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Research Article

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESILATE AND INDAPAMIDE IN COMBINED PHARMACEUTICAL FORMULATION

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ABSTRACT

Three simple, rapid, precise and accurate spectrophotometric methods have been developed for simultaneous analysis of Amlodipine Besilate (AML) and Indapamide (IND) in their combined dosage form. Method I, zero crossing first derivative spectrophotometry involves measurement of amplitudes at 240.6 nm (for AML) and 236.8 nm (for IND) in first derivative spectra. Method II, absorbance correction method, involves measurement of absorbance at 360.2 nm for estimation of AML and measurement of corrected absorbance at 241.2 nm for estimation of IND. Method III, ratio derivative spectrophotometry, involves division of spectra of AML by one selected standard spectrum of IND and then measuring amplitudes at 332.8 nm in ratio derivative spectra for estimation of AML. Similarly, spectra of IND are divided by one selected standard spectrum of AML and then amplitudes at 299.4 nm in ratio derivative spectra are measured for estimation of IND. Developed methods were validated according to ICH guidelines. The calibration graph follows Beer's law in the range of 5 to 30 µg/ml for AML and 1.5 to 9 µg/ml for IND with R square value greater than 0.999. Accuracy of all methods was determined by recovery studies and showed % recovery between 98 to 102%. Intraday and interday precision was checked for all methods and mean %RSD was found to be less than 2 for all the methods. The methods were successfully applied for estimation of AML and IND in marketed formulation.

INTRODUCTION

Amlodipine (as besylate, mesylate or maleate), 3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-l,4-dihydro-6-methyl-3,5-pyridinedicarboxylate (Fig. 1.), is a long-acting calcium channel blocker (dihydropyridine (DHP) class) used as an anti-hypertensive and in the treatment of angina. It acts by relaxing the smooth muscle in the arterial wall, decreasing total peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart muscle. Indapamide, 4-chloro-N-(2-methyl-2,3-dihydroindol-1-yl)- 3-sulfamoyl-benzamide (Fig. 2.), is a thiazide diuretic drug, used in the treatment of hypertension as well as decompensated cardiac failure. It acts by inhibiting transmembrane ionic influx and stimulating synthesis of the vasodilatory hypotensive prostaglandin PGE2. At dose above 2.5 mg/day, the diuretic action of Indapamide predominates.

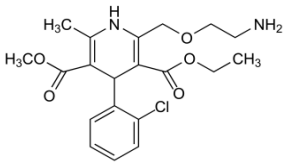

Fig. 1: Amlodipine

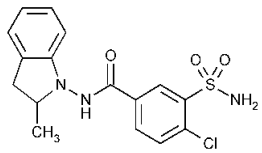

Fig. 2: Indapamide

Objective of the study

Spectrophotometric methods for simultaneous determination of Amlodipine besylate with Perindopril erbumine and with Atorvastatin calcium have been reported[1,2]. A stability indicating UPLC method for simultaneous determination of Amlodipine besylate, Telmisartan and Hydrochlorthiazide has also been reported[3]. HPLC methods for determination of Amlodipine besylate with Atorvastatin, with Hydrochlorthiazide and with Metoprolol succinate have also been reported[4,5,6]. RP-HPLC methods for simultaneous determination of Indapamide with Atenolol and with Telmisartan have been reported[7,8]. The objective of the present study was to develop easy, economic, accurate, specific and precise analytical methods for simultaneous estimation of Amlodipine and Indapamide in combined pharmaceutical formulations.

MATERIALS AND METHODS

Apparatus and Software

Shimadzu UV-1700 double beam spectrophotometer connected to a computer loaded with Shimadzu UVProbe 2.10 software was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1 cm quartz cells over the range of 200-400 nm.

Reagents and Chemicals

Methaol for UV Spectroscopy (Spectrohem Pvt. Ltd, Mumbai, India) was used as solvent and vehicle.

