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Research Article

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Development and validation of analytical method for simultaneous estimation of Azilsartan medoxomil and Amlodipine besylate in synthetic mixture

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Abstract A simple, rapid, accurate and precise RP-HPLC method was developed and validated for simultaneous estimation of Azilsartan medoxomil and Amlodipine besylate in synthetic mixture. The chromatographic separation was carried out on Hypersil ODS C18 column (250×4.6 mm; 5µ) with mixture of 0.2% ortho-phosporic acid in water :acetonitrile (35:65% v/v) as a mobile phase; at a flow rate of 1.0 mL/min. UV detection was performed at 232 nm. The retention time was found to be 5.496 min and 3.255 min for Azilsartan medoxomil and Amlodipine besylate, respectively. Calibration plots were linear over the concentration range of 80.3-120.5 µg/mL and 20.2-30.2 µg/mL of both Azilsartan medoxomil and Amlodipine besylate, respectively. The method was validated for linearity, accuracy, precision, recovery, repeatability, LOD and LOQ. The proposed method was successfully used for quantitative analysis of Azilsartan medoxomil and Amlodipine besylate used for hypertension. No interference from the components of pharmaceutical dosage form was observed. Good percentage recovery and low relative standard deviation confirm the suitability of the proposed method for routine estimation of Azilsartan medoxomiland Amlodipine besylatein tablet dosage form.

Keywords RP-HPLC, Azilsartan medoxomil, Amlodipine besylate

Introduction

Azilsartan medoxomil (AZL) belongs to the category of angiotensin II antagonist. AZL Chemically (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 2-ethoxy-1-([2'-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]methyl)-1H-benzimidazole-7-carboxylate (Figure-1). AZL is an angiotensin II receptor blocker (ARB) that lowers blood pressure by blocking the action of angiotensin II, a vasopressor hormone. AZL is used to treat high blood pressure helps prevent stokes, heart attacks and kidney problems. AZL belongs to a class of drugs called angiotensin receptor blocker. It works by relaxing blood vessels so that blood can flow more easily. Amlodipine besylate is belongs to category dihyphridine calcium antagonist. Chemically 3-ethyl-5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5dicarboxylateb monobenzenesulfonate (Figure-2). It is a dihyphridine calcium antagonist that inhibits the transmembrane influx of calcium ions in to vascular smooth muscle. It also prevents the gliazone induced weight gain without interfering with its insulin- sensitizing properties.

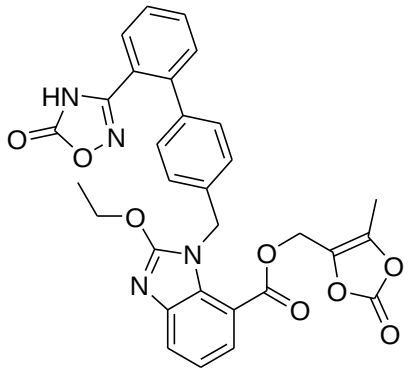

Figure 1: *Chemical structure of Azilsartan medoxomil*

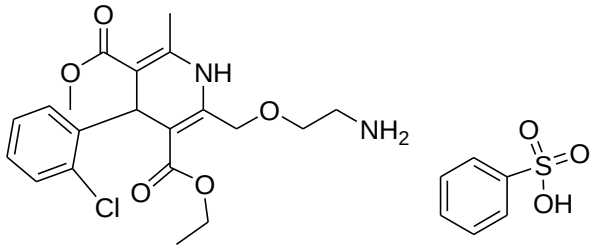

Figure 2: *Chemical structure of Amlodipine besylate*

Aims and Objectives

Various methods have been given for determination of Azilsartan [1-2] and its combination with other drugs [3-5]. Methods have also been developed for determination of Amlodipine alone [8-9] and in combination with other drugs [10-14]. But no method has been developed for the determination of Azilsartan and Amlopdipine in combination. The following method has been developed method.

