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SPECTROPHOTOMETRIC DETERMINATION OF METALAZONE AND IN ITS COMMERCIAL FORMULATIONS

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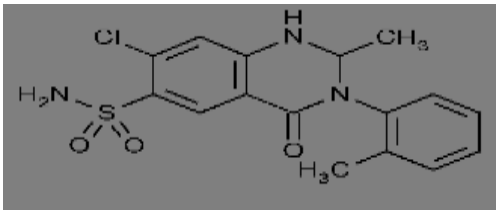
ABSTRACT

A simple, précise, rapid sensitive and accurate spectro phoptometric methods have been developed for the estimation of Metalazone in pure form and its formulations and Spiked vegetables and water samples. This method is based on complex formation of Metalazone with 2,2-BP in the presence of Ferric chloride to form coloured product with maximum of 620 nm. The product obeyed Beer's law in the concentration range 0.4--2.4 ml (4-24 µgml^{-1}) with molar absorptivity of 0.4029x10^4. Sandells sensitivity 0.00945. The assay of results was found to be good agreement with label claim.

KEYWORDS: Metalazone. UV. Validation.

INTRODUCTION

Metolazone is chemically 7-chloro-2-methyl-3-(2-methylphenyl)-4-oxo-1, 2-dihydroquinazoline- 6-sulfonamide [3] (Structure-1). Metolazone is an oral diuretic drug, commonly classified with the thiazide diuretics. It is primarily used to treat congestive heart failure and high blood pressure. Metolazone indirectly decreases the amount of water reabsorbed into the bloodstream by the Kidney, so that blood volume decreases and urine volume increases. This lowers blood pressure and prevents excess fluid accumulation in heart failure. The emperical formula for Metolazone is $C_{16}H_{16}ClN_3O_3S$ and the molecular weight is 365.84 grams.



Metolazone (Structure-1)

Literature review reveals that methods have been reported for analysis of Metolazone. Ultra-Violet and Derivative spectrophotometric methods for estimation of Metolazone in pharmaceuticals [2&5-8]. A Validated UV spectrophotometric method of Metolazone in Bulk and its Tablet Dosage forms [3&9-11]. Validated RP-HPLC method for simultaneous quantitation of Losartan potassium and Metolazone in bulk drug and formulation[4&12-17]. Validated HPTLC Method for Simultaneous Estimation of Ramipril and Metolazone in Bulk Drug and formulation [1&18-21]. LC-MS-MS development and validation for simultaneous quantitation of Metolazone with other drug in human plasma.

There is however no reported HPLC and UV- Visible spectrophotometric method for the analysis of Metolazone in its technical grade and formulations. This chapter describes a validated HPLC and UV-Visible spectrophotometric methods for the quantitative determination of Metalazone Functional group used for color development of Metalazone was primary amine group. The results obtained in this method was based on reaction of Metalazone with complex formation with 2, 2- Bipyridine/ Ferric chloride.. An attempt has been made to develop and validate all methods to ensure their accuracy, precision, repeatability, reproducibility and other analytical method validation parameters as mentioned in the various guidelines.

METHOD (D): (U.V- VISIBLE SPECTROPHOTOMETRIC METHOD BY 2, 2 - BIPYRIDINE REAGENT).

Experimental

A. Preparation of Standard Calibration curve of pure drug.

Solvent

Dimethylsulfoxide was used as solvent.

Preparation of calibration curve

Fresh aliquots of Metolazone ranging from 0.4 to 2.4 ml were transferred into a series of 10 ml volumetric flasks to provide final concentration range of 4 to 24 µg ml^{-1}. To each flask 1 ml of (0.01M) 2, 2-Bipyridine solution was added followed by 1ml of (0.2%) Ferric chloride solution and resulting solution was heated for 15 min and finally 1ml (0.2M) Orthophosphoric acid solution was added. The solutions were cooled at room temperature and made up to mark with distilled water. The absorbance of orange red colored chromogen was measured at 520 nm against the reagent blank. The color species was stable for 24h. The amount of Metolazone present in the sample solution was computed from its calibration curve.

Procedure for formulations

Twenty tablets containing Metolazone were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 100 mg of Metolazone was dissolved in a 100 ml of Dimethyl sulfoxide and mixed for about 5 min and then filtered. The Dimethylsulfoxide was evaporated to dryness. The remaining portion of solution was diluted in a 100 ml volumetric flask to the volume with Dimethylsulfoxide up to 100 ml to get the stock solution A. 10 ml of aliquots was pipette out into 100 ml volumetric flask and the volume was made up to the mark with Dimethylsulfoxide to obtain the final concentration of 100 µg ml^{-1} (Stock solution).

