METHOD DEVELOPMENT AND VALIDATION OF METFORMIN HYDROCHLORIDE BY RP-HPLC

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ABSTRACT: A new simple, economical, sensitive, less-time consuming was developed and validated for the determination of metformin hydrochloride. The analysis complied with Beer's law in the concentration range of 8-13 µg/mL at 233 nm for metformin hydrochloride by RP-HPLC & UV method Methanol, Acetonitrile, the proposed method was validated as per ICH guidelines parameters like linearity, specificity, precision, accuracy, robustness, degradation studies, absorption maximum, LOD & LOQ. The accuracy of methods was evaluated by recovery studies and good recovery results were obtained. The maximum absorption of metformin hydrochloride was found to be 236.40nm, in Methanol, Acetonitrile, Water (1:3:6). The linearity was found to be with the range of 0.005-1.0. The limits of accuracy were found to be within the limits of 98-110%. The results obtained showed a good agreement with the declared contents in case of pharmaceutical formulations. The method was also applied for the degradation studies. The development method is successfully validated for the estimation of metformin hydrochloride in bulk and dosage forms.

KEYWORDS: Metformin HCl, UV Visible spectroscopy, RP-HPLC method, ICH, validation, degradation studies, accuracy, precision.

INTRODUCTION:

Metformin hydrochloride is chemically 1,1-Dimethylbiguanide hydrochloride is an antihyperglycemic agent that belongs to the class of biguanide. The empirical formula is ($C_4H_{11}N_4$.HCl) and the molecular weight is 165.62g/mole. The structure formula is

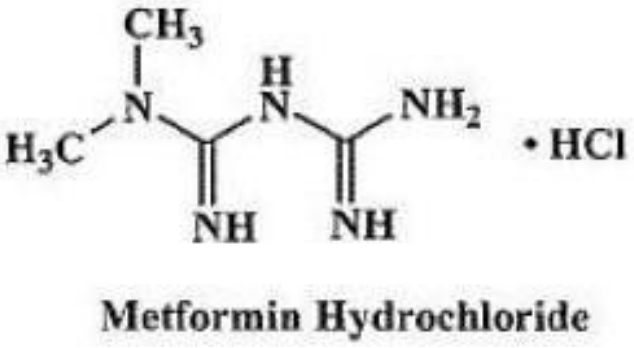

Figure No.1: Structure of metformin HCl

Metformin hydrochloride is the first line medication for the treatment of type 2 3diabetes, particularly in the people who are overweight. It is also used in the treatment of polycystic ovary syndrome it is generally well tolerated common adverse effects include diarrhoea, nausea, abdominal pain. It works by decreasing glucose production in the liver, increasing the insulin sensitivity of body tissues, and increasing GDF15 secretion which reduces appetite and calorie intake. It decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization.

