Development and Validation of Stability-Indicating RP-HPLC Method for Determination of Indapamide and Amlodipine Besylate

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ABSTRACT

A new simple, accurate, precise and selective stability- indicating high performance liquid chromatographic (HPLC) method was developed and validated for simultaneous estimation of Amlodipine Besylate and Indapamide in tablet dosage form. An isocratic, reverse phase HPLC method was developed and validated using NUCLEOSIL C18 (250 x 4.6 mm, 5 μm) column and 0.01 M potassium dihydrogen phosphate buffer pH 3 and methanol (30:70 v/v) as mobile phase and detection is carried out at a wavelength of 241 nm. The retention time for IND and AMLO were 3.84 ± 0.02 and 5.96 ± 0.09 m. respectively. The method was validated with respect to linearity, precision, accuracy and robustness. The drugs were subjected to stress condition of hydrolysis (acid, base), oxidation, photolysis and thermal degradation.

Keywords: Indapamide, Amlodipine besylate, HPLC, Stability.

INTRODUCTION

Amlodipine besylate (AMLO), chemically, 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydro-pyridine-3,5-dicarboxylate benzenesulfonate (Fig. 1) is a long-acting calcium channel blocker of the dihydropyridine (DHP) class used as an antihypertensive and in the treatment of angina pectoris.[1] It is official in Indian pharmacopoeia[2] and British pharmacopoeia.[3] Indapamide (IND), 4-chloro-N-(2-methyl-2, 3-dihydroindol-1-yl) - 3-sulfamoyl-benzamide (Fig. 2) is widely used in the treatment of hypertension, as well as decompensated cardiac failure.[4] It is official in United State Pharmacopeia[5] and British Pharmacopoeia.[3]

The literature survey reveals that several UV-VIS Spectrophotometric[6–8], HPLC[9–23] and ion pair liquid chromatographic[24] methods have been reported for the analysis of AMLO and IND as a single drug or in combination with other drugs in pharmaceutical dosage form.

No reports were found for stability-indicating HPLC method for simultaneous determination of AMLO and IND in tablet dosage form. This paper describes simple, precise, accurate and sensitive HPLC method development and validation as well as stability study (hydrolysis, oxidation, photo-degradation and thermal degradation) as per international conference on harmonisation guidelines.[25,26]

EXPERIMENTAL

Reagents and chemicals

Authentic sample of AMLO and IND were obtained from Shreya pharmaceuticals

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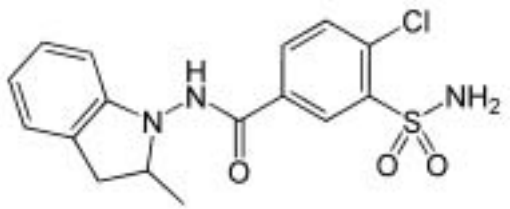

Figure 1: Structure of Indapamide

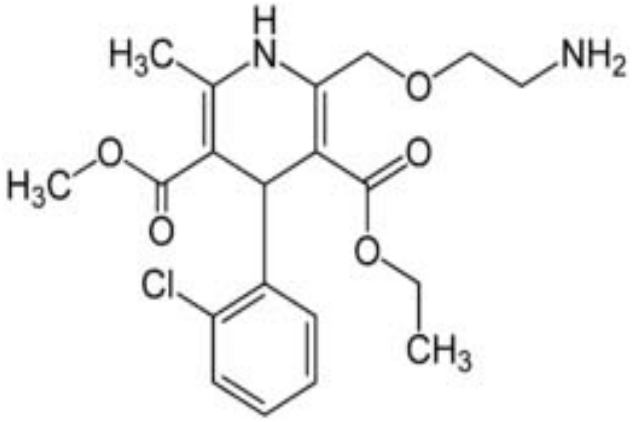

Figure 2: Structure of Amlodipine besylate

(Aurangabad) and Mylan Laboratories Ltd (Hyderabad), respectively. The brand of tablet AMLODAC D (Manufactured by- Zydus cardia) labelled to contain Amlodipine Besylate (IP) equivalent to Amlodipine 5 mg and IND (USP) 1.5 mg were procured form local market. Methanol (HPLC grade) was obtained from S. D. fine chem. Limited (Mumbai, India), HPLC grade water is collected at college using ELGA water purification system, potassium hydrogen phosphate, sodium hydroxide, o- phosphoric acid (all are AR grade) were purchased from S. D. fine chem. Limited (Mumbai, India).

