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Quantitative Estimation and Validation of Chlorthlidone and Azilsartan Medoximil in Bulk and Tablet Dosage Form by using RP-HPLC

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ABSTRACT

The first reversed phase high performance liquid chromatographic method for Stability Indicating of, Azilsartan and chlorthalidone has been developed and validated to be a simple, sensitive, rapid, specific, precise, and accurate method. Chromatographic separation was achieved on Zorbax XBD-C8, 250mm × 4.6mm,5µm.Buffer pH5.5 : Methanol (60:40) as a mobile phase at flow rate of 1 ml/min. UV detection was operated at 234 nm and injection volum was 25 µl.. The proposed method showed good linearity, accuracy, precision and was successfully applied for determination of the drugs in laboratory prepared pharmaceutical dosage forms.

Keywords: Azilsartan and chlorthalidone, RP-HPLC, Stability Indicating.

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INTRODUCTION:

Azilsartan Medoximil[1] :

Azilsartan is used in the treatment of hypertention. It is a angiotensin II receptor antagonist. Its mechanism of action is blocking the angiotensin receptor by vasopressor harmone that stops vasoconstriction and thus decreases the blood pressure. Its IUPAC name is (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl2-ethoxy-1-([2'-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3- yl)biphenyl-4-yl]methyl)-1H-benzimidazole-7-carboxylate and molecular formula $C_{30}H_{23}KN_4O_8$.Azilsartan was practicaly insoluble in water but soluble in DMSO and methanol. Pka of the drug was
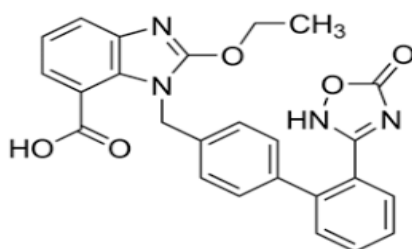

Fig. 1 Structure of Azilsartan Medoxomil

Pharmacokinetics: Azilsartan medoxomil is quickly absorbed from the gut, independently of food intake. Maximal blood plasma concentrations are reached after one to three hours. The liver enzyme CYP2C9 is involved in the formation of the two main metabolites, which are pharmacologically inactive; they are the *O*-deethylationand decarboxylation products of azilsartan.

Adverse Drug Reaction: nausea,diarrhea,fatigue,cough.

Chlorthlidone :

. Chlorthalidone is used in the treatment of hypertension, it is a thiazide diuretic drug which inhibits Na^+ and Cl^- ions re-absorption in the distal convoluted tubule by blocking the Na^+ /Cl^-Symporter. IUPAC name was (*RS*)-2-Chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl)benzene-1-sulfonamide with molecular formula $C_{14}H_{11}ClN_2O_4S$. Chlorthalidone was soluble in Methanol, water and DMSO. Pka found was 8.76. According to literature two methods were available in which madhu et al., the retention time for Chlortalidone and AzilsartanMedoxomil were 7min and 11 min respectively. Naazneen et al., the retention time for Chlortalidone and AzilsartanMedoxomil were 2.36±0.1 mins and 5.54±0.5 minsrespectively.
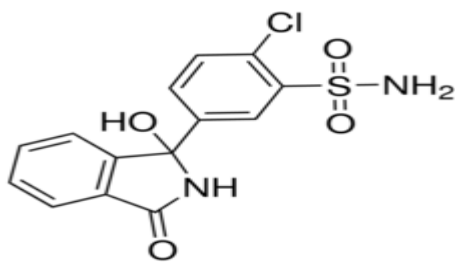
Fig. 2 Structure of Chlorthalidone

Pharmacokinetics: Chlortalidone is slowly absorbed from the gastrointestinal tract after oral ingestion. It has a long half-life and therefore a prolonged diuretic action, which results in continued diuretic effects despite a skipped dose. This prolonged action of chlortalidone despite missing doses may account for the higher efficacy of chlortalidone compared to the shorter half-life medication, hydrochlorothiazide. Chlortalidone is eliminated from the body mostly by the kidney, as unchanged drug.[2]

