Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [2] January, 2023: 171-179

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Method development and validation of related substances in API and tablet formulation for Amlodipine and Indapamide by RP HPLC

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ABSTRACT

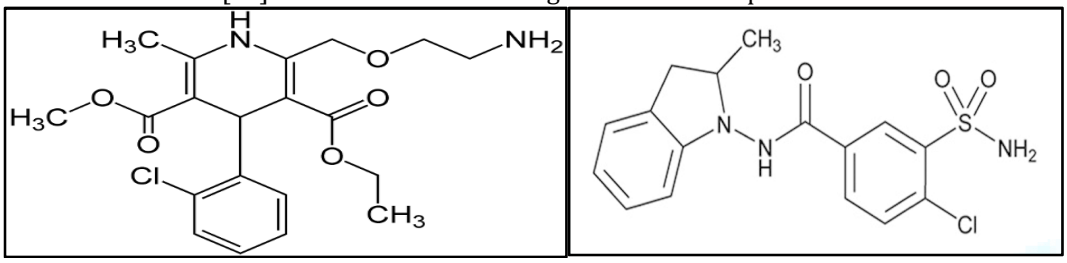
The current study aimed to develop and verify an accurate, precise, and fast technique for estimating related material for amlodipine and indapamide in API and tablet formulation using RP-HPLC. The created approach was discovered to be straightforward, accurate, exact, and sensitive. The separation was accomplished using an Isocratic High Pressure Liquid Chromatography (HPLC). HPLC analysis was carried out with the aid of AGLIET (1100) and CHEMSTATION software. AGLIET (1100) system with G1310A ISO PUMP, G-13148 (DAD) Detector, 0.001 to 5 ml discharge rate, 400 bar pressure limit range, 5% pressure display accuracy, 04 mobile phase, 0 to 100% mixing ratio range, pump unit HP-1100 reciprocating pump, and C-8 column (4.6 mm 250 mm, 5 m) used as stationary phase. The method was validated for precision, linearity, and accuracy, as well as LOD and LOQ parameters, in accordance with ICH guidelines. The recovery range was within the range, and the method could be successfully applied for routine drug substance analysis. Statistical analysis demonstrated that the procedure was exact, repeatable, selective, specific, and accurate for analysing amlodipine and indapamide as well as their impurities.

Keywords: *RP HPLC, Method Development, related substance, tablet, Impurities, amlodipine, indapamide*

Received 20.08.2022 Revised 13.11.2022 Accepted 15.12.2022

INTRODUCTION

3-ethyl amlodipine besylate (AMLO) is a chemical compound. 5-[4RS]-2-[(2 aminoethoxy) methyl] -4-(2-chlorophenyl) 6-methyl Dihydro-1,4 pyridine Benzenesulphonate -3, 5-dicarboxylate [1] (Figure 1 a) is a calcium channel blocker that is used to treat hypertension [2]. It is officially described in IP, BP, and USP, with IP [3], BP [4], and USP [5] describing the HPLC technique for its determination. A review of the literature revealed UV spectrophotometry [6], RP-HPLC [7], spectrophotometric [8] technique for simultaneous measurement of AMLO with other medication and RP-HPLC [9] method for simultaneous determination of AMLO in pharmaceutical dosage forms as well as biological fluids. Indapamide (INDA) is a 4-Chloro-N-[(2RS)-2-methyl-2,3-dihydro1H-indol-1-yl] compound. [10] -3-sulpha moylbenzamide Thiazide diuretics (Figure 2) are used to treat hypertension [11]. Indapamide is approved by IP [12], BP [13], and USP [14]. IP, BP, and USP define the HPLC procedure for estimating it. A review of the literature reveals the LC-MS [15], spectrophotometric [16], and HPLC [17] techniques for simultaneous quantification of INDA in whole human blood, the RP-HPLC [18] method for simultaneous estimation of INDA, and the LC-ESI-MS [19] methods for determining INDA in human plasma.



a. AMLODIPINE [20] b. INDAPAMIDE [21]

Fig 1: Structure of API

Related substance

Table 1: related substances of Amlodipine and Indapamide

SR NO	API	Impurity
1	Amlodipine	Amlodipine Related Compound D
2	Amlodipine	Amlodipine Related Compound A
3	Indapamide	Indapamide Related compound B(USP)
4	Indapamide	5-Hydroxy Indapamide

