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RP-HPLC Analytical Method Development and Validation of Metformin Hydrochloride Tablets Assay

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Abstract
An easy, definite and sensitive RP-HPLC method assay was developed and validated for Metformin Hydrochloride in pure and tablet dosage forms. The method uses Hypersil ODS C18, 25cm x 4.6mm x 5µm column and isocratic elution. Mobile phase was by adding 65% acetonitrile and 35% phosphate buffer and P^H was adjusted to 5.75 with 85v/v Ortho phosphoric acid was used at a flow rate of 1.0 ml/min. The UV detection was programmed at 233 nm. All statistical values are within the acceptable range and parameters are validated by statistical methods. The new method of development was efficient for quantitate estimation of the pure and Metformin Hydrochloride tablet.

Keywords
Metformin hydrochloride, RP-HPLC.

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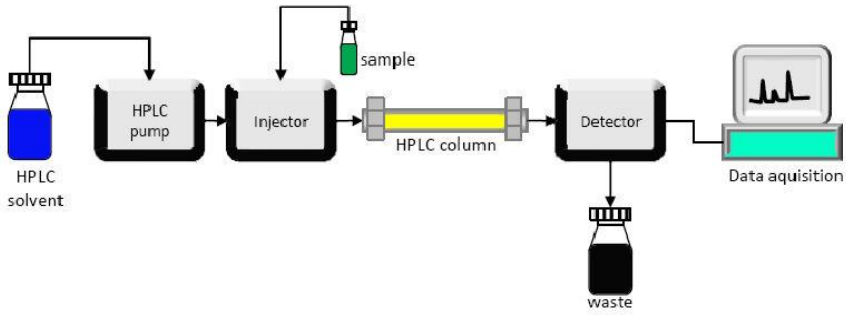
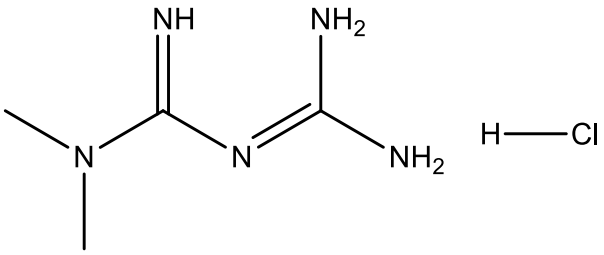
INTRODUCTION
The term "chromatography" was coined from Greek words chromo means color and graphein means writing. Chromatography is one of the best methods using for separation of dyes from plants from past centuries ago. The chromatography was discovered by Russian scientist Tswett separated the pigments from leaves. The noble prize was awarded to Martin and Synge for their extraordinary work on liquid-liquid chromatography.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:
NOW a day's HPLC is one of the mode of chromatography and common analytical techniques for separation, identification and quantification of pharmaceuticals in dosage forms. HPLC can be defined as technique of transferring mass between two phases (stationary and liquid mobile phase). The ratio of different solvents cause mobility of solutes with different speeds results in separation of mixtures. The instrumentation of HPLC
was shown in the below fig



DETAILS OF DRUG:

Drug Structure



IUPAC name:
3-(diaminomethylidene)-1, 1-dimethylguandine; hydrochloride
Mol formula: $C_4H_{11}N_5$
Mol weight: 129.164 g/mol
Storage: Store in a well closed container, between 15°C-30°C. Protect from Moisture

Solubility: It's water soluble, slightly soluble in alcohol and insoluble in acetone and methylene chloride.
Description
Metformin is mainly used for first line of treatment for TYPE-2 diabetes along with polycystic ovary syndrome treatment and not preferable in cardio and cancer patients.

MATERIALS AND METHODS:

Method development by RP-HPLC method for Metformin hydrochloride
↓
Selection of suitable related impurities for the estimation
↓
From physicochemical properties select detector λmax
↓
Selection of suitable stationary phase
↓
Development of suitable mobile phase
↓
Perform forced degradation experiments to challenge method
↓
Separation of each related substance finely
↓
Validation of the developed method

CHEMICALS AND REAGENTS: Potassium Dihydrogen ortho phosphate (Merck, AR grade), Ortho phosphoric acid (Merck, AR grade), Acetonitrile (Merck, HPLC grade), Water (Merck, HPLC grade), Hydrochloric acid (Merck), Sodium hydroxide (Merck), Hydrogen peroxide (Merck), 0.45 µm Nylon filter (Zodiac Life Sciences, Lot #100428027), 0.45 µm PVDF filter (Zodiac Life Sciences, Lot #100430019), Metformin hydrochloride (working standard LOT #3267,Baroque pharmaceuticals private limited), Diphenhydramine tablets BP 50mg (PL 1 602810010, Senate Laboratories), Metformin Hydrochloride (drug substances, CAS1115, Monark Biocare private limited)

INSTRUMENT/EQUIPMENT DETAILS: HPLC (Waters - Alliance 510 with UV- 484 Data Ace software), HPLC (Agilent 1100 Series with Chromeleon software), HPLC (Analytical column Hypersil - C18, 25cm x 4.6mm x 5µm), Analytical weighing balance (Mettler Toledo B204S), Millipore membrane 0.2µm (Sartorius ME235P), Sonicator (Ultrasonic Cleaner Power sonic 420), Vacuum oven (Wadegati; WIL-190), Refrigerator (Samsung RT41MASW)

RESULTS:

Validation of Analytical Method for the Assay of Metformin Hydrochloride

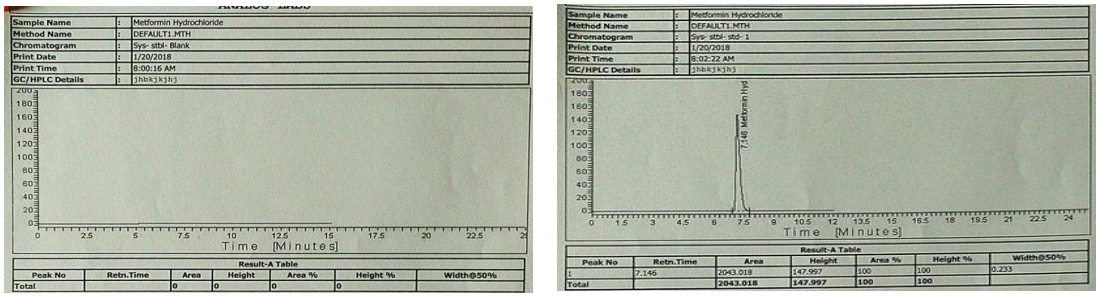
Validation id required to establish documentary evidence that the method meets the acceptance criteria, as the method given for assay is a pharmacopeial method and developed in-house. Typical Analytical Parameters used in Assay validation are System Suitability, Selectivity, Linearity and Range for standard, Method Precision, Intermediate Precision, Accuracy (% Recovery), Robustness, and Stability of Analytical Solution

