

## Absorbance correction method for simultaneous estimation of Amlodipine besylate and Simvastatin in synthetic mixture

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### ABSTRACT

A simple, accurate and precise spectroscopic method was developed for simultaneous estimation of Amlodipine besylate and Simvastatin in synthetic mixture using Absorbance correction method. At 360.80 nm ( $\lambda_{max}$  of Amlodipine besylate) Simvastatin has zero absorbance so Amlodipine besylate is directly estimate at 360.80 nm. At 237.60 nm ( $\lambda_{max}$  of Simvastatin) both drugs have some absorbance so Simvastatin is estimate at 237.60 nm using absorbance correction method. The method was found to be linear ( $r^2 > 0.999$ ) in the range of 5-10  $\mu\text{g/ml}$  for Amlodipine besylate at 360.80 nm. The linear correlation was obtained ( $r^2 > 0.999$ ) in the range of 5-10  $\mu\text{g/ml}$  for Simvastatin at 237.60 nm. The limit of determination was 0.17  $\mu\text{g/ml}$  and 0.10  $\mu\text{g/ml}$  for Amlodipine besylate and Simvastatin, respectively. The limit of quantification was 0.54  $\mu\text{g/ml}$  and 0.32  $\mu\text{g/ml}$  for Amlodipine besylate and Simvastatin, respectively. The accuracy of these method were evaluated by recovery studies and good recovery result were obtained greater than 99%. The method was successfully applied for simultaneous determination of Amlodipine besylate and Simvastatin in binary mixture.

**Keywords:** Amlodipine besylate, Simvastatin, Absorption correction Method

### INTRODUCTION

Hypertension (HTN) or high blood pressure, is a very common disorder. Blood pressure is summarised by two measurements, systolic and diastolic, which depend on whether the heart muscle is contracting (systole) or relaxed between beats (diastole). Both the drug used for cholesterol induced hypertension disease.<sup>[1]</sup>

Amlodipine besylate and Simvastatin combination was approved on 28 feb, 2008<sup>[8]</sup>

#### A. Amlodipine besylate<sup>[2-4]</sup>

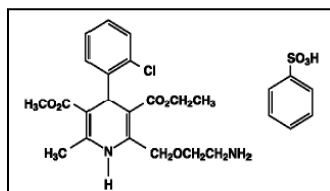
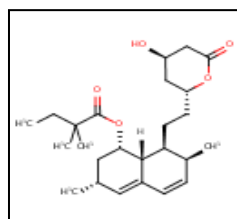


Figure 1 : Chemical Structure of Amlodipine besylate

Amlodipine besylate is long-acting dihydropyridine-type (DHP) calcium channel blocker used to lower blood pressure and to treat anginal chest pain.

IUPAC name of Amlodipine besylate is 3-ethyl 5-methyl(4RS)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl -1,4-dihydropyridine -3,5-dicarboxylate benzene sulphonate. Amlodipine besylate acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By inhibiting the influx of calcium in smooth muscle cells, amlodipine prevents calcium-dependent myocyte contraction and vasoconstriction.



#### B. Simvastatin<sup>[5-7]</sup>

Figure 2: Chemical structure of Simvastatin

Simvastatin is HMG-CoA reductase inhibitor (3-hydroxy-3-methylglutaryl-coA Reductase Inhibitor). Simvastatin is used along with a proper diet to help lower "bad" cholesterol and fats (such as LDL,

triglycerides) and raise "good" cholesterol (HDL) in the blood.

Simvastatin used In Hyperlipidaemia Inhibitor of HMG – CoA Reductase Enzyme, HMG-CoA reductase catalyzes the conversion HMG to mevalonate, which is the rate determining step in the biosynthesis of cholesterol, thus inhibition leads to a reduction in the concentration of cholesterol in the liver.

