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Development and Validation of RP-UPLC method for Simultaneous Estimation of Amlodipine and Indapamide in Their Combined Tablet Dosage Form

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ABSTRACT:

High efficiency and less run time are the basic requirements of high-speed chromatographic separations. To fulfill these requirements, a new separation technique, ultra-performance liquid chromatography (UPLC), has shown promising developments. A rapid, specific, sensitive, and precise reverse-phase UPLC method is developed for the determination of Amlodipine (AMLO) and Indapamide (INDP) in tablet dosage form. The chromatographic separation was achieved on Acquity UPLC BEH C18 column (2.1 mm× 100 mm, 1.7 µm) using mobile phase of Buffer (1% Glacial acetic acid) : Acetonitrile (58:42, v/v), at a flow rate of 0.25 mL/min at an ambient temperature. UV detection was carried out at 240 nm with injection volume of 2 µl. The retention time for Amlodipine (AMLO) and Indapamide (INDP) was found 1.56 min and 2.58 min respectively. Linearity was observed in range of 10-50 µg/ml and 3-15 µg/ml for AMLO and INDP respectively. The method is validated according to the ICH guidelines and is applied successfully for the determination of both the rugs in tablet formulation as well as bulk.

KEYWORDS: Amlodipine, Indapamide , Simultaneous estimation, RP- UPLC method, Validation

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INTRODUCTION:

A new category of separation technique, UPLC can be regarded as new invention for liquid chromatography. UPLC refers to Ultra Performance Liquid Chromatography. UPLC brings dramatic improvements in sensitivity, resolution and speed of analysis can be calculated. It has instrumentation that operates at high pressure than that used in HPLC & in this system uses fine particles(less than 2.5µm) & mobile phases at high linear velocities decreases the length of column, reduces solvent consumption & saves time. The comparative study of features of HPLV and UPLC is given in Table 1. In the present work, this technology has been applied to the method development, validation, and assay determination of AMLO and INDP in tablet dosage form.

Amlodipine(AMLO) (Figure 1) is a Dihydro pyridine calcium antagonist that inhibits the movement of calcium ions into vascular smooth muscle cells and cardiac muscle cells. Chemically is a *(RS)*-3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate[1-4]. Indapamide(INDP) (Figure 1) is Thiazide diuretic class drug and act as a Diuretic and Vasodilator and chemically 4-chloro-N-(2-methyl-2, 3-dihydroindol-1-yl)-3-sulfamoyl-benzamide[1-4]. The medication can help with water retention and lower blood pressure by increasing the amount of salt and water that the kidneys remove from the blood. Amlodipine and Indapamide are official in Indian pharmacopoeia[5], British Pharmacopoeia[6] ,United state Pharmacopoeia[7] and European pharmacopoeia[8] but there combination is not official in any of the Pharmacopoeia. Literature survey indicate some spectrophotometric[9-12,15,20,22,23,25-28,33], HPLC [10,13,16,17,20,22,29-31,34-36,38],

Table 1: Comparison between UPLC and HPLC

Characteristics	HPLC	UPLC
Particle size	3 to 5m	Less than 2m
Maximum backpressure	35-40 MPa	103.5 MPa
Analytical column	Alltima C_{18}	Acquity UPLC BEH C_{18}
Column dimensions	150 X 3.2 mm	150 X 2.1 mm
Column temperature	30 °C	65 °C
Injection volume	5µL(Std.In100% MeOH)	2µL(Std.In100% MeOH)

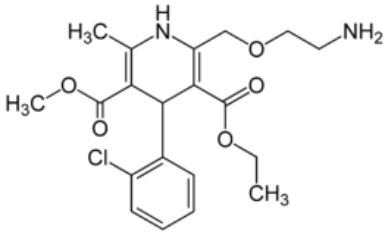
HPTLC [14,19,32], stability indicating HPLC [37], LC-MS [18,21], stability indicating UPLC [24] methods for estimation of AMLO either individually or in combination with other drugs . Literature survey also reports HPLC [39,41], spectrophotometric[42-43], stability indicating HPLC [40] methods for estimation of INDP individually or in combination with other drugs. However there is one HPLC [44] ,one stability indicating HPLC [45] and HPTLC [46] method reported for simultaneous estimation of both drugs in their combined tablet dosage form. From the literature survey it was revealed that there is no UPLC method reported for simultaneous estimation of both the drugs. Present work describes rapid, simple, sensitive, accurate and reproducible RP-UPLC method .

