



DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR DETERMINATION OF INDAPAMIDE AND AMLODIPINE BESYLATE

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ABSTRACT

A new simple, accurate, precise and sensitive stability- indicating high performance thin layer chromatographic (HPTLC) method was developed and validated for simultaneous estimation of Indapamide and Amlodipine besylate in bulk and in tablet dosage form. The chromatographic development was carried out on precoated silica gel 60 F₂₅₄ aluminium plates using mixture of Chloroform: Glacial acetic acid: Methanol (8.5: 1: 0.5 v/v) as mobile phase and densitometric evaluation of bands at 241 nm using Camag TLC Scanner-3 with win CAT 1.4.3 version software. The R_f value of Indapamide and Amlodipine besylate were found to be 0.69 ± 0.02 and 0.29 ± 0.02 ,

respectively. The method was validated with respect to linearity, accuracy, precision and robustness. The calibration curve was found to be linear over a range of 100 - 1000 ng/ band for Indapamide and 500 – 3000 ng/ band for Amlodipine Besylate. The drugs were subjected to stress condition of hydrolysis (acid, base, neutral), oxidation, photolysis and thermal degradation.

KEYWORDS: Indapamide, Amlodipine besylate, HPTLC, Stability.

INTRODUCTION

Indapamide (IND), 4-chloro-N-(2-methyl-2, 3-dihydroindol-1-yl)-3-sulfamoyl-benzamide (Fig 1, I) is widely used in the treatment of hypertension as well as decompensated cardiac failure.^[1]

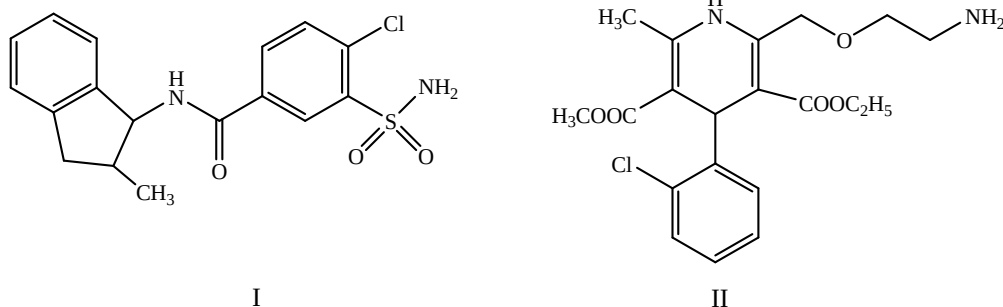


Fig 1: Structure of Indapamide (I) and Amlodipine besylate (II)

It is official in United State Pharmacopeia^[2] and British Pharmacopoeia.^[3] Amlodipine besylate (AMLO), chemically, 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulfonate (Fig 1, II) is a long-acting calcium channel blocker of the dihydropyridine (DHP) class used as an antihypertensive and in the treatment of angina pectoris.^[4] It is official in Indian pharmacopoeia^[5] and British pharmacopoeia.^[3]

The literature survey reveals that several UV-VIS Spectrophotometric^[6-8], HPLC^[9-14], ion pair liquid chromatographic^[15] and HPTLC^[16-23] methods have been reported for the analysis of IND and AMLO as a single drug or in combination with other drugs in pharmaceutical dosage form. No reports were found for stability-indicating HPTLC method for simultaneous determination of IND and AMLO in tablet dosage form.

This paper describes simple, precise, accurate and sensitive HPTLC method development and validation as well as stability study (hydrolysis, oxidation, photo-degradation and thermal degradation) as per international conference on harmonisation guidelines.

EXPERIMENTAL

Reagents and chemicals

Authentic sample of IND and AMLO were obtained from Mylan Laboratories Ltd (Hyderabad) and Shreya Pharmaceuticals (Aurangabad), respectively. The brand of tablet AMLODAC D (Manufactured by- Zydus cardia) labelled to contain Amlodipine Besylate

(IP) equivalent to Amlodipine 5 mg and Indapamide (USP) 1.5 mg were procured from local market. Methanol, Chloroform, Glacial Acetic acid, sodium hydroxide, o-phosphoric acid (all AR grade) were purchased from S. D. fine chem. Limited (Mumbai, India).