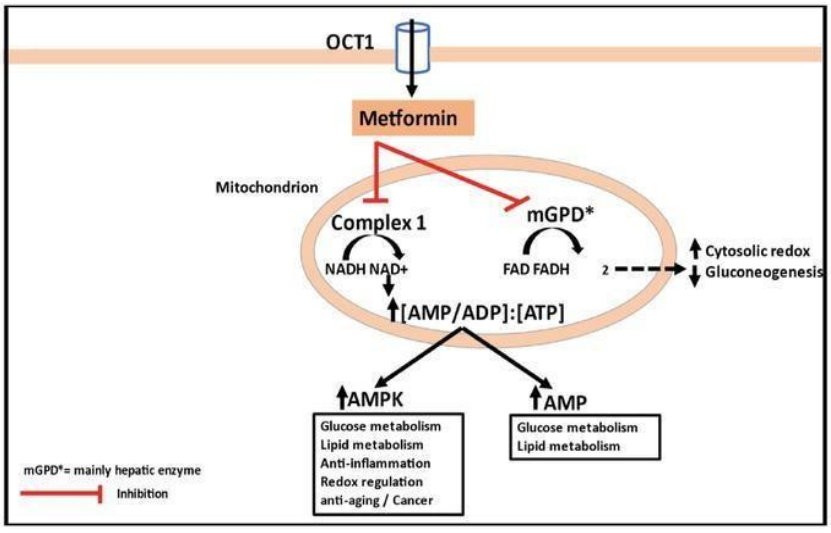

Figure No.2: Mechanism of Action of Metformin HCl

It has oral bioavailability of 50-60%, under fasting conditions metformin has Pk_a values of 2.8 and 11.5. the half-life in plasma 6.2-hour, renal clearance value is 510±120ml/min. Approximately 90% of the drug is eliminated in 24 hours in those with

healthy renal function and is excreted unchanged in the urine, metformin clearance is approximately 3.5 times that of creatinine clearance including the tubular secretion is the primary mode of metformin UV, RP-HPLC was reported for the determination of metformin.

EXPERIMENTAL

Apparatus

The liquid chromatographic equipped with auto sampler and DAD or UV detector with an injecting volume of 20 ml. the analytes were observed at 297nm. Chromatographic analysis was performed on Ambient C_{18} having 4.6 × 150nm i.d. and 5 nm particle size.

Reagents and materials

The drugs used were of AR grade. The drug of metformin in tablet (solid dosage form) were obtained from ESIC supply.

Selection Of Detection Wavelength

Solutions of drug was scanned over the range of 200nm. It was observed that the absorbance of metformin

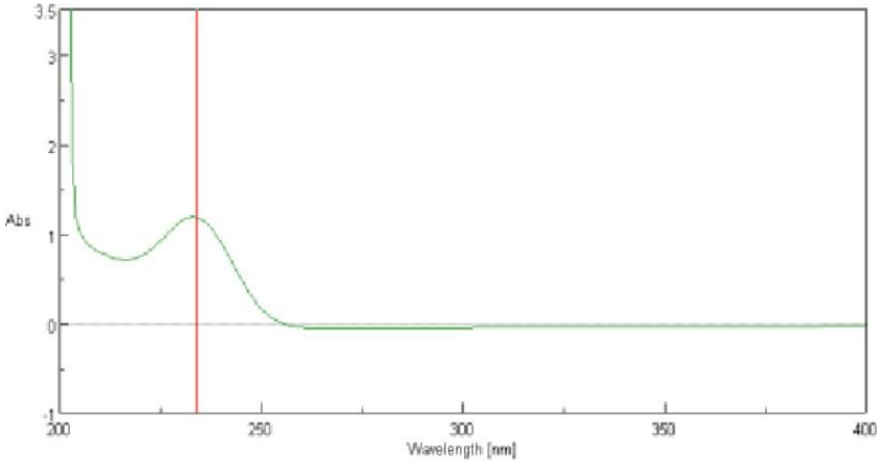

Figure No.3: UV spectrum of metformin HCl at 234nm

Chromatographic Conditions

The Ambient C_{18} (4.6× 150nm) with 5 mm particle size. Acetonitrile, methanol, water used in the ratio (1:2:3) (v/v/v). The flow rate was maintained at 0.8ml/min, detection wavelength at 297nm and the injection volume is 20ml, and run time was kept at 5 minute.

Preparation Of Standard Solution

Accurately weigh and transfer 10mg of metformin working standard into 100 ml volumetric flask add about 10 ml of diluent and sonicate to dissolve it completely make volume up to the mark with same solvent. (Stock solution).

Further pipette out 0.7 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Preparation Of Working Standard

Aliquot from the stock solution of metformin were appropriately diluted with distilled water to obtain working standard of metformin.

Sample solution Preparation

Weigh 5 metformin tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of metformin into a 100 ml volumetric flask. Add about 10ml of dilute and sonicate to dissolve it completely and make up to the mark with diluent. Mix well and filter through 0.45µm filter.

Further pipette out 0.7 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter**.**

METHOD DEVELOPMENT

Lots of mobile phase and their different proportions were tried and finally was selected as 0.02M Potassium dihydrogen phosphate (KH2PO4), Acetonitrile, Methanol in the ratio of 50:25:25 (v/v/v) at pH 4.3 appropriate mobile phase which gave good resolution and acceptable system suitability parameters. The chromatogram of working standard solution.

