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Stability indicating assay method for estimation of Metformin in Metformin Hydrochloride extended-release tablets

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ABSTRACT: Background: Metformin hydrochloride is an oral antihyperglycemic drug used in the management of type 2 diabetes. However, gastrointestinal (GI) intolerance may limit use in the same patients. Extended-release Metformin improves GI tolerability and allows once-daily dosing. **Aim:** To develop and validate an accurate, precise, sensitive, and economical method for the estimation of metformin hydrochloride in extended-release tablets dosage form. **Methods:** The chromatographic separation was achieved on the reversed-phase column, µ Bondapack, C18, 10µm, 125A°, 3.9 × 300 mm, a mobile phase comprising sodium chloride and 1-Heptane Sodium Sulfonic acid salt buffer (pH 3.85): Acetonitrile (90:10 v/v). A detection wavelength of 218 nm as λmax, 1.5 ml/min flow rate, 30°C column oven temperature, and methanol, acetonitrile, and water were assessed as a solvent for sample preparation which for the determination of metformin. **Results:** The developed method resulted in the elution of metformin hydrochloride at 4.810 min. Metformin concentration range 0.015-0.045 mg/ml (r^2=0.9999). The mean recovery of Metformin was found to be 99.27 (% RSD 0.12), 99.88 (% RSD 0.20) and 99.93 (% RSD 0.07) at levels 50, 100, and 150 % respectively. The solution stability of Metformin in standard solution and sample solution were found to be 68 h at operating condition, at refrigerator condition the standard solution and sample solution found stable up to 68 hrs and 66 h respectively. The developed method was validated according to ICH guidelines and values of precision, accuracy, and other statistical analyses were found to be in good accordance with the prescribed values. **Conclusion:** Thus, the proposed methods were successfully applied for the determination of metformin in routine industrial work.

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INTRODUCTION:

Metformin hydrochloride is an oral antihyperglycemic drug used in the management of type 2 diabetes. Metformin hydrochloride (N, N-dimethyl imido dicarbo nimidic diamide hydrochloride) is not chemically or pharmacologically related to any other classes of oral anti-hyperglycaemic agents [1]. The structural formula is shown in Fig 1. A fixed-dose containing metformin hydrochloride is available in the market in the tablet dosage form. Several methods have been reported for the

estimation of metformin hydrochloride such as high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection [2-6] or fluorescence detection [7] and capillary electrophoresis (CE) with ultraviolet (UV) detection [8,9]. Most of the methods are tedious and time-consuming, involving complex sample preparations. The developed method can be applied successfully for quality control and stability testing purpose. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines [10-14].

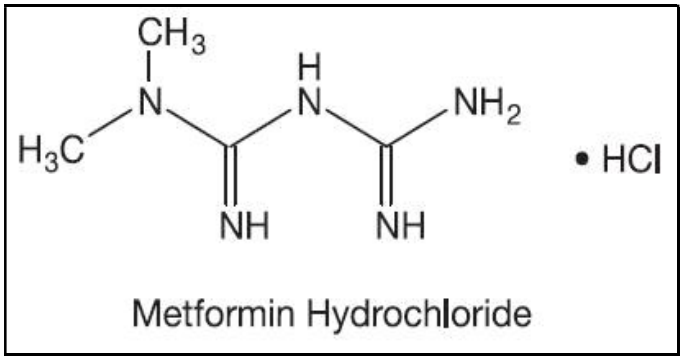

Fig 1. Molecular structure of metformin hydrochloride.

MATERIALS AND METHODS:

Chemicals and reagents:

Metformin Hydrochloride working standard and commercial pharmaceutical preparation of Metformin HCl ER tablets, containing 750 mg of metformin hydrochloride was made available by Oman Pharmaceutical Products LLC. Acetonitrile and Methanol were procured from Merck Ltd. and Lobachemie respectively. A 0.45 µm nylon filter (pall life sciences, India) was used. All other chemicals and reagents used were analytical grade.

