

Simultaneous Determination of Amlodipine Besylate and Indapamide in Tablet Dosage Form by Absorption Correction Method and First-Order Derivative UV Spectrophotometry

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Abstract: Two simple, accurate, precise, reproducible UV Spectrophotometric methods have been developed for the simultaneous estimation of Amlodipine Besylate and Indapamide in tablet dosage form. First method employed was Absorption Correction Method which involves direct estimation of Amlodipine Besylate at 360 nm, as at this wavelength Indapamide has zero absorbance and shows no interference. For estimation of Indapamide, corrected absorbance was calculated at 242 nm due to the interference of Amlodipine Besylate at this wavelength. The second method was first order derivative spectrophotometry, wavelengths selected for quantitation were 242 nm for Amlodipine Besylate (zero crossing point for Indapamide) and 238 nm for Indapamide (zero crossing point for Amlodipine Besylate). In both methods linearity was observed in the concentration range of 10-70 µg/ml for Amlodipine Besylate and 2-16 µg/ml for Indapamide. The results of analysis have been validated statistically and by recovery studies. The proposed method was successfully applied for the simultaneous estimation of both drugs in commercial tablet dosage form.

Key Words: Amlodipine Besylate, Indapamide, Absorption Correction method, First order derivative UV spectrophotometry.

INTRODUCTION

A combined fixed dose formulation containing Amlodipine besylate (ADB) and Indapamide (INDA) is available as tablet dosage form for treatment of hypertension and angina pectoris. ADB is chemically known as (4 R, S)-3ethyl-5-methyl 2-(2-amino-ethoxymethyl)-4 (2-chlorophenyl)-1, 4-dihydroxy-6-methylpyridine-3, 5 dicarboxylate monobenzene sulphonate.

B.P¹ and I.P² describes a Reversed Phase High Performance liquid chromatographic (RP-HPLC)

method for the determination of ADB in bulk and pharmaceutical formulations. The literature survey reveals numbers of methods are reported for the quantitative determination of ADB alone or in combination with other anti hypertensive drugs including Spectrophotometry³⁻⁸ and Chromatographic methods⁹⁻¹². INDA, chemically is a 4-chloro-N-(2methyl-2,3-dihydroindol-1-yl)-3-sulfamoyl benzamide used as an antihypertensive agents and as a diuretics, which inhibits the reabsorption of sodium and calcium at the beginning

of distal convoluted tubules. U.S.P¹³ describes a Reversed Phase High Performance liquid chromatographic (RP-HPLC) method for the determination of INDA in bulk and pharmaceutical formulations. The literature survey reveals that, Spectrophotometry¹⁴⁻¹⁶, HPLC¹⁷⁻¹⁹, Liquid chromatography – electrospray tandem mass spectroscopy method²⁰ are reported for the estimation of INDA. However, there is no evidence in literature for simultaneous determination of ADB and INDA. Hence present work describes two spectrophotometric methods for estimation these two drugs simultaneously from tablet dosage form.

EXPERIMENTAL

MATERIALS AND REAGENTS

Pure ADB and INDA were obtained from Zydus Cadila, Ahmedabad, India, and Dishman Pharma, Ahmedabad respectively as a gift samples. The tablets Amlodac-D of the said combination were purchased from a local pharmacy (The label claim for both contained 5 mg of ADB and 1.5 mg of INDA). Methanol was used of analytical grade.

INSTRUMENT

All Absorbance measurements were made on Shimadzu model UV 1800 double beam UV - Visible spectrophotometer with matched quartz cuvettes.

STANDARD STOCK SOLUTION

The standard stock solution of ADB and INDA were prepared by dissolving 50 mg of each drug in 50 ml of Methanol. Stock solutions of ADB and INDA were further diluted in Methanol to get standard solutions of concentration of 100 µg/ml.

METHOD

ABSORPTION CORRECTION METHOD

a) Study of Beer's –Lamberts law

The standard solutions of ADB (10 µg/ml) and INDA (10 µg/ml) in Methanol were scanned in the entire UV range to determine λ_{max} of both the drugs. The λ_{max} of ADB and INDA were found to be 360 nm and 242 nm, respectively (fig. 1). A series of standard solutions were prepared having concentration range of 10-70 µg/ml for ADB and 2-16 µg/ml for INDA in Methanol using working standard solution (100 µg/ml). The absorbance of resulting solutions were measured at 360 nm and 242 nm, and calibration curves plotted at these wavelengths. Both the drugs obeyed linearity individually and combination within the concentration range of 10-70 µg/ml for ADB and 2-16 µg/ml for INDA. Linear regression analysis of calibration curves were showed in Table 1.