System Suitability

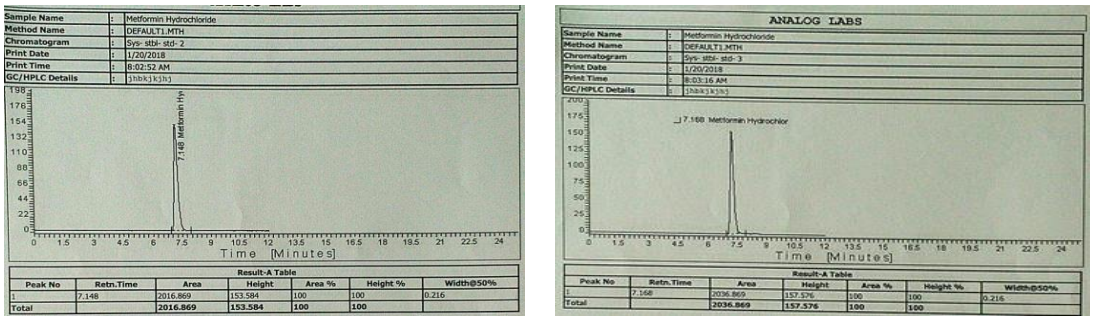
System suitability tests were carried out on five replicate injections of the standard solution containing Metformin Hydrochloride. Various parameters such as theoretical plates per meter, tailing factor were obtained.

System suitability Table:1

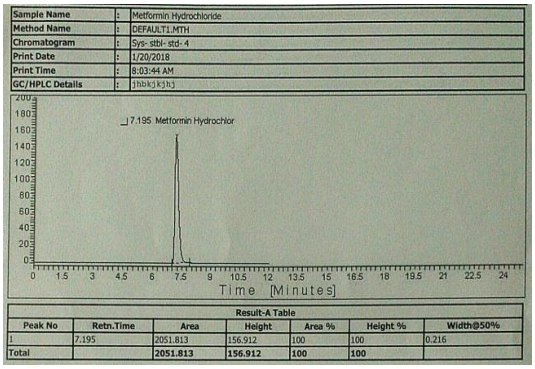
Sr. No.	Area of Metformin HCl
1	2043.01
2	2016.86
3	2036.86
4	2051.81
5	2042.45
Mean	2038.20

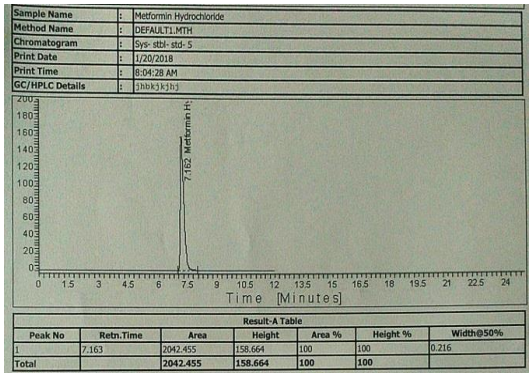


SYSTEM SUITABILITY -TYPICAL CHROMATOGRAPHY OF BLANK AND STD-1



TYPICAL CHROMATOGRAM OF STANDARD-2 AND 3





TYPCAL CHROMATOGRAM OF STANDARD-4 AND 5

Standard Deviation (±)	**13.07**
(%) Relative Standard Deviation	**0.64**

Selectivity:

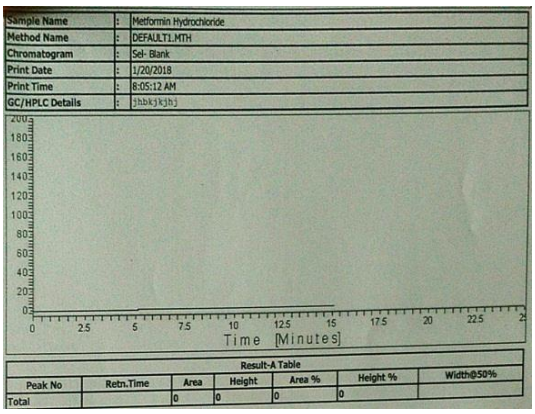
Selectivity was performed by injecting the diluent blank solution, system suitability solution, test solution. The Metformin Hydrochloride peak should be well resolved from any other peak and from each other. The diluent blank solution, excipient blend solution should not show any peak at the retention time of the Metformin Hydrochloride.

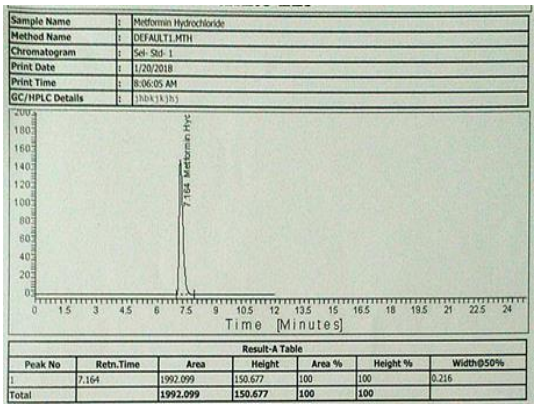
RESULTS:

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. All the injections were processed at the wavelength provided in the method. There was no interference observed from diluent blank solution, excipient blend solution with Metformin Hydrochloride peak. The method is selective.

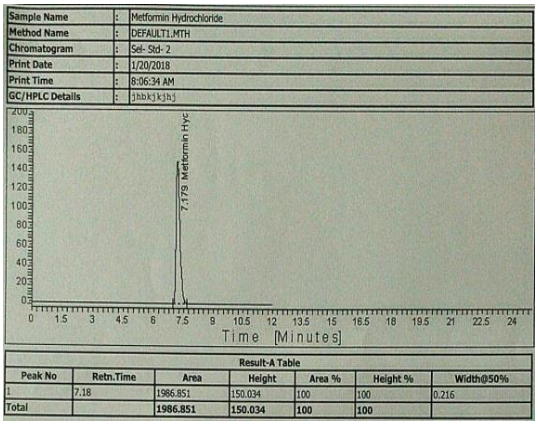
Selectivity Table :2

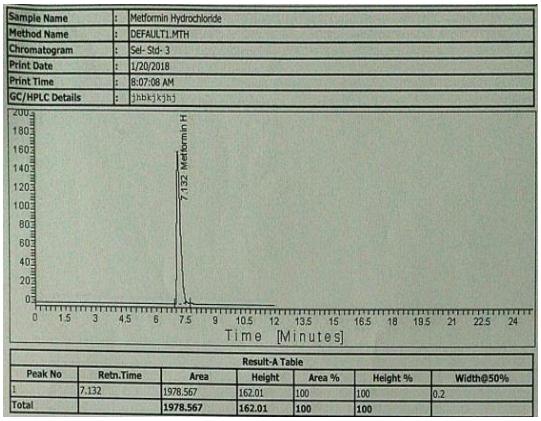
Sr. No.	Area of Metformin HCl in std
1	1992.09
2	1986.85
3	1978.56
4	1957.98
5	1979.95
Mean	1979.09
Standard Deviation (±)	13.00
(%) Relative Standard Deviation	0.66