Instrumentation:

The proposed work was carried out on Water's HPLC (Model: e2695 separation module) equipped with a PDA detector. All weighing was done by electronic balance (Model: Mettler Toledo). A fast-clean ultrasonic cleaner was used for degassing the mobile phase and sample preparation.

Selection of solvent:

Based on the solubility study methanol, acetonitrile, and water were selected as the solvent for dissolving metformin hydrochloride and associate excipients [15].

Preparation of diluent:

About 900 ml of double distilled water, 80 ml of Acetonitrile, and 20 ml of Methanol were mixed well.

Preparation of Metformin Hydrochloride Standard Stock Solutions:

Accurately weighed 30 mg of Metformin Hydrochloride working standard was taken in a 100 ml volumetric flask. It was diluted to volume with diluent to obtain a solution of the strength of 0.3 mg/ml.

Metformin hydrochloride standard solution:

About 5 ml of Metformin hydrochloride standard stock solution was transferred into a 50 ml volumetric flask and diluted up to the mark with diluent to prepare the solution of Metformin hydrochloride of strength 0.03 mg/ml.

Preparation of diluted Ortho-phosphoric acid:

About 1 ml of Ortho-phosphoric acid was diluted into a 20 ml volumetric flask with water.

Preparation of buffer solution for mobile phase:

About 0.5 g each of Sodium 1-Heptanesulfonate sodium salt and Sodium chloride were dissolved in 900 ml of mili q water. The pH of the solution was adjusted to 3.85 ± 0.02 with diluted Ortho-phosphoric acid solution, and finally, the solution was diluted to 1000 ml with mili q water.

Preparation of mobile phase:

The Acetonitrile and buffer solution were mixed in a ratio of 100:900 v/v respectively. The mobile phase solution was degassed by sonication for about 10 to 15 min.

Preparation of blank solution:

The blank solution was prepared by using the diluent.

Preparation of Sample solution:

An accurately weighed sample equivalent to 750 mg of Metformin HCl was taken into a 500 ml volumetric flask. About 150 ml methanol was added and sonicated for 15 min with intermittent swirling. Then, 250 ml acetonitrile was added and further sonicated for 15 min with intermittent swirling.

The flask was removed from the sonicator and the solution was allowed to cool at room temperature. Then the solution was diluted to volume with water and mixed well. Further, the solution was centrifuged at 3000 rpm for 5 min.

About 2 ml of the supernatant solution was transferred into a clean and dry 100 ml volumetric flask and diluted with diluent. The sample solution was filtered through 0.45µ PVDF filter in a HPLC vial after discarding 5 ml of the filtrate.

Calculation formula:

The content of the drug in the solution was assayed by using the following equation 1.

Assay (%) = [(AT×WS×5×500×100×P×100×AW)/ (AS×100×50×WT×2×100×LC)] ....(1)

Where; AT and AS area of metformin in sample solution and the standard solution respectively. WS and WT weight of metformin hydrochloride standard and weight of the sample. P, purity of metformin hydrochloride standard. LC, lable claim of metformin hydrochloride in the marketed product. AW, the average weight of the sample.

System suitability criteria:

The % RSD of five replicate injections of the standard solution for peak area response of Metformin should not be more than 2.0 and the cumulative % RSD for five replicate injections of the standard solution including the Bracketing standard should not be more than 2.0. Tailing factor of Metformin peak in standard solution peak should not be more than 2.0. Theoretical plates for Metformin peak in standard solution should not be less than 2000.

Validation of proposed method:

The metformin in Metformin HCl extended-release tablet was validated by using chromatographic method for the parameters like system precision, rudggedness, specificity, force degradation, linearity and range, accuracy, solution stability, and Robustness as per the standard procedure mentioned in pharmacopoeia [16-20].

RESULTS AND DISCUSSIONS:

System Precision:

Evaluated system suitability results for all the method validation parameters (Precision, Intermediate precision, Linearity, accuracy).

Table 1. The System suitability.