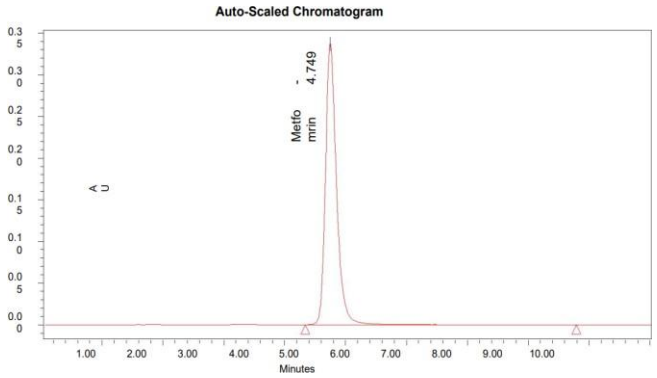

Figure No.4: Chromatogram of metformin

Procedure

Inject 20 ml of the standard sample into the chromatographic system and measure the area for the metformin peak and calculate the % Assay by using the formulae.

Linearity

According to ICH guidelines linearity should be calculated by taking 5-8 non zeros concentration values and R^2 (Correlation Coefficient) should be within the range of 0.995 – 1.0, In this proposed method it was found to be within the limits, table no. 6.3 and figure no 6.3 shows the result.

Table No.1: Linearity

S. No.	**Linearity level**	**Concentration ($\mu g/ml$)**	**Absorbance**
1	50	0.90	0.095
2	75	5.00	0.295
3	100	9.00	0.492
4	125	14.50	0.781
5	150	18.00	0.933

Figure No.5: colour formation for linearity

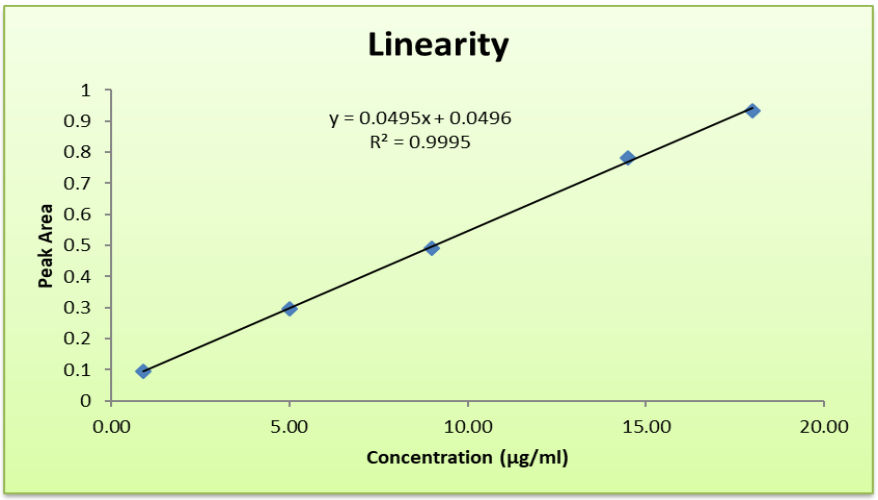

Figure No.6: Linearity for Metformin HCl

Precision

According to ICH guidelines, inter-day and intra-day precision was performed in 3 consecutive days & 3 times in a same day respectively and %RSD in all the cases were found to be less than 2

Table No.2: Inter-Day Precision

S. No.	**Day 1**		**Day 2**		**Day 3**	
	Absorbance	**% Assay**	**Absorbance**	**% Assay**	**Absorbance**	**% Assay**
1	0.491	99.80	0.489	99.39	0.488	99.19
2	0.485	98.58	0.487	98.98	0.49	99.59
3	0.487	98.98	0.49	99.59	0.486	98.78
4	0.489	99.39	0.485	98.58	0.491	99.80
5	0.490	99.59	0.488	99.19	0.483	98.17
6	0.492	100.0	0.486	98.78	0.49	99.59
Average	0.49	99.39	0.49	99.09	0.49	99.19
Std dev	0.003	0.53	0.002	0.38	0.003	0.62
% RSD	0.53	0.53	0.38	0.38	0.62	0.62

