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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF 2-CHLOROMETHYL-1H BENZIMIDAZOLE IMPURITY IN AZILSARTAN MEDOXOMIL BULK AND FORMULATION

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ABSTRACT

The process related impurity of Azilsartan Medoxomil i.e: 2-Chloromethyl-1 H benzimidazole was synthesized**,** characterized and quantified in bulk and formulation. The synthesis of intermediate was carried out by benzimidazole synthesis process using O-phenylenediamine, chloroaceticacid, in presence of 5N HCl. The percentage yield was found to be 85%. Recrystallization and purification of impurity was done. The preliminary evaluation was done on laboratory scale via melting point, elemental analysis and TLC. The melting point of impurity was found to be 153-155^{0}C. The TLC of impurity was carried by using Chloroform: Methanol (9:1) and the R_f was found to be 0.68. The process impurity was synthesized, purified and characterized by IR, NMR and UV method was developed for quantification of synthesized impurity. The method was validated as per ICH Q2B guidelines. The UV method was found to be linear, precise, accurate, robust and rugged. Finally 2-Chloromethyl-1H-Benzimidazole impurity was quantified from Azilsartan

medoxomil bulk and its marketed tablet formulation.

KEYWORDS: Validation, Azilsartan Medoxomil, Impurity.

1. INTRODUCTION

Azilsartan medoxomil is (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. It is an angiotensin-II receptor antagonist has been widely used for the treatment of hypertension. Its empirical formula is $C_{25}H_{20}N_4O_4$ and having the molecular weight 456.46 gm.[1]

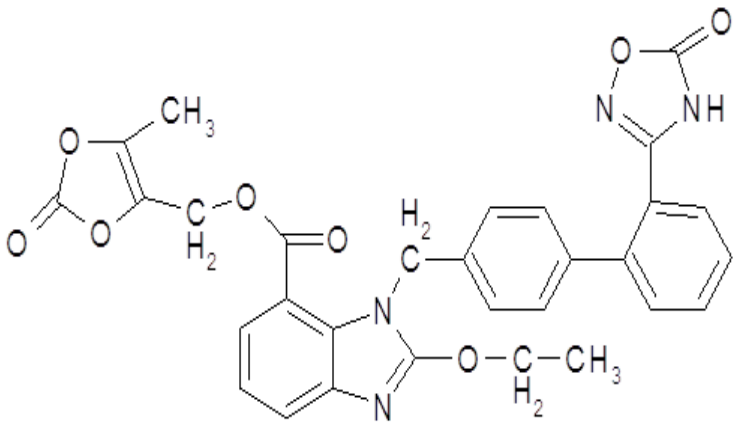

Figure 1: Chemical structure of Azilsartan Medoxomil.

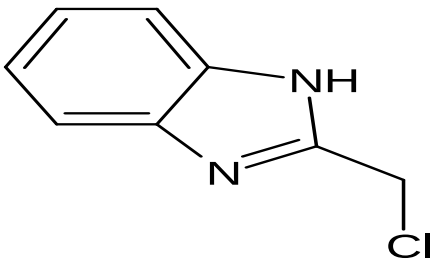

Figure 2: Chemical structure of Azilsartan Medoxomil Impurity.

ICH defines impurities profile of a drug materials is, "A description of the identified and unidentified impurities present in a new drug substance." For Pharmaceutical products, impurities are defined as, "substance in the product that are not the API itself or the excipient used to manufacture it" i.e. impurities are unwanted chemical that remains within the formulation or API in small amounts which can influence Quality, Safety and Efficacy, thereby causing serious health hazards.[2,3] An organic impurity within the manufacturing process along with a good control strategy is an integral part of the quality control of drug substance.

2. MATERIALS AND METHODS

O-phenylenediamine (AR), Chloroacetic acid (AR), Hcl, Methanol of AR grade was purchased from merck fine chemicals (Mumbai, India) Azilsartan medoxomil drug was obtained from Hetero labs ltd Hyderabad, India. Azilsartan medoxomil tablets were procured from pharmacy.

3. INSTRUMENTS

3.1 UV-Visible Spectrophotometer

The wavelength of Azilsartan medoxomil impurity was found to be 242 nm by using UV-Vis Spectrophotometer (UV-1650 PC) SHIMADZU INC.

3.2 FT-IR

The IR spectra were recorded by using Fourier Transform Infrared Spectrophotometer Model No. 8400S SHIMADZU by KBr press pellet technique.

3.3 NMR

Characterization of impurities was achieved by using Varian NMR Mercury 300 MHz spectrometer, using DMSO as a solvent and TMS as an internal reference standard for the proton experiment. All experiments were conducted at 25°C and no shift relaxation agents were employed. The ^{1}H and ^{13}C NMR chemical shift values were reported on the δ scale in ppm.