Injec-tions	SAM	PCM	TFM
1	598698	8446	1.1
2	597562	8517	1.1
3	599264	8656	1.2
4	598875	8714	1.1
5	599583	8672	1.1
Mean	598796		
SD	770.875		
% RSD	0.13		

SAM - Standard Area of metformin, PCM - Plate count of metformin, TFM - Tailing Factor metformin.

Reported system suitability results from method precision parameters as % RSD, tailing factor, and theoretical plate count of metformin standard solution was found to be 0.13, 8449, and 1.1 respectively. Data is given in Table 1 and 2.

Table 2. System suitability criteria.

Criteria	Limit	Results
The % RSD of five replicate injections of the standard solution for peak area response of metformin should not be more than 2.0	NMT 2.0	0.13
Tailing factor of metformin peak in standard solution peak should not be more than 2.0	NMT 2.0	1.1
Theoretical plates for metformin peak in standard solution should not be less than 2000	NLT 2000	8446

Method precision:

Six samples were prepared as per the test methodology and injected in duplicate. The mean assay and % RSD of metformin in the sample solutions were found 100.39 % and 0.54 respectively as data given in Table 3.

Table 3. The method Precision results.

Sample Name	SW (mg)	Mean Area	% Assay
P-1	1140.15	614932	101.1
P-2	1140.31	612600	100.7
P-3	1115.77	599090	100.7
P-4	1114.64	593280	99.8
P-5	1117.55	597123	100.2
P-6	1118.48	595366	99.8
Mean			100.39
SD			0.542
%RSD			0.54

P – Preparation, SW – Sample weight.

Ruggedness:

The ruggedness of the proposed method was determined by the analysis of the same lot of samples by different analysts using the same operational and environmental conditions on different days and different chromatographic systems. The mean assay and

% RSD of six sample preparations was found to be 100.3 % and 0.25, and the cumulative mean assay of 12 sample preparations with cumulative % RSD was found to be 100.3 % and 0.4. The data are given in Tables 4 to 6.

Table 4. Method Intermediate Precision Study.

Sample Name	SW (mg)	Mean Area	% Assay
P-1	1115.08	595378	100.3
P-2	1114.14	594828	100.3
P-3	1115.43	597987	100.7
P-4	1116.00	595942	100.3
P-5	1115.06	593192	99.9
P-6	1115.64	596313	100.4
		Mean	100.3
		SD	0.248
		%RSD	0.25

P – Preparation, SW – Sample weight.

Table 5. Comparison table between precision and intermediate precision.

Analyst 1		Analyst 2	
Sample name	% Assay	Sample name	% Assay
Sample-1	101.1	Sample-1	100.3
Sample-2	100.7	Sample-2	100.3
Sample-3	100.7	Sample-3	100.7
Sample-4	99.8	Sample-4	100.3
Sample-5	100.2	Sample-5	99.9
Sample-6	99.8	Sample-6	100.4

Table.6. Cumulative results between precision and intermediate precision.

Cumulative Average	100.3
Cumulative SD	0.405
Cumulative % RSD	0.4

Specificity:

Blank solution, placebo solution, sample solution as such, sample solution spiked with known impurities at 1.0%of target concentration (1000 ppm – related substances test)and individual impurities were analyzed. To check the interference at the retention of metformin, peak purity was evaluated in standard and sample solutions. The retention time of metformin and related compounds A, B, C, and D were found to be 4.81, 2.07, 3.79, 4.19, and 10.82 min respectively. For metformin in standard solution and sample solution – as such and spiked sample, purity angle was found less than the purity threshold. The data is given in Tables 7 and 8 and reference chromatograms are presented in Fig 2 to 6 respectively.

