<u>**Research Article**</u>

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND INDAPAMIDE IN COMBINED DOSAGE FORM

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ABSTRACT

Two simple, accurate, rapid and precise UV spectrophotometric methods have been developed for simultaneous estimation of Amlodipine Besylate and Indapamide in combined tablet formulation. Amlodipine Besylate and Indapamide at their respective λmax 237nm and 240 nm shows linearity in a concentration range of 5-25 µg/ml and 2-10 µg/ml respectively. The methods employed were (A) Absorbance ratio and (B) Area under curve (AUC). Method-A involves measurement of absorbance at 310.0nm (iso-absorptive point) and 237.0 nm (λmax of Amlodipine Besylate). Method–B involves measurement of area at two wavelength range 232.0-242.0 nm and 235.0-245.0 nm. The linearity for Amlodipine Besylate and Indapamide were found over the concentration range of 5-25 µg/ml and 2-10 µg/ml respectively. Both the methods were found to be rapid, specific, precise and accurate, can be successfully applied for the routine analysis of Amlodipine Besylate and Indapamide in bulk, and combined dosage form without any interference by the excipients. The method was validated according to ICH guidelines.

Keywords: Amlodipine Besylate, Indapamide, Absorbance ratio method, Area under curve method

INTRODUCTION

Amlodipine Besylate (AMLO), chemically, [3-ethyl-5-methyl(4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-methyl-1-dihydropyridine-3,5-dicarboxylate benzenesulfonate[1] (fig.-1), is a long acting calcium channel blocker which is used as an antihypertensive agent. Amlodipine is official in I.P.,B.P.,U.S.P. Amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing total peripheral resistance, hence reducing blood pressure; in angina it increases blood flow to the heart muscle[2].
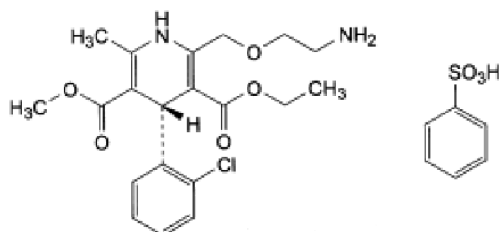

Fig. 1: Structure of Amlodipine Besylate

Indapamide (INDA),chemically,3-(Aminosulfonyl)-4-chloro-N-(2,3-dihydro-2-methyl-1H-indol-1-yl)benzamid,is a diuretic which is used as an antihypertensive agent[3](fig.-2). Indapamide is official in B.P. and U.S.P. Indapamide, in vitro, directly inhibits pressor stimuli probably through a reduction of calcium flux in vascular smooth muscle, whilst diuretics are inactive. Indapamide does have mild diuretic activity at therapeutic doses, has both diuretic and vasodilator properties. A low urinary excretion and specific accumulation into arterial smooth muscle of this lipophilic molecule may provide a rationale for this dual activity [4].
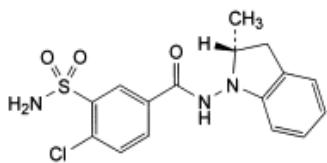

Fig. 2: Structure of Indapamide

Literature survey revealed that a number of methods have been reported for estimation of AMLO and INDA individually or in combination with other drugs [5-16]. However, there is no Q-absorbance ratio and Area under curve method reported for the simultaneous estimation of AMLO and INDA in a combined dosage formulation. Present work describes two simple, accurate, reproducible, rapid and economical methods for simultaneous estimation of Amlodipine Besylate and Indapamide in tablet formulation.

MATERIALS AND METHOD

Instrument

A double-beam Shimadzu UV/Vis spectrophotometer, 1800 with spectral bandwidth of 2 nm, wavelength accuracy of ±0.5 nm and a pair of 1-cm matched quartz cells, was used to measure absorbance of the resulting solution. All weighing were done on electronic balance (Model Shimadzu AUW-220D), Ultrasonicator model 5.5L150H were used.

Chemicals and reagents

AR grade of methanol was used, purchased from Astron Chemicals LTD.