Subsequent dilutions of this solution were made with Dimethylsulfoxide to get concentration of 4 to 24 µg ml^{-1} and were prepared as above and analyzed at the selected wavelength, 520 nm and the results were statistically validated.

Procedure for blood sample

After collection of Blood sample it will be centrifuged. For isolation of **Metolazone** from plasma sample, Dimethylsulfoxide was used for protein precipitation. Liquid- Liquid extraction was performed with plasma by alkalinization with 1M NaOH, followed by extraction with 30% dichloromethane in Hexane. The upper organic layer was evaporated to dryness and dry residue 100 mg was dissolved in 100 ml of Dimethylsulfoxide (1000 µg ml^{-1}). From the above solution 10 ml is taken into a 100 ml of volumetric flask and made up to the mark. (100 µg ml^{-1})

From the above solution ranging from 0.5-3 ml (5-30 µg ml^{-1}) were transferred into 10 ml volumetric flask and to the each flask 1 ml of (0.01M) 2,2-Bipyridine solution was added followed by 1ml of (0.2%) Ferric chloride solution and made up to the mark with Dimethylsulfoxide. Then the resulting solution was heated for 15 min and finally 1ml (0.2M) Orthophosphoric acid solution was added. The solutions were cooled at room temperature and made up to the mark with distilled water. The absorbance of orange red colored chromogen was measured at 520 nm against the reagent blank. The color species was stable for 24 h. The amount of Metolazone present in the sample solution was computed from its calibration curve.

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Fig-7.11: Absorption spectrum of Metolazone with 2,2-Bipyridine/$FeCl_3$