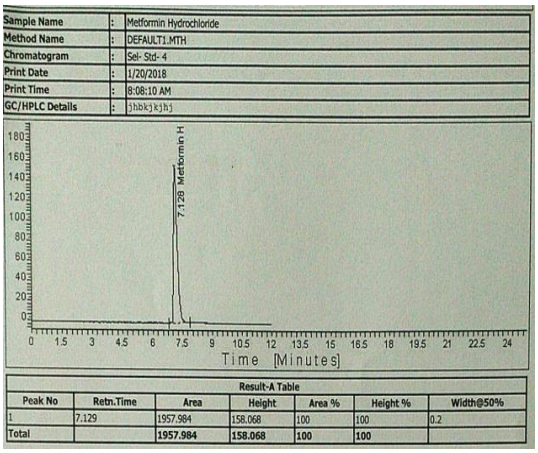


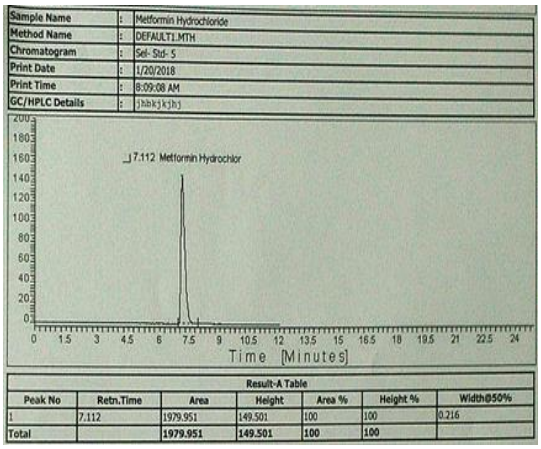
SELECTIVITY-TYPICAL CHROMATOGRAM OF BLANK & STD-1





TYPICAL CHROMATOGRAM OF STANDARD -2 AND 3





TYPICAL CHROMATOGRAM OF STANDARD-4 AND 5

All the injections were processed at the wavelength provided in the method. There was no interference observed from diluent blank solution, excipient blend solution with Metformin Hydrochloride peak. The method is selective.

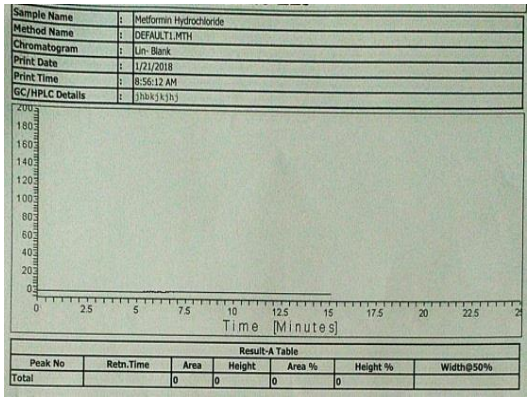
C. Linearity of Standard

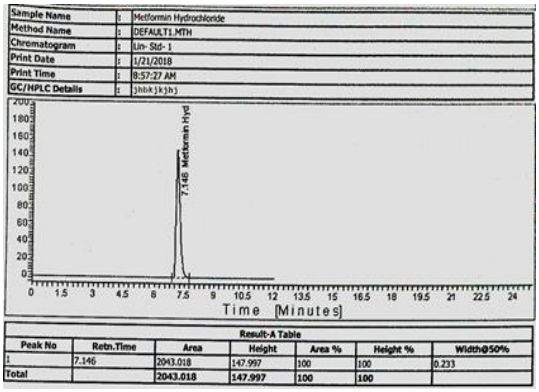
Demonstrate the linearity of the analytical method for assay by injecting the various concentrations of Standard preparation prepared in the range of 50% - 150% of test concentration, into the chromatograph, covering 5 different concentrations. Draw a plot between the concentrations in ppm Vs. Peak response of Metformin Hydrochloride WS. Report the slope, intercept and regression coefficient from the plot obtained for Concentration Vs. Peak response of Metformin Hydrochloride WS in standard preparation

Prepare a series of standard preparations (five preparations) of Metformin Hydrochloride over a range starting from 50% to at least 150% of the specified limits of assay.

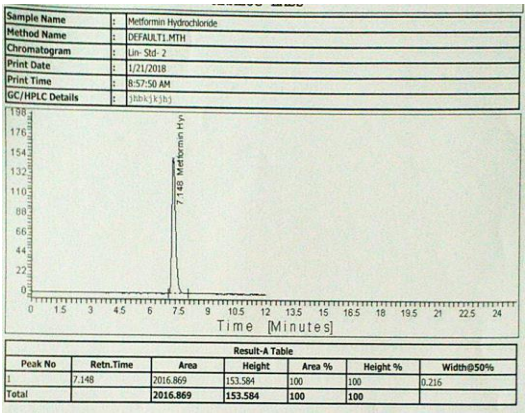
Dilutions for linearity of standard Table: 3

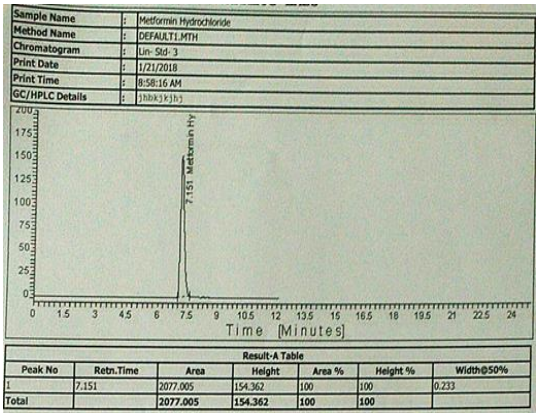
Linearity level	Sample conc	Amount of stock solution preparation of Metformin HCl to be transferred (ml)	Volume made up to (ml) with Diluent	Concentration of Metformin HCl (ppm)
Level – 1	50%	2.5 ml	50	50
Level – 2	75%	3.7 ml	50	75
Level – 3	100%	5.0 ml	50	100
Level – 4	125%	6.7 ml	50	125
Level – 5	150%	7.5 ml	50	150



