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Research Article

METHOD DEVELOPMENT AND VALIDATION OF FORCED DEGRADATION STUDIES OF METFORMIN HYDROCHLORIDE BY USING UV SPECTROSCOPY

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ABSTRACT

Metformin hydrochloride is the biguanide class of oral antihyperglycemics improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. Metformin was freely soluble in Methanol, pH4 and pH7 buffer. Methanol was choosen as a solvent. The drug has maximum absorbance at 237nm.The optical characteristic of drug was found to be Beer's law limits 1-10 µg/ml, Correlation coefficient is 0.9989, Std error is 0.012089, Molar Absorbance is 37058.33.The drug sample was analyzed by UV spectroscopy using methanol as solvent and the average content of drug present in the formulation was found to be 100.4%. The % RSD of accuracy studies was found to be 99.7±0.352. The %RSD of precision was found to be 0.0209 to 0975.The% recovery of ruggedness was found to be 99.17±0.1636 and 99.69±0.5953.The pH degradation studies of tablet formulation were found to be less at pH 6-8. The force degradation studies of metformin tablet formulation was done on Stress degradation by hydrolysis under alkaline condition by using 0.1N NaOH was found to be 8.07% for 60min, 11.95% for 90min. Stress degradation by hydrolysis under acidic condition by using 3N HCl and product degradation was found to be 9.75% for 60min and 12.79% for 90 min. Dry heat induced degradation was done by using 70^0c temperature was found to be 20.94% for 48 hrs. Oxidative degradation was done by using hydrogen peroxide and product degradation was found to be 12.65%for 15 min. Photolytic degradation was found to be 10.53% for 3hrs and 14.36% for 6hrs.

Keywords: Metformin Hydrochloride, UV Spectroscopy, P^H degradation, Forced Degradation.

INTRODUCTION

Metformin hydrochloride (MET) is chemically (N, Ndimethyl imidodicarbonimidic diamide hydrochloride) is a member of the biguanide class of oral antihyperglycemics improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose and improves insulin sensitivity by increasing peripheral glucose uptake and utilization.[1-2] the chemical structure of Metformin Hydrochloride was shown in Fig-1. Literature survey reveals that methods like HPLC[3-5] and different Spectrophotometric[6-8] have been reported for estimation of the Metformin hydrochloride in pharmaceutical formulations and biological fluids. Official method includes UV Spectrophotometric method for estimation of the drug from the tablets. There is a need for a simple, rapid, cost effective and reproducible method for assay of Metformin in its dosage forms But there was no reported method for the Forced degradation studies of Metformin hydrochloride by using UV spectroscopy. So the present work is to carry out the force degradation studies along with its pH degradation studies. The method was

validated according to the ICH (Q2A1995) guidelines[9] Forced degradation studies may help facilitate pharmaceutical development as well in areas such as formulation development, manufacturing, and packaging, in which knowledge of chemical behaviour can be used to improve a drug product. The available regulatory guidance provides useful definitions and general comments about degradation studies[10] The International Conference on Harmonization (ICH) guidelines[11-12] indicates that stress testing is designed to determine the intrinsic stability of the molecule by establishing degradation pathway in order to identify the likely degradation products and to validate the stability indicating power of the analytical procedure used. ICH guidelines stability testing of new drug substances and products' Q1A (R2)[13] and (Q1B)[14] requires that stress testing should be carried out to elucidate the substance. It suggests that the degradation products that are formed under the variety of condition should include the effect of temperature, appropriate oxidation, photolysis and susceptibility.

EXPERIMENTAL

Instrument

Absorption spectral measurements were carried out with a UV – Visible spectrophotometer (Shimadzu Model 1700) using UV Probe software version 2 was employed with spectral bandwidth of 1 nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 5 cm matched quartz cells.

Chemicals

Metformin HCl (MET) supplied by Aribindo pharmaceuticals, India as gift sample and used as such. Methanol used was from Qualigen fine chemicals Ltd, India. Water used was generated by double distillation.

Preparation of standard stock solution

Standard stock solution of Metformin was prepared by dissolving 10mg of drug in methanol and making up the volume to 10ml to get 1mg/ml.

