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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR THE SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND PIOGLITAZONE HYDROCHLORIDE

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Abstract:

Amlodipine Besylate and Pioglitazone Hydrochloride in bulk and pharmaceutical dosage form were estimated using a simple to utilize, accurate, and economical UV and RP-HPLC approach that was developed and validated. The method was validated as per ICH guidelines by using various validation parameters such as Linearity, accuracy, precision, specificity and robustness. This present work provides a very simple and accurate method for simultaneous estimation of Antihypertensive drug and anti-diabetic drugs. Pharmaceutical drugs studies, standardization, and quality control all use precise and subtle analytical techniques. Quality is essential because the medicinal product involves human life. Strong primary healthcare programs must concentrate on proper drug production and quality management on a global basis. The methods are validated according to ICH guidelines and all validation parameters were studied for the proposed method like linearity, precision, range.

Keywords: HPLC, UV, Validation, Amlodipine Besylate and Pioglitazone Hydrochloride.

Introduction:

In the discovery of drugs, analytical methods development and validation for impurities, active pharmaceutical ingredients, etc. are important aspects. The quality of the analytical data play very critical and important role as analytical methods for the success of drugs and formulation development programs. Therefore, it is necessary that when performing quantitative determination of any drug forms the tablet formulations or dosage forms, exact quantity of known drug to be added during the process of analysis to ensure accurate determination of unknown drug determined in the tablet formulations. Therefore, the objective of the work is to develop the simultaneous analysis method with internal standard, which is economical, logical, simple, and precise and to validate the method in terms of specificity, precision, linearity, ruggedness, robustness, solution stability, stability studies.

Amlodipine Besylate:

Amlodipine Besylate (AB), 2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl) 1,4-dihydro-6-methyl-3,5-pyridine-dicarboxylic acid-3 ethyl-5 methyl ester, is a calcium channel blocker. It is used in the treatment of hypertension and angina.

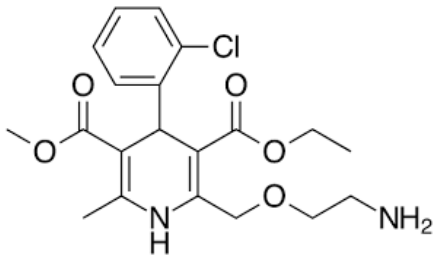

Fig. 1 Structure of Amlodipine Besylate

Pioglitazone Hydrochloride:

Pioglitazone hydrochloride, (±)-5-{[[4-2-(5-ethyl-2-pyridinyl) ethoxy] phenyl]methyl}-2,4-thiazolidinedione monohydrochloride, is an oral anti-hyperglycemic agent which acts primarily by decreasing insulin resistance and was developed by Takeda chemicals. It is used in the treatment of type-II diabetes.

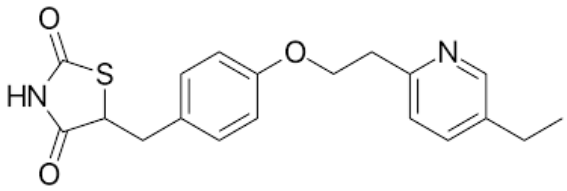

Fig. 2 Structure of Pioglitazone Hydrochloride

Material & Methods:

Determination of Λ Max (Selection of Wavelength)

The standard solutions were scanned separately between 400nm to 200nm. From the spectrum show high absorbance.

Simultaneous Equation Method

From the overlain spectra of the two drugs Amlodipine and Pioglitazone shows absorbance at 234nm and 285 nm which is the λmax of Amlodipine and Pioglitazone respectively. Working standard solutions were analyzed in concentration range 5-30µg/ml for both Amlodipine and Pioglitazone respectively, concentration was selected 20 µg/ml to measure the wavelength.

$Cx = (A2\ ay1 - A1\ ay2) /(ax2\ ay1 - ax1\ ay2)$

$Cy = (A1\ ax2 - A2\ ax1) / (ax2\ ay1 - ax1\ ay2)$

Selection of Solvent

The solubility of drugs was determined in a variety of polar and non-polar solvents as per IP specification. The common and stable solvent was found to be Water: methanol and dilutions were made with same Water: methanol 20:80 v/v for the analysis of Amlodipine and Pioglitazone for the proposed method.