Chromatographic condition

HPLC system used was JASCO system equipped with Model PU 2080 Plus pump, Rheodyne sample injection port (20 µl), MD 2010 PDA detector and Borwin- PDA software (version 1.5). A chromatographic column NUCLEOSIL C18 (250 x 4.6 mm, 5µm, Sr. No. E7060354) was used. Separation was carried out at flow rate of 1 ml/m using 0.01 M potassium dihydrogen phosphate buffer pH 3 adjusted by o-phosphoric acid and methanol (30: 70 v/v) and detection at 241 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 each in mobile phase separately (B). From this further dilution was made in mobile phase to get final solution of IND (10 µg/ml) and AMLO (10 µg/ml), separately.

Selection of Detection Wavelength

From the standard stock solution further dilutions were done 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 (Fig. 3.)

Preparation of sample solution (Tablet Formulation Analysis)

Ten tablets each containing 1.5 mg of IND and 6.93 mg AMLO (equivalent to Amlodipine 5 mg) was weighed and powdered. Powder equivalent to 10 mg of AMLO (2.16 mg of IND) was transferred to 10 ml volumetric flask and was diluted with methanol, sonicated for 10 m and volume made to 10 ml (216 µg/ml of IND and 1000 µg/ml of AMLO) with methanol. Solution was filtered and further dilutions were made with mobile phase to get the final concentration of µg/ml of IND and µg/ml of AMLO. Sample solutions were injected and the contents of drugs in tablet were determined by the proposed method using the calibration curve.

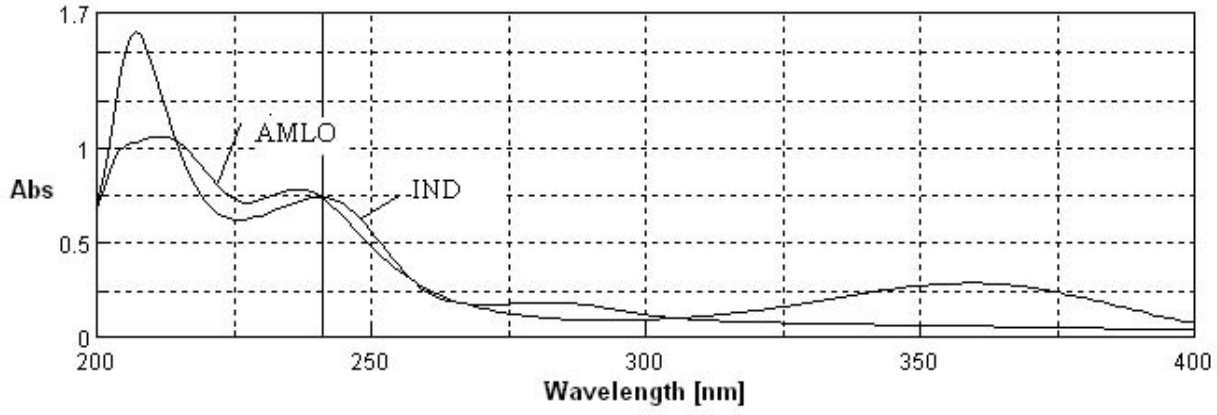

Figure 3: Overlaid UV-Vis Spectra of IND (10 µg/ml) and AMLO (10 µg/ml)

STRESS DEGRADATION STUDIES OF BULK DRUG

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

Alkaline treatment

1 ml working standard solution of IND (100 µg/ml) was mixed with 1 ml of 0.1 N NaOH (methanolic) and 8 ml of methanol. Solution was kept for 24 h in dark place. AMLO was treated in similar manner to IND.

Acid treatment

1 ml working standard solution of IND (100 µg/ml) was mixed with 1 ml of 0.1 N HCl (methanolic) and 8 ml of methanol. Solution was kept for 24 h in dark place. AMLO was treated in similar manner to IND.