MATERIAL AND METHODS

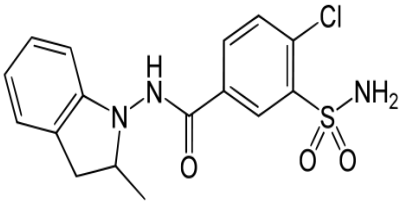
Quantitative RP-UPLC method was performed on waters Acquity UPLC. Acquity UPLC BEH C18 column (2.1 mm× 100 mm, 1.7 µm) was used for separation of drugs. Amlodipine and Indapamide standards were obtained from Accuprec Research Labs Pvt. Ltd., Ahmedabad, Gujarat, India. The combination product (Amlodac-D, Zydus cadila) was procured from market. Acetonitrile, Methanol and Glacial acetic acid (HPLC grade from Merck Ltd, Mumbai, India), Sodium hydroxide (GR grade), Concentrate hydrochloric acid (GR grade) was used in study. High purity water for injection was used in the study.

Preparation of Standard stock solution

An accurately weighed standard powder of 50 mg of AMLO and 15 mg of INDP were transferred in 10 ml volumetric flask, dissolved and diluted up to the mark with diluents (mobile phase) to get final concentration 5000 µg/ml of AMLO and 1500 µg/ml of INDP. From this standard stock solution, 5 ml was diluted upto 25 ml with diluents to get concentration 1000 µg/ml of AMLO and 300 µg/ml of INDP. This solution was used as a working standard solution (WSS).



AMLODIPINE



INDAPAMINE

Preparation of calibration curve

From the above WSS solution appropriate dilutions were made with diluent to get concentration in range of 10-50 µg/ml for AMLO and 3-15 µg/ml for INDP. 2 µl aliquots from these solutions were injected into UPLC system under prescribed chromatographic condition. Calibration curve was prepared by plotting graph of area vs. concentration.

Chromatographic condition:

Column : Acquity UPLC BEH C18 1.7 UM (2.1mm X 100 mm)

Flow rate : 0.25 ml/min

Mobile Phase : Buffer(1% Glacial acetic acid) : Acetonitrile (58:42)

Column Oven Temperature : Ambient

Injection volume : 2 µl

Detection wavelength : 240 nm

Diluents : Mobile Phase

Procedure for Analysis of Tablet Formulation

Twenty tablets were weighed and powdered. An accurately weighed tablet powder equivalent to 2.5 mg of AMLO and0.75 mg of INDP was transferred in to 25 ml volumetric flask. To this 20 ml of diluent was added and sonicated for 15 min. Volume was made up to the mark with diluent to get

concentration 1000 µg/ml of AMLO and 300 µg/ml of INDP . Solution was filtered through whatman filter paper no.41. Then the sample solution was filtered through 0.45 µm cellulose acetate filter paper (0.45 µm) before diluting further. From this stock solution, different aliquots were transferred into 10 ml volumetric flask and volume was made up to the mark with diluent. AMLO and INDP were separated at 1.56 min and 2.58 min respectively **(figure 2, 3 & 4)**. Results of analysis of tablet formulation are shown in **Table 5**.

METHOD VALIDATION [47-55]

The proposed methods were validated accordance to ICH Q2(R1) guidelines for linearity, precision, accuracy, limit of detection, limit of quantification and system suitability. The results are shown in **Table 2, 3 & 4**.