Instrumentation and chromatographic conditions

The samples were spotted in the form of bands of width 4 mm with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F₂₅₄ (10 × 10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 3 mm × 0.45 mm and scanning speed of 10 mm/sec was employed. The linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber (CAMAG, Muttens, Switzerland) using mixture of Chloroform: Glacial acetic acid: Methanol (8.5: 1: 0.5 v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 15 min. The length of chromatogram run was 80 mm. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG TLC scanner 3 at 241 nm for all developments operated by WINCATS software version 1.4.3. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of Standard stock solution

Standard stock solution of IND and AMLO were prepared separately by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. From the respective standard stock solution, working standard solution was prepared containing 100 µg/ml of IND and AMLO separately in methanol.

Selection of Detection Wavelength

From the standard stock solution further dilutions were made using methanol and scanned over the range of 200 - 400 nm and the spectra was obtained. It was observed that both the drug showed considerable absorbance at 241 nm.

Preparation of sample solution (Tablet Formulation Analysis)

Ten tablets each containing 1.5 mg of Indapamide and 6.93 mg Amlodipine besylate (equivalent to Amlodipine 5 mg) was weighed and powdered. Powder equivalent to 2.16 mg of Indapamide (10 mg of Amlodipine besylate) was transferred to 10 ml volumetric flask and was diluted with methanol, sonicated for 10 min and volume made to 10 ml (216 µg/ml of Indapamide and 1000 µg/ml of Amlodipine besylate). Solution was filtered and further

dilutions were made to get the final concentration of 21.6 µg/ml of Indapamide and 100 µg/ml of Amlodipine besylate of which 10 µl volume was applied on plate.

Stress degradation studies of bulk drug

Stability studies were carried out to provide evidence on how the quality of drug varies under the influence of variety of conditions like hydrolysis, oxidation, temperature, etc in order to establish specific storage conditions, shelf-life and retest period.

Alkaline treatment

1 ml working standard solution of IND (1000 µg/ml) was mixed with 1 ml of 0.1 N NaOH (methanolic) and 8 ml of methanol. Solution was kept for 24 hrs in dark place. AMLO was treated in similar manner to IND.

Acid treatment

1 ml working standard solution of IND (1000 µg/ml) was mixed with 1 ml of 0.1 N HCl (methanolic) and 8 ml of methanol. Solution was kept for 24 hrs in dark place. AMLO was treated in similar manner to IND.

Neutral Hydrolysis

1 ml standard solution of IND was mixed with 9 ml water. The solution was kept for 24 hrs in dark place. AMLO is treated in similar manner to IND.

Oxidation degradation

1 ml standard solution of IND (1000 µg/ml) was mixed with 1 ml of 30 % solution of H₂O₂ and 8 ml of methanol. Solution was kept for 24 hrs in dark place. AMLO was treated in similar manner to IND.

Degradation under dry heat

Dry heat study was performed by keeping IND in oven (100⁰ C) for a period of 2 hrs. A sample was withdrawn after 2 hrs, weighed and dissolved in methanol to get solution of 1000 µg/ml and further diluted with methanol to get 100 µg/ml as final concentration and was applied on TLC plate. AMLO is treated in similar manner to IND.

Photo degradation

Photolytic studies were carried out by exposure of drug to UV light up to 200 watt hrs/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux

hrs. Sample was weighed, dissolved and diluted get 100 µg/ml. as final concentration and was applied on TLC plate. AMLO is treated in similar manner to IND.

RESULT AND DISCUSSION

Optimization of chromatographic conditions

The primary objective in developing this stability indicating HPTLC method was to achieve the resolution between IND, AMLO and its degradation products. R_f found was 0.69 ± 0.02 and 0.29 ± 0.02 for IND and AMLO, respectively (Fig 2). The % assay was found to be 98.79 ± 0.77 for IND and 99.79 ± 1.39 for AMLO. Forced degradation study showed the method is highly specific and no degradation products were retained at R_f of drugs.