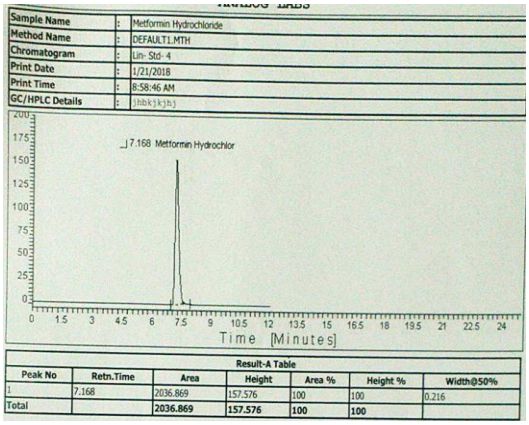


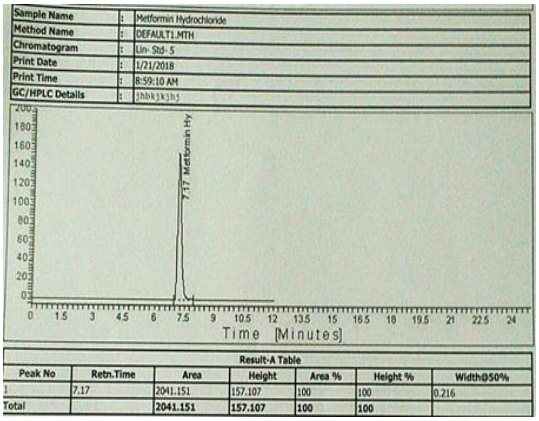
LINEARITY- TYPICAL CHROMATOGRAM OF BLANK AND STD-1





TYPICAL CHROMATOGRAM OF STANDARD-2 AND 3

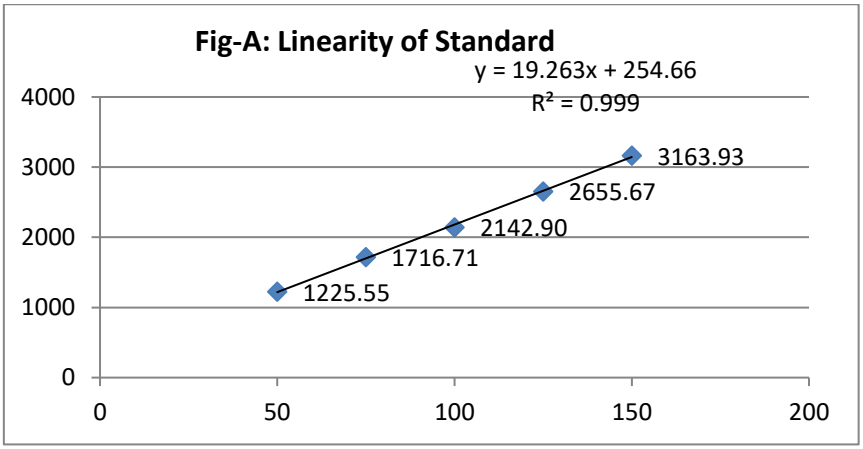




TYPICAL CHROMATOGAM OF STANDARD-4 AND 5

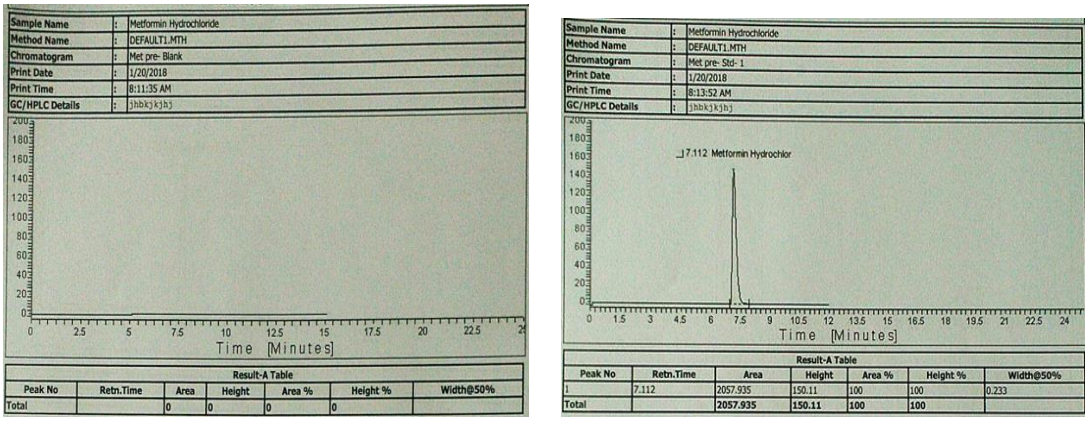
Linearity Level	Sample Concentration	Sample Concentration	Average Area (n = 2)	Correlation Coefficient
Level – 1	50	50	1225.55	
Level – 2	75	75	1716.71	
Level – 3	100	100	2142.90	0.999
Level – 4	125	125	2655.67	
Level – 5	150	150	3163.93	

Linearity of standard Table:4

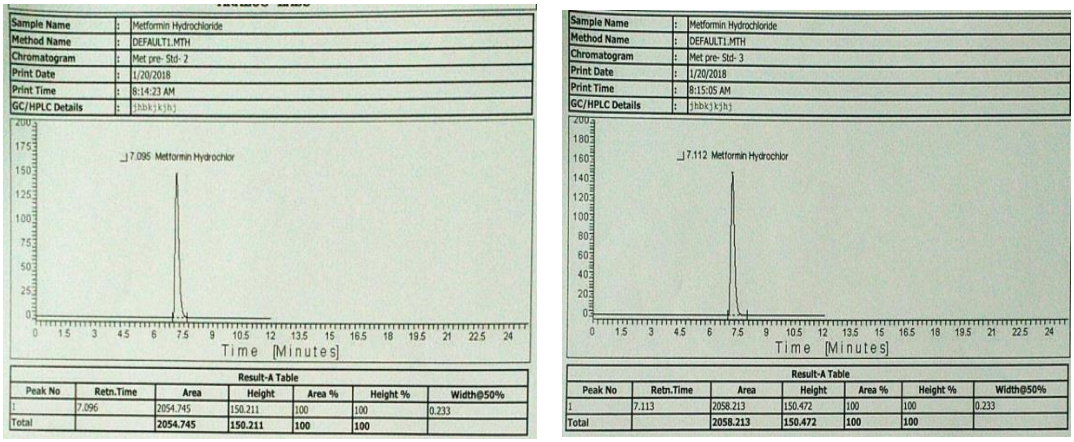


A linearity graph of the average area at each level against the concentration (%) is plotted and is found to be a straight line graph. The correlation coefficient is found to be more than 0.999. Hence it is concluded that, the method is found to be linear in the range of 50% to 150% of the working concentration. The range for the analytical method is 50 ppm to 150 ppm.