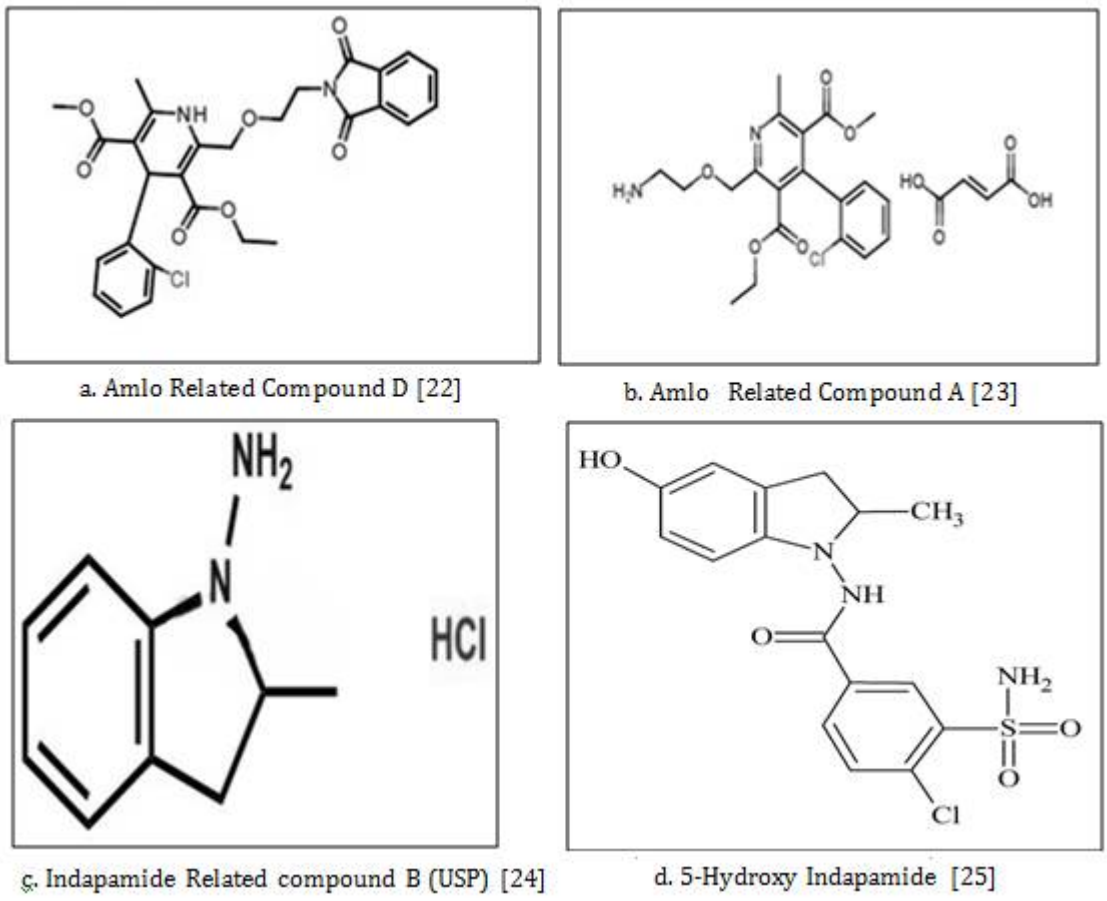


Fig 2: Related substances of Amlodipine and Indapamide

The present manuscript describes method development and validation of related substances in API and tablet formulation for amlodipine and indapamide by RP-HPLC.

MATERIAL AND METHODS

Materials and Chemical

Amlodipine API was supplied by Zydus Cadila PVt Ltd, Goa and Indapamide API was supplied by Supra chemicals, Thane. Related substances were supplied by Karpschem Lab, Nagpur. All other ingredient were used analytical grade.

Instrumentation

HPLC Analysis was performed using AGLIET (1100) and CHEMSTATION software.AGLIET (1100) system equipped with G1310A ISO PUMP , G-13148 (DAD) Detector, 0.001 to 5 ml discharge rate , 400 bar pressure limit range , 5 % pressure display accuracy , 04 no of mobile phase , 0 to 100 % mixing ratio range ,pump unit HP-1100 reciprocating pump and C-8 column (4.6 mm × 250 mm, 5 µm)used as stationary phase

Chromatographic conditions

The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation. Chromatographic separation was performed on, C-8 column (4.6 mm × 250 mm, 5 µm) with a c18 as stationary phase. Mobile phase were used as methanol:0.1%OPAPH 3.0 in 65:35 ratio respectively at 0.9 ml/min flow rate and particle size was 20 µ ml at ambient temperature.

Preparation of standard sample solution

The standard solution of amlodipine and indapamide were prepared by adding 10 mg and 3 mg in 10 ml of methanol respectively. Stock solutions were prepared as 1000 µgm/ml AMLA and 300 mcg IND. From stock solution 0.1,0.2,0.3,0.4 and 0.5 add in 10 ml of mobile phase to get 3,6,9,12,15 µ gm/ml IND and 10,20,30,40,50 mcg AMLA, respectively.

Tab solution Preparation:-

Tab Assay:-

For tablet assay total 20 tablets powdered, calculated and used in stock solution.23 mg in 20 ml of methanol was used to get 1000 µgm/ml AMLA and 300 mcg IND. Take 0.4 from stock solution and make up volume 10 ml wit mobile pahese in volumetric flask to get 12 µgm /ml IND and 40 mcg AMLA.

Method Validation

In house method validation for the current method was carried as per ICH Q2R1 guidelines. Validation was done through linearity, system suitability, and limit of quantification, limit of detection, range, accuracy, recovery, precision, robustness, solution stability, specificity and ruggedness.