Standard gift sample of Amlodipine Besylate was obtained from CPL, Chemicals SBU, Ankleshwar, Unit – 1, Gujarat, India and Indapamide from Ami Life Sciences PVT. LTD. 82/B, ECP, canal Rd.Karkhadi, Padra, Baroda, Gujarat, India.

Amlodipine Besylate and Indapamide combination tablets (NATRILAM, 5 mg Amlodipine Besylate and 1.5 mg Indapamide; is manufactured by Serdia Pharmaceuticals, Mumbai, India), (AMLODAC-D, 5 mg Amlodipine Besylate and 1.5 mg Indapamide; is manufactured by Zydus Cadila, Ahmedabad, India), were purchased from the local pharmacy.

Preparation of standard stock solution (100 µg/ml)

The stock solution (100 µg/ml) of AMLO and INDA were prepared separately by dissolving accurately about 10 mg of drug in Methanol and the volume was made up to 100 ml with Methanol to prepare standard stock solution (100 µg/ml).

Preparation of Calibration curve of AMLO and INDA

The standard stock solution (100 µg/ml) of AMLO and INDA were further diluted with methanol to obtain the final concentration 5, 10, 15, 20, 25 µg/ml and 2, 4, 6, 8, 10 µg/ml respectively. The standard solution of both drug were scanned in the spectrum mode from 200 nm to 400 nm against solvent methanol and spectra was recorded. λmax of AMLO and INDA was found 237.0 nm (Fig.3) & 240.0 nm(Fig.4) respectively.
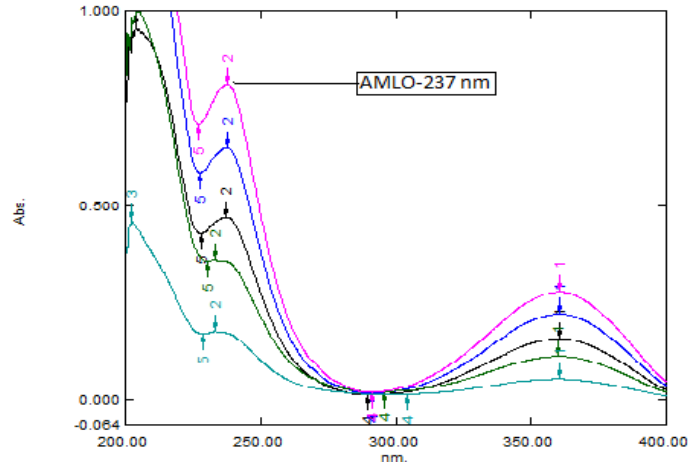
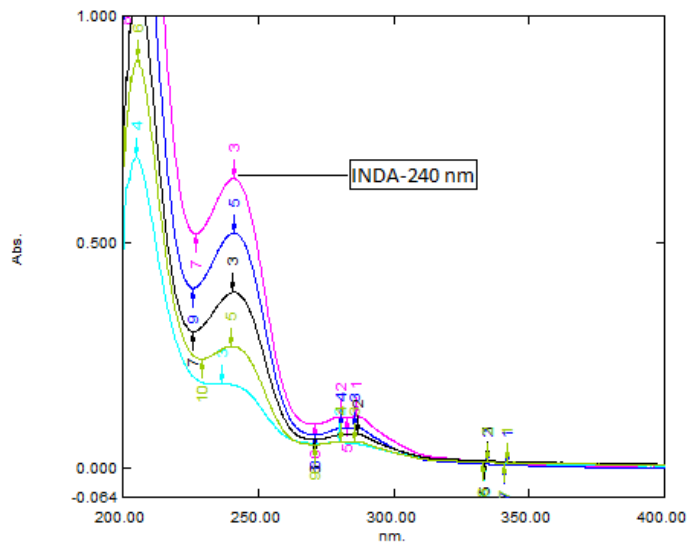**Fig. 3: Overlay spectra of Amlodipine Besylate at 237.0 nm**.