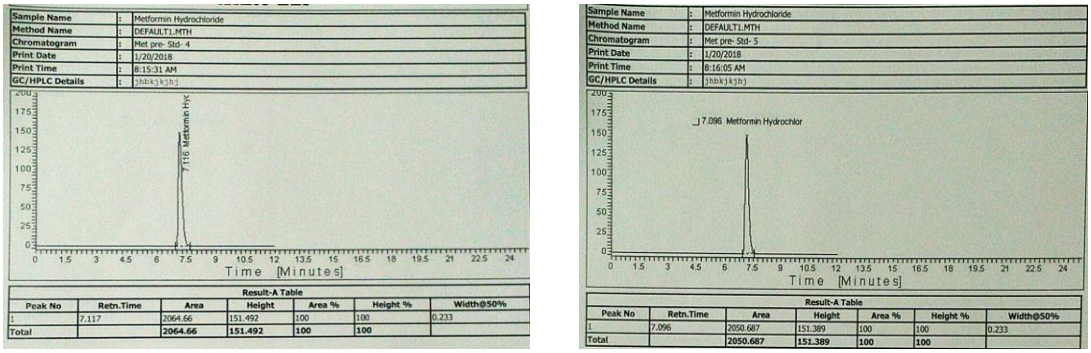
Method Precision: Six test solutions of Metformin Hydrochloride in Metformin Hydrochloride Tablets were prepared as per the analytical method.



METHOD PRECISION-TYPICAL CHROMATOGRAM BLANK AND STD-1



TYPICAL CHROMATOGRAM OF STANDARD-2 AND 3



TYPICAL CHROMATOGRAPH OF STANDARD-4 AND 5

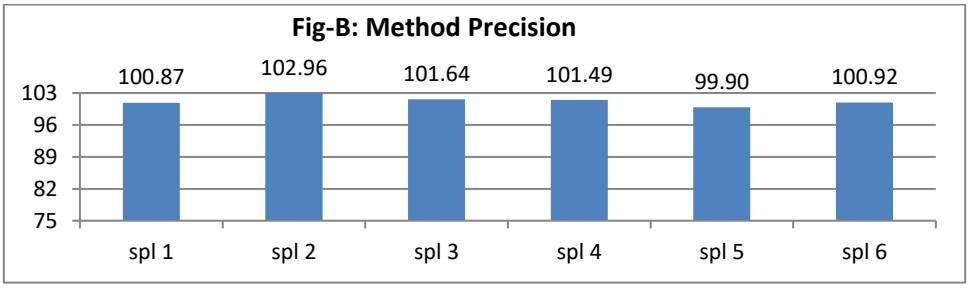
The % RSD of assay of six test solutions was calculated. % RSD of the results of six test solutions should not be more than 2.0%. The system suitability criterion was found to meet the pre-established acceptance criteria as per the analytical method. The results of assay obtained from six test solutions preparations are presented in below table.

System suitability for method Precision Table:5

Sr. No.	Area of Metformin HCl
1	2057.93
2	2054.74
3	2058.21
4	2064.66
5	2050.68
Mean	2057.24
Standard Deviation (±)	5.14
(%) Relative Standard Deviation	0.25

Results of Method Precision Table:6

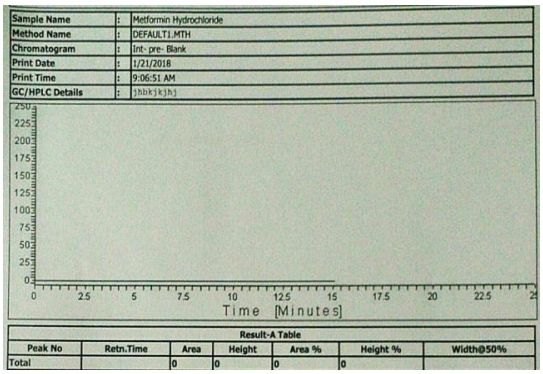
Test Solution	% Assay of Metformin HCl
1	100.87
2	102.96
3	101.64
4	101.49
5	99.90
6	100.92
Mean	101.30
Standard Deviation (±)	1.02
(%) Relative Standard Deviation	1.01

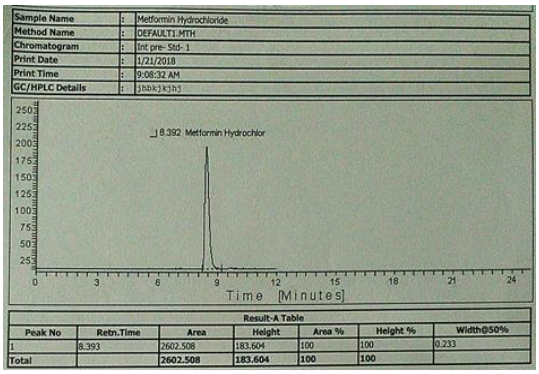


The % RSD of the six assay results is found less than 2.0% and meets the pre-established acceptance criteria. Hence, it is concluded that the method is precise.

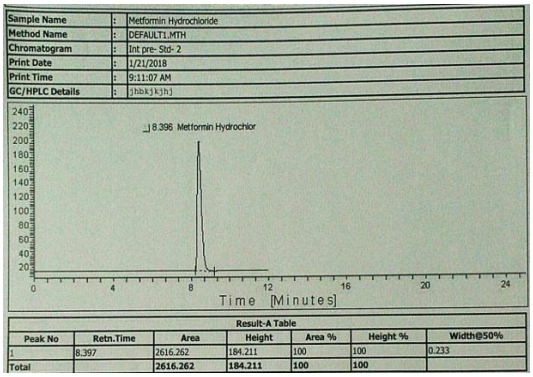
3.2 Intermediate Precision:

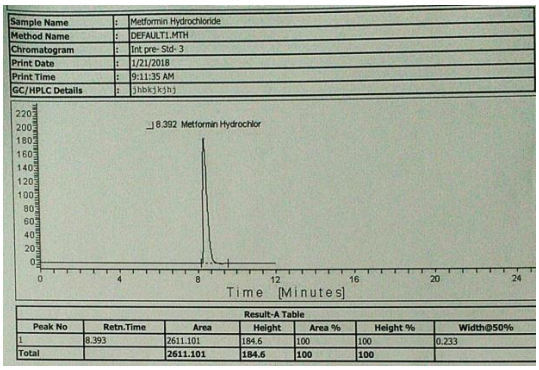
Six test solutions of Metformin Hydrochloride Tablets and were prepared as per the analytical method on different day. These test solutions were analyzed by a different analyst using different HPLC column of same make but having different serial number and different HPLC system. The % RSD of % assay results of twelve test solutions (six samples from method precision and six samples from intermediate precision.