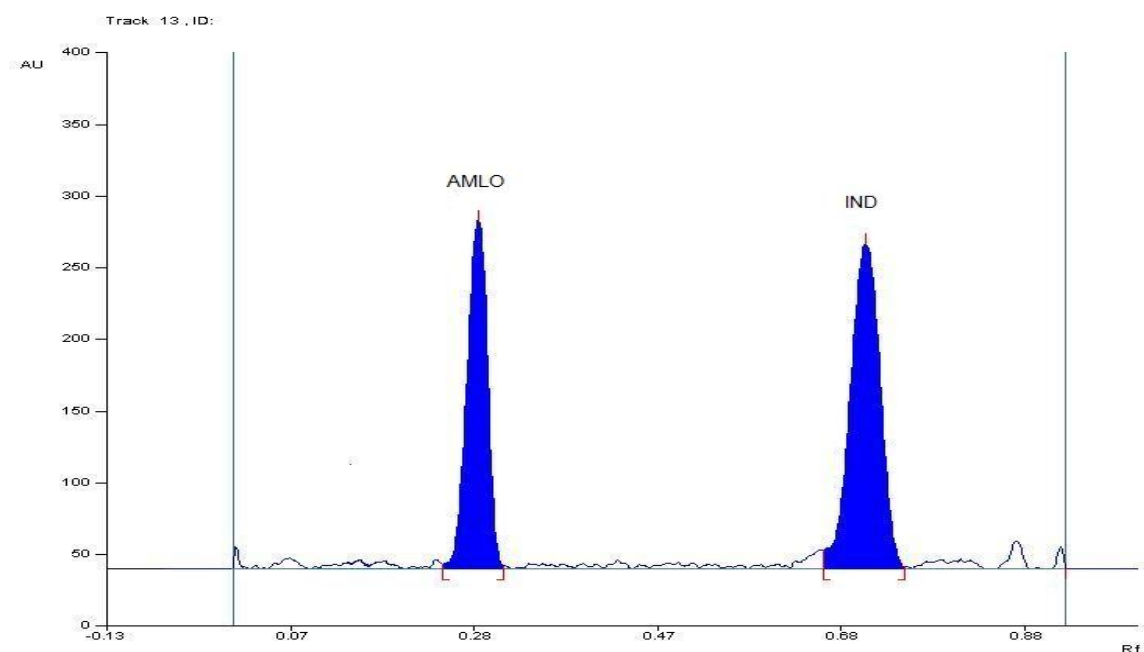


Fig 2: Densitogram of mixed standard solution of IND (200 ng/band) and AMLO (1000 ng/band)

