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SIMULTANEOUS ESTIMATION OF AMLODIPINE AND INDAPAMIDE IN COMBINED DOSAGE FORM USING DERIVATIVE SPECTROPHOTOMETRIC METHOD

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Abstract: A simple and reproducible spectrophotometric method, requiring no prior separation, has been developed for the estimation of Amlodipine and Indapamide in combined dosage form. First order derivative spectroscopy method was adopted to eliminate spectral interference, using 237.40 nm and 241.40 nm as zero crossing points for Amlodipine and Indapamide respectively. Methanol was used as a solvent. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method.

Keywords: First order derivative spectroscopy, Amlodipine, Indapamide

INTRODUCTION

A simple, accurate, and reproducible UV spectrophotometric method for simultaneous estimation of two component drug mixture of Amlodipine and Indapamide in combined dosage form has been developed. Amlodipine is (RS)-3- ethyl 5-methyl 2-[(2-aminoethoxy)methyl-4-(2-chlorophenyl)-6-mthyl-1,4-dihyrdropyridine-3,5 dicarboxylate (Figure 1), which is best known as an anti-hypertensive drug. Indapamide is 4-chloro-N-(2-methyl-2,3-dihyrro-1H-indol-1-yl)-3-sulfamoylbenzamide (Figure 2) and it is used as antihypertensive and diuretic drug [2, 3]. Amlodipine is officinalis chemically 4-(of Amlodipine and 1.5 mg of Indapamide is available in market (AMLODAC-D*, zydus cadilla, Ahmedabad). A survey of literature revealed that few chromatographic, HPTLC and Spectrophotometric and methods are reported for determination of Amlodipine[8-12] and Indapamide individually or in combination with other drugs. However there is no method reported so far its simultaneous determination of Amlodipine and Indapamide [13-25] from combined dosage form. The present work describes a validated, simple, precise and accurate spectrophotometric method for simultaneous estimation of Amlodipine and Indapamide from combined tablet dosage form.[26-37]

MATERIALS AND METHODS

Materials

Reference Standards of Amlodipine and Indapamide were obtained as gift samples from the Torrent research centre, Gandhinagar. The drug sample, AMLODAC-D* marketed by zydus cadilla,Ahmedabad were procured from local market. All other reagents were of analytical grade for Spectrophotometric method.

Preparation of Standard Solution

Accurately weighed AMLO (10 mg) and IND (10 mg) standards were transferred to a 10 ml volumetric flask, dissolved in and diluted to the mark with methanol to obtain standard stock solution for AMLO (1000 µg/ml) and IND (1000 µg/ml). Aliquot of the solution (10 ml) was transferred to a 10 ml volumetric flask, and diluted to the mark with methanol to obtain working standard solution for AMLO (100 µg/ml) and IND (100 µg/ml).

Selection of Analytical wavelength

Solution of AMLO (20 µg/ml) was prepared in methanol and spectrum was recorded between 200-400 nm. First-derivative spectrum for above concentration was obtained. Similarly, Solution of IND (6 µg/ml) was prepared in methanol and spectrum was recorded between 200-400 nm and first derivative spectrum was obtained. The overlain derivative spectrum of AMLO (20 µg/ml) and IND (6 µg/ml) show the zero crossing point (ZCP) 237.4 nm and 241.4 nm, respectively, which were selected for measurement of IND and AMLO respectively.

Preparation of calibration curve

From the working standard solution appropriate volume of aliquots were transferred to different volumetric flask of 10 ml capacity. The volume was adjusted to the mark with methanol to obtain the concentration of 5,10,15,20,25 and 30 µg/ml for AMLO and 2,4,6,8,10 and 12 µg/ml for IND. The samples were scanned between 200-400 nm using SCHIMADZU UV/Visible double beam spectrophotometer (UV-1800) with 1cm matched quartz cells. And spectrums were converted into first order derivative form. Absorbances of AMLO and IND solutions were measured at 241.4 nm and 237.4 nm, respectively using first order derivative spectrophotometric method. The graph of absorbance versus respective concentration was plotted.

Methods

Twenty tablets were accurately weighed and average weight per capsule was calculated. Powder equivalent to 5 mg AMLO and 1.5 mg IND was accurately weighed and transferred to a 10 ml volumetric flask containing methanol (10 ml). The flask was sonicated for 5 min. The flask was shaken and the volume was diluted to the mark with methanol. The above solution was filtered through Whatman filter paper no. 41. The aliquot 1 ml was transferred to 10 ml volumetric flask and volume adjusted to the mark with methanol. The first derivative response of this solution was measured at 241.4 nm and 237.4 nm for quantification of AMLO and IND, respectively. First order derivative absorbances at these wavelengths were substituted in regression equation representing the calibration curves for AMLO and IND, with correction for dilution, to calculate the amounts of drug present.