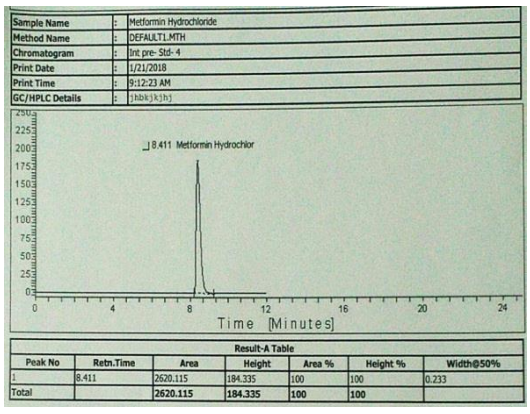


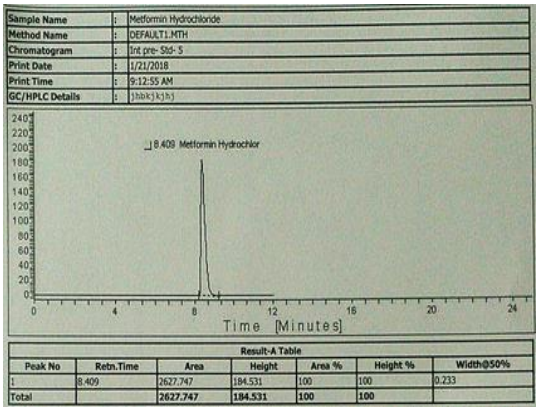
INTERMEDIATE PRECISION-TYPICAL CHROMATOGRAM OF BLANK AND STD-1





TYPICAL CHROMATOGRAM OF STANDARD-2 AND 3





TYPICAL CHROMATOGRAM OF STANDARD-4 AND 5

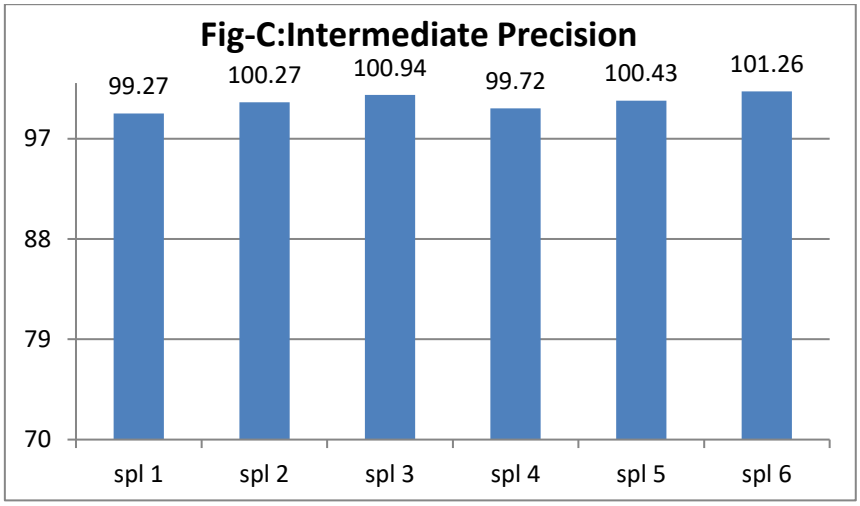
was calculated. % RSD of the results of twelve test solutions (six of method precision and six of intermediate precision) should not be more than 2.0%. The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. The results of assay obtained from six test solutions are presented in below table.

System suitability for Intermediate Precision Table:7

Sr. No.	Area of Metformin HCl
1	2602.50
2	2616.26
3	2611.10
4	2620.11
5	2627.74
Mean	2615.54
Standard Deviation (±)	9.49
(%) Relative Standard Deviation	0.36

Results of intermediate precision Table:8

Test Solution	% Assay of Metformin HCl
1	99.27
2	100.27
3	100.94
4	99.72
5	100.43
6	101.26
Mean	100.32
Standard Deviation (±)	0.74
(%) Relative Standard Deviation	0.74



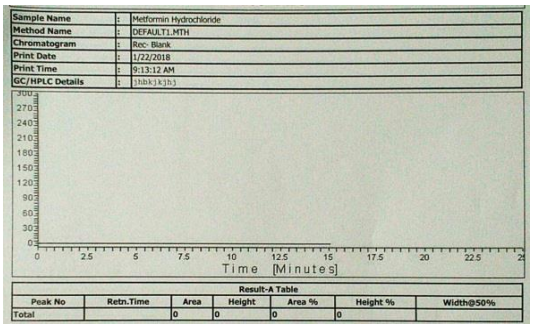
The analysis was carried out on six test solutions of the same lot of the drug product by two different analysts using two different equipment's within the same laboratory using two different columns of the same make but having different serial numbers on two different days.

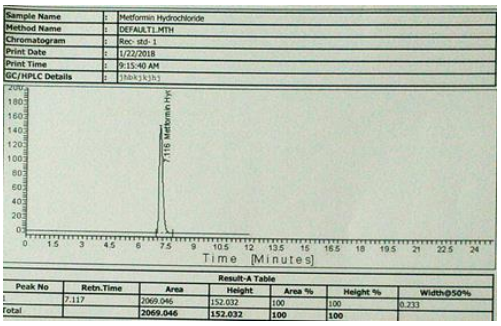
The % RSD of the six assay results is found to be less than 2.0%. Thus, the method is found to be rugged and precise.

Accuracy

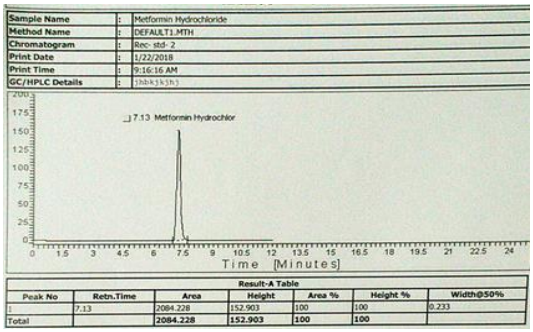
Accuracy study was performed by analyzing Metformin Hydrochloride test solutions which were prepared by mixing Metformin Hydrochloride API with excipient blend. These test solutions were prepared by adding a quantity of Metformin Hydrochloride API to excipient blend to produce three different concentration solutions equivalent to 50%, 75%, 100%, 125% and 150% of test concentration. Mean recovery at each concentration level should be between 97.0% and 102.0%. The results of accuracy study obtained are presented in below table