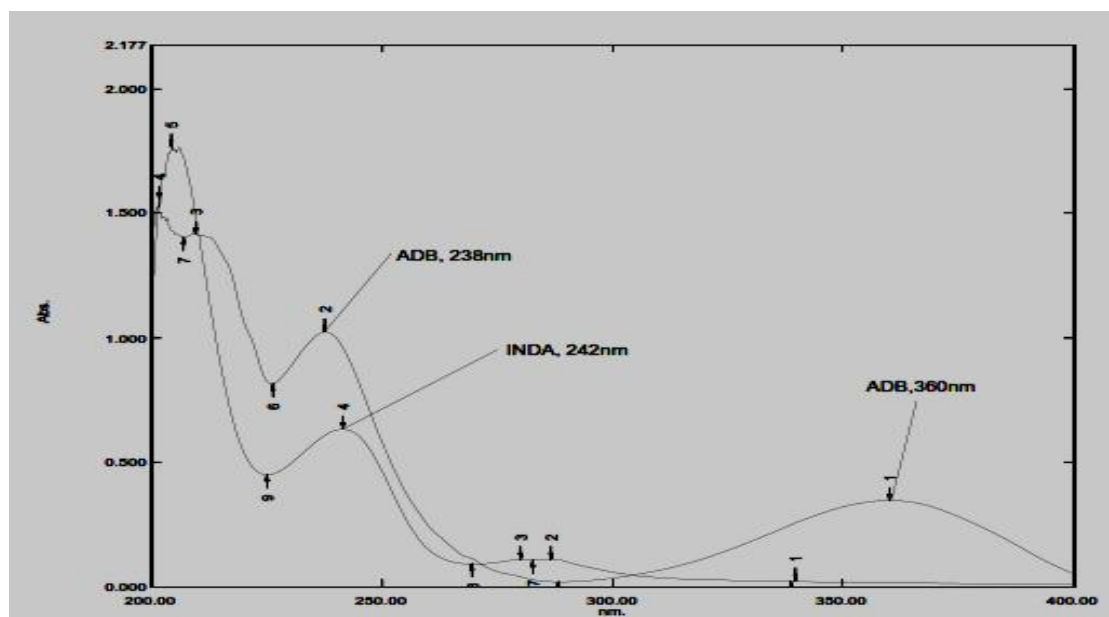


Figure.1. Zero Order Overlain Spectra of ADB (10 µg/ml) and INDA (10 µg/ml)