Amlodipine serves as an anti-hypertensive medicine and also increases the lipid-lowering activity of simvastatin through synergistic activity with the lipid-lowering agent. Simvastatin serves as a lipid-lowering agent and also has an activity of decreasing blood pressure through a synergistic effect with amlodipine.<sup>[8]</sup>

#### MATERIALS AND METHODOLOGY<sup>[9]</sup>

- Amlodipine besylate and Simvastatin were obtained as gift samples from Prudence pharmachem, Ankleshwar. And Praveen Laboratories, Surat. Synthetic Mixture contains 10mg of Amlodipine besylate and 10mg of Simvastatin.
- A double beam UV/Visible spectrophotometer (Shimadzu model 2450, Japan) with spectral width of 2 nm, 1 cm quartz cells was used to measure absorbance of all the solutions.
- Spectra were automatically obtained by UV-Probe system software.
- An analytical balance (Sartorius CD2250, Gottingen, Germany) was used for weighing the samples.
- Sonicator(D120/2H, TRANS-O-SONIC)
- Class 'A' volumetric glassware were used (Borosillicite)

#### Materials and reagents

##### Preparation of stock solution of AML

Accurately weighed quantity of Amlodipine besylate 10 mg was transferred to 100 ml volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution having strength of 100µg/ml.

##### Preparation of stock solution of SIM

Accurately weighed quantity of Simvastatin 10mg was transferred to 100 ml volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution having strength of 100µg/ml.

#### Preparation of Standard Mixture Solution (AML + SIM):

1ml of standard stock solution of AML (100µg/ml) and 1ml of standard stock solution of SIM(100µg/ml) were pipetted out into two 10ml volumetric flasks and volume was adjusted to the mark with methanol to get 10µg/ml of AML and 10µg/ml of SIM.

#### Preparation of test solution:

The preparation of synthetic mixture was as per patent:

- Amlodipine besylate : 10 mg
- Simvastatin : 10 mg
- Sodium Starch glycolate: 20 mg
- Starch : 20 mg
- Talc : 40 mg

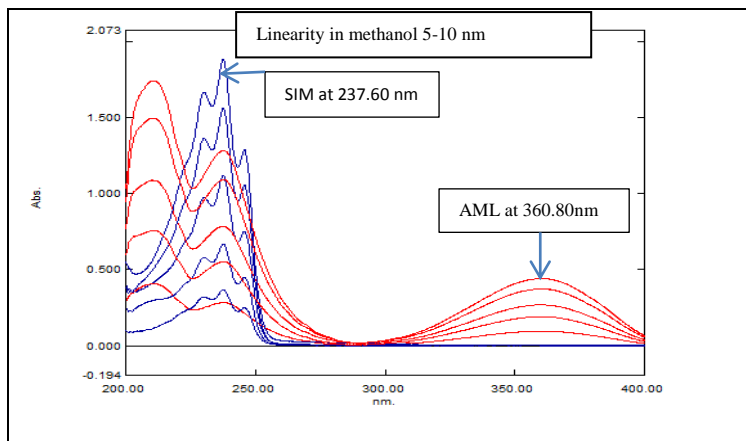
All the excipients were mixed in 100ml volumetric flask. make up the volume with Methanol up to 25 ml. and sonicated for 15min .The solution was filtered through Whatman filter paper. and make up the volume up to 100 ml with methanol. Finally the solution had concentration 100µg/ml for Amlodipine besylate and 100µg/ml for Simvastatin.

#### RESULT AND DISCUSSION

##### SELECTION OF WAVELENGTH AND METHOD DEVELOPMENT FOR DETERMINATION OF AMLODIPINE BESYATE AND SIMVASTATIN

The standard solution of AML and SIM were scanned separately between 200-400nm, and zero-order spectra were not showed overlapping peaks.

- From spectra at 360.80 nm ( $\lambda_{max}$  of Amlodipine besylate) Simvastatin has zero absorbance so Amlodipine besylate is directly estimate at 360.80 nm.
- At 237.60 nm ( $\lambda_{max}$  of Simvastatin) both drugs have some absorbance so Simvastatin is estimate at 237.60 nm using absorbance correction method.