Preparation of Amlodipine Standard stock solution

An accurately weighed quantity about 20 mg of Amlodipine standard was transferred to 200 mL volumetric flask. Add 150 mL of diluent, sonicate to dissolve and dilute up to the mark with diluent and mixed. Further transferred 5ml of above solution in a 25 ml volumetric flask added diluent up to the mark and mixed well.

Preparation of Pioglitazone Standard stock solution

An accurately weighed quantity about 20 mg of Pioglitazone standard was transferred to 200 mL volumetric flask. Add 150 mL of diluent, sonicate to dissolve and dilute up to the mark with diluent and mixed. Further transferred 5ml of above solution in a 25 ml volumetric flask added diluent up to the mark and mixed well.

Preparation of sample solution

A sample was prepared by taking 5ml of each Amlodipine and Pioglitazone from stock solution in 10ml volumetric flask, mix well & make up volume up to mark then measure the absorbance of this sample solution at respective wavelength using double-beam Uv-visible.

Method Validation:

The following parameters were considered for the analytical method validation of title ingredients.

- System Suitability.
- Specificity.
- Linearity.
- Accuracy.
- Precision.
- System Precision.
- Method Precision.
- Intermediate Precision.
- Robustness.

SYSTEM SUITABILITY:

System suitability test is a Pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done.

Table 1 System suitability test of Amlodipine and Pioglitazone

	Amlodipine	**Pioglitazone**
Symmetry factor	**1.2**	**1.0**
Theoretical plates	**10207**	**16825**
S. No.	**Area**	**Area**
1	441287	38756874
2	435394	39336548
3	434464	38226258

4	443345	38957249
5	440435	39100240
6	437547	39265475
Mean	**438745**	**38940440**
%RSD	**0.8**	**1.0**

The tests were performed by collecting data from Single injection of blank (Diluent) and six replicate injections of Standard solution were injected into the chromatograph. The data obtained is summarized in Table 1.

Specificity: (Identification, Interference & Peak Purity)

Inject Blank (Diluent), standard solution, and sample solution. The data obtained is summarized in Table 2.

Table 2 Specificity of Amlodipine (Identification and Interference)

Component	**Retention time (min)**	**Theoretical plates**	**Tailing factor**	**Purity angle**	**Purity threshold**
Blank	-		-	-	-
Standard solution	6.911	11157	1.2	1.32	3.27
Sample Solution	6.917	11003	1.2	1.49	3.76

Table 3 Specificity of Pioglitazone (Identification and Interference)

Component	**Retention time (min)**	**Theoretical plates**	**Tailing factor**	**Purity angle**	**Purity threshold**
Blank	-		-	-	-
Standard solution	11.693	16239	1.0	1.84	4.00
Sample Solution	11.716	16530	1.1	1.90	3.62