Calibration curve of amlodipine and Indapamide

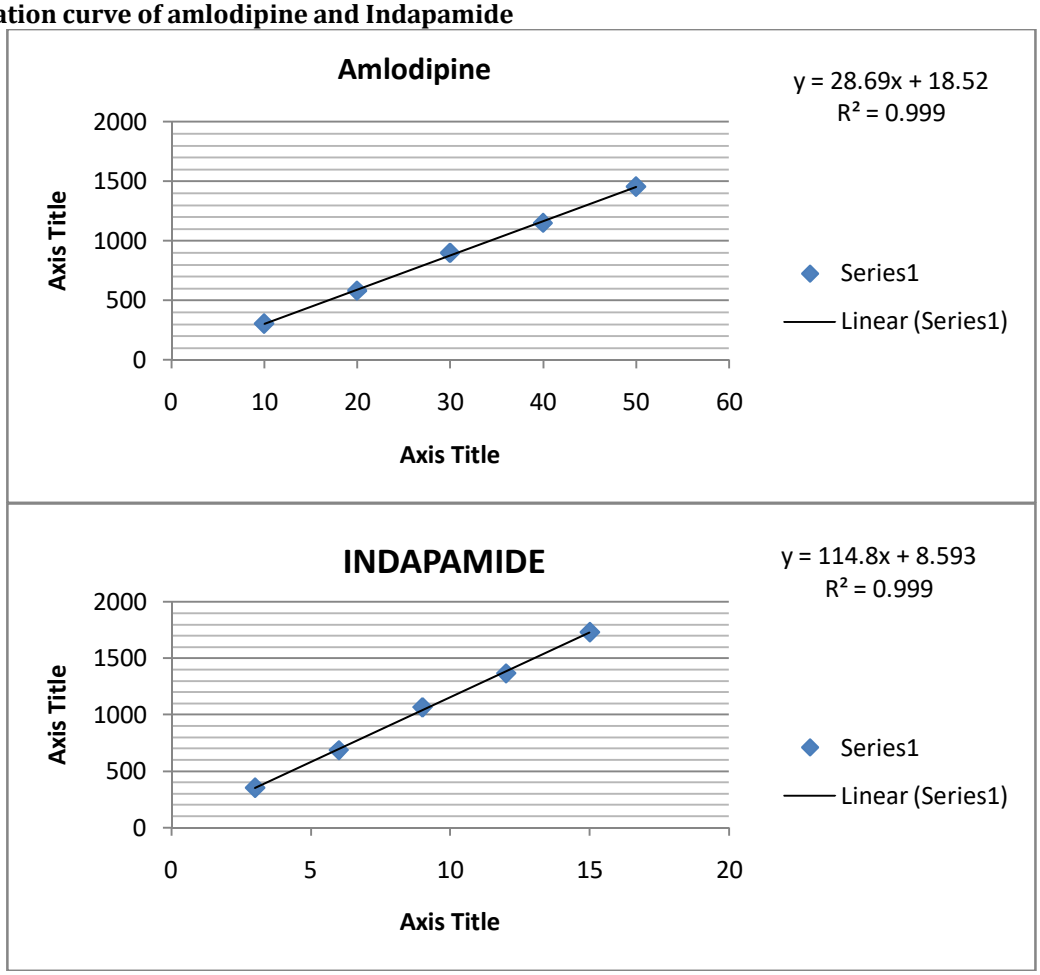


Fig 3: Calibration curve of Amlodipine and Indapamide

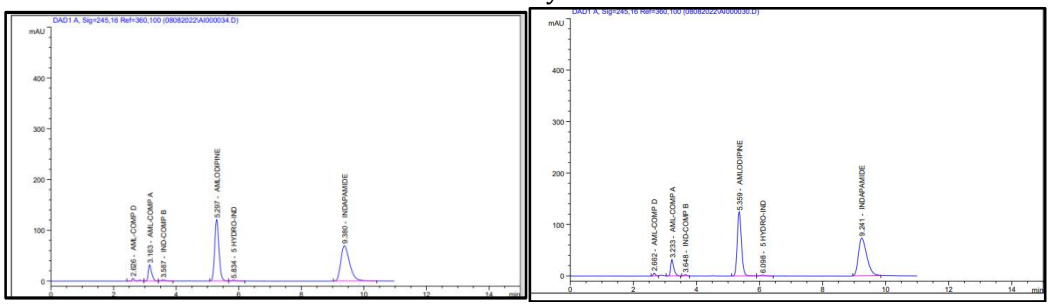
Amlodipine		Indapamide	
CON	ABS	CON	ABS
10	306.06 ±251.06	3	352.52± 297.52
20	582.5±527.50	6	686.98± 631.98
30	900.04±845.04	9	1067.38± 1012.38
40	1151.55±1096.55	12	1368.53± 1313.53
50	1456.017 ±1401.07	15	1733.84± 1733.84

Table 2:Calibration curve of amlodipine and Indapamide

ASSAY:

Conc	Area	Amt Found	% Label Claim
12.00	1361.613	11.78589	98.22
12.00	1353.688	11.71685	97.64
Mean	1357.65	11.75	97.93
SD	5.604	0.049	0.407
%RSD	0.413	0.415	0.415