Preparation of solutions

Accurately weighed AML and IND (in quantities of 25 mg and 7.5 mg respectively) were transferred to two separate 25 ml volumetric flasks, dissolved with the use of methanol and volume was made up to the mark with methanol. From this, standard stocks solutions of AML (100 µg/ml) and IND (30 µg/ml) were prepared by transferring 2.5 ml aliquots to other 25 ml volumetric flasks and making up the volume with methanol. From this, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml were transferred to 10 ml volumetric flasks and volume were made up to the mark with methanol. This gives 5 to 30 µg/ml of AML and 1.5 to 9 µg/ml of IND.

Method I: Zero crossing first derivative spectrophotometry

The solutions of standard AML and IND were prepared in the range of 5 to 30 µg/ml and 1.5 to 9 µg/ml respectively. The absorption spectra of the solutions of AML and IND were recorded in the range of 220 nm to 400 nm and were stored in the memory of the instrument and transformed to first derivative with $\Delta\lambda$ = 8nm and scaling factor 50 (Fig. 3.). At 240.6 nm, IND is having zero crossing point and AML can be determined. At 236.8 nm, AML is having zero crossing point and IND can be determined. The amplitudes at 240.6 nm were plotted against respective concentrations of AML and the amplitudes at 236.8 nm were plotted against the respective concentrations of IND for the preparation of calibration graph. Calibration graph for AML and IND are shown below (Fig. 4.).

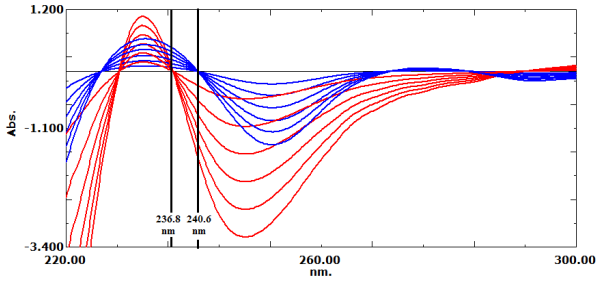

Fig. 3: First derivative overlain spectra of AML (5, 10, 15, 20, 25, 30 µg/ml, red) and IND (1.5, 3, 4.5, 6, 7.5, 9 µg/ml, blue)

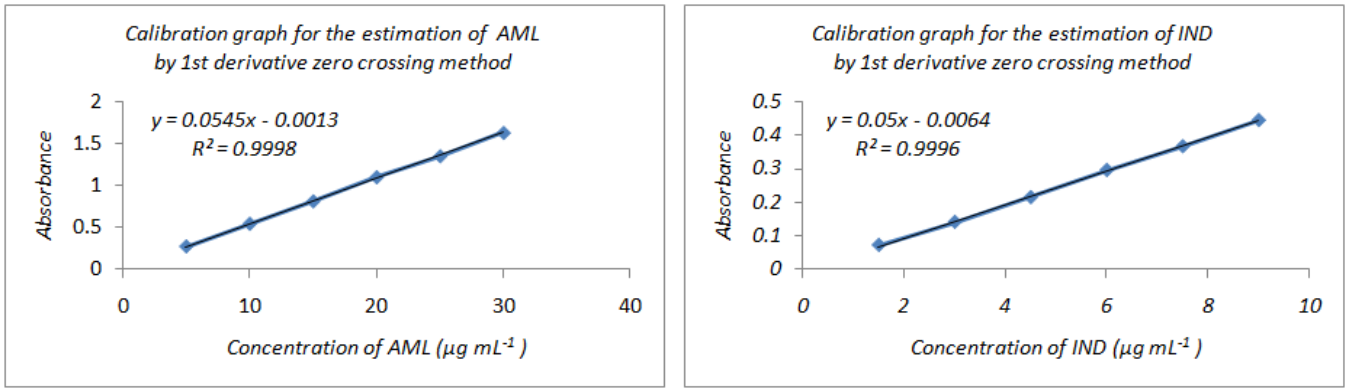

Fig. 4: Calibration graphs of AML and IND by zero crossing first derivative method