Fig. 4: Overlay spectra of Indapamide at 240.0 nm

Procedure

Method A (Absorbance ratio method)

It uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that AMLO and INDA show an isoabsorptive point at 310.0 nm. The second wavelength used is 237 nm, which is the λ-max of AMLO. Five working standard solutions having concentration 5, 10, 15, 20, 25µg/ml for AMLO and 2, 4, 6, 8, 10 µg/ml for INDA were prepared in methanol and the absorbances at 310.0 nm (isoabsorptive point) and 237.0 nm (λ-max of AMLO) were measured and absorptivity coefficients were calculated using calibration curve.

Absorptivity = Absorbance/ Concentration of that component in gm/100 ml.

The concentration of two drugs in the mixture can be calculated using following equations.

$C_A = [(Q_M - Q_I) / (Q_A - Q_I)] \times A_1/aX_1$ .... (1)

$C_I = (A_1/aX_1) - C_A$ .... (2)

Where, A_1 and A_2 are absorbances of mixture at 310.0 nm and 237.0 nm;

aX_1 and aY_1 are absorptivities of AMLO and INDA at 310.0 nm;

aX_2 and aY_2 are absorptivities of AMLO and INDA respectively at 237.0 nm;

$Q_M = A_2 / A_1$,

$Q_A = aX_2 / aX_1$ and

$Q_I = aY_2 / aY_1$.

Validation data for this method are given in Table-1.

Method B (Area under curve)

From the overlay spectra of both the drugs, wavelengths range 242.0-232.0 nm (for AMLO) and 255.0-245.0 nm (for INDA) were selected for the analysis. The calibration curves for AMLO and INDA were prepared in the concentration range of 5-25 µg/ml and 2-10 µg/ml at both the wavelength range, respectively. The absorptivity values were determined for both the drugs at both the wavelength range and following Equations were used,

$A_1 = 0.01392C_{AMLO} + 0.0106C_{INDA}$ .... (3)

$A_2 = 0.012C_{AMLO} + 0.0265C_{INDA}$ .... (4)

where A_1 and A_2 are area under curve of the sample at 242.0-232.0 nm and 255.0-245.0 nm, respectively,

0.01392 and 0.012 are absorptivities of AMLO at 242.0-232.0 nm and 255.0-245.0 nm, respectively,

0.0106 and 0.0265 are the absorptivities of INDA at 242.0-232.0 nm and 245.0-235.0 nm, respectively.

C_{AMLO} is the concentration of AMLO

C_{INDA} is the concentration of the INDA. The mixture concentration was determined by using the Equations 3 and 4.

Validation data for this method are given in Table-2.

Procedure for the Analysis of Tablet formulation

Twenty tablets containing label claim of 5mg of AMLO and 1.5 mg of INDA were weighed and finely powdered .The average weight was calculated. Weight of the powder equivalent to 5 mg Amlo was accurately weighed, transferred into a 25 ml flask, dissolved in methanol and sonicated for 20 min. The volume is adjusted up to the mark with methanol. The solution was then filtered through Whatman filter paper no. 41.

The solution was suitably diluted with methanol to get a final concentration of 10 µg/ml of AMLO and 3 µg/ml of INDA.

Concentrations of both AMLO and INDA were determined by measuring the absorbance of the sample at 310.0 nm (isoabsorptive point-Fig.5) and 237.0 nm (λ-max of AMLO-Fig.6) respectively, and ratios of absorbance were calculated, i.e. A_2/A_1. (method-A) and at 242.0-232.0 nm(Fig.7) and 245.0-235.0 nm(Fig.8) (method B) in the spectrum mode and values were substituted in the respective formula to obtain concentrations. Results of the tablet analysis were analyzed against the calibration curve in quantitation mode(Table 3).The analysis procedure was repeated three times with tablet formulation. Overlain spectra for both methods (Fig.9 and Fig.10).Recovery studies was performed (Table-4)
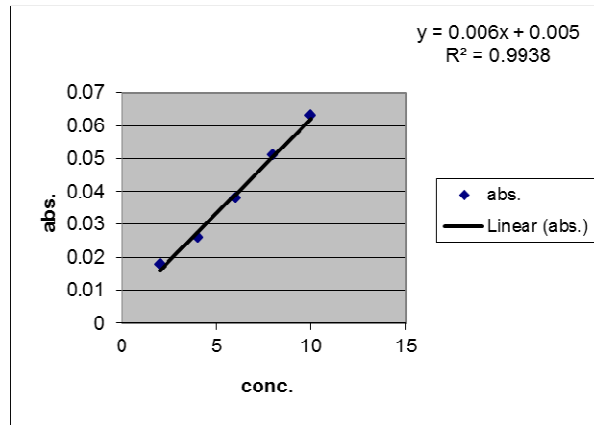