Adverse   Drug
Reaction:Hyperuricemia,Hyperglycemia,Hyperlipidemia

MATERIAL:

DRUG:

Table 1: Drug and drug product samples suppliers and manufacturers

Name of drug and drug product	Supplier and manufacturer by
Chlorthalidone	Amoli organics pvt ltd,mumbai
Azilsartan Medoxomil	Honour lab limted hydrabad
Chlorthalidone and Azilsartan Medoxomil Tablet	Ipca pharmaceutical pvt ltd,Gujrat

REAGENTS:

Table 2: List of Reagent[3]

Sr.No	Chemical	Make
1	Water	Rankem
2	Acetonitrile	Merck life science
3	Phosphoric acid 88%	Merck life science
4	Potassium dihydrogen phosphate	Merck life science
5	Sodium hydroxide	Merck life science
6	Triethylamine	Merck life science
7	0.45 µ Nylon membrane disc filter	Mdi
8	0.45µ PVDF Syringe Filter	Mdi

INSTRUMENTS:

Table No 3: HPLC

Make	Waters e2695
Pump	Reciprocating Water-510
Detector	Waters 2695 PDA
Software	Empower PRO
Column	X-Bridge

SPECTROPHOTOMETER: Double beam UV-visible spectrophotometer with 10mm Matched quartz cells

Model	UV1700
Make	Thermo scientific

ANALYTICAL BALANCE: Digital Analytical balance

Model	XS205D0
Make	Mettler Toledo

PH METER: Digital pH Meter

Make	Thermo Scientific
Model	Orian Star A211

METHOD

UV SPECTROSCOPIC2 SELECTION OF WAVELENGTH

Preparation of Chlorthalidone Standard solution: An accurately weighed quantity about 15 mg of Chlorthalidone 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.

Preparation of Azilsartan Medoxomil Standard solution: An accurately weighed quantity about 20 mg of Azilsartan Medoxomil standard was transferred to 100 mL volumetric flask. Add 70 mL of diluent, sonicate to dissolve and dilute up to the mark with diluent and mixed.

Preparation of Chlorthalidone Standard stock solution: Weigh accurately about 50 mg of Chlorthalidone and transfer it into 50 mL amber colored volumetric flask. Add about 30 mL of diluent, sonicate to dissolve and make up to mark with diluent (1.0mg/mL).

Preparation of Azilsartan Medoxomil Standard stock solution: Weigh accurately about 80 mg of Azilsartan Medoxomil working standard and transfer it into 50 mL amber colored volumetric flask. Add about 30 mL of diluent, sonicate to dissolve and make up to mark with diluent (1.6 mg/mL ppm).

Further dilute 5 mL each of Chlorthalidone stock solution and Azilsartan Medoxomil Standard stock solution to 50 mL with diluent. (0.10 mg/mL Chlorthalidone and 0.16 mg/mL of Azilsartan Medoxomil).

Note: Prepare standard solution in duplicate as 1st standard solution and 2nd standard solution.

Preparation of Sample solution:

Chlorthalidone and Azilsartan Medoxomil) stock solution:

Weigh and transfer 10 tablets into 1000 mL amber colored volumetric flask. Add about 100 mL of water and sonicate to disperse the tablets then add 600 mL of diluent, sonicate for about 60 minutes along with intermittent shaking for complete disintegration of tablets. Allow it to cool and make up to volume with diluent. Centrifuge the solution for about 10 minutes at 3000 rpm. Filter through 0.45µ Nylon membrane syringe filter. Inject stock solution for Chlorthalidone (0.1 mg/mL of Amlodipine).Further dilute 5 mL of sample stock solution to 50 mL with diluent (0.16 mg/mL of Azilsartan Medoxomil).[2]