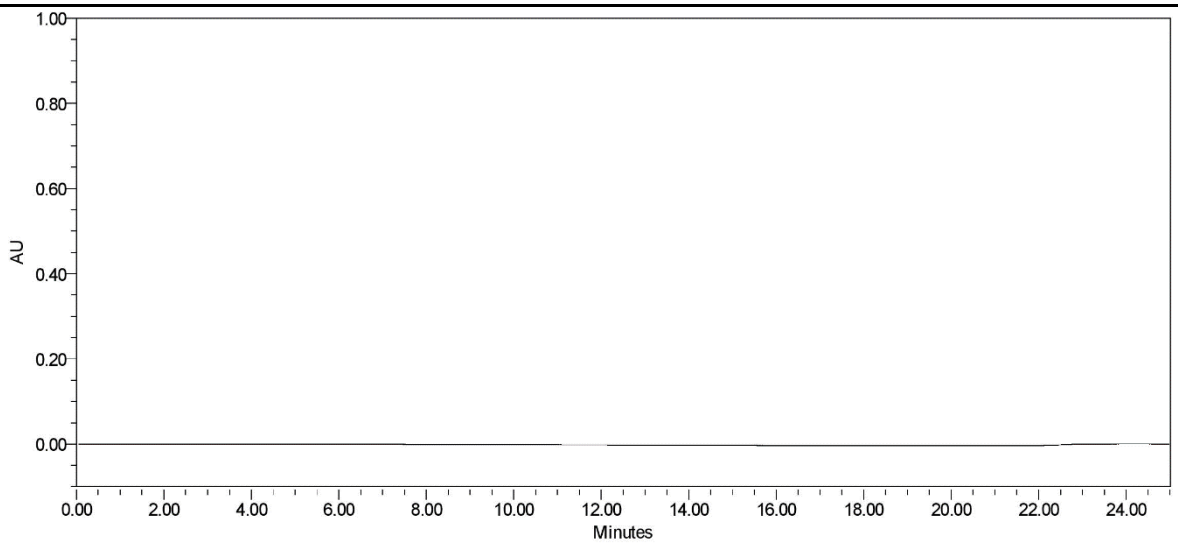

Fig 3 Chromatogram of Blank

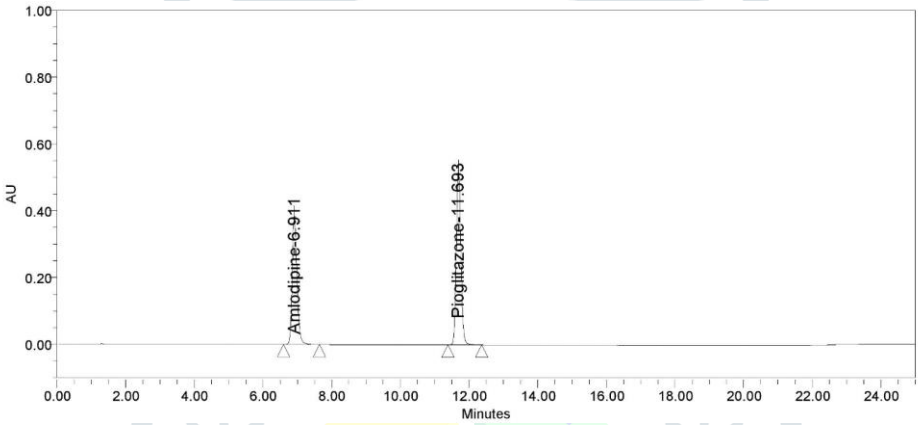

Fig 4 Chromatogram of Standard solution

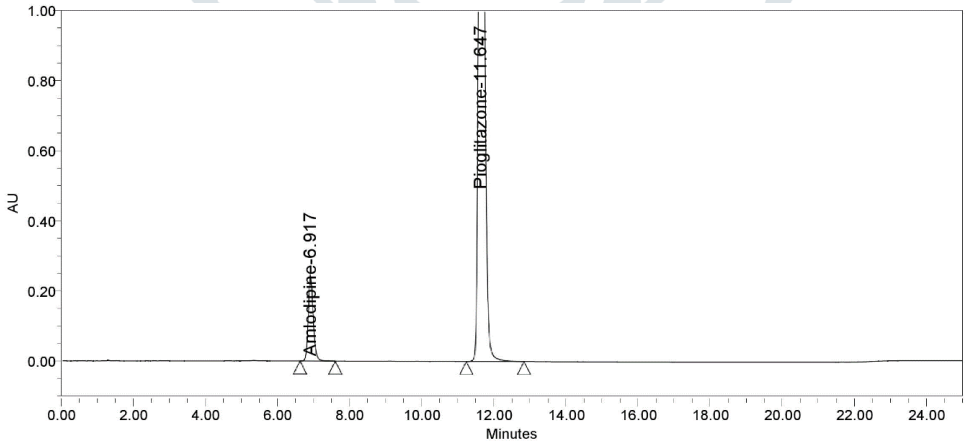

Fig 5 Chromatogram of Amlodipine Sample

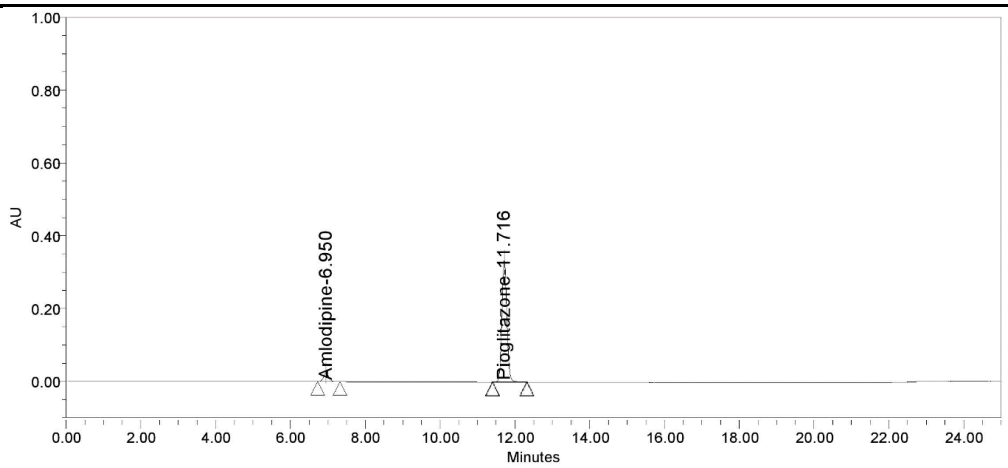

Fig 6 Chromatogram of Pioglitazone Sample

LINEARITY

Linearity was evaluated in the range of 50% to 150% of the working concentration level. As the working concentration level of Amlodipine is 20µg/mL and Pioglitazone is 40µg /mL. The range proposed is 50% to 150% of 20 mg/mL for Amlodipine and 50% to 150% of 40 mg/mL for Pioglitazone.

Table 4 System suitability for Amlodipine and Pioglitazone

	Amlodipine	**Pioglitazone**
Symmetry factor	**1.3**	**1.0**
Theoretical plates	**10057**	**16027**
S. No.	**Area**	**Area**
1	445802	39000987
2	440156	38740568
3	438625	38965811
4	439254	39012225
5	437681	38752666
6	438468	38669588
Mean	**439998**	**38856974**
%RSD	**0.7**	**0.4**

Table 5 : Linearity of Amlodipine

Level (%)	**Concentration (ppm)**	**Amlodipine Area**		
		Injection- 1	**Injection- 2**	**Mean**
50	10	219990	220624	2203
75	15	335962	336410	336186

100	20	440568	441257	440913
125	25	555814	556327	556071
150	30	659858	661207	660533
Co-relation coefficient (R)				**0.9993**
SLOPE				**21666.38**
Y-INTERCEPT				**885.8**
WORKING LEVEL AREA				**440913**
%LIMIT OF Y-INTERCEPT (± 2 OF WORKING LEVEL)				**0.20**