Fig-7.12: Beer's law plot of Metolazone with 2, 2-Bipyridine/$FeCl_3$

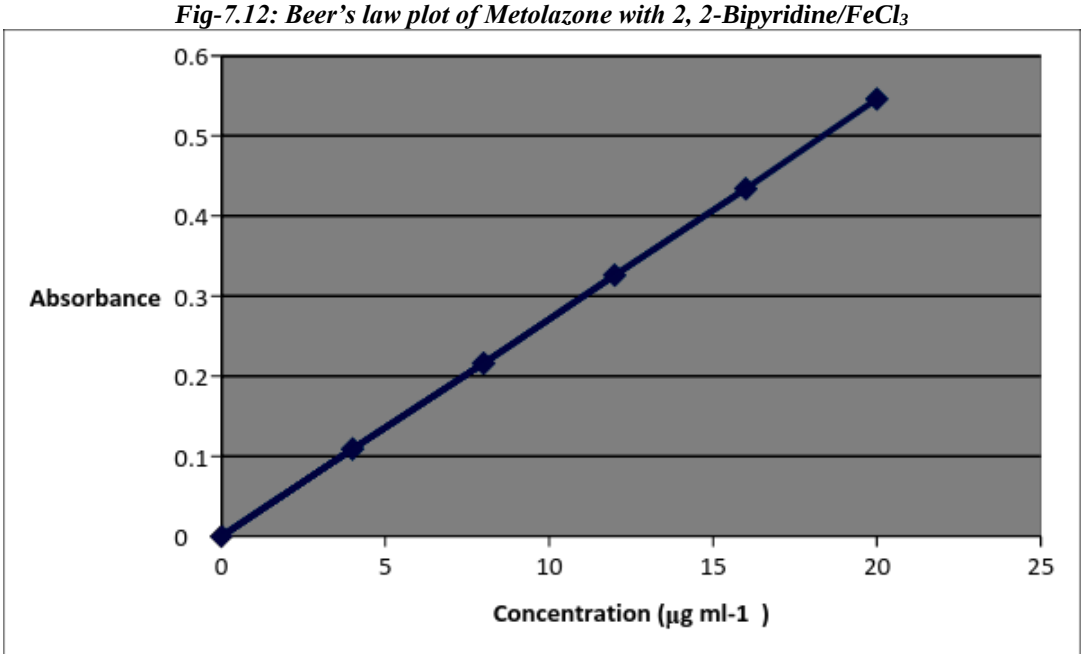

Fig-7.13: Beer's law plot for Metolazone in Blood sample

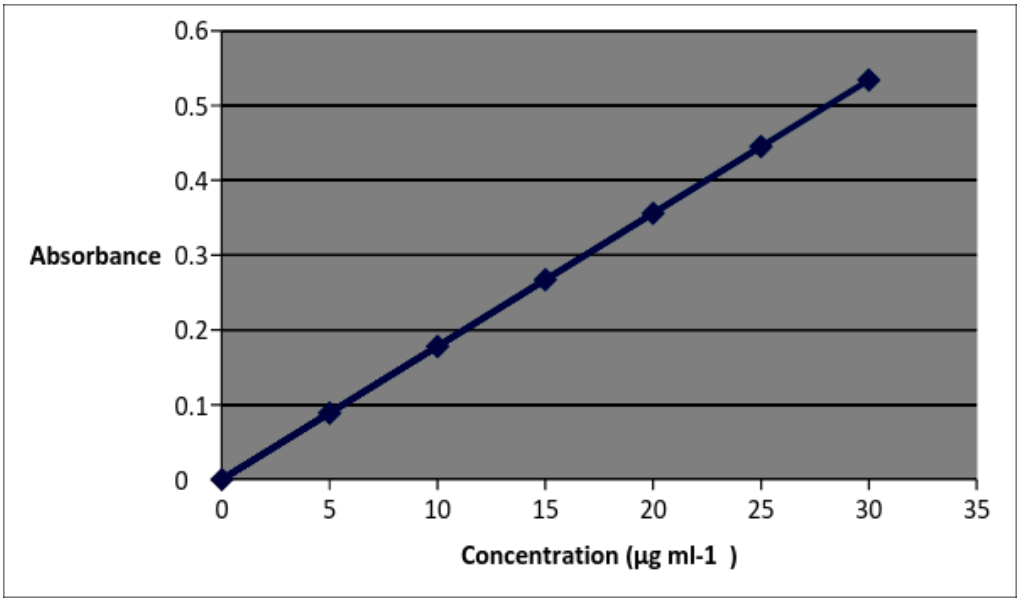

Fig-7.14: A Schematic reaction Mechanism of Metolazone with

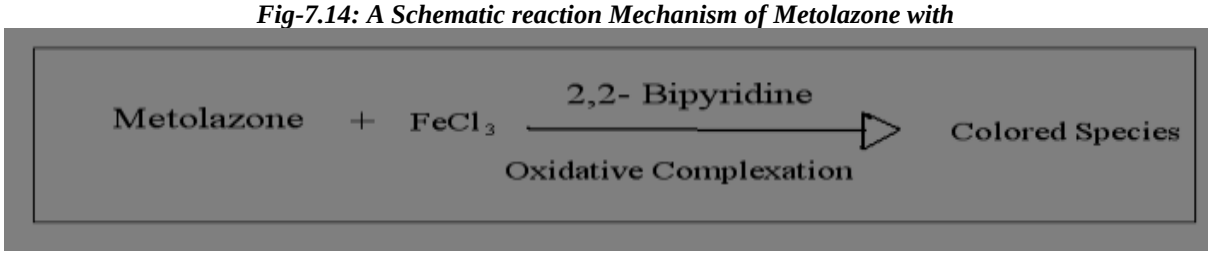

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Table -7.22: Optical characteristics and precision by (2, 2-B.P)

Parameter	**Visible method**
Absorption maxima (nm)	520
Beer's law limits (µg ml^{-1})	4-24
Molar absorptivity (l $mol^{-1}cm^{-1}$)	$0.4029x10^4$
Sandell's Sensitivity (µg cm^{-2})	0.09
Regression equation (Y*)	
Slope (b)	0.0273
Intercept(a)	0.00193
Standard deviation(SD)	0.0009
Correlation coefficient (r^2)	0.9999
%RSD (Relative Standard deviation)*	0.236
Range of errors	
Confidence limits with 0.05 level	0.0007
Confidence limits with 0.01 level	0.0009
Limits of detection (LOD)(µg ml^{-1})	0.0989
Limits of quantification (LOQ) (µg ml^{-1})	0.3296

*RSD of 6 independent determinations.

Table-7.23: Assay results of Metolazone in formulations by visible method

Name of the Formulation	**Formulation in (mg)**	**Amount found by the proposed method (mg)**	**Amount found by the reference method[27,28] (mg)**	**% Recovery**
Zyntanix	250	249.25 t=0.00396* F=1.7627*	239.5	95.92

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ZAROXOLYN	250	249.37 t=0.00398* F=1.7635*	241.25	96.63

*t and F- values refer to comparison of the proposed method with reference method.
*Theoretical values at 95% confidence limits t= 0.00296 and F= 1.6647.