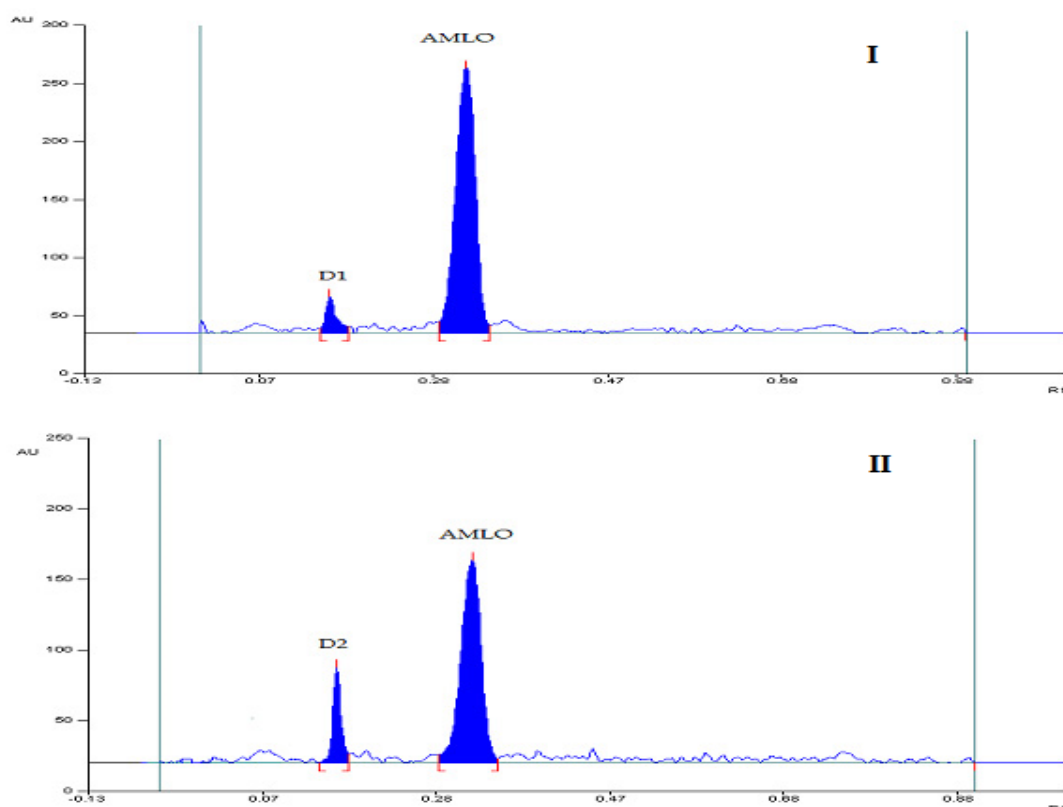
Result of forced degradation studies

Degradation was studied for IND and AMLO samples under stress conditions like base, acid, neutral, oxidation, dry heat and light. Results of the stress degradation studies are presented in table 1.

Table 1: Summary of stress degradation of IND and AMLO.

Stress Degradation Condition	IND			AMLO		
	Peak Area	Percent Recovery (%)	R _f of degradation product	Peak Area	Percent Recovery (%)	R _f of degradation product
Initial	4302.85	-	-	4113.88	-	-
Base (0.1 N NaOH, kept for 24 hrs)	3613.4	85.14	-	3472.4	83.52	0.17
Acid (0.1 N HCl, kept for 24 hrs)	3879	92.94	-	3184.3	75.66	-
H ₂ O ₂ 30 % (kept for 24 hrs)	3703.4	87.78	-	3265.9	77.88	-
Neutral (kept for 24 hr)	3625.6	85.5	-	3464.9	83.31	0.15
Heat dry (100 °C, 2 hrs)	3819.8	91.20	-	3336.2	79.80	-
Photo stability(UV, 200 watt hrs/square meter and Florescence 1.2 million Lux. Hrs)	3918.1	94.09	-	3747.3	91.01	0.04

Although % recovery is reduced for Indapamide under all stress conditions but no peak of degradation product was observed in chromatograph. A well resolved peak was observed for product of alkali induced degradation of AMLO (D1) at R_f 0.17, product of neutral degradation (D2) at R_f 0.15, and product of photo degradation (D3) at R_f 0.04. The only representative chromatographs of drugs showing degradation are presented in Fig 3.



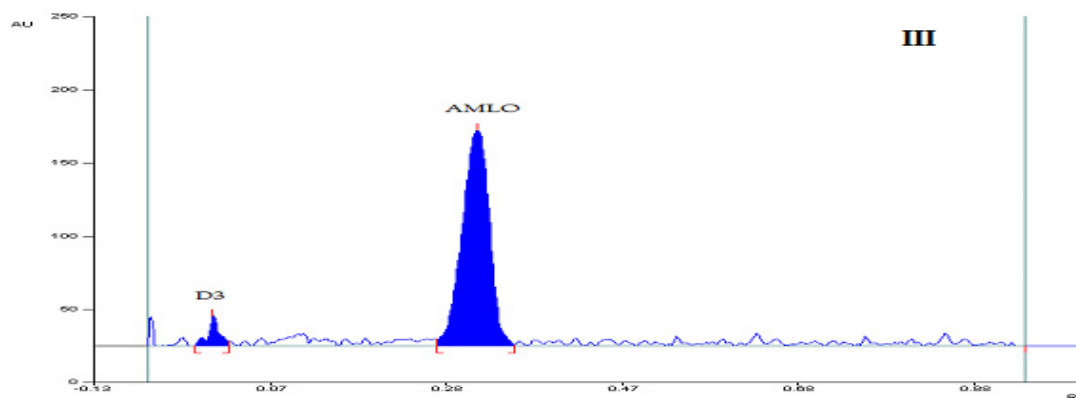


Fig 3: Densitogram of AMLO: I- Base degradation, II- Neutral degradation, III- Photo degradation.

METHOD VALIDATION^[24, 25]

Linearity

The calibration curve was obtained in the range of 100 - 1000 ng/band for IND and 500-3000 ng/band for AMLO by applying different volumes of stock solution (100 µg/ml) on TLC plate. Each standard in six replicates was analyzed and peak areas were recorded. Standard calibration graph was plotted of peak area Vs concentration applied. The equation of the calibration curve found for IND as $y = 8.508x + 715.9$ and for AMLO as $y = 3.665x + 411.0$, respectively. The coefficient of determination (r^2) was found to be 0.996 for IND and 0.998 for AMLO respectively (Fig 4).