Method II: Absorbance Correction Method

Absorbance spectra of AML (5 to 30 µg/ml) and IND (1.5 to 9 µg/ml) in the range of 220 to 400 nm were taken. Overlain zero order spectra of both drugs are shown below (Fig. 5.). This method involves measurement of absorbance at 360.2 nm and 241.2 nm. At 360.2 nm, IND shows no absorbance and AML can be estimated directly without any interference of IND. IND shows maximum absorbance at 241.2 nm where AML is having considerable interference. So, absorbance of AML at 241.2 nm is corrected from total absorbance and then it is related to concentration of IND. Calibration graphs are prepared at 360.2 nm and 241.2 nm for AML and IND respectively (Fig. 6.).

$$CA_{IND,\ 241.2\ nm} = A_{241.2\ nm} - A_{AML,\ 241.2\ nm}$$

$CA_{IND,\ 241.2\ nm}$ = Corrected absorbance for IND at 241.2 nm

$A_{241.2\ nm}$ = Absorbance at 241.2 nm

$A_{AML,\ 241.2\ nm}$ = Absorbance of AML at 241.2 nm

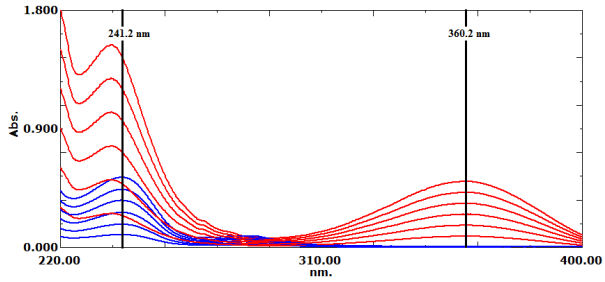

Fig. 5: Zero order overlain spectra of AML (5, 10, 15, 20, 25, 30 µg/ml, red) and IND (1.5, 3, 4.5, 6, 7.5, 9 µg/ml, blue)