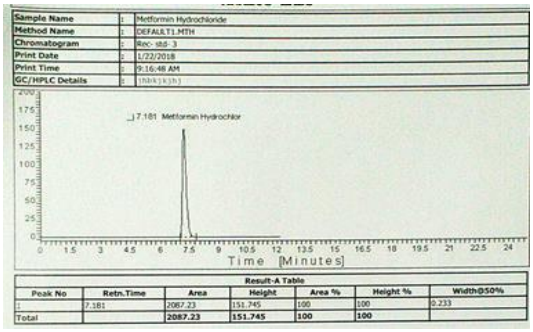
Accuracy (%Recovery) – results



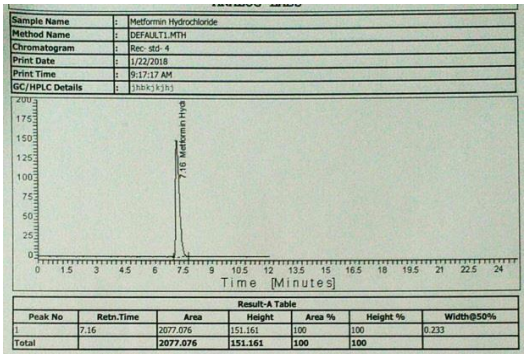


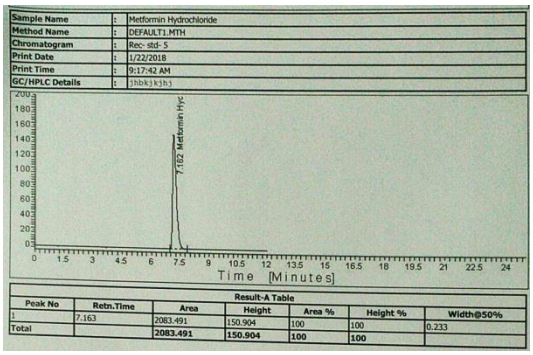
ACCURACY-TYPICAL CHROMATOGRAM OF BLANK AND STD-1





TYPICAL CHROMATOGRAM OF STANDARD-2 AND 3



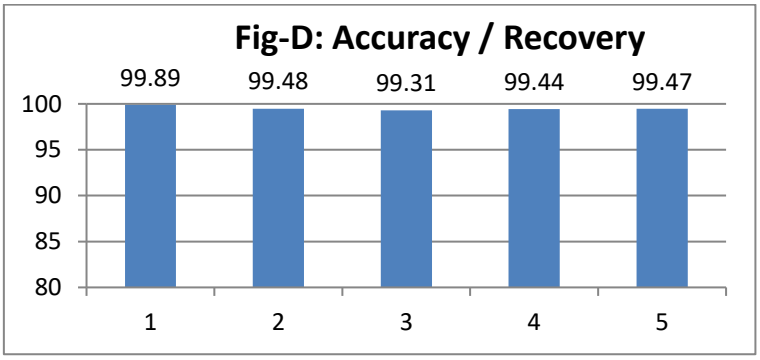


TYPICAL CHROMATOGRAM OF STANDARD-4 AND 5

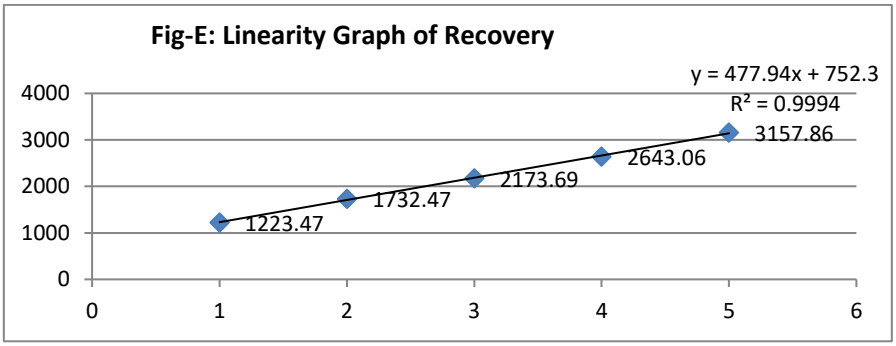
Accuracy Table :9

Level of addition	Amount of Metformin HCl added in mg	Amount of Metformin HCl found in mg	Recovery (%)
First Level (Rec-50 %)	28.2	28.17	99.89
Second Level (Rec-75)	40.1	39.89	99.48
Third Level (Rec-100%)	50.4	50.05	
Fourth Level (Rec-125%)	61.2	60.86	99.31
Fifth Level (Rec-150 %)	73.1	72.71	99.44
Mean			99.52
Standard Deviation (±)			0.22
(%) Relative Standard Deviation			0.22

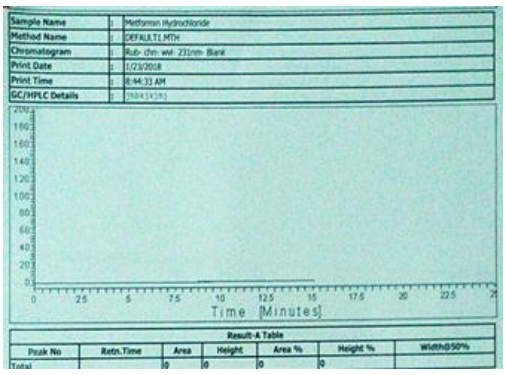
The percentage recovery for Metformin Hydrochloride at each level lies between 97.0% and 102.0%. % RSD at each recovery level is less than 2.0%. The analytical method meets the pre-established acceptance criteria for recovery study as per protocol. Hence, it is concluded that the method is accurate. System suitability criteria should pass as per analytical method and the % RSD between results obtained with changed condition and average result of method precision, should not be more than 2.0%.

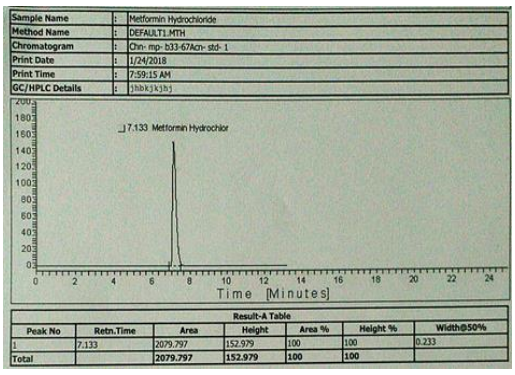


System suitability criteria should pass as per analytical method and the % RSD between results obtained with changed condition and average result of method precision, should not be more than 2.0%. For the linearity study five standard solutions of Metformin Hydrochloride were prepared from the range starting from 50% to 150% of the theoretical concentration of assay preparation. The system suitability solution and the linearity solutions were injected as per the protocol. The linearity graph of concentration against peak response was plotted and the correlation coefficient was determined. Correlation coefficient should be greater than or equal to 0.999.