Materials and Methods

Chromatographic separation was performed with Shimadzu high performance liquid chromatography having C18, ODS (250mm×4.6mm),5µm analytical column with photodiode array detector. Chromatographic data were recorded by LC Solution software.

Standard preparation of Azilsartan medoxomil

10 mg of AZL was weighed and transferred to 2 mL of volumetric flask, dissolved and volume was made up with diluent (water: acetonitrile (50:50%v/v)).

Standard preparation of Amlodipine besylate

Fourty mg of AML was weighed and transferred to 20mL of Volumetric flask, dissolved and volume made with diluent (water: acetonitrile (50:50%v/v)).

Sample preparation

Accurately transferred 5 tablets into 100 mL volumetric flask, added 60 mL of diluent into it, sonicated for 30 minutes with intermittent shaking, cooled to attain room temperature and made up to volume with diluent and mixed well. It was filtered through 0.45 µg/mL syringe filter. 5 mL of obtained filtrate was further diluted to 50 mL with diluent, mixed well and it was injected.

Table 1: Optimized Conditions

Column	C18 ODS (250x4.6mm, 5µ)
Mobile Phase	0.2%OPA in Water:Acetonitrile (35:65%v/v)
Flow rate	1.0mL/min
Column Temperature	Ambient
Detection	232
Injection vol.	10µl
Runtime	10 min
Diluent	Water:Acetonitrile (40:60%v/v)

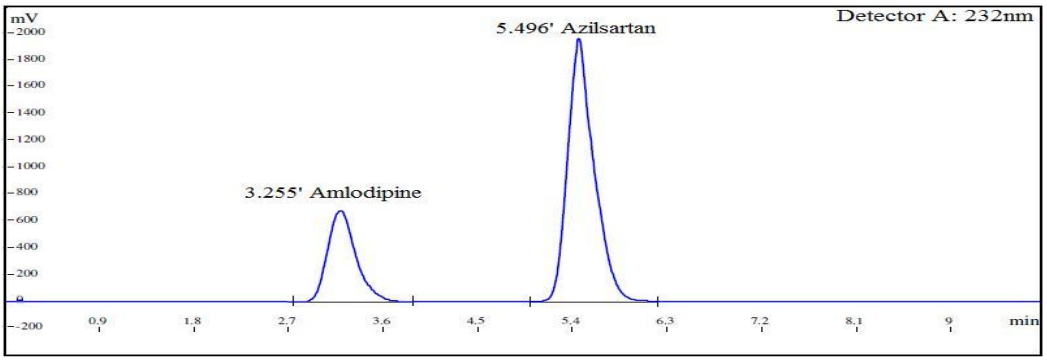

Figure 3: *Typical chromatogram for AML and AZL*

Chromatographic conditions: For HPLC a number of preliminary trials were conducted with combinations of different organic solvents, compositions, and flow rate to check the retention time, shape, resolution, and other chromatographic parameters. Among all tried experiments, the mobile phase combination of Mobile phase is 0.2%. OPA in water: acetonitrile (35:65%v/v). The instrumental settings area flow rate of 1.0mL/min; the column temperature is ambient, and detector wavelength is 232 nm found to be most suitable. Best resolution and sensitivity of the method were obtained for AZL and AML. Typical chromatogram with optimized condition gives sharp and symmetric peak with retention time of 10 min.

Results and Discussion

System suitability

The standard solution was analyzed six times as per chromatographic conditions and injected the start of study and acceptance criteria area as follows:

Table 2: Acceptance criteria of system suitability

Name of compound	Mean peak area (cm^{-1})	%RSD peak Area	Mean theoretical Plates of peak	Mean tailing factor of peak
Azilsartan medoxomil	6276275	0.3	12558	0
Amlodipine besylate	2535865	0.2	6292	-
Limit	N	NMT2.0	NLT2000	NMT2.0

Linearity

Azilsartan medoxomi and Amlodipine besylate

The linearity was determined at five levels the range of 80 % to120 % of the sample concentration. The graph of mean area versus concentration in µg/mL was plotted and regression equation was determined.

