RESEARCH ARTICLE

Standardized Analytical Procedure for Routine Testing of Amlodipine Besilate and Enalapril Maleate Simultaneously in *In-vitro* Samples

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ABSTRACT

This study develops a standardized methodology for routine analysis of in-vitro samples of amlodipine besilate and enalapril maleate concurrently in immediate-release tablet dosage forms. UV-detection was performed at absorption maxima of 237.20 nm and 217.00 nm for amlodipine besilate and enalapril maleate, respectively; 6.8 pH phosphate buffer was used as dissolution media for 30 mintues, on USP II (Paddle) dissolution apparatus, at 50 RPM and 37.0 ± 0.5ºC.

The fallouts of the analysis were endorsed statistically. Results were satisfactory in the arrays of 1–19 µg mL^{-1} for amlodipine besilate and 1–9 µg mL^{-1} for enalapril maleate. The analysis results exhibited that the projected simultaneous method is modest, swift, precise, and accurate and used for the repetitive analysis of dissolution samples of amlodipine besilate and enalapril maleate in combined pharmaceutical dosage forms (tablets).

Keywords: Anti-hypertensive, *In-vitro*, Screening, UV-spectrophotometric, Validated.

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INTRODUCTION

Amlodipine besilate is an official drug that behaves as a dihydropyridine calcium antagonist, a long-acting calcium channel blocker.[1] It blocks the influx of extracellular calcium across the myocardial and vascular smooth muscles. It acts as a peripheral arterial vasodilator, leading to a reduction in peripheral vascular resistance and reduction in blood pressure.[2-5]

Enalapril maleate is an official drug listed in Merck Index.[6] It is a prodrug; and gets bioactivated by hydrolysis of the ethyl ester to enalaprilat, acting as an active angiotensin-converting enzyme (ACE) inhibitor.[2-5]

Various spectrophotometric and reversed-phase ultra performance liquid chromatography (RP-UPLC) methods have been reported for the assay of amlodipine besilate and enalapril maleate in combination. However, there is no UV-spectrophotometric or RP-HPLC method for simultaneous dissolution profiling of amlodipine besilate and enalapril maleate in the combined dosage form.[7-10]

The research presents a modest, swift, accurate, and replicable UV-spectrophotometric method[11] for discernment of amlodipine besilate and enalapril maleate from combined tablet dosage form in dissolution media, helpful in the simultaneous determination of amlodipine besilate and enalapril maleate during dissolution study.

MATERIALS AND METHODS

Materials

Amlodipine besilate (purity- 99.37%) and enalapril maleate (purity-98.64%) were received as a gift sample from Glenmark Pharmaceuticals Ltd., Goa, India Tirupati Medicare Limited, Ponta Sahib, Himachal Pradesh, India, respectively. Analytical grade chemicals used were purchased from a local supplier. The amlodipine besilate and enalapril maleate tablets (Amta E, Intas Pharm Ltd. India) were purchased from the local market.

Methods

Preparation of Standard Stock Solution

Amlodipine besilate and enalapril maleate stock solution (100 µg mL^{-1}) were prepared by accurately dissolving about 10 mg of pure drug in 25 mL of methanol, shaking for 15 minutes making up the volume to 100 mL with phosphate buffer pH 6.8 (dissolution media).

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Table 1: Dissolution parameters for the *in-vitro* dissolution of amlodipine besilate and enalapril maleate[8]

Parameters	*Description*
Dissolution apparatus type	USP II (paddle)
Dissolution media	6.8 phosphate buffer, 900 mL
Rpm	50
Time points	5, 10, 15, 20, 25, 30 min
Temperature	37.0°C ± 0.5°C
Sample volume	10 mL
Filter	0.45µm Teflon* membrane syringe

*Teflon use is indicated in USP (revision bulletin 2011) monograph of amlodipine and for enalapril maleate in US patent no. US20110104241A1.

Table 2: Calculation of the ratio (A2/A1)/(ax2/ax1) and (ay2/ay1)/(A2/A1)

	A	*Absorptivity*	*Condition to be fulfilled*	*Observation*
ax1	0.6310	0.1262	(A2/A1)/(ax2/ax1)	2.044022404
ax2	0.7120	0.1424	(ay2/ay1)/(A2/A1)	12.51172077
ay1	0.0280	0.0056	Criteria: Should lie outside the range of 0.1–2.0	
ay2	0.8080	0.1616		
A1	0.2637		λ1= 237.00nm	λ2= 217.00 nm
A2	0.6082			

ax1, ax2, and ay1, ay2 are the absorptivity of amlodipine besilate and enalapril maleate, respectively, at λ1 and λ2, respectively, while A1 and A2 are the absorbances of sample solution at λ1 and λ2, respectively.

Table 3: LoD and LoQ for amlodipine besilate and enalapril maleate.