Preliminary solubility study of drug

Solubility of the drug was determined at 28±1 C. A small quantity of standard drug was dissolved in different solvents like distilled water, methanol, ethanol, acetonitrile, isopropyl alcohol, dimethyl sulfoxide, dimethyl formamide, 0.1 N HCl, chloroform, acetonitrile and pH 4, 7, 9.2 buffer solutions. The results are reported in table 1.

Solvent Selection

Various solvents were selected for the solubility studies and it was found that Metformin was soluble in the following solvents; Distilled water, methanol, pH4 and pH 7 buffer. In the present investigation methanol was selected as a solvent.

Selection of analytical wavelength and absorption maxima

Appropriate 10µg/ml dilutions were prepared for drug from the standard stock solution and the solutions were scanned in the wavelength range of 200-400 nm. The absorption spectra thus obtained was derivatized for zero order spectroscopy. This zero order spectrum was selected for the analysis of the drugs. The absorption maximum was found at 237 nm which can be further used for analysis as shown in Fig.2

Preparation of stock solutions

Standard Metformin 100mg was weighed and transformed to a 100 ml volumetric flask and dissolved in 25 ml of methanol. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 1000 µg/ml (Stock solution A). From this stock solution A, pipette out 5 ml and place into 50 ml volumetric flask. The volume was made up to the mark with methanol to give a solution containing 100 µg/ml (Stock solution B)

Selection of analytical concentration range

From the standard stock solution B of Metformin, appropriate aliquots 1, 2, 3, 4 and 5 ml were pipette out in 10 ml volumetric flasks and dilutions were made with methanol to obtain working standard solutions of concentrations from 1-10µg/ml. Absorbance for these solutions were measured at 237 nm. For standard solution analytical concentration range was found to be 1-10 µg/ml and overlain spectra was obtained and optical characteristic and linearity data was reported in table 2.

Calibration curve for the Metformin

Appropriate volumes of aliquots from standard Metformin stock solution B were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with methanol to obtain concentrations of 2, 4, 6 ,8 and 10µg/ml. Absorbance value of each solution against methanol as a blank were measured at 237 nm. From that absorbance value, regression equation and correlation coefficient (r^2) are determined and presented Fig.3.

Analysis of Metformin from Tablet Dosage form

Twenty tablets of formulation were weighed and finely powdered. The powder equivalent to 100 mg of Metformin was accurately weighed. It was then transferred to volumetric flask of 100 ml capacity containing 25 ml of methanol and sonicated for 30 min. The flask was shaken and the solution was filtered through Whatmann filter paper (No. 41) into 100 ml volumetric flask. Volume was made up to the mark with methanol to give a solution of 1000 µg/ml (Stock solution A). From this solution 5 ml was taken and placed in 50 ml volumetric flask. The volume was made up to the mark using methanol to give a solution of 100 µg/ml (Stock solution B). From the stock solution B, 5.0 ml was taken and diluted to 10 ml to give 50µg/ml and it was further used for the estimation of Metformin. The result was reported in Table 3.

METHOD VALIDATION

Validation parameters

The method was validated with reference to accuracy, precision, and ruggedness.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of the drug to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods; the results are reported in Table 4.

Precision

Precision of the methods was studied as intra-day, interday and repeatability. Intra-day study was performed by analyzing, the three different concentration of drug for three times in the same day. Inter-day precision was performed by analyzing three different concentration of the drug for three days in a week. Repeatability was performed by analyzing same concentration of drugs for six times. The results are reported in Table 5.

Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by different analysts using similar operational and environmental conditions. The results are reported in Table 6.

pH DEGRADATION STUDIES

The pH effect on the drug was carried out by using 0.1N Hydrochloric acid, 2N Hydrochloric acid, 0.1N Sodium Hydroxide and 2N Sodium Hydroxide solution. The drug solutions (100µg/ml) from pH 0-14 were prepared in the manner as shown in table 7 and these were allowed to stands for 4 hours. Finally the absorbances were measured at 237nm. The K value for 1st order kinetics was determined by using the formula:

$$K = (2.303/t) \log (Co/C)$$

Where,

K =1st order rate constant,

Co = initial drug concentration,

C = final drug concentration

The results were reported in table 8.