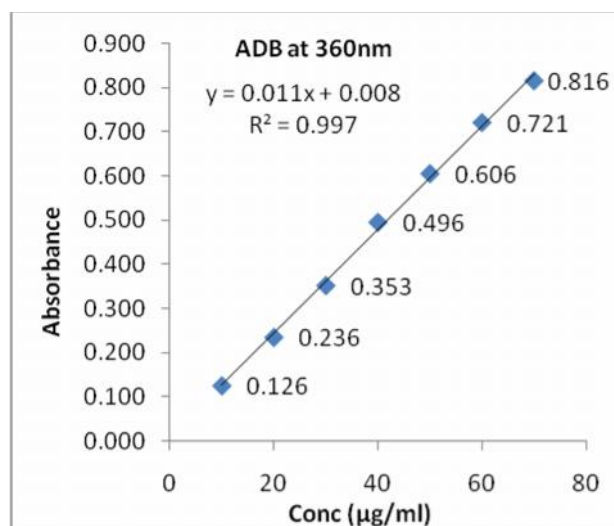


Figure.2. Calibration curve of ADB at 360nm

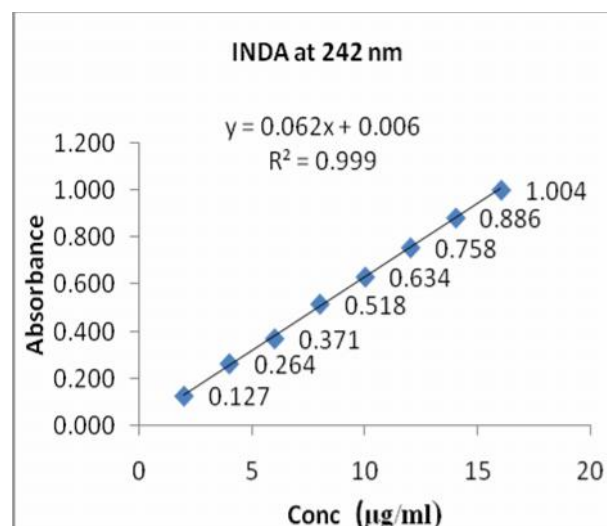


Figure.3. Calibration curve of INDA at 242nm

Table.1. Linear regression analysis of calibration curves*

Parameter	Method I**		Method II***	
	ADB	INDA	ADB	INDA
Beer's law limit (µg/ml)	10-70	2-16	10-70	2-16
Correlation Coefficient	0.9979	0.9995	0.9983	0.9973
Slope	0.0118	0.0627	0.0118	0.0106
Intercept	0.0086	0.0063	0.0102	0.0012

*Average of six Determinations

**Absorption Correction Method

*** First Order Derivative Spectrophotometry

b) Determination of A (1%, 1cm) value at selected wavelength

The A (1%, 1cm) value of ADB and INDA were calculated at 360 nm and 242 nm. A (1%, 1cm) value for ADB were found to be 120 and 320 at 360nm and 242nm respectively, and 631 at 242nm for INDA

Two equations were formed using these values as given below.

$$C_x = A_{360\text{nm}} / A_{(1\%, 1\text{cm})\text{ADB at } 360\text{nm}}$$

$$C_y = A_{242\text{nm}} - (A_{(1\%, 1\text{cm})\text{ADB at } 242\text{nm}})(C_x) / A_{(1\%, 1\text{cm})\text{INDA at } 242\text{nm}}$$

Where,

C_x and C_y = Concentration of ADB and INDA (gm/100ml)

$A_{360\text{nm}}$ and A_{242} = Absorbance of sample at 360nm and 242nm respectively

$A_{(1\%, 1\text{cm})\text{ADB at } 360\text{nm}}$, $A_{(1\%, 1\text{cm})\text{ADB at } 242\text{nm}}$, $A_{(1\%, 1\text{cm})\text{INDA at } 242\text{ nm}}$ = Absorptivity of ADB and INDA at 360nm, 242nm respectively.

FIRST ORDER DERIVATIVE SPECTROPHOTOMETRY

a).Determination of Zero Crossing Point(ZCP) and selection of suitable wavelength

Standard solutions of both drugs were scanned separately in the range of 200-400 nm. These spectrums were converted to first derivative spectra by using derivative mode with 2 delta point and scaling factor 10. The two spectra were overlain and it appeared that ADB showed zero crossing at 238 nm, while INDA showed zero crossing at 242 nm. At the zero crossing point (ZCP) of ADB (238 nm), INDA showed a first-derivative absorbance, whereas at the ZCP of INDA (242 nm), ADB showed a first-derivative absorbance. Hence 242nm and 238 nm was selected as analytical wavelengths for determination of ADB and INDA respectively. These two wavelengths can be employed for the determination of ADB and INDA without any interference from the other drug in their combined dosage formulations.