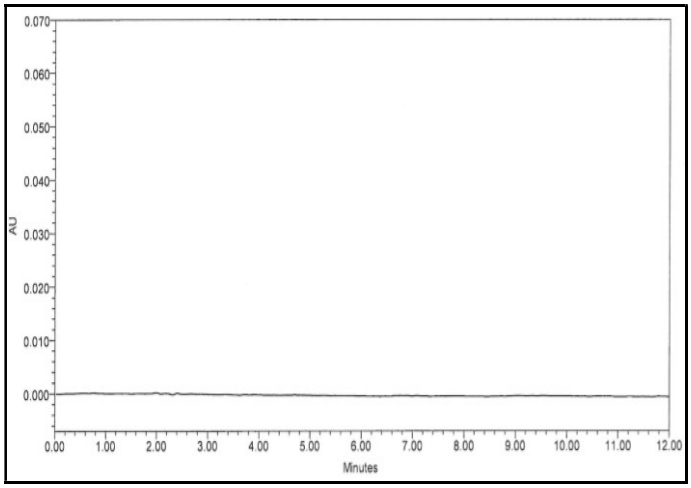

Fig 2. Reference chromatogram of Blank Solution.

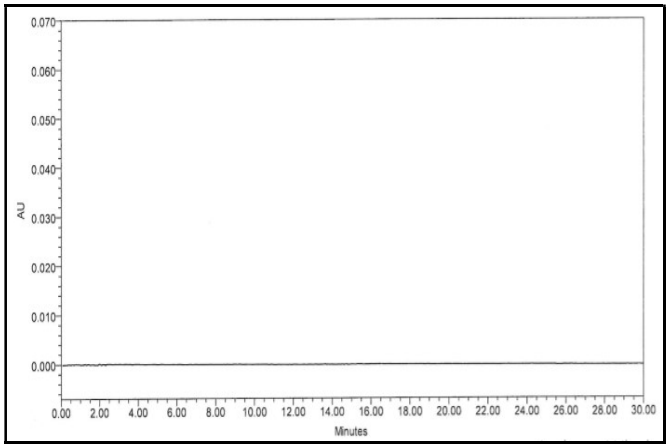

Fig 3. Reference chromatogram of Placebo Solution.

Table 7. Observation of Peak Purity Results.

Condition	Purity angle	Purity threshold
Standard Solution	0.278	0.408
Control Sample	0.281	0.468
Spiked Sample	0.191	0.668

Table 8. Specificity results.

Name of Component	Retention time (min)						
	Blank	Placebo	Impurity A	Impurity D	Impurity B	Spike Sample	Impurity C
Blank	ND	ND	ND	ND	ND	ND	ND
Placebo	ND	ND	ND	ND	ND	ND	ND
Impurity A	ND	ND	2.07	ND	ND	2.058	ND
Impurity D	ND	ND	ND	3.794	ND	3.773	ND
Impurity B	ND	ND	ND	ND	4.19	4.167	ND
Metformin	ND	ND	ND	ND	ND	4.81	ND
Impurity C	ND	ND	ND	ND	ND	10.717	10.817

***ND: Not Detected.**

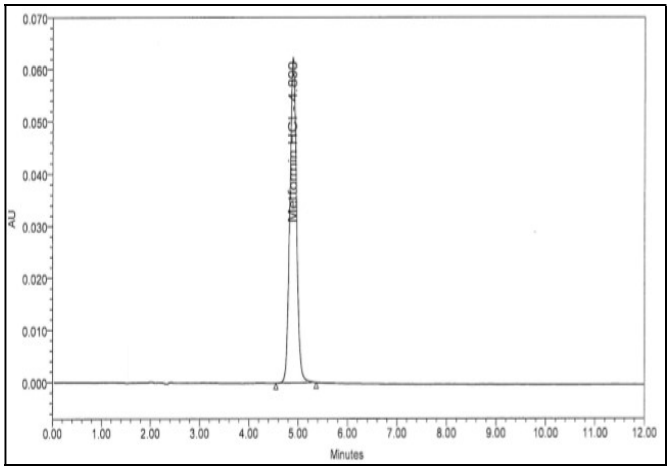

Fig 4. Reference chromatogram of Standard Solution.