Degradation Studies

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Metformin hydrochloride using the method developed.

Stress degradation by hydrolysis under acidic condition

To 3 ml of stock solution(1000µg/ml) of Metformin, 1 ml of 3 N HCl was added in 10 ml of volumetric flask and the volume was made up to the mark with methanol. Then, the volumetric flask was kept at normal condition for 90 minutes. After 60 min. time interval, 1 ml of solution was pipette out from this flask, neutralised and diluted with methanol in order to make the volume up to 10 ml and the dilution was carried out to achieve the appropriate concentration (30µg/ml). This solution was taken in cuvette. For the blank, 0.5 ml solution of 3N HCl and 0.5 ml solution of 3N NaOH were diluted with methanol in 10 ml of volumetric flask. After 90 minutes, again 1ml of the solution was pipetted out from the flask and the above procedure was repeated

Stress degradation by hydrolysis under alkaline condition

To 3 ml of stock solution of Metformin 1 ml of 0.1 N NaOH was added in 10 ml of volumetric flask and made up the volume to the mark with methanol. Volumetric flask was kept at normal condition for 90 min. After 60 min time interval, 1 ml of solution was pipette out from this flask, neutralized and diluted with methanol in order to make the volume up to 10 ml and the dilutions were carried out to achieve the appropriate concentration (20µg/ml). The solution was then taken in cuvette. For the blank, 0.5 ml solution of 0.1N HCl and 0.5 ml

solution of 0.1N NaOH diluted with methanol in 10 ml of volumetric flask. After, 90 minutes 1ml of solution was again pipette out from the flask and the above procedure was repeated.

Dry heat induced degradation

Metformin sample was taken in a petriplate and exposed to a temperature of 70°c for 48 hours in an oven. After 48 hours, 10 mg of the sample was diluted with methanol in order to make the volume up to 10 ml. From this solution, dilutions were carried out to achieve the appropriate concentration (20µg/ml) and the solution was taken in cuvette for the UV-VIS Analysis.

Oxidative degradation

To 1.5 ml of the stock solution of Metformin (1000µg/ml), 1 ml of 30% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with methanol. The volumetric flask was then kept at room temperature for 15 min. For the blank, 1 ml of the 30 % w/v of hydrogen peroxide was kept at normal condition for overnight in 10 ml of volumetric flask. Both solutions were heated on boiling water bath to remove the excess of hydrogen peroxide. Finally, after 15 minutes dilutions were made from the stock solution to achieve the required concentration (30µg/ml). The solution was then taken in a cuvette and analysed in UV.

Photolytic degradation

Sample of Metformin was exposed to near ultraviolet lamp in photostablity chamber providing illumination of not less than 1.2 million lux hours. Ten milligrams sample was dissolved in methanol and volume made up to 10 ml. From this solution appropriate dilution (30µg/ml) was made using methanol and taken in cuvette for the U.V analysis. The results were reported in table 9.

RESULTS AND DISCUSSION

Metformin was freely soluble in Methanol, pH4 and pH7 buffer. Methanol was choosen as a solvent. The drug has maximum absorbance at 237nm.The optical characteristic of drug was found to be Beer's law limits 1-10 µg/ml, Correlation coefficient is 0.9989, Std error is 0.012089, Molar Absorbance is 37058.33.The drug sample was analyzed by UV spectroscopy using methanol as solvent and the average content of drug present in the formulation was found to be 100.4%. The % RSD of accuracy studies was found to be 99.7±0.352. The %RSD of precision was found to be 0.0209 to 0975.The% recovery of ruggedness was found to be 99.17±0.1636 and 99.69±0.5953.The pH degradation studies of tablet formulation were found to be less at pH 6-8. The force degradation studies of metformin tablet formulation was done on Stress degradation by hydrolysis under alkaline condition by using 0.1N NaOH was found to be 8.07% for 60min, 11.95% for 90min. Stress degradation by hydrolysis under acidic condition by using 3N HCl and product degradation was found to be 9.75% for 60min and 12.79% for 90 min. Dry heat induced degradation was done by using 70^{0}c temperature was found to be 20.94% for 48 hrs. Oxidative degradation was done by using hydrogen peroxide and product degradation was found to be 12.65%for 15 min. Photolytic degradation was found to be 10.53% for 3hrs and 14.36% for 6hrs.