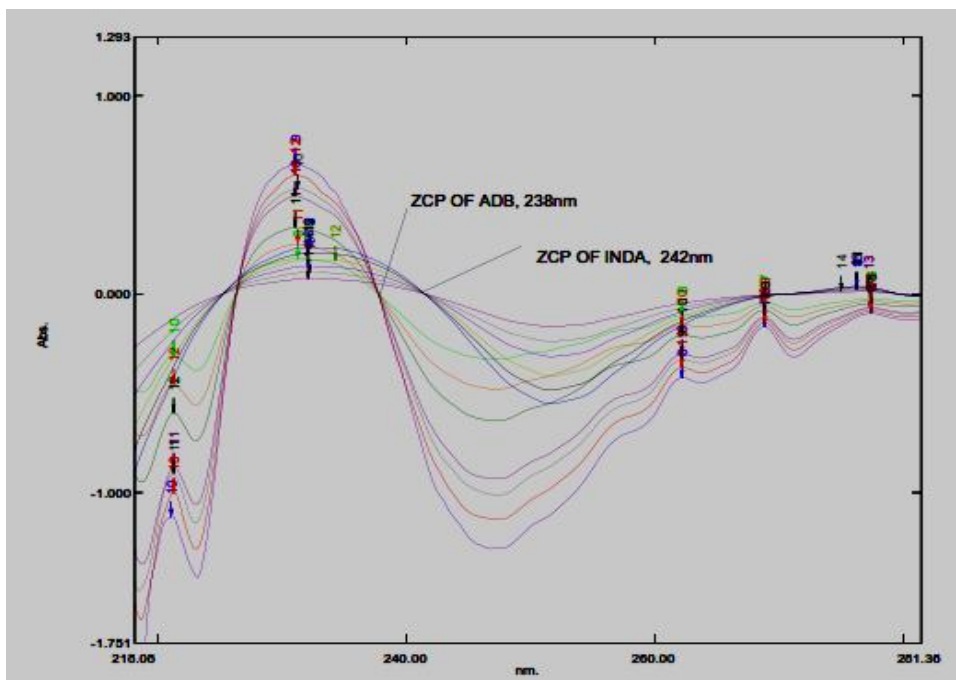


Figure 4. Overlain Spectra of First Order Derivative Spectra of ADB and INDA

b) Study of Beer's –Lamberts law

A series of standard solutions were prepared having concentration range of 10-70 µg/ml for ADB and 2-16 µg/ml for INDA in Methanol using working standard solution(100 µg/ml). the First Order Derivative absorbance of resulting solution was measured at 242 nm and 238 nm, and calibration

curves plotted at these wavelengths. Both the drugs obeyed linearity individually and combination within the concentration range of 10-70 µg/ml for ADB and 2-16 µg/ml for INDA. Linear regression analysis of calibration curves were showed in Table.1.

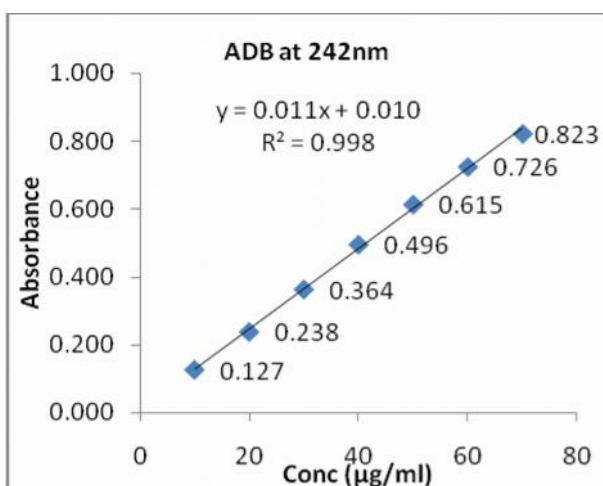


Figure 5. Calibration curve of ADB at 242nm

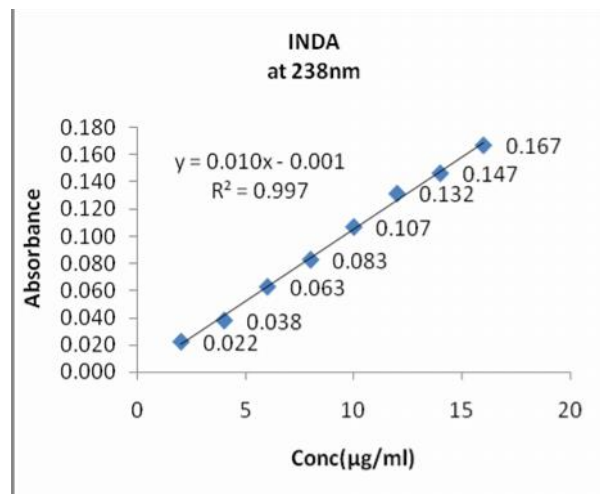


Figure 6. Calibration curve of INDA at 238nm

Table.2.Recovery studies of ADB and INDA*

Drug	Method	Amount Added (µg/ml)	Total Amount Found (µg/ml)	% Recovery	Average % Recovery	%RSD
ADB (20µg/ml)	Method I	10	30.13	100.43	100.99	0.55
		20	40.47	101.18		
		30	50.18	100.36		
	Method II	10	30.24	100.82	101.05	0.48
		20	40.64	101.61		
		30	50.36	100.72		
INDA (6µg/ml)	Method I	3	9.13	101.5	100.98	1.39
		6	11.92	99.39		
		9	15.31	102.06		
	Method II	3	9.14	101.51	101.06	0.39
		6	12.09	100.75		
		9	15.14	100.93		