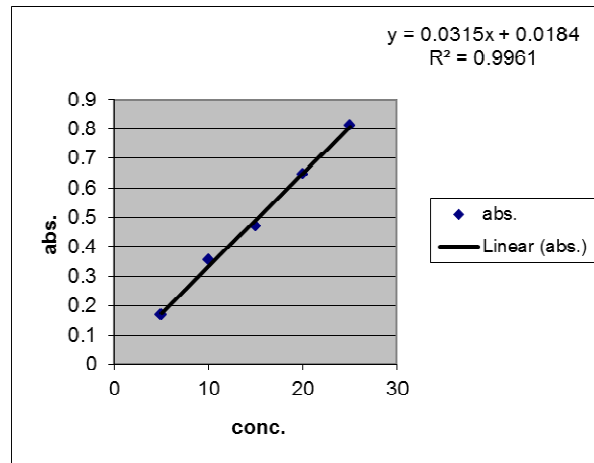**Fig. 5: Calibration curve at iso absorptive point 310.0 nm**
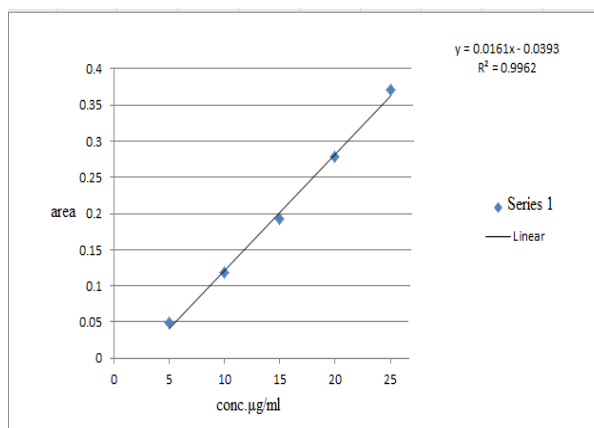

Fig. 6: Calibration curve of AMLO at 237.0 nm

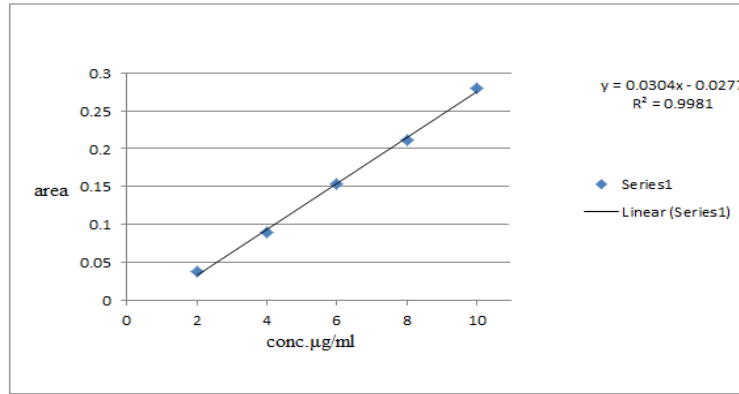
Fig. 7: AUC of AMLO at 232-242nm
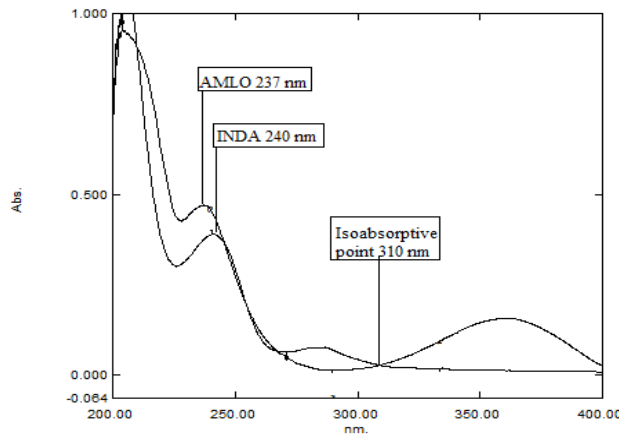
Fig. 8: AUC of INDA at 235-245 nm