Table 3 : Assay of tablet



40+12 mcg-01 40+12 mcg-02

Fig 4: Assay of tablet

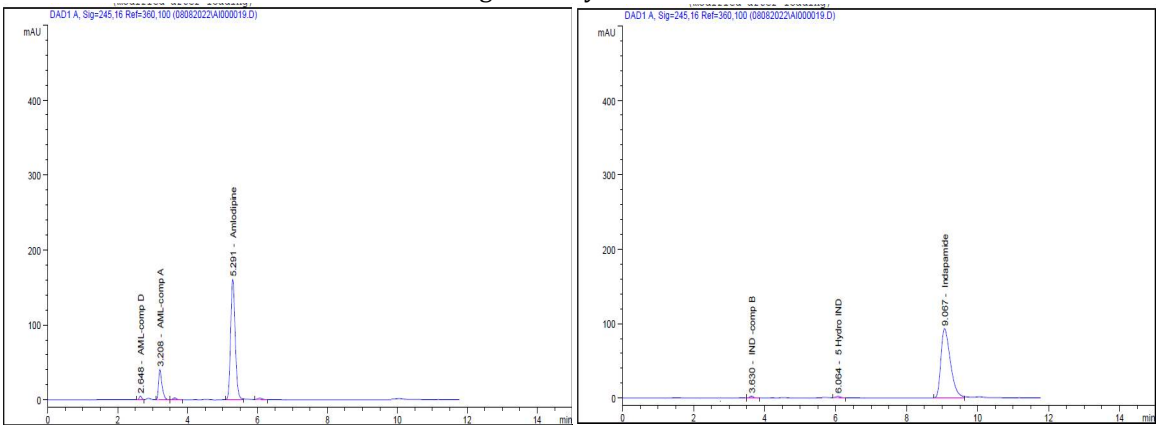
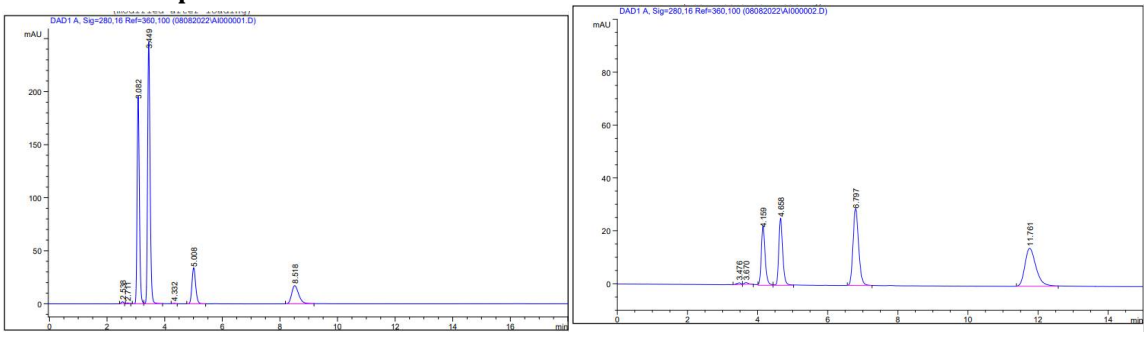
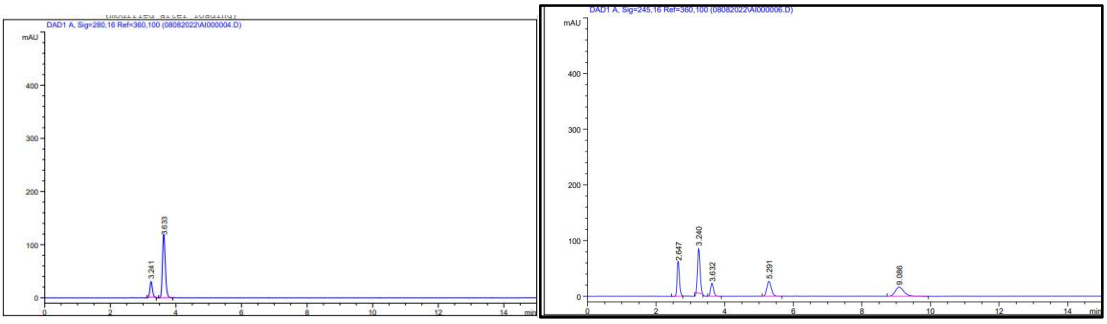