Parameter	Amlodipine	Enalapril	Remarks
LoD	0.018583	0.009042	3.3(SD*/Slope)
LoQ	0.056314	0.0274	10(SD*/Slope)

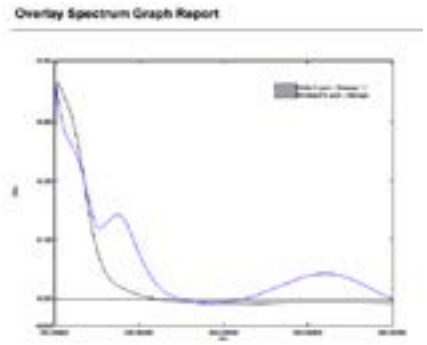

Figure 1: Overlay spectrum graph report of amlodipine besilate and enalapril maleate.

Dissolution media

According to official records,[12] the dissolution media for amlodipine besilate and enalapril maleate is monobasic sodium phosphate buffer pH 6.8 (Table 1).

Study of UV-spectrum

Suitable dilutions of standard stock solution were made in dissolution media to yield a solution of 5 µg mL^{-1} of both drugs, and this solution was scanned in the wavelength range of 200–400 nm. Overlay spectra of the two drugs were observed to select the point of maximum absorbance of each one (λ1, λ2). These wavelengths were selected for developing a simultaneous equation method through this graph.

Table 4: Precision by repeatability for amlodipine besilate and enalapril maleate.

Absorbance	*Amlodipine*	*Enalapril*
1	0.2573	0.6054
2	0.2703	0.6175
3	0.2784	0.6507
4	0.2639	0.6196
5	0.2891	0.6648
6	0.2596	0.6104
Average	0.26977	0.62807
SD	0.01218	0.0240
%RSD*	0.0452	0.0382

%RSD should be less than 1.0

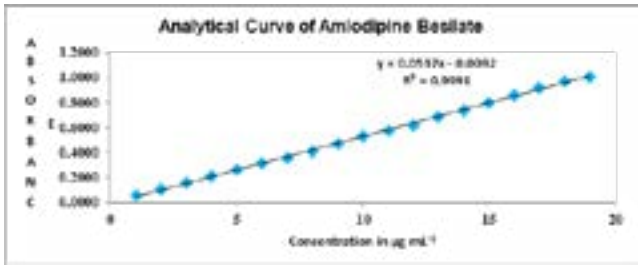

Figure 2: The analytical curve of amlodipine besilate.

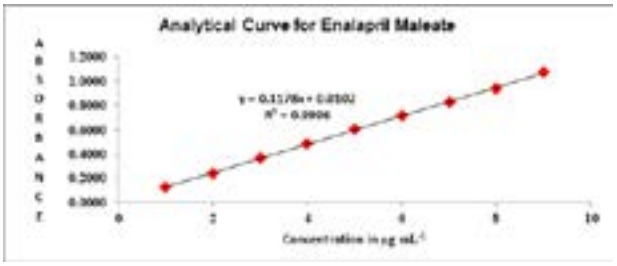

Figure 3: The analytical curve of enalapril maleate.

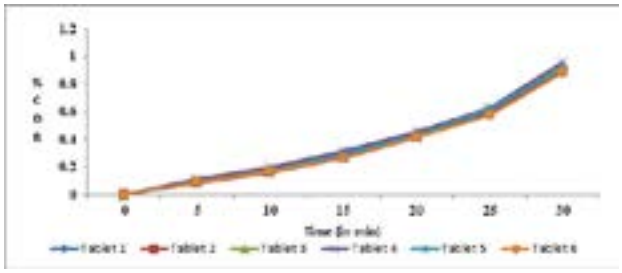

Figure 4: Dissolution profile for amlodipine besilate.

Preparation of Sample Solution

Twenty tablets containing 5 mg of amlodipine besilate and 5 mg of enalapril maleate were individually weighed and crushed. Suitable aliquots of the sample solutions of concentrations

Table 5: Data for Recovery Studies for amlodipine besilate and enalapril maleate

Conc. (µg mL^{-1})	*Conc. before spiking (µg mL^{-1}) C1*	*Conc. of std. added C_2*	*Conc. after spiking (µg mL^{-1}) C3*	*%Recovery (C3-C1) *100/C2*	*Mean ± SD*	*%RSD*	*SE**
Amlodipine besilate							
5	4.9999	1	6.0048	100.49			
	4.9982	2 6.9967 6.9989	6.9938	99.78	100.0356 ± 0.393567	0.00393	0.13119
	5.0002	3 8.0023 7.9976	8.0007	100.0167			
Enalapril maleate							
5	4.997	1 5.9972 5.9993	5.998	100.1		0.00236	0.07855
			100.02				
			100.23				
	5.001	2 6.9995 6.9984	6.9899	99.445			
			99.925				
			99.87				
	4.999	3 8.0051 7.9975	8.0019	100.097			
			100.203				
			99.95				