Selection of Wavelength:
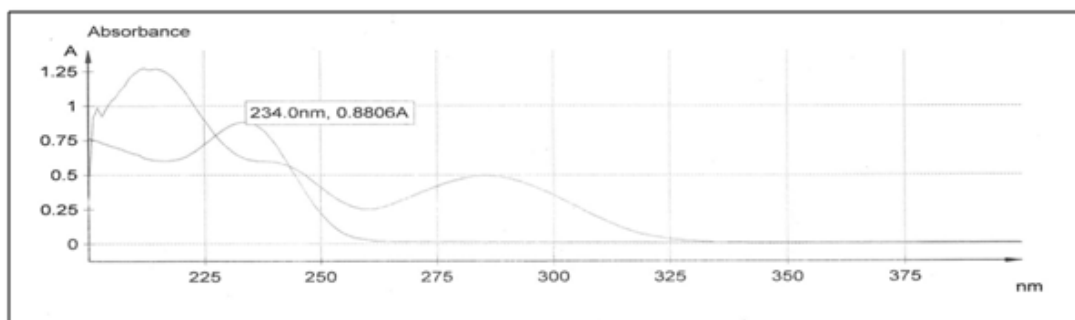
Fig 3 Spectra showing λ max of Chlorthalidone & Azilsartan Medoxomil

Optimized Chromatographic Condition:

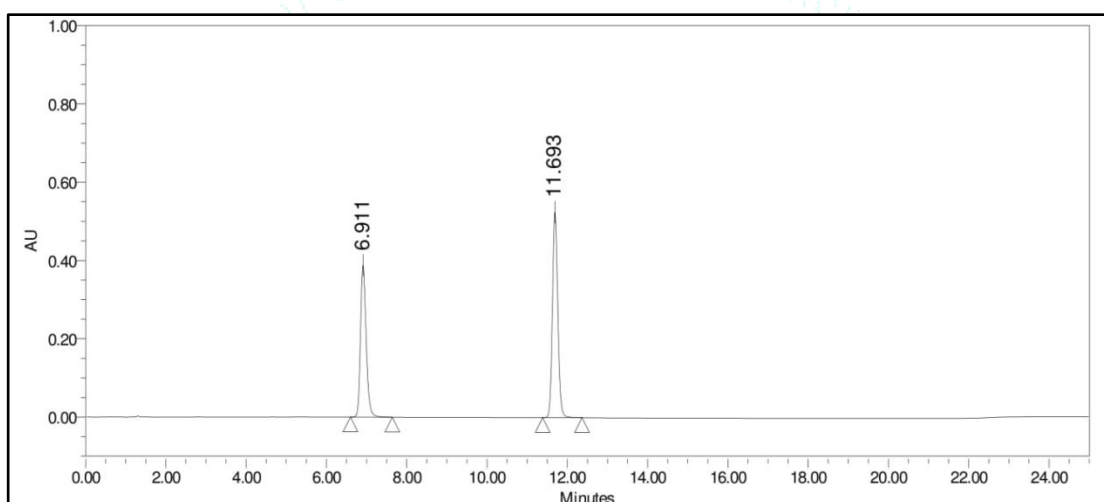| Column	:	Zorbax XBD-C8, 250 mm x 4.6 mm, 5µm or equivalent Part No.990967-906
Mobile Phase		Buffer pH5.5::methanol (60:40/v)
Flow Rate	:	1.0 mL/min
Injection Volume	:	25 µL
Wavelength	:	234 nm

Fig. 4 Typical Optimised chromatogram Azilsartan Medoxomil, Chlorthalidone

Result and Discussion –

Table 3: Result of linearity.

Conc.of Chlorthalidone HCL(conc in ppm)	Area	Conc. of AzilsartanMedoxomil. (conc in ppm)	Area
50.56	1245690	80.010	1593708
76.8	1875783	121.615	2419267
101.12	2470595	160.020	3184787
127.41	3200029	201.625	4069226
151.68	3819248	240.030	4857134