Table 3: Linearity study for Azilsartan medoxomil

S. No.	Concentration (µg/mL)	Mean Peak Area (cm^{-1})
1	80.3	4913265
2	90.4	5578036
3	90.4	6318345
4	110.5	6917123
5	120.5	7551962
Slope		65884.794
Y-intercept		360734.516
R^2		0.999

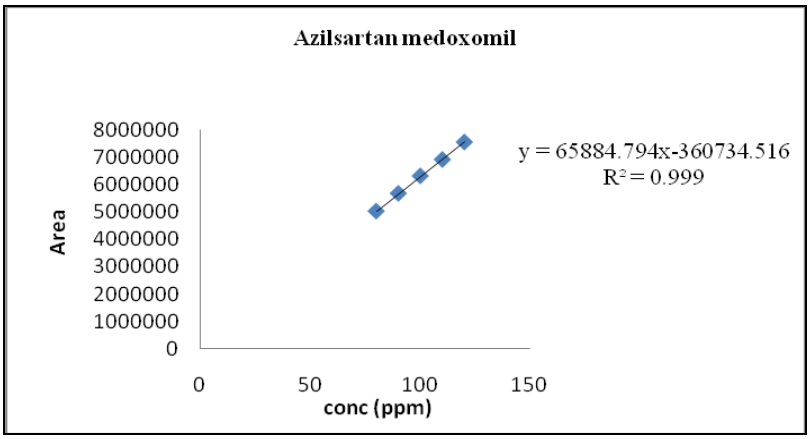

Figure 4: *Linear graph of Azilsartan medoxomil*

Table 4: Linearity study for Amlodipine besylate

S. No.	Concentration (µg/mL)	Mean Peak Area (cm^{-1})
1	20.2	1981155
2	22.7	2249208
3	25.2	2547720
4	27.7	2789162
5	30.2	3045146
Slope		105870.463
Y-intercept		145457.467
R^2		0.999

Acceptance criteria

The correlation coefficient should not be less than 0.999.

% RSD of response factor should not be more than 2.

Result

The correlation coefficient was found to be well within limits.

% RSD of response factor was found to be well within limits.

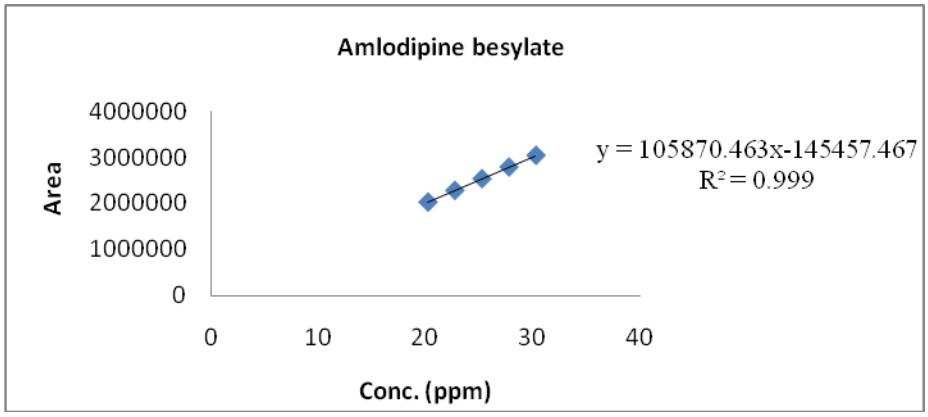

Figure 5*: Linear graph of Amlodipine besylate*

LOD & LOQ

Table 5: LOD and LOQ for Azilsartan medoxomil and Amlodipine besylate

Parameter	**AZL**	**AML**
LOD (µg/mL)	1.23	1.03
LOQ (µg/mL)	3.75	3.15

Acceptance Criteria

- LOD S/N > 2 or 3
- LOQ S/N > 10

Results

LOD & LOQ was found to be well within limits.