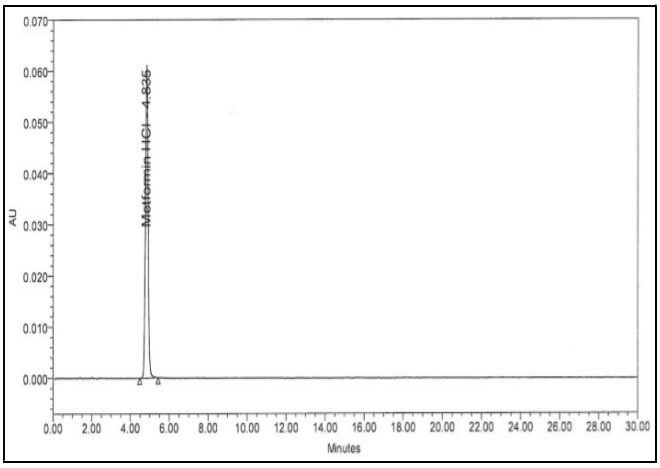

Fig 5. Reference chromatogram of Control Sample Solution.

Force degradation:

In addition, to prove there was an absence of interference of degradation products, the degradation study was conducted and the product was exposed to degradation conditions such as acidity, alkalinity, oxidation, temperature, and light exposure, in a similar stage purity of metformin was established. The proposed study was conducted in three stages to achieve the desired degradation ranges from 5 to 30 %. The data is given in Table 9.

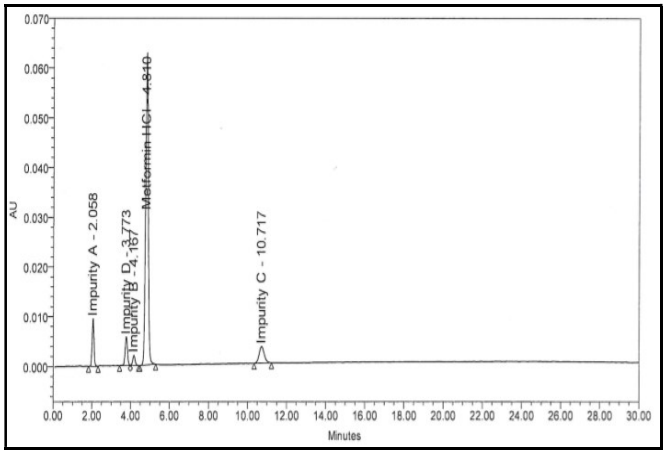

Fig 6. Reference chromatogram of Spike Sample Solution.

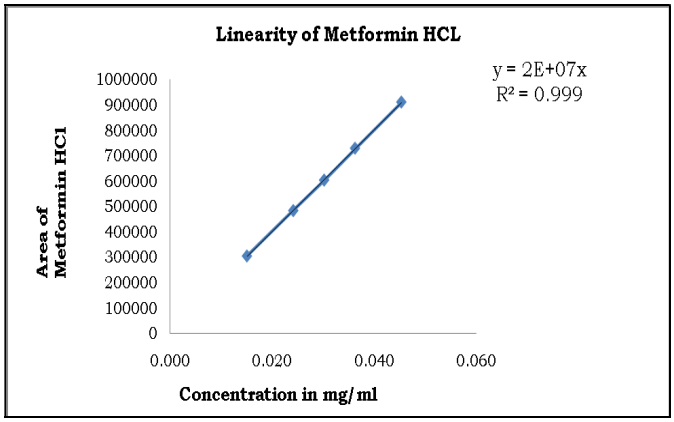

Fig 7. Linearity plot for Metformin HCl.

Linearity and Range:

The linearity study was performed to encompass the range of the method. Linearity levels at concentrations of 50, 80, 100, 120 and 150 % of the target concentration were prepared and each solution was injected in duplicate. For the range experiment, 50 and 150 % concentration solution were injected in six replicates.

Table 9. Force degradation results.