*Average of Three Determinations

Table.3.Summary of Validation Parameter for ADB and INDA

Parameter	Method I		Method II	
	ADB	INDA	ADB	INDA
Linearity(µg/ml)	10-70	2-16	10-70	2-16
Correlation Coefficient	0.9979	0.9995	0.9983	0.9973
Intraday precision % RSD	0.47	0.79	0.47	1.10
Interday precision % RSD	0.70	0.78	0.90	1.13
LOD µg/mL	0.54	0.38	1.09	0.22
LOQ µg/mL	0.38	1.14	3.31	0.68

VALIDATION OF ANALYTICAL METHODS

The proposed methods were validated as per the ICH guidelines²¹⁻²². The methods were validated in terms of linearity, accuracy, precision. Recovery studies were carried out to study the accuracy of the proposed method and ascertained by standard addition method. A known amount of drug was added to preanalysed tablet powder, at three levels and the percentage recoveries were calculated. Recovery studies of ADB and INDA were showed in Table.2. Precision was found to be lower than 2%. Summary of validation parameter was showed in the Table.3.

ASSAY OF TABLET FORMULATION

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to about 5 mg of ADB was taken in 50 ml volumetric flask and dissolved in 25 ml of Methanol. It was sonicated for 20 min. It was further diluted up

to the mark with same solvent. The solutions were then filtered. Necessary dilutions are made with methanol to give final concentration 40µg/ml and 12 µg/ml of ADB and INDA respectively.

a).By Absorption Correction Method

The absorbance's values of sample were read at 360nm and 242 nm and concentration was obtained by solving the absorption correction equations. Results of analysis of tablet formulation are shown in Table 4.

b).By First Order Derivative Spectrophotometry

The first order derivative absorbance's values of sample were read at 242nm and 238 nm and concentration was obtained by solving the First Order Derivative equations. Results of analysis of tablet formulation are shown in Table 4.

Table.4.Analysis of Marketed Formulation(Amlodac-D)*

Method	Drug	Labeled Claim(mg/Tablet)	%Labeled Claim	%RSD
Method I	ADB	5	100.75	1.24
	INDA	1.5	101.39	1.05
Method II	ADB	5	100.25	0.66
	INDA	1.5	101.27	1.28

*Average of three Determinations

RESULTS AND DISCUSSION

Tablets were analyzed and amount of drug determined by proposed method was in good agreement with the labelled claim. The results of the marketed formulations were found to be 100.75 ± 1.24 and 101.39 ± 1.05 for ADB and INDA respectively by absorption correction method and 100.25 ± 0.66 and 101.27 ± 1.28 for ADB and INDA respectively by First Order Derivative Spectrophotometry. Linearity was determined at different concentration, ADB and INDA shows linearity in the concentration range of 10-70 $\mu\text{g/ml}$ for ADB and 2-16 $\mu\text{g/ml}$ for INDA. Limit of detection (LOD) and Limit of quantitation (LOQ) were determined by standard deviation of response and slope of calibration curve. The recovery of drug was determined at 50, 100 and 150 % levels. The percent recovery was shown in Table.2. for both the

drugs. It is within the range 98-102% which indicate the method is accurate. The results show the % RSD values $< 2.0\%$ signifies the precision of the method.

CONCLUSION

The proposed methods for simultaneous estimation of ADB and INDA in combined dosage form were found to be simple, accurate and rapid and can be employed for estimation of pharmaceutical formulations in quality control departments.

ACKNOWLEDGEMENT

The authors are grateful to Zydus Cadila, Ahmedabad, India, and Dishman Pharma, Ahmedabad for providing gift samples of ADB and INDA respectively.

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