Linearity:

The linearity of proposed methods were evaluated by linear regression analysis, which was calculated by least square method. The drugs were linear in the concentration range of 10-50 µg/ml for AMLO and 3-15 µg/ml for INDP (**Figure 5 & 6**).

Accuracy:

Accuracy of the methods were determined at three different concentration levels i.e.50%, 100% and 150% in triplicate for each drug as per ICH guidelines. From the total amount of drug found, the percentage recovery was calculated. The results were shown in **Table 2**.

Precision:

Intraday Precision of the UPLC method was determined by analyzing six replicate measurements at 100% at concentration of drugs for three times in the same day. Inter-day precision was conducted during routine operation of the system over a period of 3 consecutive days. The precision of an analytical method is expressed as %RSD of a series of measurements. The results were shown in **Table 3**.

Robustness

The robustness of the method was verified by making deliberate changes in the chromatographic conditions, viz. change in flow rate by ±0.1 ml/min and change in the ratio of mobile phase (±2% absolute). The method was demonstrated to be robust over an acceptable working range of UPLC operational parameters. The results were shown in **Table 4**.

RESULTS AND DISCUSSION

The proposed method for simultaneous estimation of AMLO and INDP in tablet dosage forms was found to be simple, accurate, economical and rapid. The method was validated as per the ICH Q2(R1) guidelines. Standard calibration curves for AMLO and INDP were linear with correlation coefficients (r^2) values in the range of 0.9940- 0.996 at the selected wavelength and the values were average of three readings with relative standard deviation in the range of 0.06 –1.94. The retention time for AMLO and INDP was found to be 1.56 min and 2.58 min respectively under prescribed chromatographic condition **(figure 3)**. The values of %RSD are within the prescribed limit of 2 %, showing high precision of method and recovery was close to 100% for both the drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed method are suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. The comparative study of RP-HPLC[45] and RP-UPLC methods has been also done which indicate that RP-UPLC method is found to be more accurate, fast and economic as compared to RP-HPLC method (**Table 6**). Hence, the above method can be applied successfully in simultaneous estimation of AMLO and INDP in marketed formulations.

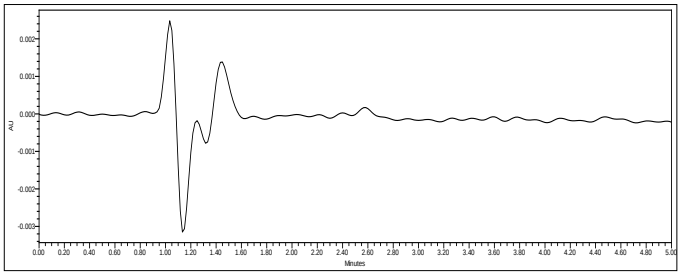


Figure 2: UPLC chromatogram of blank

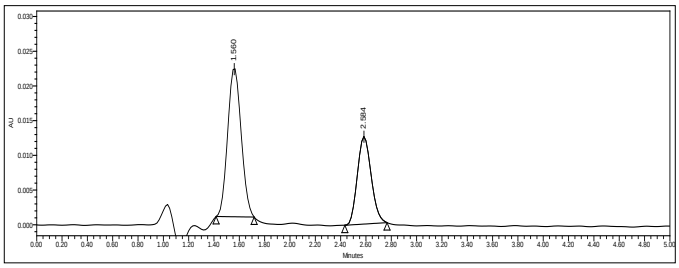


Figure 3: UPLC chromatogram of standard mixture of AMLO (30 µg/ml) and INDP (9µg/ml)