Fig 5: Chromatogram of standard and sample solution

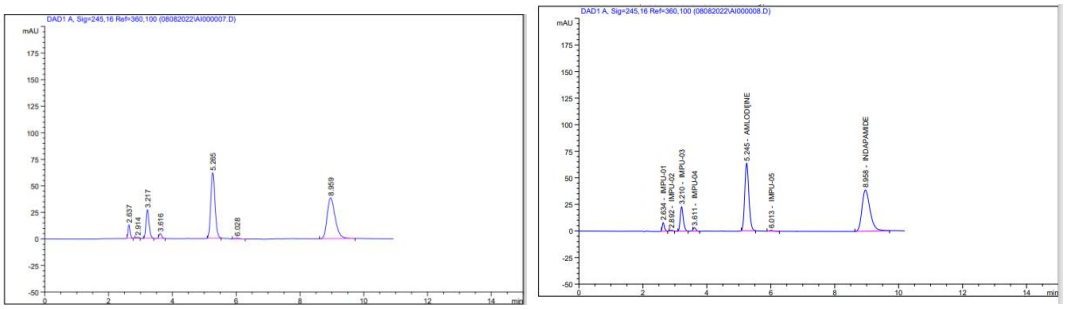
Method development:



a **b**



c **d**



e **f**

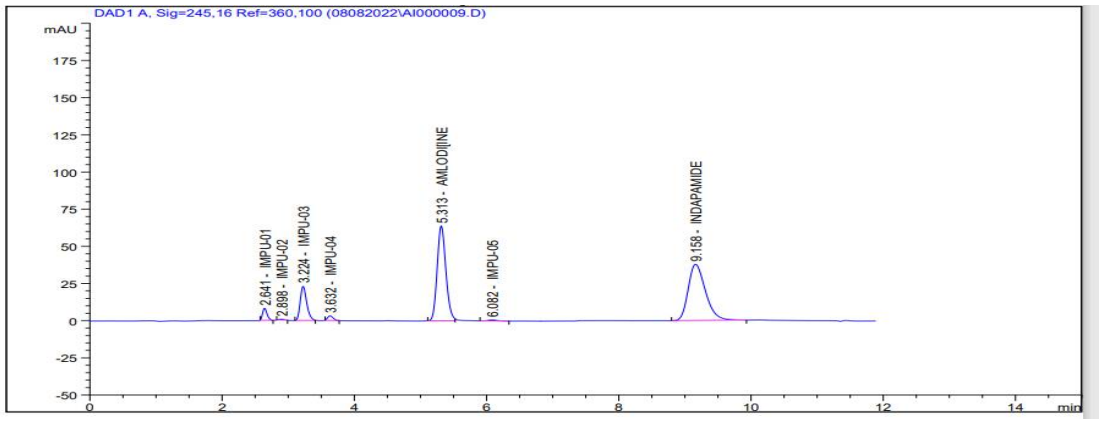


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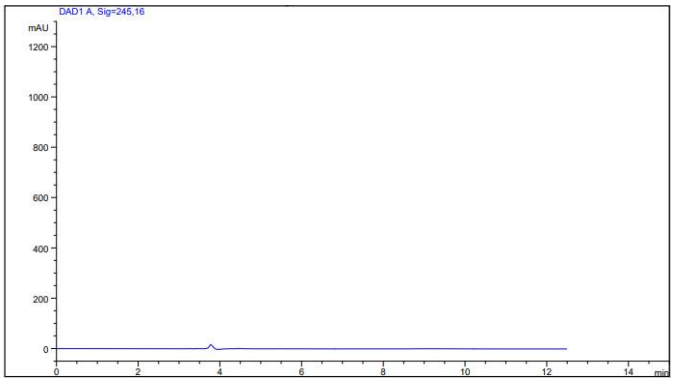
Fig 6: method development trials

Linearity:

The ability of the method to obtain test results proportional to the concentration of the analyte within a given range. It was evaluated by linear regression analysis, which was calculated by the least square regression method.

Table 4 :linearity

Amlodipine				**Indapamide**			
CONC	**Mean**	**SD**	**%SD**	**CON**	**Mean**	**SD**	**%SD**
10	306.06	0.15	0.05	3	352.52	0.03	0.01
20	582.50	3.71	0.64	6	686.98	3.62	0.53
30	900.04	7.08	0.79	9	1067.38	1.51	0.14
40	1151.55	4.67	0.41	12	1368.53	11.90	0.87
50	1456.07	0.82	0.06	15	1733.84	2.35	0.14
Avg SD		3.29		**Avg SD**	3.88		