D Stage's	Sample Name	% Assay	% Degradation	Purity	
				Purity angle	Purity Threshold
FD Stage 1	Control Sample	99.6	-	0.281	0.468
	30% H_2O_2_1 h_100°C	97.5	2.1	3.588	24.959
	1N NaOH_1h_100°C	83	16.7	0.38	0.551
	1N HCl_1h_100°C	97.7	1.9	0.284	0.526
	105°C_5 Days	101.8	2.2	0.289	0.429
	1.2 million Lux.	99.4	0.2	0.293	0.465
FD Stage 2	Control Sample	100.2	-	0.361	0.519
	Conc.HCl_1_h_100°C	101.7	1.4	0.363	0.49
	30 % H_2O_2_2 h_100°C	92.6	7.6	2.812	23.937
FD Stage 3	Control Sample	100	-	0.407	0.563
	Conc.HCl_7 Days	86.9	13.1	0.377	0.665
	30% H_2O_2_4h_100°C	68.9	31.1	3.06	25.431

Plot the concentration of the analyte versus the response using a linearity spreadsheet and determine the correlation coefficient, intercept, slope and bias.

Table 10. Linearity results.

Linearity Level	Concentration (mg/ml)	Mean Area
Linearity- 50%	0.015	303960
Linearity- 80%	0.024	482737
Linearity- 100%	0.03	602840
Linearity- 120%	0.036	728189
Linearity- 150%	0.045	910049

Table 11. Linearity results.

Observation	Result
Correlation coefficient	0.999963718
Intercept	-1575.041379
Slope	20077997.81
Bias	-0.261270434

Table 12. Range results.

Prepara-tions	50% Level Conc. Area	100% Level Conc. Area
1	304269	912183
2	303650	907915
3	303316	908779
4	303558	908071
5	303330	908361
6	303479	911901
Avg	303600	909535
STD	352.142	1966.081
% RSD	0.12	0.22

For range, the calculated % RSD of each of the six replicate injections of 50 and 150 % concentration solution was found to be 0.12 and 0.22 respectively. The data is given in Tables 10 to 12 and Fig 7.

Accuracy:

The accuracy study of the proposed method was ascertained based on the recovery study performed by the standard addition method. A known amount of standard solutions were added to the placebo powder to make the final concentration in the range of 50, 100, and 150 %, and each level was prepared in triplicate. The preparations were then analyzed by the proposed method. Each preparation was injected in duplicate. The recovery was calculated using a formula as given in equation 2. Recovery (%) = (ADR/ AA) …(2)

Where ADR - Amount of drug recovered and AA – Amount added.

The mean recovery for metformin was found to be 99.27±0.12, 99.88±0.2, and 99.93±0.07 % at 50 %, 100 % and 150 % respectively. The data is given in Tables 13 to 15.

Solution Stability:

Standard and sample solutions were prepared as per the proposed methodology and these shall be considered the initial time points samples (0 h). Further, the standard and sample solutions were divided into three parts. The vials were kept at respective temperature conditions i.e., working conditions (benchtop) and refrigerator (2 to 8 ºC). The stability of Metformin in the standard solution was estimated to be 68 h at operating temperature conditions and refrigerator conditions (2 to 8 ºC) was 68 h

Table 13. Accuracy 50 % level.

Level	Weight of API (mg)	Amount added	Amount recovered	% Recovery
Accuracy 50% - 1	376.4	15	14.9	99.4
Accuracy 50% - 2	376.16	15	14.9	99.23
Accuracy 50% - 3	376.46	15	14.9	99.17
			Mean	99.27
			SD	0.12
			%RSD	0.12

Table 14. Accuracy 100 % level.

Level	Weight of API (mg)	Amount added	Amount recovered	% Recovery
Accuracy 100% - 1	751.42	30	29.9	99.65
Accuracy 100% - 2	751.39	30	30	99.96
Accuracy 100% - 3	751.47	30	30	100.03
			Mean	99.88
			SD	0.2
			%RSD	0.2

Table 15. Accuracy 150 % level.