Table-7.24: Determination of accuracy of Metolazone

Amount of METL in formulation (mg)	**Amount of Standard METL added (mg)**	**Total amount found (mg)**	**% Recovery**
247.58 246.66 246.55	200 200 200	445.64 443.98 439.86	99.03 98.66 97.74
247.58 246.75 245.77	250 250 250	495.16 493.5 491.54	99.03 98.7 98.3
247.28 247.91 248.53	300 300 300	544.01 545.40 546.76	98.91 99.16 99.41

Table-7.25: Statistical data for accuracy determination

Total amount found (mean)	**Standard deviation**	**% RSD**
246.93	0.565	0.2288
246.7	0.906	0.3672
247.9	0.625	0.2521

The results are the mean of five readings at each level of recovery.

Table-7.26: Repeatability data for METL at 520 nm

Conc. (µg ml^{-1})	**Abs 1**	**Abs2**	**Abs3**	**Mean**	**Std. deviation**	**(%) RSD***
4	0.109	0.108	0.106	0.107	0.0015	0.9345
8	0.216	0.217	0.218	0.217	0.001	0.4608
12	0.326	0.325	0.327	0.326	0.001	0.3067
16	0.434	0.435	0.436	0.435	0.001	0.2298
20	0.546	0.547	0.545	0.546	0.001	0.1831
24	0.654	0.656	0.655	0.655	0.001	0.1526

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*RSD of six independent determinations.

Table -7.27: Color stability data for 2, 2- Bipyridine Method

Conc. in µg ml^{-1}	Time in Hours							
16	4	8	12	16	20	24	28	32
	0.435	0.435	0.436	0.436	0.437	0.438	0.317	0.298

Table-7.28: Assay results of Metolazone in Blood sample

Name of the Formulation	**Formulation in (mg)**	**Amount found by the proposed method in (mg)**	**Amount found by the reference method[27,28] (mg)**	**% of Recovery**
Zyntanix	2.5	1.625 t=0.00296* F=1.9392*	1.54	94.48
ZAROXOLYN	2.5	1.615 t=0.00297* F=1.9382*	1.52	93.75

*t and F values refer to comparison of the proposed method with reference method.
*Theoretical values at 95% confidence limits t=0.00178 and F=1.324.

Table-7.29: Determination of accuracy of Metolazone

Name of the Formulation in (mg)	**Amount of Drug in Blood sample (mg)**	**Amount of Standard Drug added in (mg)**	**Total amount found (mg)**	**% Recovery**
2.5	1.625	2.5	3.25	65.00
2.5	1.615	2.5	3.23	64.6

The results are the mean of five readings at each level of recovery.

Table-7.30: Repeatability data for Metolazone at 520 nm

Concentration in (µg ml^{-1})	**Abs1**	**Abs2**	**Abs3**	**Mean**	**Std. Deviation**	**(%) RSD***
5	0.089	0.088	0.087	0.088	0.0001	0.0011
10	0.178	0.179	0.178	0.178	0.0005	0.280
15	0.267	0.268	0.269	0.268	0.001	0.373
20	0.356	0.358	0.357	0.357	0.001	0.2801
25	0.445	0.446	0.447	0.446	0.001	0.2242
30	0.534	0.536	0.534	0.534	0.001	0.1872

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*RSD of 6 independent determinations.

RESULTS AND DISCUSSION

Optical parameters

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) formed in UV spectrophotometric method (Reference method – A) and of the colored species formed in each so the four visible spectrophotometric methods, specified amount of Metolazone in final solution 5 µg ml^{-1} (method A), 5 µg ml^{-1} (method B), 10 µg ml^{-1} (method C) and 4µg ml^{-1} (method D) were taken and the colors were developed following the above mentioned procedures individually. The absorption spectra were scanned on spectrophotometer in the wavelength region of 200-400 nm (for method A) and 380-800 nm against corresponding reagent blanks. The reagent blank absorption spectrum of each method was also recorded against distilled water /Dimethylsulfoxide. The results are graphically represented in (fig 7.1, 7.3, 7.7 & 7.11)

Parameters fixation

In developing these methods, a systematic study of the effects of various relevant parameters in the methods concerned were under taken by verifying one parameter at a time and controlling all other parameter to get the maximum color development , reproducibility and reasonable period of stability of final colored species formed. The following studies ware conducted.