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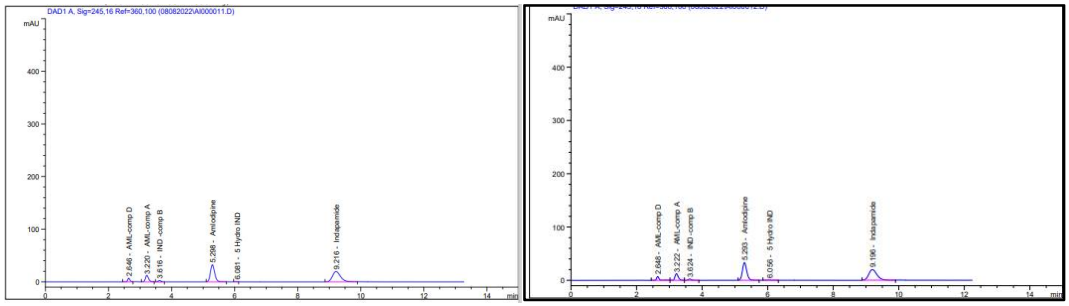
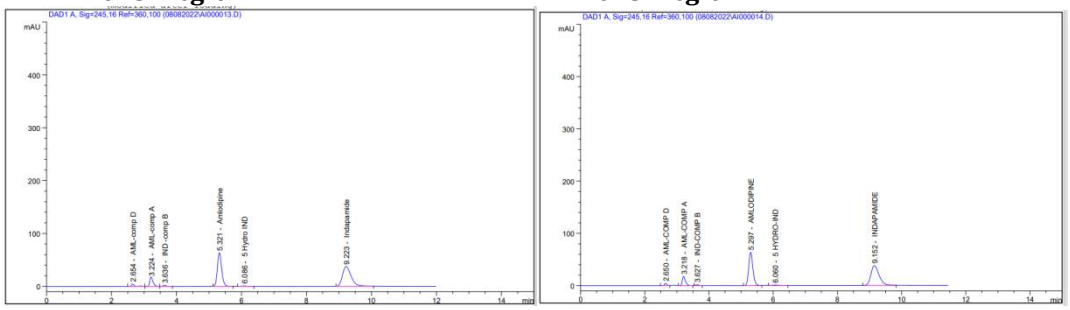
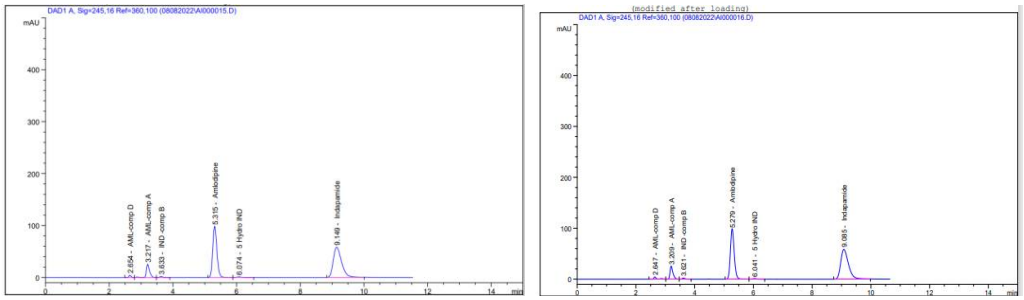


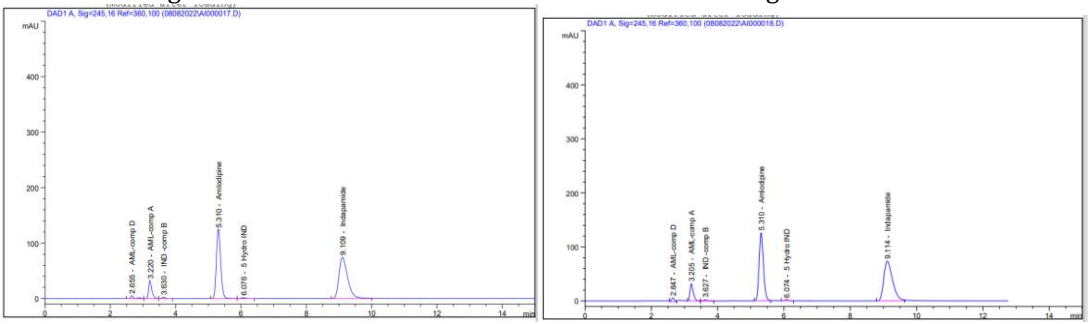
10+3 mcg-01 **10+3 mcg-02**



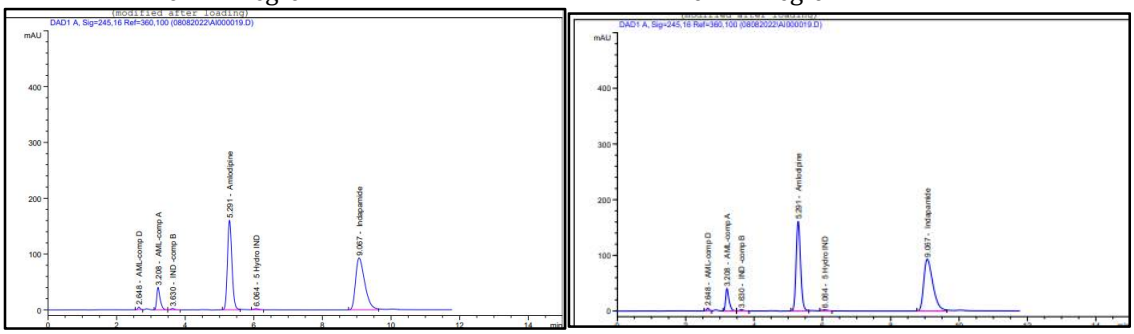
20+6 mcg-01 20+6 mcg-02



30+9 mcg-01 30+9 mcg-02



40+12 mcg-01 40+12 mcg-02



50+15 mcg-01 50+15 mcg-02

Fig 7: Linearity

Limit of detection

The limit of detection (LOD) **i**s the lowest concentration of analyte in a sample that can be detected but not necessary quantified. The obtained LOD values of specified impurities and API is tabulated in Table-7.

$$\mathbf{LOD = 3.3 \times \sigma / S}$$

Where, σ = the standard deviation of the response and S= slope of the calibration curve 5.5