Table 1: Solubility data for Metformin hydrochloride

S. No.	Solvent	Solubility status
1.	Distilled water (HPLC and spectroscopic grade)	Freely soluble
2.	Ethanol	In soluble
3	Methanol	Freely soluble
4.	Acetonitrile	Insoluble
5.	Iso propyl alcohol	Insoluble
6.	Buffer P^H 4 solution	Freely soluble
7.	Buffer P^H 7 solution	Freely soluble

Table 2: UV Optical characteristic and linearity data

Parameters	Metformin
λ max (nm)	230
Beer's law limits in µg/ml	1-10
Correlation coefficient	0.9989
Regression equation Y=mx+c	Y=0.0956x+0.0128
Intercept(c)	0.0128
Slope	0.0956
Std error	0.012089
Molar Abs	37058.33
Sandeel's	0.01046

Table 3: Analysis data of Tablet formulation by UV

Drug	Label claim mg/tab	Amount found mg/tab	Label claim (%)	S.D.*	% COV	S.E*
MET	500	500.5	100.4	0.057	0.033	0.009

MET: Metformin, S.D: Standard deviation,
COV: Coefficient of variation, S.E: Standard error

Table 4: Results of Accuracy studies by UV spectroscopy

Level of recovery	Amount of sample (µg/ml)	Amount of drug added (µg/ml)**	Amount of drug recovered (µg/ml)**	% Recovery ± S.D**
80%	10	8.0	7.98	99.7±0.352
100%	10	10.1	9.92	99.2±0.207
120%	10	12.0	11.97	99.7±0.215

** is average of six determinations

Table 5: Precision study data of Metformin by UV spectroscopy

Concentration (µg/ml)	Inter-day Absorbance mean ± SD**	% RSD	Intra-day Absorbance mean ± SD**	% RSD
1	0.120±0.00041	0.342	0.120±0.000117	0.975
2	0.227±0.00052	0.224	0.227±0.00052	0.225
3	0.332±0.00063	0.192	0.332±0.00041	0.124
4	0.446±0.00041	0.089	0.446±0.00052	0.113
5	0.558±0.00052	0.092	0.558±0.00117	0.209

** is average of six determinations.

Table 6: Ruggedness study data of Metformin by UV

Sample	Label claim(mg)	Analyst 1		Analyst 2	
		Amount found (mg)	% Recovery ±SD**	Amount found (mg)	% Recovery ±SD**
MET	500	495.89	99.17 ±0.1636	498.45	99.69 ±0.5953

MET-Metformin ** is average of six determinations

Table 7: Preparation of sample solution of pH 0-14 for pH stability

pH	Amount of Drug solution added (100 µg/ml) in ml	Amount of 0.1N NaOH solution added(ml)	Amount of HCl / NaOH added in ml
0	3.5	3.5	13.5 of 2N HCl
1	3.5	3.5	1.35 of 2N HCl
2	3.5	3.5	1.35 of 0.2N HCl
3	3.5	3.5	1.35 of 0.02N HCl
4	3.5	3.5	1.35 of 0.002N HCl
5	3.5	3.5	1.35 of 0.002N HCl
6	3.5	3.5	1.35 of 0.002N HCl
7	3.5	3.5	-
8	3.5	3.5	13.5 of 2N NaOH
9	3.5	3.5	1.35 of 2N NaOH
10	3.5	3.5	1.35 of 0.2N NaOH
11	3.5	3.5	1.35 of 0.02N NaOH
12	3.5	3.5	1.35 of 0.002N NaOH
13	3.5	3.5	1.35 of 0.0002N NaOH
14	2.5	3.5	1.35 of 0.00002N NaOH