RESULTS AND DISCUSSION

Selection of wavelength for simultaneous estimation of AMLO and IND

UV spectra of AMLO completely overlaps that of IND so, absorbance effect of IND is suppressed in the mixture. Therefore simultaneous estimation in zero order spectra was not successful. So it was thought of interest to develop the first order derivative spectrophotometric method for simultaneous estimation of AMLO and IND from capsule dosage form. Individual first order derivative spectra were recorded for both drugs and zero crossing points were selected (Figure 4). First order derivative spectrum for AMLO was taken and it showed zero crossing point 237.40 nm, was selected for determination of IND in the mixture. Similarly, first order derivative spectrum for IND was taken and it showed zero crossing point 241.40 nm, was selected for estimation of IND in mixture since it showed adequate absorbance at this wavelength.

Validation of the proposed Method

The method is validated as per ICH (International conference on harmonization) Guidelines as follows:

Linearity and Range

The linearity range for both AMLO and IND was found to be in the range of 5-30 µg/ml and 2-12 µg/ml respectively (Figure 5). Correlation co-efficient for calibration curve of AMLO and IND was found to be 0.9993 and 0.9994 respectively.

The regression line equation for AMLO and IND are as following,

$Y_{AMLO} = 0.002X-0.002$ …… (1)

$Y_{IND} = 0.001X-0.001$ ..... (2)

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of AMLO and IND by the standard addition method. Known amount of standards of AMLO (08, 10 and 12 µg/ml) and IND (4.8, 6 and 7.2 µg/ml) were spiked to a prequantified sample (10 and 6 µg/ml for AMLO and IND, respectively) and the mixtures were analyzed again. The amounts of AMLO and IND were determined by measuring the absorbances and by fitting these values into the regression equation of the calibration plots. The % recovery was found in the range of 98.00 - 100.42 % for AMLO (Table 2) and 98.60 – 101.15 % for IND (Table 3).

Precision

Repeatability

The repeatability of measurement of absorbance was checked by repeatedly

measuring (n = 7) absorbance of same concentration of AMLO (30 µg/ml) and IND(12 µg/ml). The relative standard deviations for the same are 0.47 for AMLO; and 0.96 for IND, respectively (Table 4).

Intermediate precision

The Intermediate precision of the proposed method was assessed by estimating the corresponding responses (n = 3) for 5 different concentrations (5,10,15,20,25 µg/ml) for AMLO and (2,4,6,8,10 µg/ml) for IND on the same day (Intraday) (Table 5), and on the different days (Interday) (Table 6). The results are reported in terms of relative standard deviation.

LOD and LOQ

The limits of detection (LOD) and quantification (LOQ) were calculated from the standard deviation (SD) of y-intercepts and slope (S) of the calibration plots using equations LOD = 3.3 × SD/S and LOQ = 10 × SD/S as per International Conference on Harmonization (ICH) guidelines. The detection and quantification limits obtained by this method were 0.208 and 0.622 µg for AMLO; while 0.301 and 0.325 µg for IND, respectively, which indicates the sensitivity of the method (Table 7).

Simultaneous estimation of AMLO and IND in pharmaceutical dosage form

The proposed method was applied to analyze the combined capsule dosage form of AMLO and IND. Marketed preparation was analyzed by the proposed method. The amount of AMLO and IND was found to be 99.84 ± 0.2666 and 99.41 ± 0.4055% of the labeled amount respectively. Thus, the developed first order derivative spectrophotometric method is simple, rapid, precise, accurate and economical. It can be applied for routine analysis of AMLO and IND combined dosage forms

CONCLUSION

The proposed first order derivative Spectrophotometric method is accurate, simple, rapid and selective for simultaneous estimation of ASP and IND in capsule dosage form. Hence it can be conveniently adopted for routine quality analysis of the capsules.

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work and also to the Principle Dr Ragin Shah, Arihant School of pharmacy and bio-research institute, Adalaj, Dist-Gandhinagar for providing facilities.

Table 1.

Linear Regression data of the calibration plots for AMLO and IND (n=3)

Parameter	AMLO	IND
Linearity range (µg/ml)	5-30	2-12
Correlation coefficient (r)	0.9993	0.9994
Slope	0.002	0.002
Intercept	0.001	0.001

Table 2.

Results of recovery studies for AMLO (n=3)

Amount of AMLO in sample (µg/ml)	Amount of Std AMLO added (µg/ml)	Total amount of AMLO (µg/ml)	Spicked amount of AMLO (µg/ml) (n=3)	% Recovery Mean ± SD	% C.V.
10	8	18	7.84	98.58 ± 0.6186	0.63
	8	18	7.88		
	8	18	7.93		
10	10	20	9.82	99.48 ± 1.1150	1.12
	10	20	9.98		
	10	20	10.04		
10	12	22	11.94	99.83 ± 0.3350	0.33
	12	22	11.98		
	12	22	12.02		

Table 3.