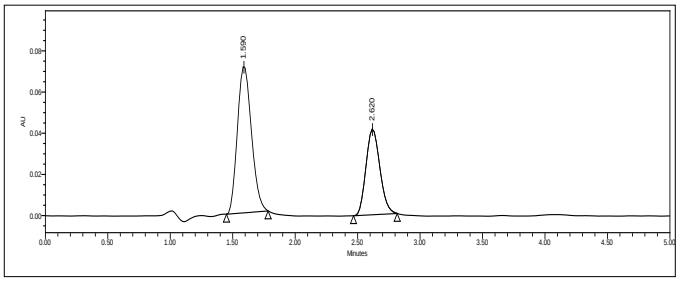


Figure 4: UPLC chromatogram of test of AMLO (30 µg/ml) and INDP (9µg/ml)

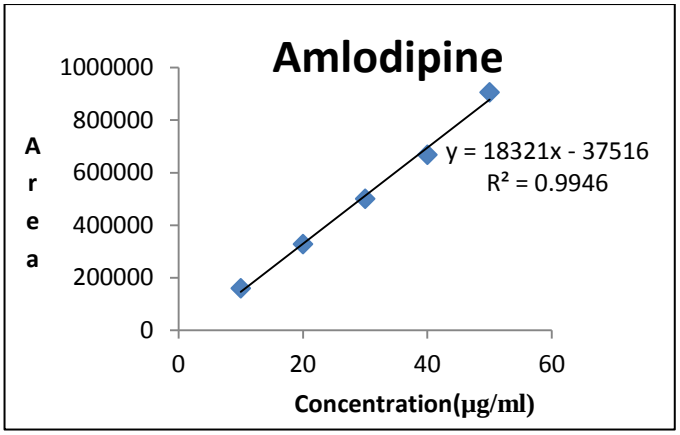


Figure 5: Calibration Graph for Amlodipine

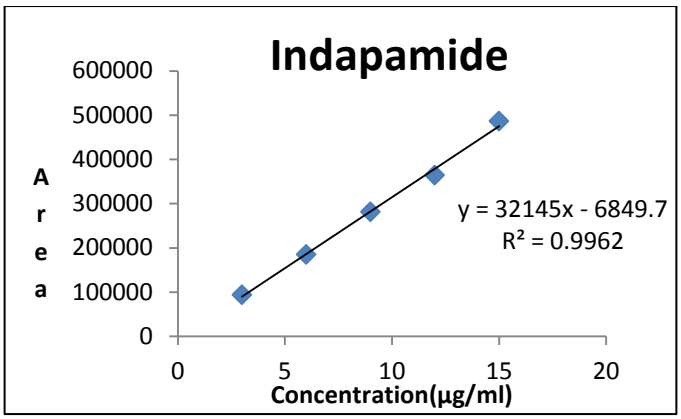


Figure 6: Calibration Graph for Indapamine

Table 2: Results of Recovery Study (Accuracy)

	AMLO				INDP			
%Level	Amount taken (µg/ml)	Area (n=3)	Amount found (µg/ml)	%Recovery	Amount taken (µg/ml)	Area (n=3)	Amount found (µg/ml)	%Recovery
50%	15	282080.7	14.47	96.47	4.5	160918	4.39	97.59
100%	30	581703	29.84	99.47	9	317035.667	8.65	96.13
150%	45	890307.7	45.67	101.49	13.5	494050.333	13.48	99.87
AVG				**99.14**				**97.87**

Table 3: Results of Precision Study

Sr. No.	Intraday (n=3)				Interday (n=3)			
	AMLO		INDP		AMLO		INDP	
	Conc. found (µg/ml)	%Assay	Conc. found (µg/ml)	%Assay	Conc. found (µg/ml)	%Assay	Conc. found (µg/ml)	%Assay
1	29.25	97.51	9.64	107.17	28.83	96.12	9.12	101.37
2	29.40	98.00	9.73	108.19	28.00	93.34	9.24	102.68
3	29.50	98.34	9.53	105.89	28.48	94.93	9.09	101.03
4	29.23	97.44	9.68	107.64	28.65	95.52	9.13	101.49
5	28.95	96.51	9.37	104.13	28.83	96.11	9.10	101.19
6	29.25	97.53	9.41	104.58	29.04	96.82	9.12	101.37
Avg		**97.56**		**106.27**		**95.48**		**101.53**
SD		**0.62**		**1.67**		**1.23**		**0.59**
% RSD		**0.63**		**1.57**		**1.283**		**0.58**