Neutral Hydrolysis

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

Oxidation degradation

1 ml working standard solution of IND (100 µg/ml) was mixed with 1 ml of 30 % v/v solution of H_2O_2 and 8 ml of methanol. Solution was kept for 24 h 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 (100^0 C) for a period of 2 h. A sample was withdrawn after 2 h, weighed and dissolved in methanol to get solution of 1000 µg/ml and further diluted with mobile phase to get 10 µg/ml as final concentration and was injected. 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 hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux hours. Sample was weighed, dissolved and diluted get 10 µg/ml.

RESULT AND DISCUSSION

Optimization of chromatographic conditions

The primary target in developing this stability indicating HPLC method is to achieve the resolution between AMLO, IND and its degradation products. To achieve the separation, we used a stationary phase C-18 column and mobile phase 0.01 M KH_2PO_4 buffer (pH 3) and methanol in ratio 30:70 v/v. The tailing factor obtained was less than two and retention time was 3.84 ± 0.02 m and 5.96 ± 0.09 m for IND and AMLO respectively (Fig. 4). Forced degradation study showed the method is highly specific and no degradation products were eluted at retention time of drugs.

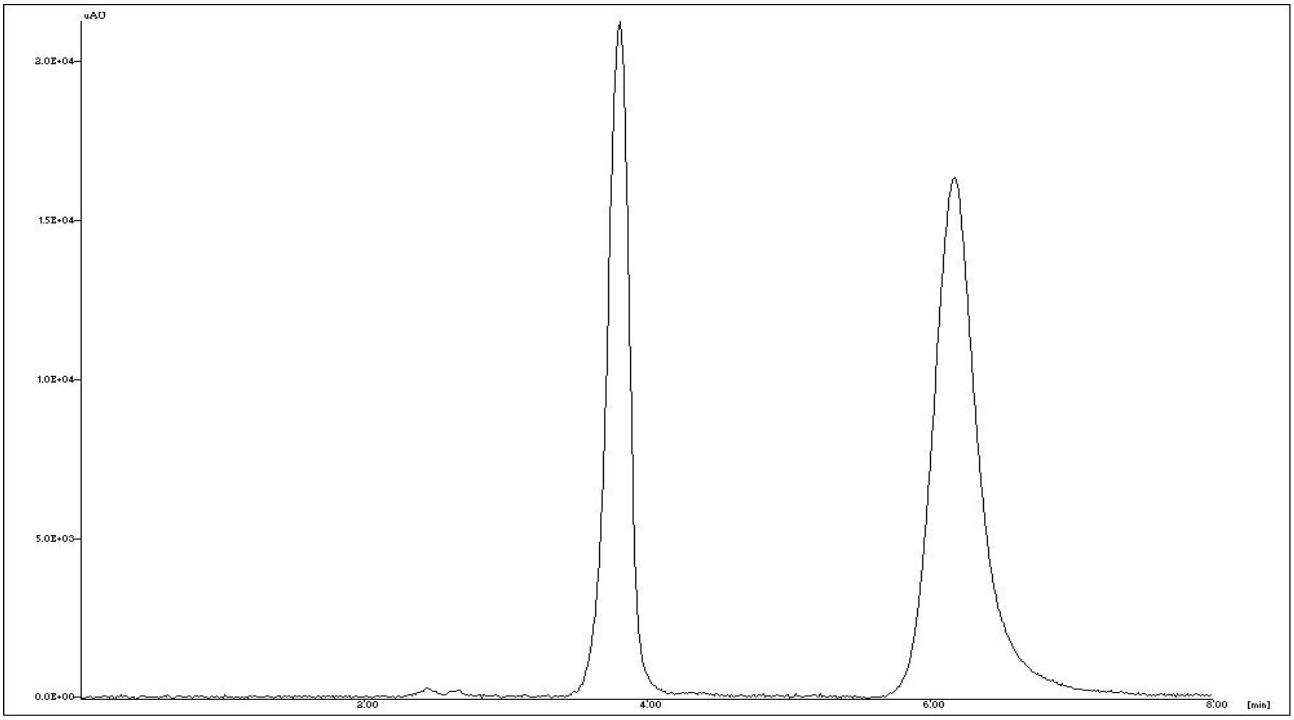

Figure 4: Chromatogram of stadard IND (10 µg/ml) and AMLO (10 µg/ml)

Result of forced degradation studies

Degradation was observed for AMLO and IND samples during stress conditions like base, acid, oxidation and dry heat except in UV and light (Fig. 5 and Fig. 6). AMLO was degraded into base and forms non-polar impurity (RT 8.76 m). Summary of stress degradation results is given in Table No. 1. Peak purity results greater than 990 indicate that AMLO and IND peaks are homogeneous in all stress conditions tested. The unaffected assay of AMLO and IND in the tablet confirms the stability indicating power of the method.