Limit of quantitation

The limit of quantitation is the lowest concentration or amount of analyte that can be determined quantitatively within an acceptable level of repeatability precision and trueness Table-7.

$$\mathbf{Limit\ of\ quantitation\ (LOQ) = 10.0 \times \sigma / S}$$

Where, σ = the standard deviation of the response and S= slope of the calibration curve Precision at LOQ is confirmed by six replicate analyses of impurities at LOQ level

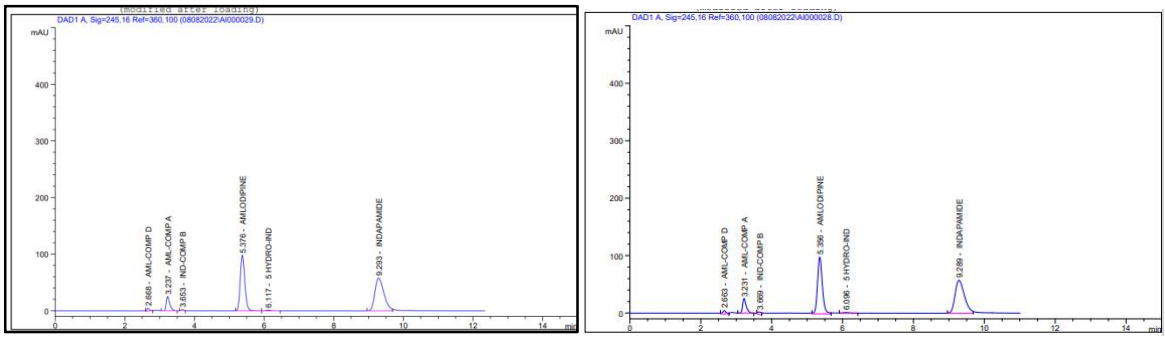
Table 5 : LOD and LOQ

	AML	**IND**
SD	3.29	3.88
LOD (µg/ml)	0.377932	0.111898
LOQ (µg/ml)	1.145247	0.338176

Precision

Table 6 : Precision

Intraday									
Amlodipine					**Indapamide**				
Conc	**Mean**	**% amt fnd**	**Sd**	**%rsd**	**Conc**	**Mean**	**% amt fnd**	**SD**	**%RSD**
20	582.79	98.34	0.87	0.15	6	686.65	98.44	3.60	0.52
30	902.35	102.69	0.44	0.05	9	1053.34	101.12	8.38	0.80
40	1156.45	99.16	0.86	0.07	12	1362.90	98.31	1.89	0.14
Interday									
Amlodipine					**Indapamide**				
Conc	**Mean**	**Mean**	**Mean**	**Mean**	**Conc**	**Mean**	**% amt fnd**	**SD**	**%RSD**
20	582.79	296.48	573.53	301.23	6	693.61	99.45	2.14	0.31
30	902.35	456.26	893.09	466.58	9	1028.54	98.72	1.33	0.13
40	1156.45	583.31	1147.19	598.06	12	1365.75	98.52	3.37	0.25



30+9 mcg-01 30+9 mcg-02

Fig 8: precision

Recovery studies:

Accuracy:

Accuracy can be defined as the closeness of agreement between a test result and the accepted reference value. Accuracy of the method was determined by recovery study. Analytical method may be considered validated in terms of accuracy if the mean value is within ± 20% of the actual value. Recovery of specified impurities was found in the range of 80.0% to 120.0%, which was well within the acceptance criteria as shown below,