Accuracy

The accuracy of method was checked by recovery of Azilsartan medoxomil and Amlodipine besylate tablet from 3 placebo preparation accurately spiked with three concentration of active ingredient. There result is reported in table. Results indicate that there no significant difference between the calculated percentage recovery and actual percentage value.

Table 6: Accuracy study for Azilsartan medoxomil

Accuracy Level (%)	Set No	Amount recovered (mg)	Amount added (mg)	%Recovery	Mean	%RSD
80 %	1	81.61	80.28	101.7	100.9	0.7
	2	80.41	79.78	100.8		
	3	81.16	80.98	100.2		
100 %	1	101.91	100.35	101.6	100.7	0.8
	2	101.55	101.35	100.2		
	3	99.70	99.55	100.2		
120%	1	121.85	120.42	101.2	100.8	0.7
	2	120.19	120.22	100.0		
	3	121.85	120.52	101.1		

Table 7: Accuracy data for Amlodipine besylate

Accuracy Level (%)	Set No	Amount recovered (mg)	Amount added (mg)	% Recovery	Mean	% RSD
80 %	1	20.29	20.32	99.9	99.9	0.6
	2	20.42	20.57	99.3		
	3	20.17	20.07	100.5		
100 %	1	25.58	25.53	100.2	99.9	0.4
	2	25.24	25.40	99.4		
	3	25.28	25.27	100.0		
120%	1	30.72	30.71	100.0	100.1	0.3
	2	30.87	30.73	100.4		
	3	30.66	30.68	99.9		

Acceptance criteria

- Recovery of Drugs should be between 97.0 % and 103.0%.
- % Relative standard deviation for recovery at each level should not be more than 3.00.
- Overall %relative standard deviation for all the levels should not be more than 3.00.

Results

- Recovery of Drugs for all the levels was found to be within the limit.
- % Relative standard deviation for %recovery at each level and overall %relative standard deviation for all the levels was found to be within the limit.
- Results indicated that calculated percentage recovery was found well within the acceptance criteria.

Precision

The precision for the developed method was determined in terms of Repeatability and intermediate precision. For repeatability evaluation, a standard solution of fixed concentration was injected at same time intervals on the same day, In addition, the intermediate precision was studied by injecting the same concentration of standard solution on consecutive days.

Table 8: Repeatability and Intermediate precision study for the Azilsartan medoxomil & Amlodipine besylate

S. No.	Repeatability (Area) (cm^{-1})		Intermediate (Area) (cm^{-1})	
	AZL	AML	AZL	AML
1.	6220527	2510663	6218158	2496003
2.	6263804	2523192	6248544	2494318
3.	6251030	2504320	6206436	2477320
4.	6286099	2519486	6224834	2490634
5.	6271550	2511913	6187010	2492139
6.	6231357	2513582	6282362	2484595
% Assay	100.1	99.9	100.4	99.4
% RSD	0.4	0.3	0.5	0.3

Acceptance criteria

% RSD of response factor should not be more than 2.

Result

% RSD of response factor was found to be well within limit.

Assay

10 µl of each standard and sample solution were injected and from the peak area of AZL and AML, amount of each drug in samples were computed. The results of the assay **Table 9** undertaken, yielded 99.5% and 99.8% of label claim of AZL and AML, respectively.

Table 9: Assay study of Azilsartan medoxomil and Amlodipine besylate

Label claim		**% Assay**	
AZL	AML	AZL	AML
5 mg	20 mg	99.5 %	99.7 %

Conclusion

The proposed HPLC method was sufficiently sensitive and reproducible for the analysis of Azilsartan medoxomil, Amlodipine besylatesynthetic mixture within a short analysis time. The method was proved to be superior to most of the reported methods. The mobile phases was simple to prepare and economical. The sample recoveries in the formulation were in good agreement with the irrespective label claim and they suggested non-interference of formulation excipients in the estimation. Hence the proposed method was found to be rapid, accurate, precise, specific, robust and economical.

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