SD – Standard Deviation, RSD *–Relative standard deviation*

Table 4: Results of Robustness Study

Parameter	AMLO		INDP		AMLO		INDP	
Flow rate (mL/min)	**0.26**				**0.24**			
	Conc. found (µg/ml)	**%Assay**	**Conc. found (µg/ml)**	**%Assay**	**Conc. found (µg/ml)**	**%Assay**	**Conc. found (µg/ml)**	**%Assay**
1	29.5	98.43	9.452	105.03	29.53	98.43	9.45	105.03
2	29.90	99.69	9.68	107.61	29.90	99.69	9.68	107.61
3	29.51	98.39	9.68	107.58	29.51	98.39	9.68	107.58
Avg	**29.65**	**98.84**	**9.60**	**106.74**	**29.65**	**98.84**	**9.60**	**106.74**
Mobile Phase	**60:40**				**56:44**			
1	29.16	97.20	9.83	109.26	29.10	97.02	9.52	105.88
2	29.25	97.51	9.73	108.19	29.17	97.25	9.48	105.40
3	29.15	97.16	9.72	108.03	28.83	96.12	9.40	104.47
Avg	**29.19**	**97.29**	**9.76**	**108.5**	**29.04**	**96.80**	**9.47**	**105.25**
Column lot change								
1	28.33	94.44	28.55	95.16				
2	28.40	94.67	28.96	96.55				
3	27.08	90.28	27.20	90.67				
Avg	**27.94**	**93.13**	**28.23**	**94.13**				

Table 5: Analysis of marketed formulation by proposed methods

Drug	Amlodac-D	% Assay ± SD
AMLO	5 mg	96.52 ± 1.47
INDP	1.5 mg	103.9. ± 3.35

Table 6: A Comparison of System Performance of HPLC and UPLC for AMLO and INDP

Parameter	RP-HPLC		RP-UPLC	
	AMLO	**INDP**	**AMLO**	**INDP**
Retention time (min)	6.44	10.24	1.56	2.58
Flow rate (mL/min)	0.8		0.25	
Injection volume (µL)	20		2	
Mobile phase Buffer (1% Glacial acetic acid) : Acetonitrile (v/v)	55:45		58:42	

Table 7: Summary of Validation parameters of UPLC Assay method of Amlodipine and IndapamideTablet

Sr. No.	Parameters	Data for Amlodipine	Data for Indapamide	Limit
1.	Linearity Range Correlation co-efficient	10-50 µg/ml r^2 = 0.994	3-15 µg/ml r^2 = 0.996	-
2.	Precision Repeatability Intraday Precision Interday precision	RSD= 1.161 RSD= 0.634 RSD= 1.283	RSD= 1.140 RSD= 1.573 RSD= 0.583	RSD < 2.0
3.	Accuracy	% Recovery 96.46-101.49%	% Recovery 96.13-99.87 %	90-110 %
4.	Specificity	Specific	Specific	Should be specific
5.	Robustness	There was no influence of small variation in flow rate and mobile phase suggested robustness.	There was no influence of small variation in flow rate and mobile phase suggested robustness.	Should be robust
6.	Stability of standard solution	Standard solution was found to be stable.	Standard solution was found to be stable.	-

CONCLUSION

The developed RP-UPLC method was found to be more accurate, precise and reproducible. The analysis of tablets containing two drugs gave the satisfactory results. The statistical parameter of these methods showed good results. The recovery studies revealed excellent accuracy and high precision of the method. The method was found to be simple & time saving. The proposed method could be applied for routine analysis in quality control laboratories.

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