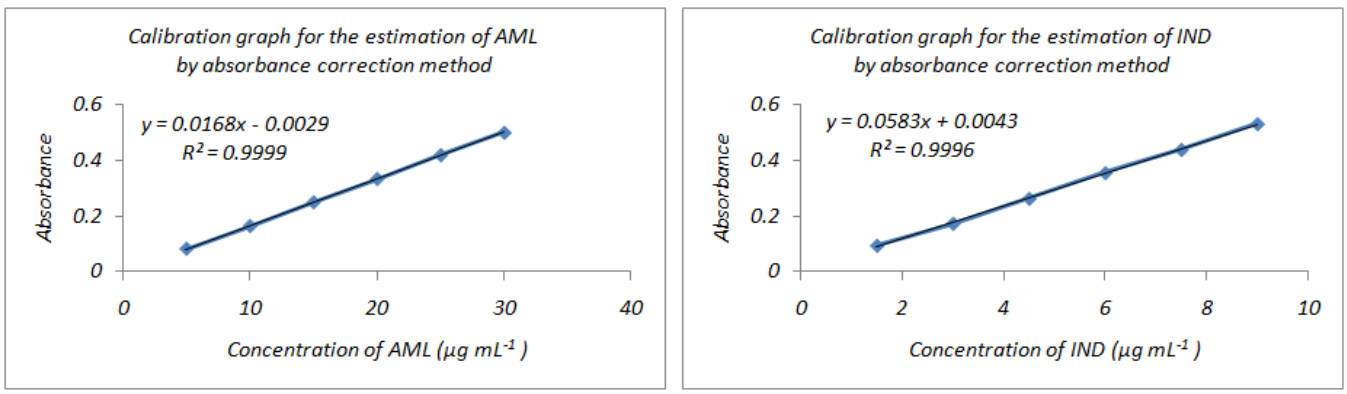

Fig. 6: Calibration graphs of AML and IND by absorbance correction method

Method III : Ratio Derivative Spectrophotometry

In this method, the spectra of AML and IND were divided by one standard spectrum of IND and AML respectively. For selecting the standard solution as divisor, appropriate concentrations of AML and IND were tested and based on better signal to noise ratio values, 25 µg/ml of AML and 9 µg/ml of IND were selected as divisor concentration. The spectra of AML ranging from 5 to 30 µg/ml were recorded in the region of 220 to 400 nm and were divided by standard spectrum of 9 µg/ml IND to obtain ratio spectra. These ratio spectra were derivatised with Δλ = 16 nm and scaling factor 5. Ratio derivative spectra are shown below (fig 7). Analytical wavelength of 332.8 nm was selected because of higher correlation co efficient for estimation of AML. Calibration graph at this wavelength is plotted and shown in fig. 8. Similarly, the spectra of IND ranging from 1.5 to 9 µg/ml were recorded and divided by standard spectrum of 25 µg/ml AML.

These ratio spectra were derivatised with Δλ = 16 nm and scaling factor 5. For estimation of IND, analytical wavelength of 299.4 nm was selected. Ratio derivative spectra (Fig. 7.) and calibration graph (Fig. 8.) are shown below.