Table 8: pH Degradation Results

PH	ABSORBANCE (at 230 nm)	CONCENTRATION (µg/ml)	% DRUG DEGRADED	k VALUE	Log k
0	0.450	17.1103	14.45	0.039	-1.4089
1	0.452	17.1863	14.07	0.0379	-1.4214
2	0.449	17.0722	14.64	0.0396	-1.4023
3	0.458	17.4144	12.93	0.0346	-1.4609
4	0.458	17.4144	12.93	0.0346	-1.4609
5	0.502	19.0875	4.56	0.0117	-1.9318
6	0.521	19.8099	0.95	0.0024	-2.6198
7	0.515	19.5817	2.09	0.0053	-2.2757
8	0.508	19.3156	3.42	0.0087	-2.0605
9	0.499	17.0722	14.64	0.0396	-1.4203
10	0.422	16.0456	19.77	0.0551	-1.2588
11	0.392	14.9049	25.48	0.0735	-1.1337
12	0.378	14.3726	28.14	0.0826	-1.083
13	0.342	13.0038	34.98	0.1076	-0.9682
14	0.331	12.5856	37.07	0.1158	-0.9363

Table 9: Results of Stress Degradation Studies

Condition	Time	%Degradation
0.1N NaOH(1ml)	60min	8.07%
	90min	11.95%
3N HCl(1ml)	60min	9.75%
	90min	12.79%
30% Hydrogen Peroxide(1ml)	15min	12.65%
Dry Heat 70°	48hr	20.94%
Photolytic	3hr	10.53%
	6hr	14.36%