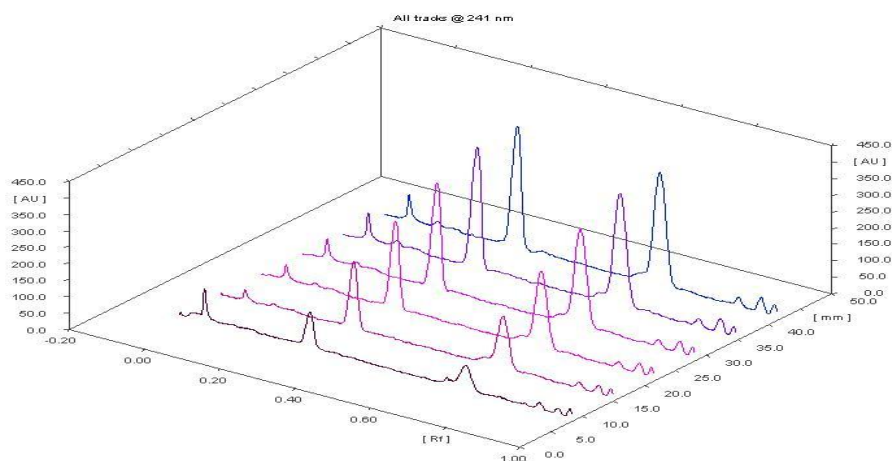


Fig 4: Densitogram of linearity of IND and AMLO.

Precision

The precision of the method was demonstrated by intra-day and inter-day studies. In the intraday study 3 replicates of 3 standard concentrations (200, 400 and 600 ng/band for IND

and 1000, 1500, 2000 ng/band for AMLO) were analyzed in a day and percentage RSD was calculated. For the inter day study 3 standard concentrations (200, 400 and 600 ng/band for IND and 1000, 1500, 2000 ng/band for AMLO) were analyzed on 3 consecutive days and percentage RSD was calculated. For intraday precision % RSD found to be 1.87 for IND and 0.47 % for AMLO. For inter-day precision % RSD found to be 0.59 for IND and 0.53 % for AMLO.

Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 50, 100 and 150%. Basic concentrations of sample chosen were 200 ng/band of IND and 1000 ng/band of AMLO. These solutions were applied on TLC plates in triplicate to obtain the densitogram. The drug concentrations of IND and AMLO were calculated by using linearity equations of IND and AMLO, respectively. The results obtained are shown in table 2 and table 3.

Table 2: Recovery studies of IND.

Level	Conc. (ng/band)		Area	Mean	Amount recovered	Mean % Recovery ± SD
	Sample	Std.				
50 %	200	100	3248.4	3243.17	297.04	100.57 ± 1.19
			3239.9			
			3241.2			
100 %	200	200	4116.1	4105.83	398.43	
			4098.5			
			4102.9			
150 %	200	300	4982.7	4996.2	501.08	
			4998.7			
			5007.2			

Table 3: Recovery studies of AMLO.

Level	Conc. (ng/band)		Area	Mean	Amount recovered	Mean % Recovery ± SD
	Sample	Std.				
50 %	1000	500	5851	5880.633	1492.07	99.45± 0.88
			5897.6			
			5893.3			
100 %	1000	1000	7701	7695.533	1987.13	
			7693			
			7692.6			
150 %	1000	1500	9632	9606.87	2508.50	
			9587.3			
			9601.3			

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.991, indicating the no interference of any other peak of degradation product, impurity or matrix at R_f value of drugs.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD of IND and AMLO were found 19.7 ng/band and 119.20 ng/band, respectively. The LOQ of IND and AMLO were 59.58 ng/band and 361.21 ng/band, respectively.

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which wavelength, chamber saturation time, time form application to development and time form development to scanning are altered and the effects on the area were noted. The results obtained are shown in table 4.

Table 4: Robustness study.

Sr. No.	Parameters	Variation	% RSD (peak area)	
			IND	AMLO
1.	Wavelength	± 2 nm	1.88	0.60
2.	Chamber saturation period	± 2 mins	0.60	0.51
3	Time form application to development	0,30,60 mins	0.32	1.60
4	Time form development to scanning	0,30,60 mins	0.77	1.07

CONCLUSION

The developed method is stability indicating where well resolved peaks were observed for analyte and degradation product. The method is specific, accurate, precise, robust and can be used for routine quality control as well as assessing the stability of IND and AMLO in bulk drug and pharmaceutical dosage form.

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