*SE should be less than 1.0

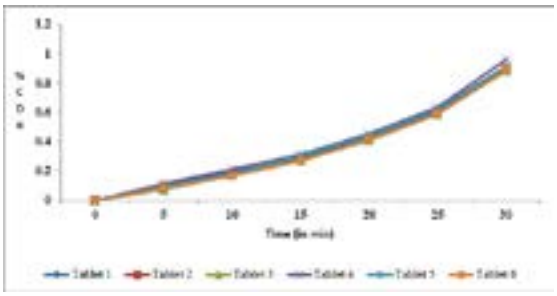

Figure 5: Dissolution profile for enalapril maleate.

within Beer-Lambert's range for both drugs were prepared by diluting with dissolution media.

Calculation of the Ratio $(A_2/A_1)/(ax_2/ax_1)$ and $(ay_2/ay_1)/(A_2/A_1)$

Since both the drugs absorb at the λmax of the other, we can determine both the drugs by the technique of simultaneous equation method.[11] However, for the applicability of the simultaneous equation method, some criteria must be fulfilled. The criterion is the ratios $(A_2/A_1)/(ax_2/ax_1)$ and $(ay_2/ay_1)/(A_2/A_1)$ should lie outside the range of 0.1–2.0.[11]

Analytical Curve

Suitable aliquots were prepared from a standard stock solution of amlodipine besilate and enalapril maleate. An analytical curve was prepared in the concentration range of 1–9 µg mL^{-1} for enalapril maleate and 1–19 µg mL^{-1} for amlodipine besilate; for both the coefficient of regression (r^2) was determined.

In-vitro Dissolution Studies

The tablet's *in-vitro* drug release rate method is official in USP. It was carried out using the USP dissolution testing protocol. The amount of drug release was determined using the simultaneous equation method.

Validation of the Analytical Method

Validate the methods according to the guidelines suggested by international conference on harmonization for analytical validation.[13]

Linearity

Linearity was evaluated by regression analysis, calculated by the least square regression method.

Limit of Quantification (LoQ) and Limit of Detection (LoD)

Estimation of LoD and LoQ was done using the following formula:

$$LoD = 3.3\times (SD/S)$$

SD stands for the standard deviation of response, and S stands for the slope of the analytical curve.

$$LoQ = 10 \times (SD/S)$$

SD stands for the standard deviation of response, and S stands for the slope of the analytical curve.

Precision

Check the precision of the UV-spectrophotometric method by repeatability measuring six replicates of the target concentration (100%) and calculating the percentage relative standard deviation (%RSD).

Accuracy

Recovery studies were performed using the standard addition method to measure the degree of accuracy of the advanced simultaneous UV-spectrophotometric method.

RESULT AND DISCUSSION

Carry out the UV detection at 237.20 nm and 217.0 nm for amlodipine besilate and enalapril maleate, respectively (absorption maxima) (Figure 1).

A simultaneous equation method for the estimation of amlodipine besilate and enalapril maleate was developed successfully. The ratio $(A_2/A_1)/(a_{x2}/a_{x1})$ and $(a_{y2}/a_{y1})/(A_2/A_1)$ was found to be acceptable with a value of 2.044022404 and 12.51172077 (acceptance criteria - should lie outside the range of 0.1–2.0), (Table 2).

Constructed the analytical curves in the concentration range of 1–19 µg mL^{-1} with a slop of 0.0537, intercept of 0.0092, and regression coefficient of 0.9991 for amlodipine besilate (Figure 2) and 1–19 µg mL^{-1} with a slope of 0.1179, the intercept of 0.0102, and regression coefficient of 0.9996 for enalapril maleate (Figure 3).

For dissolution studies, the %RSD was 2.89 at 30 minutes, and 3.17 at 30 minutes for amlodipine besilate and enalapril maleate, respectively. (Figures 4 and 5).

The method was successfully validated. The method showed a linear response with regression coefficients of 0.9991 and 0.9996 for amlodipine besilate and enalapril maleate, respectively (Figures 2 and 3). Data for range, LoD, and LoQ are present in Table 3. Repeatability showed a %RSD less than 1.0% (0.0452 and 0.0382 for amlodipine and enalapril, respectively) (Table 4). The standard error (SE) demonstrated by the method for accuracy was below the specified limit of 1.0 (0.13119 and 0.07855 for amlodipine besilate and enalapril maleate, respectively) (Table 5).

CONCLUSION

The proposed UV-spectrophotometric method was fabricated and validated as per ICH guidelines. The standard deviation and %RSD found for the given methods are low, establishing a high degree of precision. Recovery studies show a high degree of accuracy for the proposed methods. The proposed method was found to be is modest, swift, precise, accurate, and reproducible for routine analysis of amlodipine besilate and enalapril maleate in dissolution media.

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CONFLICT OF INTEREST

The author(s) declare(s) no conflict of interest.

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