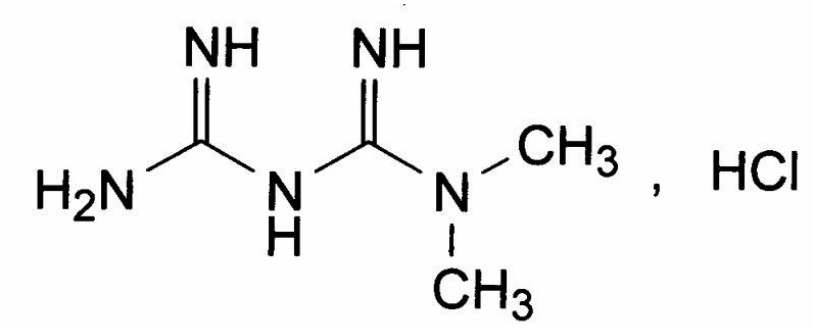

Fig. 1: chemical structure of Metformin

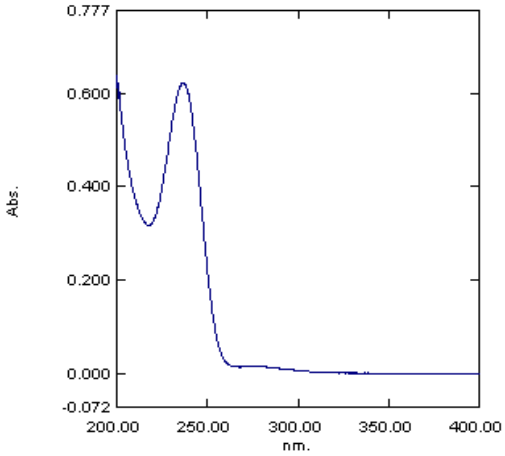

Fig. 2: UV Spectra of Metformin

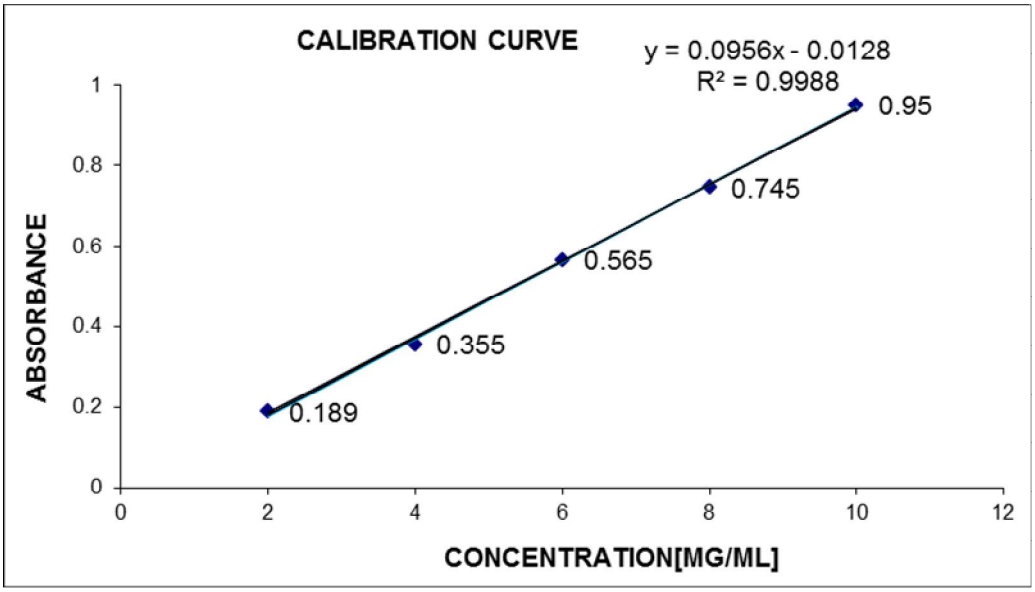

Fig. 3: UV Linearity graph of Metformin

REFERENCES

1. The Indian Pharmacopoeia, 4th ed.vol. I, New Delhi: The controller of publications, 1996:469.
2. Budavari .S. editors. In; The Merck index.13th edition, Whitehouse Station Merck &Co.Inc:2001:998.
3. Cheng CLO, Chou Ch. HPLC method for determination of Metformin in human plasma. *J Chromatography B Biomed Sciences Appl*.2001: 762:51-7.
4. Vesterquist O, Nabbie F and Swanson BJ. Determination of Metformin in plasma by HPLC after ultrafiltration. *J Chromatography B Biomed Sci Appl.* 1998;176:229-304.
5. Benzi L, Marchetti P, Cecchetti P, Navalesi R. determination of Metformin and phenformin in human plasma and

urine by RP-HPLC. *J Chromatogr Biomed appl.* 1986;9:184-6

6. Patil Sudarshan .S, Bonde C.G **(**2009) Development and Validation of analytical method for Simultaneous estimation of Glibenclamide and metformin HCl in bulk and tablets using UV – visible spectroscopy *International Journal of ChemTech Research*,1, 4, 905-909.
7. Sujana .K, Swathi.G, Rani, Bhanu Prasad .M, Saheethi.M (2010) Simultaneous estimation of Pioglitazone hydrochloride and metformin hydrochloride using UV Spectroscopic Method**,** *Journal of Biomed Science and Research*. 2 (2), 102-107.
8. Lakshmi.K.S., Rajesh.T, Sharma.S, Lakshmi.S (2009) Development and Validation of Liquid Chromatographic and UV Derivative Spectrophotometric Methods for the Determination of Metformin, pioglitazone and glimepiride in pharmaceutical formulations. *Der Pharma Chemica*; 1 (1) 238-246.
9. International conference on harmonization "Validation of analytical procedures Methodology",14, Federal Register Nov.1996, 1-8.
10. Reynolds DW, Facchine KL, Mullaney JF, Alsante KM, Hatajik TD, Motto MG, Available Guidance and Best Practices for Conducting Forced Degradation Studies. Pharmaceutical Technology, 2002; 2:48-56.
11. ICH, Q1A (R2) Stability testing of new drug substances and products, International Conference on Harmonization, 2003.
12. ICH, Q1B Stability testing: photo stability testing of new drug substances and Products,1996.
13. Chopde VV, Sensitive Analytical Methods for Determination of Stability of Drugs in Pharmaceutical Dosage Forms, 2008.
14. http://www.pharmainfo.net/reviews/sensitiveanalytical-methods-determination-stability drugs-pharmaceutical-dosage-forms.