, as per the ICH guidelines and their results as summarized in Tables 1,2 -3,4 were found to be in accordance with the ICH guidelines.

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