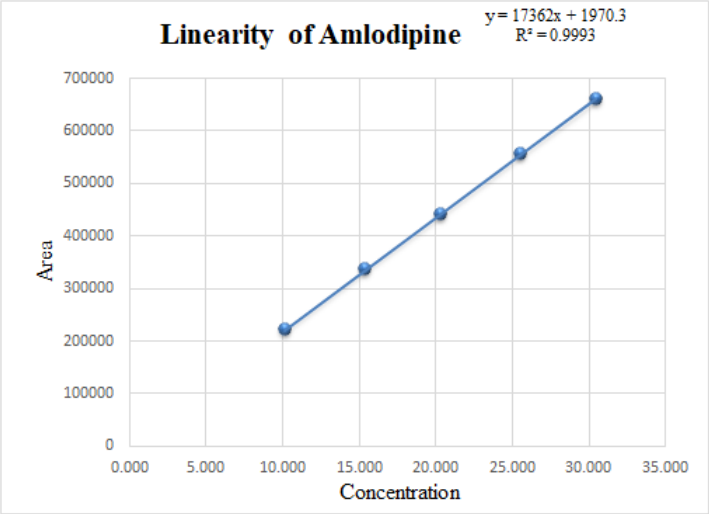

Fig 7 Linearity plot of Amlodipine

Table 6: Linearity of Pioglitazone

Level (%)	**Concentration (ppm)**	**Pioglitazone area**		
		Injection- 1	**Injection- 2**	**Mean**
50	20	19395489	19468475	19431982
75	30	29635414	29496880	29566147
100	40	38914752	38742899	38828826
125	50	49000528	48865045	48932787
150	60	58336945	58215624	58276285
Co-relation coefficient (R)				**0.999**
SLOPE				**963813.1**
Y-INTERCEPT				**29445.4**
WORKING LEVEL AREA				**38828826**
%LIMIT OF Y-INTERCEPT (± 2 OF WORKING LEVEL)				**0.08**

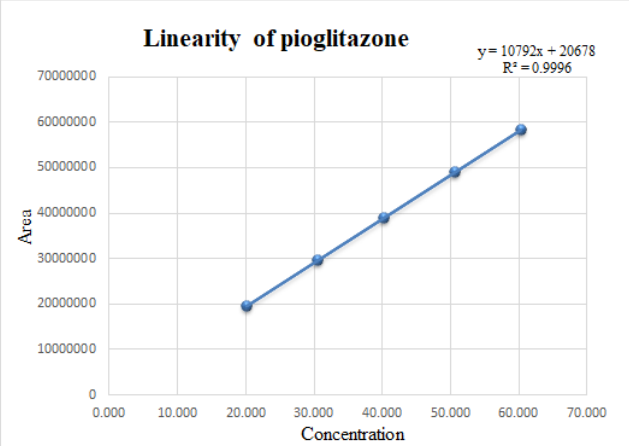

Fig 8 Linearity plot of Pioglitazone Accuracy (Recovery)

Accuracy was evaluated three levels 50%, 100% and 150% of the working concentration level for Amlodipine and Pioglitazone. As the working concentration level of Amlodipine is about 20 µg /mL, Pioglitazone is 40 µg /ml. Each level prepared in triplicates.

Table 7 System suitability Amlodipine and Pioglitazone

	Amlodipine	**Pioglitazone**
Symmetry factor	**1.2**	**1.0**
Theoretical plates	**11264**	**17094**
S. No.	**Area**	**Area**
1	442368	38667892
2	441023	38785200
3	439137	38510463
4	439999	38232230
5	441800	38323369
6	439752	38946205
Mean	**440680**	**38577560**
%RSD	**0.3**	**0.7**

Table 8 % Recovery for Amlodipine

Level (%)	**Injection-1**	**Injection-2**	**Average Peak Area**	**% Recovery**	**Mean recovery %**
50	219859	220638	220248	99.4	99.4
	221026	220462	220744	99.9	
	221364	221027	221195	100.3	
100	442356	442156	442256	100.1	100.1
	441580	442657	442118	100.1	

	439998	440625	440311	99.7	
150	662159	663245	662702	100.2	100.2
	659871	660962	660416	99.8	
	661574	661020	661297	100.0	
	Overall mean recovery				**99.9**

Table 9 % Recovery for Pioglitazone

Level (%)	**Injection-1**	**Injection-2**	**Average Peak Area**	**% Recovery**	**Mean recovery %**
50	19083657	19175300	19219479	99.0	99.0
	19252021	19190255	19221138	99.3	
	19008697	19076329	19042513	98.6	
100	38683888	38596387	38640138	100.0	100.0
	38523625	38475489	34499557	99.7	
	38495325	38456855	38476590	99.7	
150	58000684	57995882	57749772	100.1	100.1
	57706980	57792563	57749772	99.7	
	58023687	57942386	57983037	100.1	
	Overall mean recovery				**99.7**

Precision

System Precision

Single injection of Blank (Diluent) and six replicate injections of Standard solution were injected into the chromatographic system. The data obtained is summarized in Table 7.7

Table 10: System suitability for Amlodipine and Pioglitazone

	Amlodipine	**Pioglitazone**
Symmetry factor	**1.2**	**1.1**
Theoretical plates	**10268**	**16350**
S. No.	**Area**	**Area**
1	435697	38569528
2	425638	38266984

3	432156	38888276
4	440005	38374837
5	435126	38096895
6	432598	38452876
Mean	**433537**	**38441566**
%RSD	**1.1**	**0.7**

Method Precision

Single injection of blank (Diluent), Standard solution (six replicates) and sample solution (six preparations) was injected on the system.