Results of recovery studies for IND (n=3)

Amount of IND in sample (µg/ml)	Amount of Std IND added (µg/ml)	Total amount of IND (µg/ml)	Spicked amount of IND (µg/ml) (n=3)	% Recovery Mean ± SD	% C.V.
6	4.8	10.8	4.74	99.14 ± 0.3113	0.31
	4.8	10.8	4.77		
	4.8	10.8	4.75		
6	6	12	5.91	98.89 ± 0.3002	0.30
	6	12	5.93		
	6	12	5.95		
6	7.2	13.2	7.28	99.96 ± 1.0323	1.03
	7.2	13.2	7.16		
	7.2	13.2	7.14		

Table 4.

Repeatability data for AMLO and IND

Concentration(µg/ml)		% C.V. (n=7)	
AMLO	IND	AMLO	IND
30	6	0.47	0.96

Table 5.

Intraday precision data for AMLO and IND

Concentration (µg/ml)		**% C.V. (n=3)**	
AMLO	IND	AMLO	IND
5	2	1.12	1.84
10	4	1.08	0.92
15	6	0.58	1.04
20	8	0.55	1.20
25	10	0.66	0.95

Table 6.

Interday Precision data for AMLO and IND

Concentration (µg/ml)		**% C.V. (n=3)**	
AMLO	IND	AMLO	IND
5	2	1.92	1.82
10	4	1.30	1.61
15	6	0.97	1.77
20	8	1.05	1.83
25	10	1.35	1.03

Table 7.

LOD and LOQ

AMLO	IND
LOD=3.3 x (SD/Slope) **LOD=0.208 µg/ml**	LOD=3.3 x (SD/Slope) LOD=0.301 µg/ml
LOQ=10 x (SD/Slope) **LOQ=0.622 µg/ml**	LOQ=10 x (SD/Slope) LOQ=0.925 µg/ml

Table 8.

Results of Assay of marketed formulation

Brand	Formulation	AMLO content	IND content	Manufacturer	% label claim (n=3)	
					AMLO	IND
AMLODAC-D	Tablet	5 mg	1.5 mg	Zydus-cadilla	99.84 ± 0.2666	99.41 ± 0.4055