METHOD VALIDATION

Linearity

The linearity of the responses of the drugs were verified at six concentration levels, ranging from 1-10 µg/ml for IND and 2.5- 25 µg/ml for AMLO, respectively. The calibration graph was obtained by plotting peak area versus the concentration and data was treated by least-squares linear regression analysis. The equation of the calibration curve found for IND y = 85361x + 46194 and for AMLO y = 33901x + 27117 respectively. The

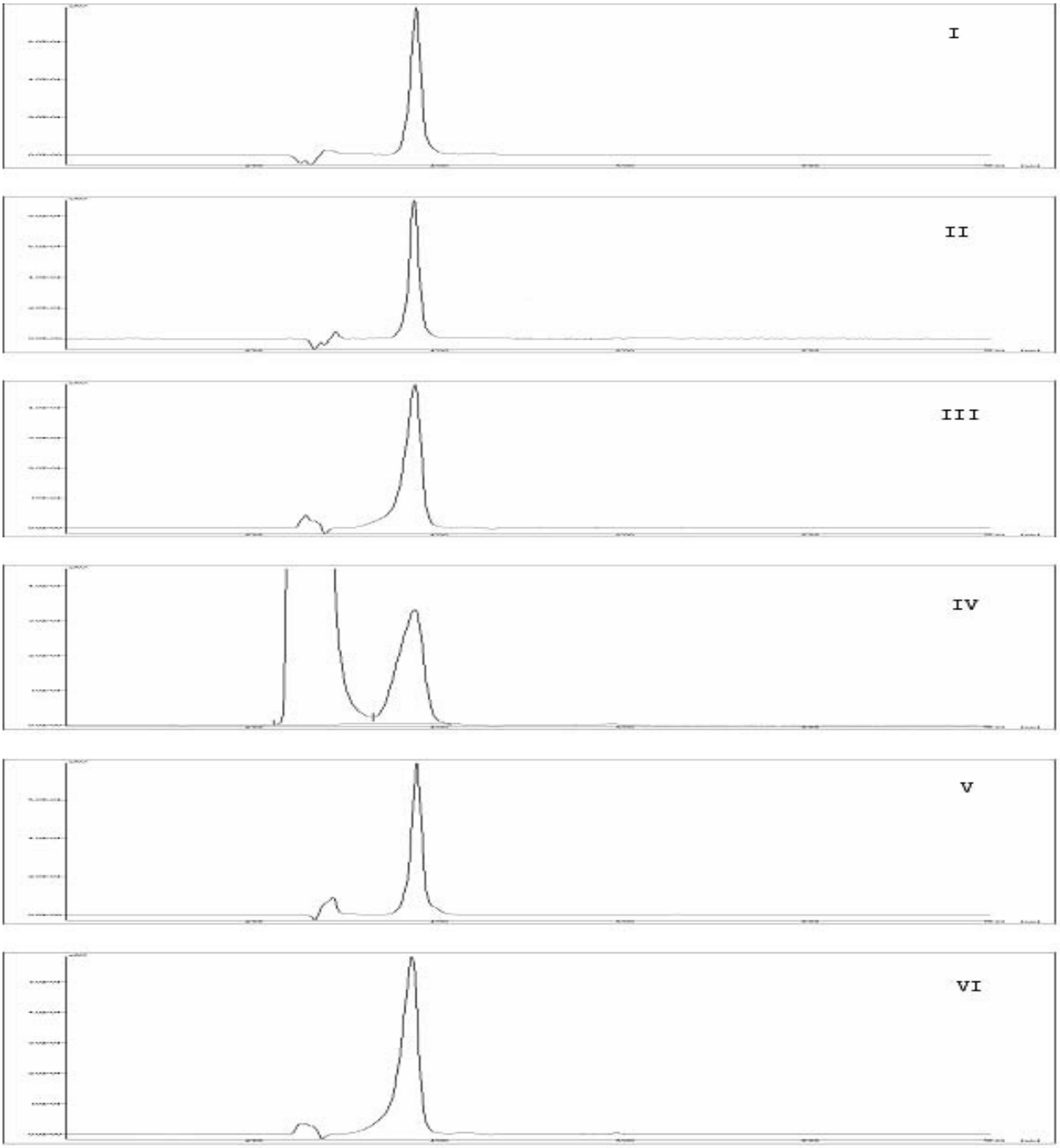

Figure 5: Chromatogram of IND I- Alkali treated, II- Acid treated, III- neutral degradation, IV- Oxidation, V- Dry heat, VI- photo degradation.