### VALIDATION OF PROPOSED METHOD<sup>[10]</sup>

Parameters to be considered for the validation of methods are:

#### 1) LINEARITY AND RANGE

##### Procedure:

The linearity response was determined by analyzing 6 independent levels of calibration curve in the range of 5-10  $\mu\text{g/ml}$  and 5-10  $\mu\text{g/ml}$  for AML and SIM respectively (n=6)

##### Calibration curve for Amlodipine besylate

This series consisted of five concentrations of standard Amlodipine besylate solution ranging from 5 to 25  $\mu\text{g/ml}$ . The solutions were prepared by pipetting out Standard Amlodipine besylate stock solution (0.5ml, 1ml, 1.5ml, 2.0ml, 2.5ml) was transferred into a series of 10 ml volumetric flask and volume was adjusted up to mark with methanol. A zero order derivative spectrum of the resulting

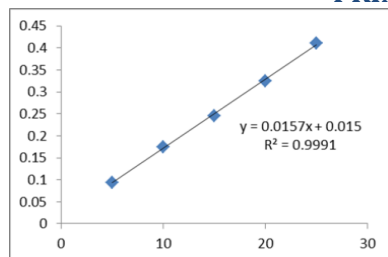
solution was recorded, measured the absorbance at 360.80 nm against a reagent blank solution (methanol). Calibration curve was prepared by plotting absorbance versus respective concentration of Amlodipine besylate.

##### Calibration curve for Simvastatin

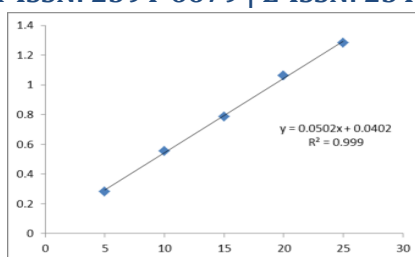
This series consisted of five concentrations of standard Simvastatin solution ranging from 5 to 25  $\mu\text{g/ml}$ . The solutions were prepared by pipetting out Standard Simvastatin stock solution (0.5ml, 1ml, 1.5ml, 2.0ml, 2.5 ml) was transferred into a series of 10 ml volumetric flask and volume was adjusted up to mark with methanol. A zero order derivative spectrum of the resulting solution was recorded, measured the absorbance at 237.60 nm against a reagent blank solution (methanol). Calibration curve was prepared by plotting absorbance versus respective concentration of Simvastatin.

AML ( $\mu\text{g/ml}$ )	ABSORBANCE* Avg $\pm$ SD	AML ( $\mu\text{g/ml}$ )	ABSORBANCE* Avg $\pm$ SD	SIM ( $\mu\text{g/ml}$ )	ABSORBANCE* Avg $\pm$ SD
5	0.094 $\pm$ 0.00098	5	0.283 $\pm$ 0.00083	5	0.356 $\pm$ 0.00089
10	0.175 $\pm$ 0.00154	10	0.553 $\pm$ 0.0016	10	0.721 $\pm$ 0.0026
15	0.246 $\pm$ 0.001095	15	0.785 $\pm$ 0.0021	15	1.116 $\pm$ 0.0037
20	0.325 $\pm$ 0.001169	20	1.061 $\pm$ 0.0010	20	1.512 $\pm$ 0.0013
25	0.411 $\pm$ 0.000816	25	1.284 $\pm$ 0.0010	25	1.851 $\pm$ 0.0015

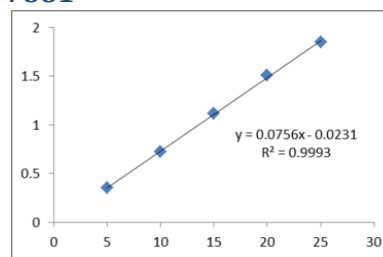
Table.1 Calibration data for mixture of AML, and SIM at 360.80nm, 237.80nm, respectively \*(n=6)



Amlodipine besylate at 360.80



Amlodipine besylate at 237.60 nm



Simvastatin at 237.60 nm

## 2) PRECISION

### i. Intraday precision

#### Procedure

- The precision of the developed method was assessed by analyzing combined standard solution containing three different concentrations 5, 15, 25 µg/ml for AML and 5,15,25 µg/ml for SIM. Three replicate (n=3) each on same day.
- For zero order spectra absorbance was measured at 360.80 nm for AML and 237.60 nm for SIM.
- The % RSD value of the results corresponding to the absorbance was expressed for intra-day precision.