Level	Weight of API (mg)	Amount added	Amount recovered	% Recovery
Accuracy 150% - 1	1125.37	44.9	44.9	100.01
Accuracy 150% - 2	1125.43	44.9	44.9	99.9
Accuracy 150% - 3	1125.76	44.9	44.9	99.88
			Mean	99.93
			SD	0.07
			%RSD	0.07

conditions (2 to 8 °C). Similarly, the solution stability established for Metformin in sample solution at operating conditions and refrigerator condition (2 to 8 °C) was 68 and 66 h respectively. The data is given in Tables 16 to 19.

Table 16. Standard Solution stability at Benchtop.

Sample Name	Standard Area	% Assay	% Difference
0 h	598698	100	-
7 h	597822	99.9	0.1
13 h	598910	100	0
18 h	598222	99.9	0.1
31 h	606049	101.2	1.2
43 h	607383	101.5	1.5
68 h	605000	101.1	1.1

Filter Study:

The standard was prepared in single preparation and sample solution in triplicate preparations as per methodology. Centrifuged from each of the triplicate preparation a small portion at 3000 rpm for 5 min.

Table 17. Sample solution stability at Benchtop.

Sample Name	Sample Area	% Assay	% Difference
0 h	615302	101.2	-
4 h	610874	100.5	0.7
11 h	610234	100.4	0.8
16 h	611714	100.6	0.6
29 h	618226	100	1.1
41 h	620904	102	0.8
66 h	622849	101.9	0.7

This would be considered the control sample which has been centrifuged and used to evaluate the filter compatibility. The remaining portion of the sample solutions was filtered using the following filters, 0.45µ PVDF syringe filter, and 0.45µ Nylon syringe filter. The filtrates were collected from individual filters after filtering 1, 3, 5, and 10 ml. Each of the filtrates was

Table 18. Standard solution stability at refrigerator conditions.

Sample Name	Standard Area	% Assay	% Difference
0 h	598698	100	-
7 h	597451	99.8	0.2
13 h	598807	100	0
18 h	599291	100.1	0.1
31 h	602715	100.7	0.7
43 h	603734	100.8	0.8
68 h	614137	102.6	2.6

Table 19. Sample solution stability at refrigerator conditions.

Sample Name	Sample Area	% Assay	% Difference
0 h	615302	101.2	-
4 h	611942	100.6	0.5
11 h	611193	100.5	0.7
16 h	612188	100.7	0.5
29 h	617562	99.9	1.2
41 h	619953	101.8	0.6
66 h	628376	102.8	1.6

Table 20. Filter Saturation results for 0.45 µ PVDF.

SV (ml)	% Assay				
	S-1	S-2	S-3	Mean	PMD
Centrifuge	98.8	98.1	100.0	99.0	NA
1 ml	99.3	99.6	99.4	99.4	0.4
3 ml	98.9	98.8	99.1	98.9	0.1
5 ml	99.4	98.8	98.6	98.9	0.1
10 ml	99.0	99.4	99.3	99.2	0.2

SV - Saturation Volume, S – Sample, PMD – Percentage mean difference, CFG – Centrifuge.

Table 21. Filter Saturation results in 0.45µ Nylon.

SV (ml)	%Assay				
	S-1	S-2	S-3	Mean	PMD
Centrifuge	100.8	100.9	100.3	100.7	NA
1 ml	100.8	100.3	100.0	100.4	0.3
3 ml	101.0	100.6	100.3	100.6	0.1
5 ml	100.9	100.4	100.1	100.5	0.2
10 ml	100.8	101.0	100.7	100.8	0.1

SV - Saturation Volume, S – Sample, PMD – Percentage mean difference, CFG – Centrifuge.

assayed against the standard and evaluated the compatibility against % Assay values obtained by centrifugation. The 0.45µ nylon and PVDF syringe filter were found compatible, and a minimum of 5 ml volume should be discarded prior to filling up of HPLC vial. The % mean differences found at 1, 3, 5, and 10 are 0.47, 0.03, 0.03, and 0.27 respectively. The data is given in Tables 20 and 21.