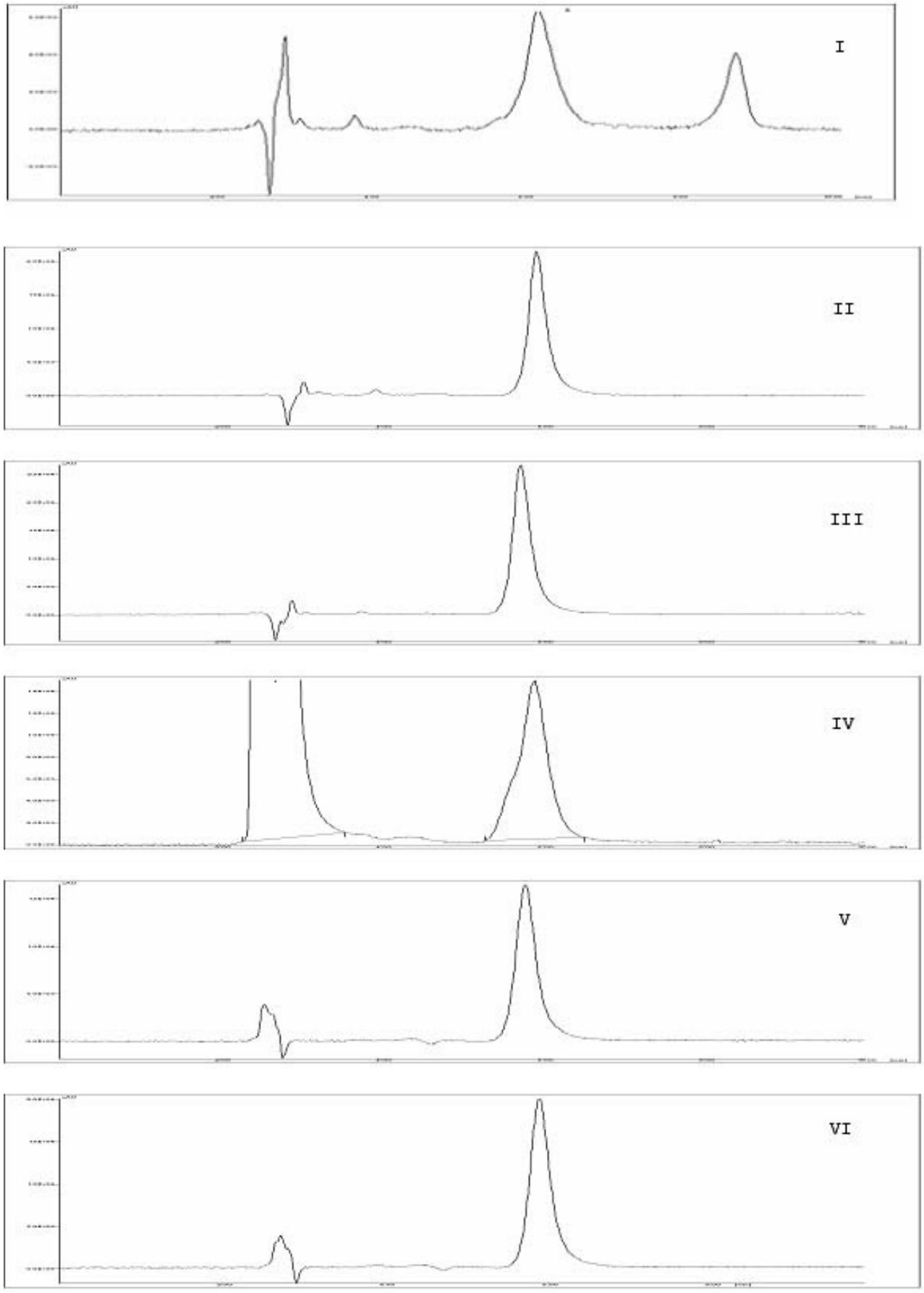

Figure 6: Chromatogram of AMLO I- Alkali treated, II- Acid treated, III- neutral degradation, IV- Oxidation, V- Dry heat, VI- photo degradation

Table 1. Summary of stress degradation results

Sr. No.	Stress Degradation Condition	% Recovery for IND (%)	% Recovery for AMLO (%)
1	Base (0.1 N NaOH Methanolic), kept for 24 h.	94.80	92.90
2	Acid (0.1 N HCl Methanolic), Kept for 24 h.	88.07	89.61
3	Neutral (kept for 24 h.)	87.39	93.40
4	H_2O_2, 30% (kept for 24 h.)	84.45	72.33
5	Dry heat (100^{0}C for 2 h.)	92.07	78.49
6	Photo stability [UV, 200 watt h/square meter Florescence , 1200 Lux. h]	95.27	99.36

calibration graphs were found to be linear in the plotted concentrations. The coefficient of determination was 0.992 for IND and 0.990 for AMLO respectively.

Precision

The precision of the method was demonstrated by Intra-day and Inter-day variation studies. In the Intra-day studies, 6 replicates of IND and (2 µg/ml) and 6 replicates of AMLO (10 µg/ml) were analyzed in a day and percentage RSD was calculated. For the inter day variation studies, 3 replicates of 3 concentrations were analyzed on 3 consecutive days and percentage RSD were calculated. For intraday precision % RSD found to be 0.93 for IND and 0.66 % for AMLO. For inter-day precision % RSD found to be 0.39 for IND and 0.92 % 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 concentration of sample chosen was 2 µg/ml of IND and 10 µg/ml of AMLO from tablet solution. The drug concentrations were calculated from respective linearity equation. The results obtained are shown in Table No. 2 and Table No. 3.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 991, indicating the no interference of any other peak of degradation product, impurity or matrix.