Fig. 9: Overlay absorption spectra of AMLO (15 µg/ml) and INDA (6 µg/ml)
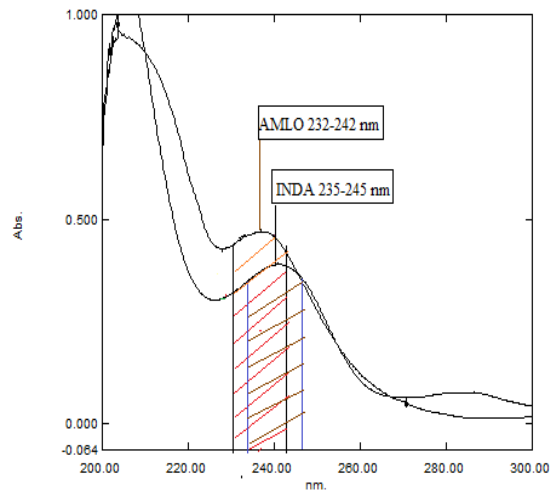
Fig. 10: Overlay absorption spectra of area of AMLO (232-242 nm) and INDA (235-245 nm)

Table 1: Validation Parameters for Absorbance ratio method

Parameters	AMLO	INDA
Beer's law range	5-25 µg/ml	2-10 µg/ml
Wavelength (nm)	237	310
Correlation Coefficient	0.996	0.9938
Slope	0.0315	0.006
Intercept	0.0184	0.005
LOD (µg/ml)	0.01	0.01
LOQ (µg/ml)	0.033	0.033
% RSD		
Intraday precision	0.29-0.93	0.22-1.3
Interday precision	0.10-0.90	1.10-1.53

Table 2: Validation Parameters for Area under curve method

Parameters	AMLO	INDA
Beer's law range	5-25 µg/ml	2-10 µg/ml
Wavelength (nm)	232-242	235-245
Correlation Coefficient	0.9962	0.9981
Slope	0.0161	0.0304
Intercept	-0.0393	-0.0277
LOD (µg /ml)	0.01	0.01
LOQ (µg /ml)	0.033	0.033
% RSD		
Intraday precision	0.29-0.93	0.22-1.3
Interday precision	0.10-0.90	1.10-1.53

LOD=limit of detection; LOQ=limit of quantification; (%RSD) = % relative standard deviation

Table 3: Results of simultaneous estimation of marketed formulation for Method A & B

Method	Label claim (mg)		Amount found (mg)*		% Label claim	
	AMLO	INDA	AMLO	INDA	AMLO	INDA
A	5	1.5	5.005	1.52	100.1	101.33
B	5	1.5	5.08	1.49	101.6	99.33

*Each value is a mean of six observations.

Table 4: Recovery studies of AMLO and INDA

Method	Level of recovery	Amount taken(µg/ml)		Amount added (µg/ml)		Total amount found (µg/ml)*		% recovery	
		AMLO	INDA	AMLO	INDA	AMLO	INDA	AMLO	INDA
1	0%	10	3	0	0	10.01	3.05	100.1	101.66
	50%	10	3	5	1.5	14.9	4.59	99.46	102.00
	100%	10	3	10	3.0	19.89	6.08	99.45	101.3
	150%	10	3	15	4.5	24.86	7.43	99.44	99.06
2	0%	10	3	0	0	10.2	3.06	102.00	102.00
	50%	10	3	5	1.5	15.25	4.46	101.66	99.11
	100%	10	3	10	3.0	19.68	6.10	98.4	101.66
	150%	10	3	15	4.5	25.5	7.64	102.00	101.86

*Each value is a mean of three observations.

CONCLUSION

These validated methods are new, rapid, accurate, precise, sensitive, reproducible and can be employed for routine analysis for simultaneous estimation of Amlodipine Besylate and Indapamide in combined dosage form

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