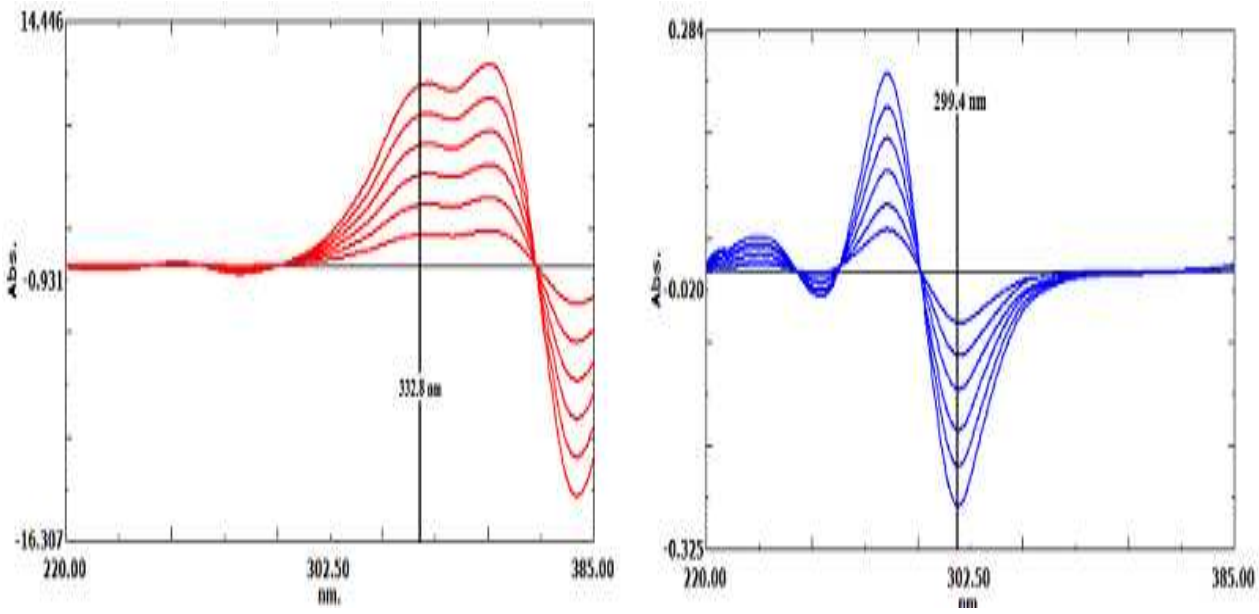

Fig. 7: Ratio derivative spectra of AML and IND

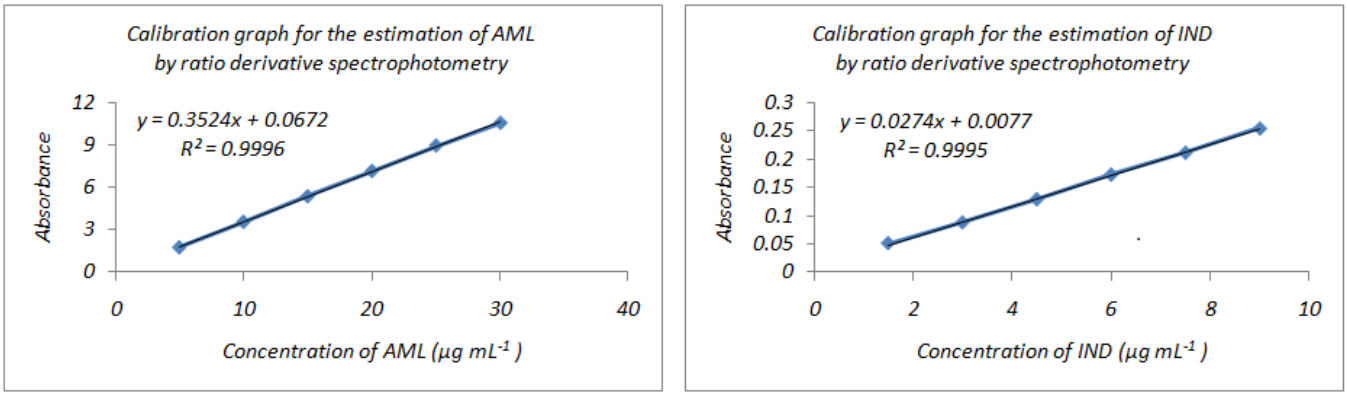

Fig. 8: Calibration graphs of AML and IND by ratio derivative spectrophotometry

Assay of Commercial Formulation by Method I, II and III

10 tablets were powdered and an amount equivalent to 5 mg AML and 1.5 mg IND was weighed and dissolved in 25 ml methanol. Solutions were filtered using whatmann filter paper grade 1. Appropriate dilutions were prepared in methanol taking suitable aliquots of the clear filtrates and subjected to analysis using all the three methods described above. The result of analysis is reported in Table 1.

Table 1: Results of Simultaneous Estimation of Marketed Formulation for Method I, II and III

Formulation :- NATRILAM **Labelled claim :- AML : IND (5 mg : 1.5 mg)** **Method**	**AML***	**IND***
I	99.58 ± 0.82 %	98.71 ± 1.34 %
II	98.67 ± 1.65 %	99.12 ± 0.97 %
III	99.85 ± 0.56 %	99.68 ± 0.77 %

* Mean value of five determinations

Table 2: Summary of Validation Parameters by Developed Methods

Parameters		Method I		Method II		Method III	
		AML	IND	AML	IND	AML	IND
Analytical wavelength (nm)		240.6	236.8	360.2	241.2	332.8	299.4
Beer's range (µg/ml)		5 to 30	1.5 to 9	5 to 30	1.5 to 9	5 to 30	1.5 to 9
Regression coefficient		0.9998	0.9996	0.9999	0.9996	0.9996	0.9995
Intraday precision (%RSD)		0.745	0.956	1.227	1.53	0.279	0.679
Interday precision (%RSD)		0.87	1.538	1.76	1.65	1.732	1.783
LOD (µg/ml)		0.052	0.045	0.057	0.041	0.064	0.051
LOQ (µg/ml)		0.156	0.135	0.171	0.135	0.192	0.153
% Recovery	80% standard addition*	98.46	97.83	98.14	102.85	100.45	99.00
	100% standard addition*	102.2	101.6	98.75	103.22	101.1	101.09
	120% standard addition*	101.42	99.67	101.14	98.38	99.98	98.44

* Mean value of three determinations

RESULTS AND DISCUSSION

Developed spectrophotometric methods for the simultaneous were validated according to ICH guidelines and data complying with the standards were obtained. The results of validation parameters for all the three methods are reported in Table 2.

CONCLULSION

Spectrophotometric methods were developed for simultaneous estimation of AML and IND in their combined formulation without prior separation. Spectra of IND were completely overlapped by AML and derivatisation was used as a powerful tool for simultaneous determination. Methods were found to be precise and accurate as can be reflected from validation data. Developed methods were successfully applied for estimation of AML and IND in marketed formulation.

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