Method :

The results obtained in this method were based on oxidation followed by complex formation reaction of Metolazone with 2,2-Bipyridine, Ferric chloride and Orthophosphoric acid to form an orange red colored chromogen that exhibited maximum absorption at 520 nm against the corresponding reagent blank. The functional group used for the color development for this method was primary amine group. A schematic reaction mechanism of METL with 2, 2-Bipyridine reagent was shown in (fig-7.16). The effect of various parameters such as concentration and volume of 2,2-Bipyridine and strength of acid order of addition of reagents, solvent for final dilution were studied by means of control experiments varying one parameters at a time.

Optical Characteristics

The reference method adhere to beer's law the absorbance at appropriate wave length of a set of solutions contains different amounts of Metolazone and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blank.

The Beer's law plot of the system illustrated graphically (fig: 7.2) least square regression analysis was carried out for the slope. Intercept and Correlation Coefficient. Beer's law limits, Molar absorptivity & Sandells sensitivity for Metolazone with each of mentioned reagents was calculated. The optical characteristics were present in the table-7.1.

In order to test whether the colored species formed in the method and the beer's law the absorbance at appropriate wavelength of a set of solutions contain different amounts of Metolazone and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blanks or distilled water. The Beer's law plots of the system illustrated graphically (fig: 7.4, 7.5, 7.8,7.9, 7.12 & 7.13) least square regression analysis was carried out for the slope, intercept and correlation coefficient, beer's law limits molar absorptivity Sandells sensitivity for Metolazone with each of mentioned reagents were calculated. The optical characteristics are presented in the Tables - 7.4, 7.13 & 7.22.

Precision

The precision of each one among the five proposed spectrophotometric methods were ascertained separately from the absorbance values obtained by actual determination of a fixed amount of Metolazone (5, 5. 10 & 4 µg/ml respectively - A,B,C&D) in final solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods and presented in Table-7.1, 7.4, 7.13 & 7.22

Analysis of formulations

Commercial formulations of Metolazone were successfully analyzed by the proposed methods. The values obtained from the proposed and reference methods were compared statistically by the t and F tests and were found that those proposed methods do not differ significantly from the reported methods and they were presented in

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Tables-7.2, 7.5, 7.14 & 7.23. The proposed methods also applied for Biological Samples (Blood) for good recoveries are obtained which were recorded in Tables-7.10, 7.19 & 7.28.

Accuracy

Recovery studies were carried by applying the method to Drugs sample present in formulations to which known amount of Metolazone of label claim was added (Standard addition method). The recovery studies were carried by applying the method to Biological sample (Blood) to which known amount of Metolazone correspond to 2 mg Formulations taken by the patient. By the follow of Standard addition method 2 mg of label claim was added. After the addition of these standards the contents were transferred to 100 ml volumetric flask and dissolved in solvent. Finally the volume was made up to the mark with solvent. The solution was filtered through Whatman No. 41 filter paper. The mixed sample solutions were analyzed and their absorbance value was determined. At each level of recovery five determinations were performed and present in Tables- 7.6, 7.15 & 7.24. The results obtained were compared with expected results and were statistically validated in Tables- 7.7, 7.16 & 7.25.

Linearity and Range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyse in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyze that have been demonstrated within a suitable level of precision, accuracy and linearity.

Specificity and Selectivity

Specificity is a procedure to detect quantitatively the analyze in the presence of components that may be expected to the present in the sample matrix. While selectivity is a procedure to detect the analyze qualitatively in presence of components that may be expected to present in the sample matrix. The excipient in formulations was spiked in a pre weighed quantity of Drugs and then absorbance was measured and calculations were done to determine the quantity of the Drugs.

Repeatability

Standard solutions of Metolazone were prepared and absorbance was measured against the solvent as the blank. The observance of the same concentration solution was measured five times and standard deviation was calculated and presented in Tables-7.3, 7.8, 7.12, 7.17, 7.21, 7.26 & 7.30.

Interferences Studies

The effect of wide range of inactive, ingredients usually present in the formulations for the assay of Metolazone under optimum conditions was investigated. None of them interfered in the proposed methods even when they are present in excess fold than anticipated in formulations.

Solution Stability

The stability of the solutions under study was established by keeping the solution at room temperature for 48 Hours. The results indicate no significant change in assay values indicating stability of Drug in the solvent used during analysis. The results are recorded in Table -7.9, 7.18, 7.27..

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