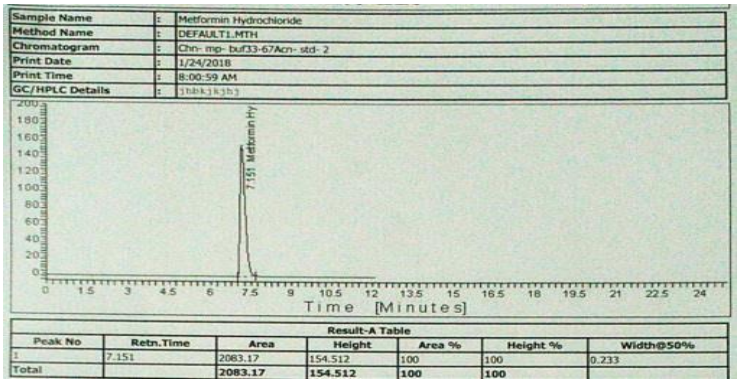


5.0 ROBUSTNESS





ROBUSTNESS-TYPICAL CHROMATOGRAM OF BLANK & STANDARD-1



Experiment:

Prepare two test solutions of the same lot of Metformin Hydrochloride in Metformin Hydrochloride Tablets 850mgas per analytical method. Inject this solution along with diluent blank solution and system suitability solution along different chromatographic conditions as shown below:

Change in flow rate (± 0.2 ml/minute)
Change in wavelength (± 2 nm)
Change in composition of mobile phase (± 20ml)

Change in Flow Rate (± 0.2mL/minute):(Normal Experimental Condition: 1.0ml/minute)

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method.

Results for change in flow rate Table: 10

Flow rate →	0.8mL/minute	1.2mL/minute
Sample	% Assay	
Test solution	100.66	100.16
Average assay result from method precision	101.3	101.3
Mean	100.98	100.73
Standard Deviation (±)	0.45	0.81
(%) Relative Standard Deviation	0.45	0.80

Change in Wavelength (± 2 nm) :(Normal Experimental Condition: 233nm)

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method.

Results for change in wavelength Table:11

Wavelength →	231nm	235nm
Sample	% Assay	
Test solution	100.49	100.66
Average assay result from method precision	101.3	101.3
Mean	100.90	100.98
Standard Deviation (±)	0.57	0.45
(%) Relative Standard Deviation	0.57	0.45

Change in composition of Mobile Phase (± 20ml)
(Normal Experimental Condition: Buffer: Acetonitrile=30:70)

The system suitability reached the criteria and % RSD is less than 2.0% between the freshly prepared test solution and the stored test solution

Results for change in composition of mobile phase Table: 12

Composition of Mobile Phase	33B :67ACN	37B :63ACN
Sample	% Assay	
Test solution	100.5	101.41
Average assay result from method precision	101.3	101.3
Mean	100.90	101.36
Standard Deviation (±)	0.57	0.08
(%) Relative Standard Deviation	0.56	0.08

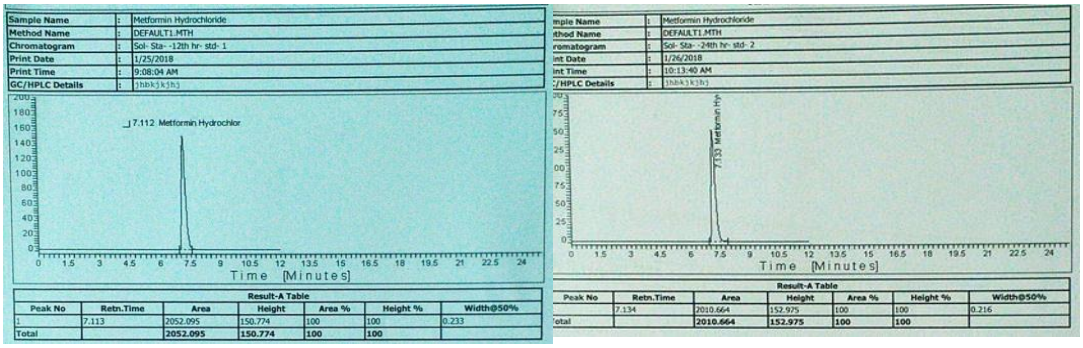
The analysis of metformin hydrochloride tablets from same lot was carried out at different conditions of column lot, flow rate, wavelength and composition of mobile phase. The system suitability reached the criteria and % RSD is less than 2.0% between the freshly prepared test solution and the stored test solutions. The analytical method meets the pre-established acceptance criteria for robustness study as per protocol. Thus, the method is robust.

Stability of Analytical Solution

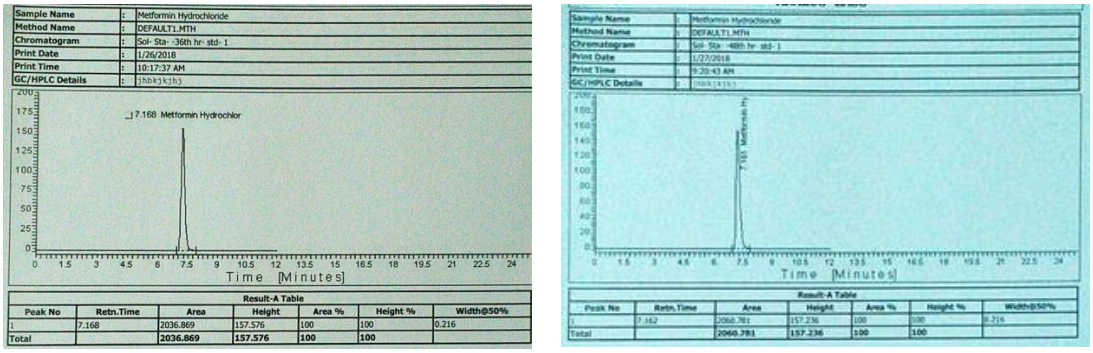
TIME	Std Area	Spl area	% Assay of Metformin HCl
0th hr	2006.86	2044.31	101.44
12th hr	2049.93	2015.23	97.90
24 hr	2008.14	2014.76	99.91
36 hr	2043.75	2040.37	99.42
48 hr	2058.48	2069.77	100.13
Mean	2033.43	2036.89	99.76
Standard Deviation (±)	24.25	22.95	1.28
(%) Relative Standard Deviation	1.19	1.13	1.28

The experiment solutions were prepared on 0th, 12th, 24th, 36th and 48th hour of and stored at a room temperature for every time interval up to 48 hrs. The test solutions are freshly prepared at the point of analysis. The analyte is assumed to be stable when there is no significant change in the percent of assay. All the criteria's were found to be to meet the initial-estimated acceptance criteria of analytical method. Stability of analytical solution Table:13

The system suitability reached the criteria and % RSD is less than 2.0% between the freshly prepared test solution and the stored test solutions. After 48 hours, significant changes were not reported in assay at room temperature. We concluded that the solution was stable up to 48hours. Solution stability-typical chromatogram of blank & 0thhr



Typical chromatography of standard at hours 12th&24th



Typical chromatogram of 36th and 48th hour

CONCLUSION:

The overall validated data mentioned in this article clearly shows the analytical method of assay of metformin hydrochloride in metformin hydrochloride tablets 850mg by HPLC is found to be suitable, selective, specific, precise, linear, accurate and robust. After 48 hours at the room temperature the analytical solution was found to be stable and it is confirmed that the method was validated for common analysis of the drug samples.

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