S. No.	Day 1		Day 2		Day 3	
	Absorbance	**% Assay**	**Absorbance**	**% Assay**	**Absorbance**	**% Assay**
1	0.491	99.80	0.489	99.39	0.488	99.19
2	0.485	98.58	0.487	98.98	0.49	99.59
3	0.487	98.98	0.49	99.59	0.486	98.78
4	0.489	99.39	0.485	98.58	0.491	99.80
5	0.490	99.59	0.488	99.19	0.483	98.17
6	0.492	100.0	0.486	98.78	0.49	99.59
Average	0.49	99.39	0.49	99.09	0.49	99.19
Std dev	0.003	0.53	0.002	0.38	0.003	0.62
% RSD	0.53	0.53	0.38	0.38	0.62	0.62

Table No.3: Intra Day Precision

Accuracy

According to ICH guide lines, accuracy was carried out by three different concentration levels & recovery was calculated, in this case it was found to be within the limits 98-102 %.

S. No	Accuracy level	Wt. of sample	Absorbance	Amount Added (µg/ml)	Amount found (µg/ml)	% recovery	Mean % recovery
1	50%	19.749	0.24	49.72	48.78	98.12	98.73%
2		19.749	0.243	49.72	49.39	99.35	
3		19.749	0.24	49.72	48.78	98.12	
4		19.749	0.242	49.72	49.19	98.94	
5		19.749	0.241	49.72	48.98	98.53	

6		19.749	0.243	49.72	49.39	99.35	
7	100%	39.498	0.487	99.43	98.98	99.55	98.87%
8		39.498	0.481	99.43	97.76	98.32	
9		39.498	0.483	99.43	98.17	98.73	
10	150%	59.247	0.727	149.15	147.76	99.07	99.19%
11		59.247	0.73	149.15	148.37	99.48	
12		59.247	0.729	149.15	148.17	99.35	
13		59.247	0.728	149.15	147.97	99.21	
14		59.247	0.727	149.15	147.76	99.07	
15		59.247	0.726	149.15	147.59	98.94	

Table No.4: Accuracy.

Robustness

Robustness was carried out by two different parameters and % RSD should be less than 2. In this case it was found to be within the limits. Table no.6.9 shows the results of robustness.

S. No	**Parameter**	**Condition**	**Absorbance**	**% Assay**
1	Wavelength (nm)	724.00	0.49	99.59
2		726.00	0.492	100.00
3		722.00	0.488	99.19

Table No.5: Robustness

Specificity

Specificity of this proposed method does not show any interference with solvents and excipients in API and tablet dosage form, figure 6.4, 6.5, 6.6 and table no. 6.4 shows the results.