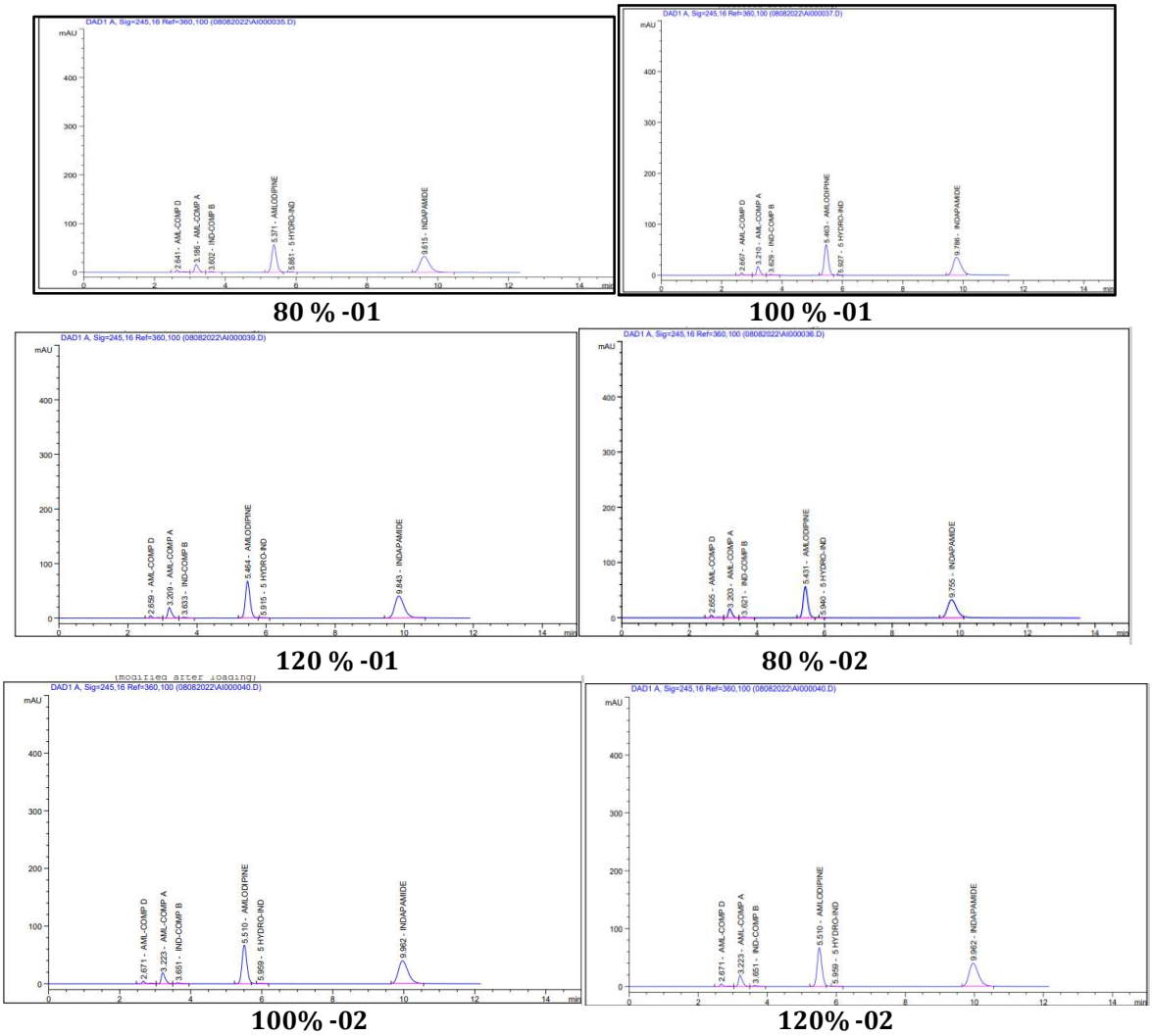


Fig 9: recovery studies; accuracy

Table 7: recovery studies; accuracy

80%				100%				120%			
Area	**Amt found**	**Amt recvd**	**% Recv**	**Area**	**Amt found**	**Amt recvd**	**% Recv**	**Area**	**Amt found**	**Amt recvd**	**% Recv**
629.78	5.41	2.41	100.46	695.11	99.65	99.65	99.65	762.38	6.566	3.566	6.59
630.43	5.42	2.42	100.70	697.25	0.44	0.44	0.44	768.49	6.619	3.619	0.038
Mean	5.41	2.41	100.58	Mean	5.99	2.99	99.65	Mean	6.59	3.59	6.59
SD	0.004	0.004	0.17	SD	0.013	0.013	0.44	SD	0.038	0.038	0.038
%RSD	0.074	0.165	0.17	%RSD	0.220	0.441	0.44	%RSD	0.571	1.048	0.571

RESULTS AND DISCUSSIONS

The quantification of related organic impurities of amlodipine and indapamide was completed following a series of experiments with various stationary phases and mobile phases. The suggested approach effectively resolves all specified and undefined contaminants.

All stated contaminants and API are linearized from LOQ to 150% with regard to test concentration. The ideal correlation coefficient for all impurities indicates that the area of impurities responds linearly with regard to concentrations as indicated in the linearity plot of peak area versus concentration of the analyte. The response factor of all specified contaminants is computed and given based on the linearity experiment. On the basis of the linearity experiment, the LOD and LOQ of all specified impurities and API are calculated and predicted, and the solution of LOD and LOQ is prepared and injected for further confirmation. LOD and LOQ are reported as previously. Six repeat assessments of all contaminants at the LOQ level demonstrate precision.

The method's accuracy is demonstrated by completing a recovery study in which known quantities of the specified contaminants are introduced into the sample at the LOQ, 50%, 100%, and 150% levels. All

required contaminants are recovered between 80.0% and 120.0%. The results are constant in spite of purposeful alterations in the different equipment, different lots of columns on separate days, and two different scientists, proving that the procedure is rough and resilient.

CONCLUSION

All validation characteristics, such as precision, specificity, accuracy, and detector response linearity, are fulfilled by the RP-HPLC analytical technique described above. All of these aspects combine to make this approach ideal for measuring impurities in amlodipine and indapamide in pharmaceutical dosage forms without interference and suited for routine analysis. As a result, it can be stated that it may be utilised in small laboratories with excellent precision and a broad linear range.

Conflict of Interest: No

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CITATION OF THIS ARTICLE

Daphal Gajanan Tulshiram, D. Umamaheswari, M. Kumar. Method development and validation of related substances in API and tablet formulation for Amlodipine and Indapamide by RP HPLC. Bull. Env. Pharmacol. Life Sci., Vol 12[2] Jan 2023: 171-179.