Table.2 Intraday precision data for estimation of AML, and SIM \*(n=3)

Precision	Conc µg/ml	AML	Conc µg/ml	SIM
Abs. ±%	5	0.093±0.22	5	0.619± 0.16
RSD	15	0.275±0.41	15	1.847± 0.11
	25	0.449±0.12	25	2.813± 0.12

### ii. Interday Precision

#### Procedure

- The precision of the developed method was assessed by analyzing combined standard solution containing three different concentrations 5,15,25 µg/ml for AML and 5, 15, 25 µg/ml for SIM

Table.4 Recovery data of AML\* and SIM\*(n=3)

Initial conc. (µg/ml)		Level of recovery	Quantity of Std. Added (µg/ml)		Total Amount (µg/ml)		Result of recovery study			
							Total Quantity Found* (µg/ml)± %RSD		% recovery ± %RSD	
AML	SIM		AML	SIM	AML	SIM	AML	SIM	AML	SIM

triplicate (n=3) per day for consecutive 3 days for inter-day precision.

- For zero order spectra absorbance was measured at 360.80 nm for AML and 237.60 nm for SIM. The % RSD value of the results corresponding to the absorbance was expressed for inter-day precision.

Table.3 Interday precision data for estimation of AML, and SIM\*(n=3)

Precision	Conc µg/ml	AML	Conc µg/ml	SIM
Abs. ±%	5	0.094±0.32	5	0.623±0.27
RSD	15	0.278±0.54	15	1.850±0.13
	25	0.451±0.22	25	2.816±0.16

## 3) ACCURACY

- Accuracy of the method was determined by recovery study from synthetic mixture at three levels (80%, 100%, and 120%) of standard addition.
- The % recovery values are tabulated in Table and Percentage recovery for AMLO and SIM by this method was found in the range of 100.37 to 102.12% and 98-101.81% respectively,
- The value of %RSD within the limit indicated that the method is accurate and percentage recovery shows that there is no interference from the excipients.

10	10	0%	-	-	10	10	10.01±0.15	10.12±0.11	100.41±0.51	100.55±0.49
10	10	80%	8	8	18	18	18.02±11	17.86±14	100.62±0.45	99.50±0.47
10	10	100%	10	10	20	20	20.14±15	20.09±21	100.80±0.16	100.65±0.29
10	10	120%	12	12	22	22	22.05±22	22.08±18	100.46±0.41	100.49±0.44
Mean of 3 Determination									100.62%	100.21%

#### 4) LOD & LOQ

- The Limit of detection and quantitation of the developed method was assessed by analyzing 10 replicates of standard solutions containing concentrations 5 µg/ml for AML and SIM.

- The LOD and LOQ were calculated as **LOD = 3.3\*σ/S**, and **LOQ = 10\*σ/S**, where σ is the standard deviation of the lowest standard concentration and S is the slope of the standard curve. % RSD was calculated.

Table.5 LOD and LOQ data of AML and SIM\*(n=10)

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Amlodipine besylate	0.17	0.54
Simvastatin	0.10	0.32

#### APPLICATION OF THE PROPOSED METHOD FOR ANALYSIS OF EDA AND ARG IN SYNTHETIC MIXTURE (ASSAY)

- A zero order derivative spectrum of the resulting solution was recorded and absorbances at 360.80nm and 237.60nm were noted for estimation of AML and SIM, respectively.

- The concentration of AML and SIM in mixture was determined using the corresponding calibration graph. The results from the analysis of synthetic mixture containing Amlodipine besylate (10mg) and

Simvastatin (10mg) in combination are presented. The percent assay shows that there is no interference from excipients and the proposed method can successfully applied to analysis of commercial formulation containing AML and SIM. The % assay values are tabulated.

Table. 6 Analysis data of commercial formulation \*(n=3)

Drugs	% Assay ± SD	% RSD(n=3)
Amlodipine besylate	100.45 ± 0.0058	0.58
Simvastatin	100.85 ± 0.0061	0.60

#### 6) Robustness

Robustness and Ruggedness of the method was determined by subjecting the method to slight change in the method condition, individually, the:

Change in Wavelength (± 0.2 nm)

AML at 360.60 nm and 361 nm and SIM at 237.40 nm and 237.80 nm

Change in instrument (UV-Vis Spectrophotometer model 1800 and 2450),

Three replicates were made for the concentration (5,15,25µg/ml of AML and 5,15,25 µg/ml of SIM) with different stock solution preparation.

% RSD was calculated.