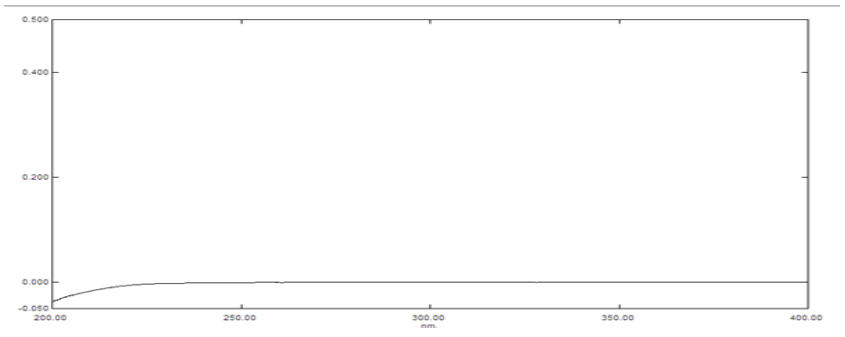

Figure No.7: Blank spectrum

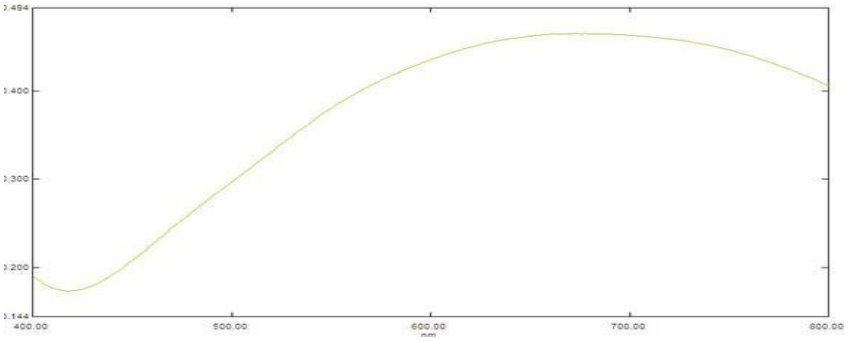

Figure No.8: Specificity spectrum of API

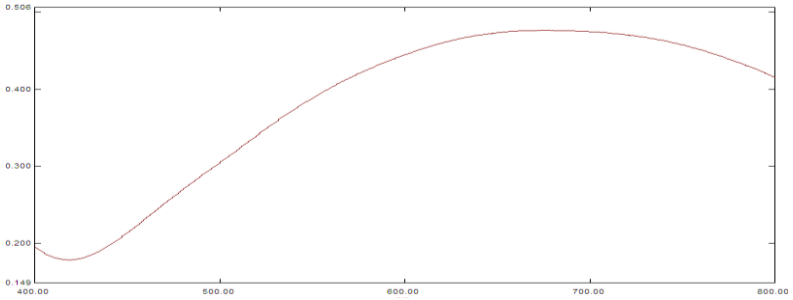

Figure No.9 Specificity spectrum of tablet

Table No.6: Specificity

Type	**Wavelength**	**Absorbance**
API	724nm	0.492
Dosage form	724nm	0.487

Degradation studies

Degradation studies were carried out in 5 different conditions. The % degradation should not be more than 10%/. In this study it was found to be less than 10 %. Table No. 6.10 shows the results of degradation studies.

Table No.7: Degradation Studies

S. No	Condition	Absorbance	% Assay	% Degradation
1	Acid (HCl)	0.45	91.46	8.54
2	Base (NaOH)	0.448	91.06	8.94
3	H_2O_2	0.443	90.04	9.96
4	UV	0.447	90.85	9.15
5	Heat	0.443	90.04	9.96

RESULTS AND DISCUSSION

UV Visible spectroscopic method and HPLC are developed and validated as per ICH guidelines. All the methods were found to be simple, sensitive, precise and accurate. These methods were tabulated with one another is given below.

Table No.8 Summary of UV – Visible spectroscopic method

Parameter	UV spectroscopy	Visible spectroscopy
Wavelength	227.40nm	270nm
Concentration range (µg/ml)	0-2µg/ml	0-1 µg/ml
Linearity	0.9982	0.9995

Assay	99.576	99.39
Inter-day precision	0.57	0.51
Intra-day precision	0.41	0.52
Accuracy	99.34%	98%
LOD (µg/ml)	0.01µg/ml	0.16µg/ml
LOQ (µg/ml)	0.04µg/ml	0.53µg/ml

Table No.9: Summary of HPLC results

Drugs	**HPLC**		
Metformin HCl	Standard	Wavelength	245nm
		Concentration	10µg/ml
		R. T value	4.735
	Sample	Wavelength	245nm
		Concentration	10µg/ml
		R. T value	4.749

CONCLUSION

An attempt was developed and validated using different spectrophotometric methods and HPLC method for the estimation of metformin hydrochloride. The method was validated and found to be simple, sensitive, accurate and precise as per ICH guidelines. The method was successfully used for determination of drugs in their pharmaceutical formulation.

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