4. SYNTHESIS OF AZILSARTAN MEDOXOMIL IMPURITY

In a 250 ml three necked flask a solution containing 7.5g (0.08 mole) of chloroacetic acid and 7.5g (0.07 moles) of 0-phenylenediamine dissolved in 60 ml of 5N HCl. The mixture was heated for 7.5 to 8 hrs with constant stirring. The reaction mixture is cooled to about $5^{0}C$. It was neutralized with aq. ammonium hydroxide or dilute NaOH. The product was filtered and washed with water to remove traces of hydrochloric acid and dried. It was re-crystallized from benzene: hexane.

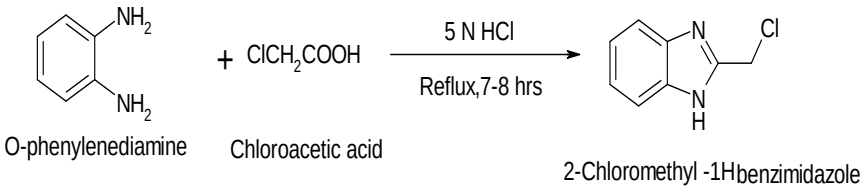

Figure 3: Synthesis Scheme of Azilsartan medoxomil impurity.

5. RESULTS

5.1 Physicochemical Properties

Table. 1.

Parameter	Result
Molecular Formula	$C_8H_7ClN_2$
Molecular Weight	166.607
Melting Point	153-155°C
R_f Value	0.68
% Yield	85%

5.2 Thin Layer Chromatography (TLC)

Mobile phase Chloroform: Methanol (9:1 v/v)

R_f Value = 0.68.

5.3 IR Data[4,5]

Table 2.

Sr.No.	v (cm^{-1})	Functional group assignment
1.	3369	–NH stretching.
2.	3063	Aromatic –CH stretching.
3.	2939	Aliphatic –CH stretching
4.	1345	C-N- stretching.
5.	780	C-Cl stretching.

5.4.1 ^{1}H NMR

Table 3.

Sr. No.	Chemical shift (δ ppm)	No. of protons	Type of peak	Assignment of peak
1.	9.18	1	s	-NH of benzimidazole.
2.	7.16-7.61	4	m	Aromatic Protons.
3.	4.98	2	s	Protons of CH_2 linkage.
4.	2.6	1	s	DMSO peak.

5.4.2 ^{13}C NMR

Table 4.

Sr.No.	δ(ppm)	Carbon Assignment
1.	151.2	1C of Imidazole
2.	114.8- 139.39	6C of Phenyl
3.	38.2	1 C of methyl

5.5 UV Method Development[4,9]

The λmax of AZI in methanol was found to be 242 nm (1) n-π^* transitions.

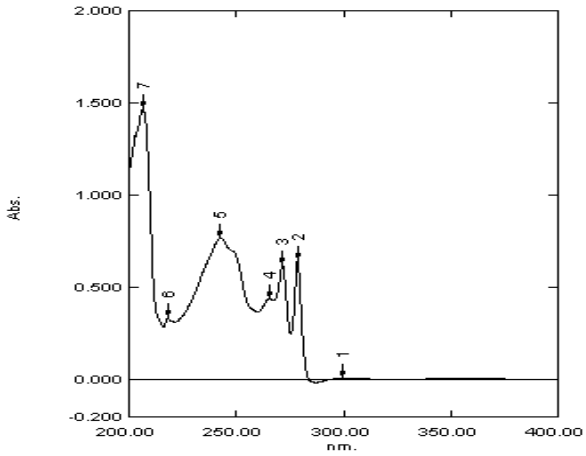

Figure 4: UV spectra of AZI.

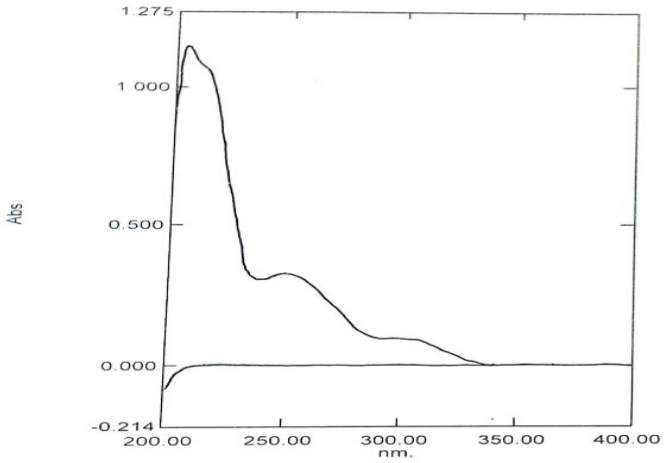

Figure 5: UV spectra of Azilsartan medoxomil.

Table 5: Linearity.