y = 25594x - 75875
R² = 0.9993

y = 20439x - 58975
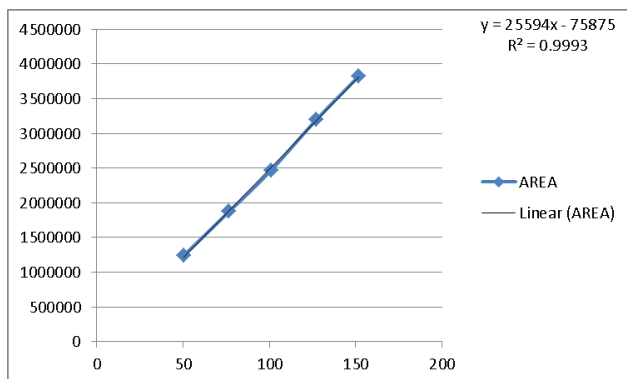R² = 0.9998

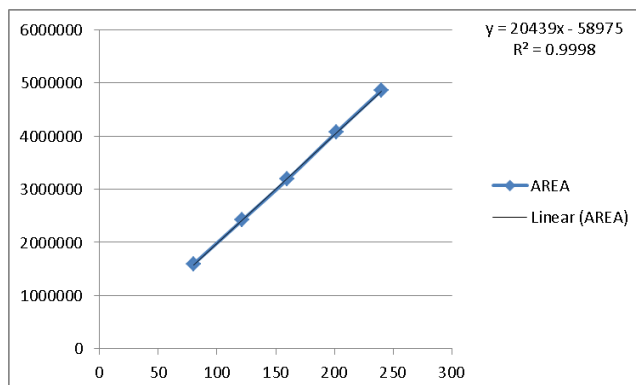**Fig.No. 5.Linearity graph for Chlorthalidone HCL.**   **Fig.No 6.Linearity graph for AzilsartanMedoxomil.**

Table 4: % Recovery data for Chlorthalidone HCL and AzilsartanMedoxomil

Drug	% Composition	% Recovery	% mean RSD
Chlorthalidone HCL	50	100.4	99.60
	100	98.01	
	150	100.02	
Azilsartan Medoxomil.	50	100.00	99.90
	100	100.5	
	150	99.01	

Robustness:

Table 5 Robustness for Chlorthalidone

Changes in parameters	Values	Retention Time of Chlorthalidonepeak	Symmetry Factor	Theoretical plates	% RSD of standard area	% Assay	Absolute difference
Control	As per method	6.757	1.17	10536	0.41	100.7	-
Flow rate (± 0.1 mL/min)	+0.1 mL/min	6.030	1.17	8824	0.65	99.9	0.6
	-0.1 mL/min	7.304	1.18	12176	0.12	100.0	0.7
Change in Wavelength (± 5 nm)	+5 nm	6.880	1.21	11758	0.20	100.3	0.4
	-5 nm	6.880	1.22	11770	0.21	100.6	0.1
Change in Column temperature (± 5°C)	+5°C	6.147	1.17	9259	0.12	98.9	1.8
	-5°C	6.404	1.18	9809	0.46	99.8	0.9

Table 6 Robustness for Azilsartan Medoxomil

Changes in parameters	Values	Retention Time of AzilsartanMedoxomil peak	Symmetry Factor	Theoretical plates	% RSD of standard area	% Assay	Absolute difference
Control	As per method	11.801	1.08	36432	0.32	100.9	-
Flow rate (± 0.1 mL/min)	+0.1 mL/min	10.997	1.08	33021	0.29	100.4	0.5
	-0.1 mL/min	12.433	1.09	40650	0.04	101.6	0.7
Change in Wavelength (± 5 nm)	+5 nm	11.638	1.11	37461	0.13	100.9	0.0
	-5 nm	11.638	1.11	37491	0.18	100.4	0.5
Change in Column temperature (± 5°C)	+5°C	11.195	1.08	35529	0.09	100.0	0.9
	-5°C	11.473	1.09	36018	0.37	101.6	0.3

CONCLUSION

The proposed simultaneous eatimation and validation method was found to be simple, precise, accurate and rapid for the determination of Chlorthalidone and AzilsartanMedoxomil.The coefficient of correlation was obtained in acceptable range .The percentage recovery obtained in acceptable range .variation in flow rate, wavelength, does not have any effect on the % RSD of standard and assay value.There lative standard deviation of main peak area ,tailing factor and therotical plate is well within the acceptable range . Hence the precision of given method is confirmed. Thus from the above result of the individual method is conclude that the analytical method is validated and found to be satisfactory.

ACKNOWLEDGMENT

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