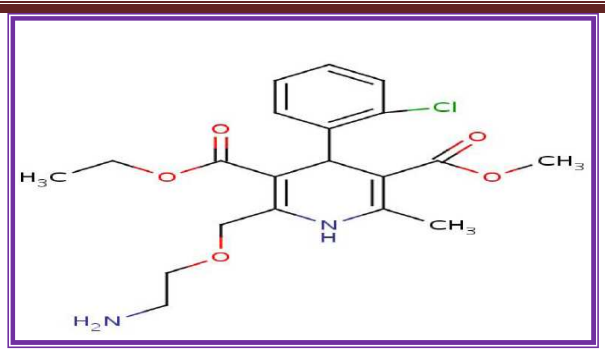

Figure 1. Chemical Structure of Amlodipine

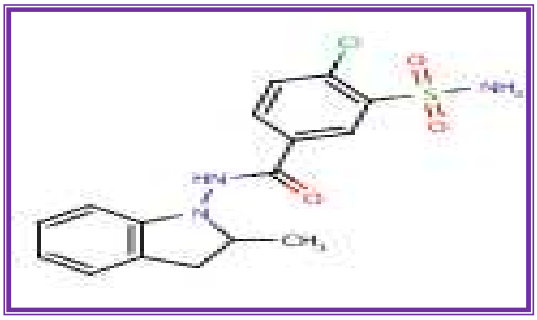

Figure 2. Chemical Structure of Indapamide

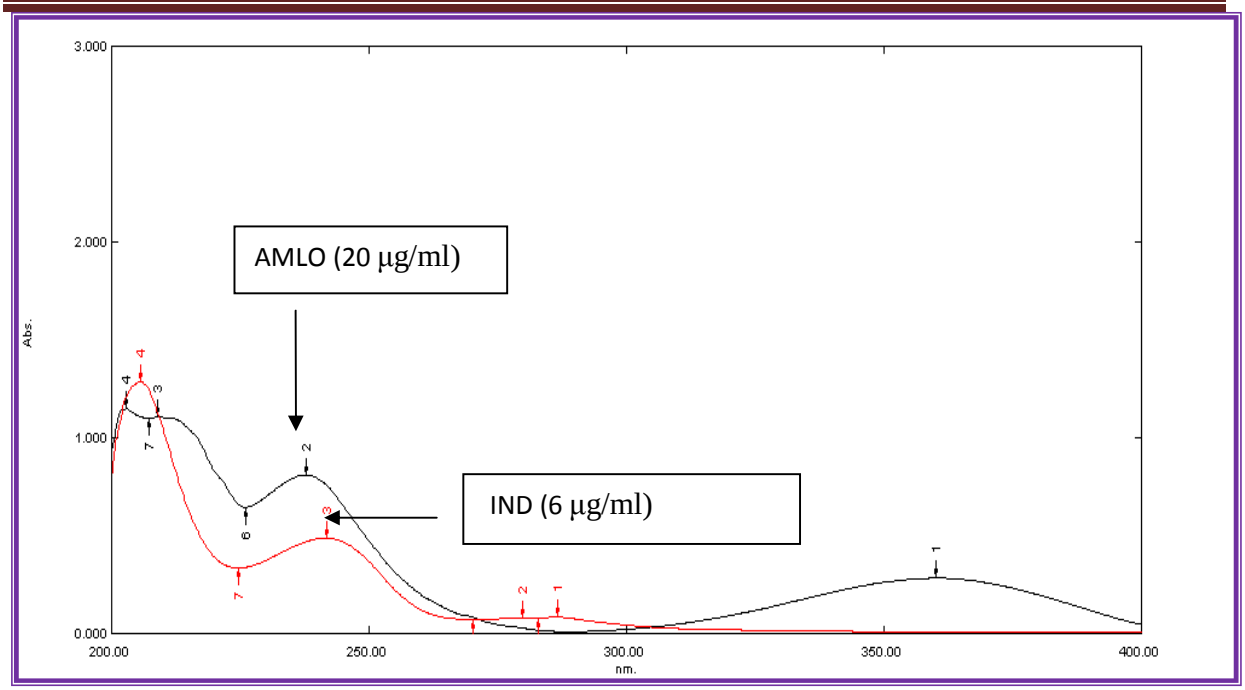

Figure 3. Overlain zero order spectra of AMLO (20 µg/ml) and IND(6 µg/ml) in methanol

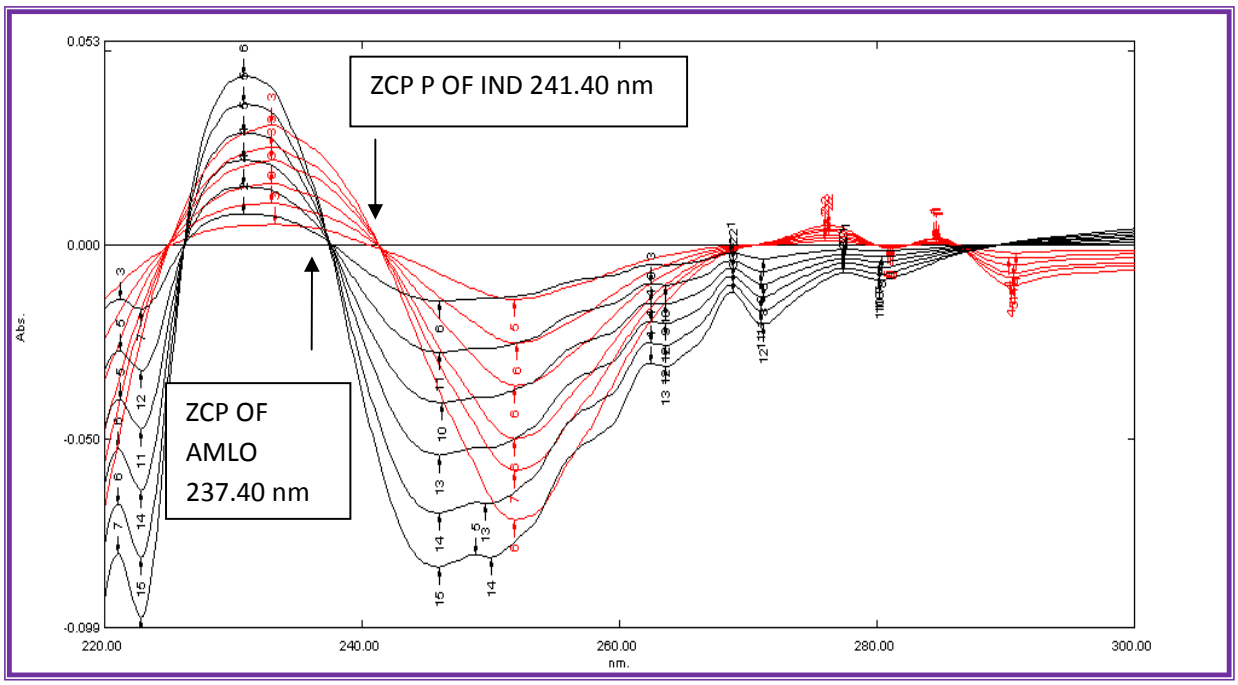

Figure 4. Overlain first order spectra of AMLO (5-30 µg/ml) and IND (2-12 µg/ml) in methanol

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