Table 11: Method precision Amlodipine

Sample No.	**Mean**	**% Assay**
1	438563	100.7
2	439006	100.4
3	440238	101.1
4	436921	100.3
5	439520	100.7
6	440124	101.2
Mean		**100.7**
% RSD		**0.4**

Table 12 Method precision Pioglitazone

Sample No.	**Mean**	**% Assay**
1	38512895	100.4
2	38405684	100.3
3	38485715	100.3
4	38384004	100.2
5	38499981	100.4
6	38480014	100.3
Mean		**100.3**
% RSD		**0.1**

Intermediate Precision:

Six independent sample preparations were prepared on different day and by different analyst and injected on the HPLC.

Table 13: System suitability of Amlodipine and Pioglitazone

	Amlodipine	**Pioglitazone**
Symmetry factor	**1.2**	**1.0**
Theoretical plates	**11964**	**16574**
S. No.	**Area**	**Area**
1	443690	39238745
2	446660	39056058
3	451190	38754200
4	449080	38996995
5	452355	39200069
6	453946	39128740
Mean	**449487**	**39062468**
%RSD	**0.8**	**0.4**

Table 14 Intermediate precision for Amlodipine

Sample No.	**Mean**	**% Assay**
1	452555	99.8
2	453206	99.5
3	454287	100.5
4	453335	99.8
5	452930	99.9
6	455637	100.7
Mean		**100.0**
% RSD		**0.5**

Table 15 Intermediate precision for Pioglitazone

Sample No.	Mean	% Assay
1	39415605	100.8
2	39480058	100.9
3	39520666	101.0
4	39556047	100.9
5	39374198	100.7
6	39504924	100.9
Mean		**100.9**
% RSD		**0.1**

Robustness:

This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.

Table 16 Robustness for Amlodipine

Changes in parameters	Values	Retention Time	% Assay	Absolute difference
Control	As per method	7.0	99.8	NA
Flow rate (± 0.1 mL/min)	+0.1 mL/min	7.2	100.1	-0.3
	-0.1 mL/min	6.7	100.0	-0.2
Change in Wavelength (± 5 nm)	+5 nm	6.9	99.3	0.5
	-5 nm	6.9	100.1	-0.3
Change in Column temp. (± 5°C)	+5°C	6.8	100.1	-0.3
	-5°C	7.1	100.2	-0.4

Table 17 Robustness for Pioglitazone

Changes in parameters	Values	Retention Time	% Assay	Absolute difference
Control	As per method	11.7	100.3	NA
Flow rate (± 0.1 mL/min)	+0.1 mL/min	11.5	100.4	0.1
	-0.1 mL/min	11.9	100.4	0.1
Change in Wavelength (± 5 nm)	+5 nm	11.6	99.2	-1.1
	-5 nm	11.7	100.1	-0.2
Change in Column temperature (± 5°C)	+5°C	11.6	100.0	-0.3
	-5°C	11.9	100.2	-0.1

Conclusion:

The High Performance Liquid Chromatographic method developed used for simultaneous determination of Amlodipine Besylate and Pioglitazone Hydrochloride drugs in the pharmaceutical formulations using standard was stability indicating as recommended by ICH guidelines and validated for Specificity, System precision, Method precision, Ruggedness, Robustness and Accuracy. The extent of the current work is to develop RP-HPLC strategy for the assessment of medication in mass. The technique was totally approved and showed good outcomes. Maintenance time and runtime was diminished, so the created technique can be utilized for synchronous determination of Amlodipine Besylate and Pioglitazone Hydrochloride.

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