Table 7: Robustness and Ruggedness data of AML and SIM\*(n=3)

Condition	Conc. (µg/ml)	Different Instrument		Wavelength (±0.2 nm)	
		UV-2450	UV-1800	360.60 nm	361 nm
AML Mean (n=3) ± % RSD	5	0.094±0.10	0.096±0.13	0.094±0.17	0.098±0.21
	15	0.276±0.17	0.274±0.15	0.275±0.19	0.278±0.16
	25	0.448±0.18	0.445±0.27	0.449±0.10	0.452±0.18
SIM Mean(n=3) ± %RSD		UV-2450	UV-1800	237.40 nm	237.80 nm
	5	0.620±0.33	0.624±0.27	0.625±0.19	0.627±0.29
	15	1.848±0.22	1.845±0.23	1.846±0.21	1.850±0.20
	25	2.812±0.20	2.814±0.32	2.817±0.17	2.820±0.25

**SUMMARY OF VALIDATION PARAMETER.**

PARAMETERS	Absorbance correction method	
	Amlodipine besylate	Simvastatin
Concentration range( $\mu\text{g/ml}$ )	5-25	5-25
Regression equation	$y = 0.0157 + 0.015x$	$y = 0.0756x - 0.0235$
Correlation Coefficient( $r^2$ )	0.9991	0.9993
Accuracy(%Recovery) (n=3)	100.62%	100.21%
Intra-day Precision (%RSD) (n=3)	0.12-0.41	0.11-0.16
Inter-day precision (%RSD) (n=3)	0.22-0.54	0.16-0.27
LOD( $\mu\text{g/ml}$ )	0.17	0.10
LOQ( $\mu\text{g/ml}$ )	0.54	0.32
Ruggedness and Robustness	0.10-0.27	0.19-0.33
% Assay	100.45%	100.85%

**CONCLUSION**

All the parameters are validated as per ICH guidelines for the method validation and found to be suitable for routine quantitative analysis in pharmaceutical dosage forms. The result of linearity, accuracy, precision proved to be within limits with lower limits of detection and quantification. Ruggedness and Robustness of method was confirmed as no significant were observed on analysis by subjecting the method to slight change in the method condition. Assay results obtained by proposed method are in fair agreement.

**ACKNOWLEDGEMENT:** We are sincerely thankful to Shree Dhanvantary Pharmacy College, Kim, Surat, for providing us Infrastructure facilities and moral support to carry out this research work. We are also thankful to SDPARC for giving us their special time and guidance for this research work. We also thank our colleagues for their helping hand.

**↓ REFERENCES**

1. Hypertension-KD Tripathi. Medical Pharmacology; 6th Edn; Jaypee Brothers Medical Publishers (p) Ltd, New Delhi, 2008, pp 539.
2. Drug profile available from: [en.wikipedia.org/wiki/Amlodipine\\_besylate](http://en.wikipedia.org/wiki/Amlodipine_besylate)
3. Drug Profile available from: [chemicalland21.com/Amlodipine\\_besylate](http://chemicalland21.com/Amlodipine_besylate)
4. Mechanism action available from: [drugsbank.com/Amlodipine\\_besylate](http://drugsbank.com/Amlodipine_besylate)
5. Drug profile available from: [drugsbank.com/Simvastatin](http://drugsbank.com/Simvastatin)
6. Introduction available on : [medicine.net.com](http://medicine.net.com)
7. Moshe FB, Itzhak EL, Naomi M, Vered R and Teva pharma. Simvastatin formulations and methods of making same. WIPO WO2005044258 A1, 2004.
8. Sung W, Sung S, Youg G, Ja-seongkod and Jin W. Combined pharmaceutical formulation with controlled-release comprising dihydropyridine calcium channel blockers and hmg-coa reductase inhibitors. WIPO WO 2008023958 A1, 2007.
9. Beckett AH, Stenlake JB, Davidson AG. Ultraviolet-Visible absorption spectrophotometry, Practical Pharmaceutical Chemistry, 4th Edn; New Delhi, CBS Publishers, 2002, pp 275-300.
10. International Conference on Harmonization, Harmonized Tripartite Guideline, Validation of Analytical Procedures Text and Methodology, ICH Q2(R1), 2005.