Filters details:

The filter detail is given in Table 21(a).

Table 21(a). The filter detail.

Filter Name	Make	LOT No.
0.45µ PVDF	Merck	R1BB02173
0.45µ PVDF	PALL Life Sciences	21877469

Robustness:

The robustness of the method was verified by altering the chromatographic conditions like mobile phase buffer pH, flow rate, and column oven temperature, and cumulative % RSD should be reported for metformin hydrochloride assay preparation from precision and robustness parameters. A deviation of ± 0.2 pH value of buffer solution, ± 0.2 ml/min in the flow rate, and ± 0.5 °C in column oven temperature were tried individually.

Table 22. Decrease in mobile phase buffer pH 3.65.

Precision buffer pH (3.85)		Low Buffer pH (3.65)	
Sample name	% Assay	Sample name	% Assay
Sample-1	101.1	Sample-1	100.8
Sample-2	100.7	Sample-2	100.6
Sample-3	100.7	Sample-3	100.8
Sample-4	99.8	Average	100.5
Sample-5	100.2	SD	0.46
Sample-6	99.8	Cumulative % RSD	0.46

Table 23. Increase in mobile phase buffer pH 4.05.

Precision buffer pH (3.85)		Low Buffer pH (4.05)	
Sample name	% Assay	Sample name	% Assay
Sample-1	101.1	Sample-1	100.3
Sample-2	100.7	Sample-2	100.4
Sample-3	100.7	Sample-3	100.7
Sample-4	99.8	Average	100.4
Sample-5	100.2	SD	0.44
Sample-6	99.8	Cumulative % RSD	0.44

Table 24. Decrease flow rate, 1.3 ml/min.

Precision buffer pH (3.85)		Low Buffer pH (4.05)	
Sample name	% Assay	Sample name	% Assay
Sample-1	101.1	Sample-1	100.3
Sample-2	100.7	Sample-2	100.4
Sample-3	100.7	Sample-3	100.7
Sample-4	99.8	Average	100.4
Sample-5	100.2	SD	0.44
Sample-6	99.8	Cumulative % RSD	0.44

Table 25. Increase flow rate, 1.7 ml/min.

Precision Flow rate (1.5 ml/min)		Decrease flow rate (1.7 ml/min)	
Sample name	% Assay	Sample name	% Assay
Sample-1	101.1	Sample-1	100.1
Sample-2	100.7	Sample-2	100.4
Sample-3	100.7	Sample-3	100.1
Sample-4	99.8	Average	100.3
Sample-5	100.2	SD	0.45
Sample-6	99.8	Cumulative % RSD	0.45

Table 26. Decrease column oven temperature (25°C).

Precision Column oven temperature (30°C)		Decrease column oven temperature (25°C)	
Sample name	% Assay	Sample name	% Assay
Sample-1	101.1	Sample-1	99.8
Sample-2	100.7	Sample-2	100.1
Sample-3	100.7	Sample-3	100.1
Sample-4	99.8	Average	100.2
Sample-5	100.2	SD	0.48
Sample-6	99.8	Cumulative % RSD	0.48

Table 27. Increase column oven temperature (35 °C).

Precision Column oven temperature (30°C)		Decrease column oven temperature (35°C)	
Sample name	% Assay	Sample name	% Assay
Sample-1	101.1	Sample-1	100.0
Sample-2	100.7	Sample-2	100.2
Sample-3	100.7	Sample-3	100.3
Sample-4	99.8	Average	100.3
Sample-5	100.2	SD	0.45
Sample-6	99.8	Cumulative % RSD	0.45

CONCLUSION:

This intended study can be concluded as: the proposed method is economical, simple, ultra-fast, sensitive, and reliable and is found to be accurate, precise, specific, stability-indicating, and rugged. All these parameters considered for validation meet the predefined acceptance criteria. So, the method is proposed for the quantitative estimation of Metformin in Metformin Hydrochloride Extended-Release tablets, for intended applications.

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