Sr. No	Concentration (ppm)	Absorbance
1	2	0.0768
2	4	0.1536
3	6	0.2604
4	8	0.3172
5	10	0.3840
6	12	0.4592
7	14	0.5376
8	16	0.6041
9	18	0.6912
10	20	0.768

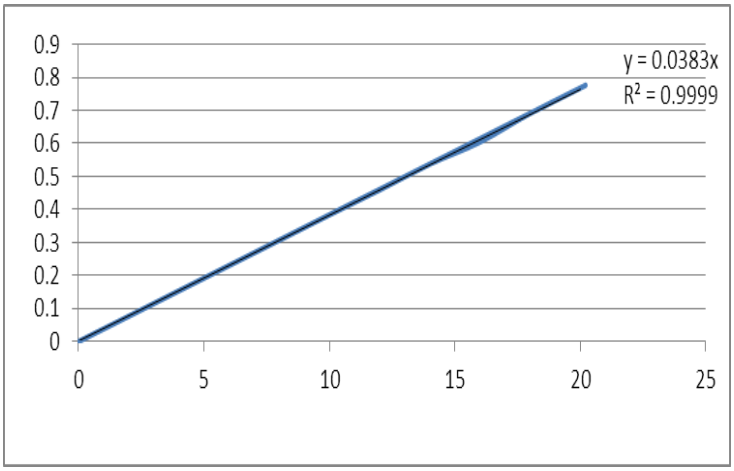

Figure 6: Calibration curve.

Table 6: Intra-day Precision.

Sr. No	Concentration (ppm)	Absorbance	SD	RSD
1	6	0.2834	0.00825	0.2802
2	6	0.2904		
3	6	0.2890		
4	6	0.2911		
5	6	0.3012		
6	6	0.2990		
7	6	0.3072		

Table 7: Inter-day Precision.

Sr. No	Concentration (ppm)	Absorbance	SD	RSD
1	6	0.3062	0.00736	0.02386
2	6	0.3140		
3	6	0.3070		
4	6	0.3151		
5	6	0.3057		
6	6	0.2951		
6	6	0.3160		

Table 8: Ruggedness.

Sr. No	Concentration (ppm)	Analyst I	Analyst II	SD I	SD II	%RSD I	%RSD II
1	6	0.2976	0.2960	0.003645	0.001632	1.22	0.55
2	6	0.2920	0.2959				
3	6	0.2951	0.2963				
4	6	0.2960	0.2961				
5	6	0.3020	0.2961				
6	6	0.3010	0.2962				
7	6	0.2945	0.2960				

Table 9: Robustness.

Sr. No	Concentration (ppm)	Absorbance I	Absorbance II	SD I	SD II	%RSD I	%RSD II
1	6	0.2990	0.2945	0.001387	0.008072	0.4648	0.2736
2	6	0.2970	0.2943				
3	6	0.2983	0.2967				
4	6	0.2992	0.2953				
5	6	0.2987	0.2951				
6	6	0.2989	0.2946				
7	6	0.2985	0.2948				

Table 10: Recovery Study.

Sr. No	Drug/Formulation	Percentage recovery			Mean	SD	%RSD
		50%	100%	150%			
1	Bulk	96.45	98.90	97.66	97.67	1.225	1.254
2	Tablet	96.93	96.46	96.29	96.93	0.9732	1.004

6. DISCUSSION

6.1 Linearity and Range

The given method was obtained in range of 1-26µg/ml. The standard Calibration curve was obtained by plotting the absorbance against its concentration measured at 242 nm. The regression coefficient was found to be 0.999 and slope was found to be 0.038.

6.2 Intra-day and Inter-day Precision

The intra-day and inter-day precision study of the developed method confirmed adequate sample stability and method reliability where all the Relative Standard Deviations were below 2%.

6.3 Ruggedness

The method was performed by changing analyst and the method was found to be rugged with standard deviation 0.00098 and relative standard deviation 0.885%.

6.4 Robustness

The robustness was performed by change in scanning speed and method was robust with standard deviation 0.00473 and relative standard deviation 0.3692%.

6.5 LOD and LOQ

The LOD 0.60 and LOQ 2.17 ensures that the method is more sensitive and selective.

6.6 Accuracy and Recovery

The results within the range 96% - 99% ensure an accurate method.

7. CONCLUSION

The Process related impurity of Azilsartan medoxomil 2-Chloromethyl-1 H benzimidazole in bulk and formulation was synthesized, characterized and the UV method was developed according to ICH Q2B guidelines for quantitation of AZI from Azilsartan medoxomil bulk and tablet formulation. The synthesis of AZI was carried out by Imidazole synthesis. The % yield was found to be 85%. The preliminary evaluation was done on laboratory scale viz. melting point, TLC and elemental analysis. The melting point of AZI was found to be 153-155^{0}C. The TLC of AZI was carried by using Chloroform: Methanol (9:1 v/v) and the R_f was found to be 0.68.The confirmation of structure of AZI was carried out by using sophisticated instruments viz, FT-IR, NMR (^{1}H and ^{13}C), A UV method was developed to identify and quantify the AZI from Azilsartan medoxomil bulk and formulation, as per ICH Q2B guidelines. The method was found to be linear, precise, robust, rugged and accurate. Finally AZI was quantified from bulk Azilsartan medoxomil and its marketed tablet formulation.

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