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

LOD and LOQ were calculated as 3.3σ/S and 10σ/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 0.13 µg/ml and 0.41 µg/ml, respectively. The LOQ of IND and AMLO were 0.44 µg/ml and 1.35 µg/ml, respectively.

Robustness studies

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, pH, flow rate were altered and the effects on the area were noted. The results are shown in Table No. 4.

CONCLUSION

The developed method is stability indicating and can be used for assessing the stability of AMLO and IND in bulk drug and pharmaceutical dosage form. The developed method is specific, selective, robust, rugged and precise.

Table 2. Results of recovery tests of IND

Level of addition (%)	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery* ± SD	Mean ± SD
50	1	1.06	101.20 ± 0.92	
100	2	1.98	99.63 ± 0.49	101.77 ± 1.004
150	3	3.04	101.49 ± 1.62	

* Average of three determinations

Table 3. Results of recovery tests of AMLO

Level of addition (%)	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery* ± SD	Mean ± SD
50	5	4.97	99.74 ± 0.53	
100	10	9.98	99.84 ± 1.09	99.62 ± 0.94
150	15	14.72	99.28 ± 0.68	

* Average of three determinations

Table 4. Robustness data for IND and AMLO

DRUG	% RSD Found For Robustness Study (peak area)*								
	MP COMPOSITION			pH			FLOW RATE		
	68:32	70:30	72:28	2.8	3	3.2	0.9	1.0	1.1
IND	1.43	0.82	0.86	1.83	0.81	0.55	0.60	0.81	0.57
AMLO	0.55	0.49	1.50	0.57	0.59	0.55	1.71	0.59	0.93

*Basic concentration of sample chosen was 2 µg/ml of IND and 10 µg/ml of AMLO

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