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Development and Validation of Stability Indicating Hplc Method for the Determination of Metformin Hydrochloride

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ABSTRACT

A new simple, precise, accurate, selective and economical RP-HPLC method for assay of metformin hydrochloride was developed for determination of metformin hydrochloride. The method was carried out by using a stainless steel column L10 CN (250 cm × 4.6 mm × 5 µm), injection volume 20 µL, and flow rate of 1.0 mL per minute, a detection wavelength of 232 nm. The column oven temperature was 34°C and a mobile phase containing: acidified water (pH-3), acetonitrile (70:30 v/v). The retention time was 1.74 min and the tailing factor was 1.1. The developed method was validated according to international conference on harmonization for precision, accuracy, sensitivity, robustness and specificity. The linearity test showed a correlation coefficient of 0.9996. The LOD and LOQ were found to be 1.389 µg/ml and 4.2102 µg/ml, respectively. The intra-day and inter-day precision were found to be 0.0408 and 1.63, respectively.

Keywords: RP-HPLC, Metformin, Mobile phase, Chromatogram.

INTRODUCTION

Metformin is indicated in the treatment of Type 2 Diabetes Mellitus (T2DM) in adults, particularly in overweight patients, when dietary management and exercise alone does not result in adequate glycemic control. Metformin is considered the first choice in the treatment for T2DM and it is widely used in the Sudan. It is also used as a second line agent for infertility in those with polycystic ovary syndrome. Metformin is the most widely used medication for diabetes taken by mouth [1-3].

There are plethora of analytical techniques for determination of metformin hydrochloride and most of these methods based on HPLC and UV or mass spectrometry detection [4,5] for detection while others employ UV/*vis* for detection. In this study, a new method for determination of metformin hydrochloride is presented and validated.

Metformin (a biguanide derivative), by controlling blood glucose level decreases these complications. Metformin works by helping to restore the body's response to insulin. It decreases the amount of blood sugar that the liver produces and that the intestines or stomach absorb.

Metformin, other than hypoglycemic activity, has been taken with diet and exercise changes to prevent diabetes in people who are at high risk for becoming diabetic. It is also used in women with polycystic ovarian syndrome. Metformin may make menstrual cycles more regular and increase fertility.

Theoretically, its use has been prohibited in a large group of patients with type 2 diabetes mellitus due to the risk of lactic acidosis. However, it has been shown that several diabetic patients who are considered to be at risk have received metformin with no increased risk of lactic acidosis.

EXPERIMENTAL

Reagents used for the study

Metformin hydrochloride standard (100% purity), Acetonirile, Orthophophoric acid 88%, deionized water.

The chromatographic procedure was carried out using stainless steel column L10 CN (250 cm × 4.6 mm × 5 µm), Injection volume 20 µL, and flow rate of 1.0 mL per minute, detection wavelength of 232 nm and column oven 34°C. The mobile phase was: Acidified water (pH-3) and acetonitrile (70:30 v/v).

Instrumentation

The development and validation of the method were performed on a Waters HPLC 2695 system equipped with quaternary pumps, an autosampler, and a photodiode array detector. Empower 2 software was applied for data collection and processing.

RESULTS AND DISCUSSION

The chromatogram obtained from injection of standard solution contains (20 µg/ml) of metformin hydrochloride shows a sharp symmetric peak at retention time of 1.1 minute (Figure 1).

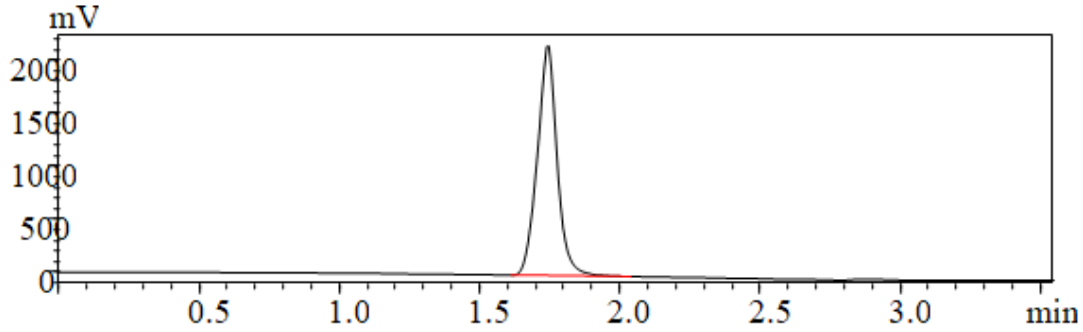

Figure 1: The chromatogram of metformin chloride.

System suitability results

The system suitability test was performed by injecting 6 injections of the standard solution (20 µg/ml). The acceptance criteria state that the number of theoretical plates (N) for metformin peaks should be not less than 2000 and the tailing factor (T) for metformin peak should be more than 2.0%. The data shown in Table 1 reveals that the method fulfills these conditions [1-6] (Table 1).

Injection	Ret. time	Peak area	Theo. plate	Tailing factor
1	1.714	523456	2135	1.2
2	1.714	523609	2139	1.2
3	1.714	523280	2133	1.2

4	1.714	524683	2135	1.05
5	1.714	523643	2132	1.05
6	1.714	522039	2135	1.2
Average	1.714	523452	2135	1.15
STD	0	849	2	0.077
RSD%	0.009	0.162	0.102	6.69

Table 1: Data of system suitability test.

Linearity

A series of five solutions of concentration ranging from 25% to 200% of the test solution concentrations were prepared from stock standard solution (200 µg/ml) of metformin hydrochloride. The proposed method shows linear relationship between the detector responses and concentration. The correlation coefficient of 0.9995 which meet the ICH guidelines for linearity (according to ICH r^2 should be not less than 0.999) (Figure 2).

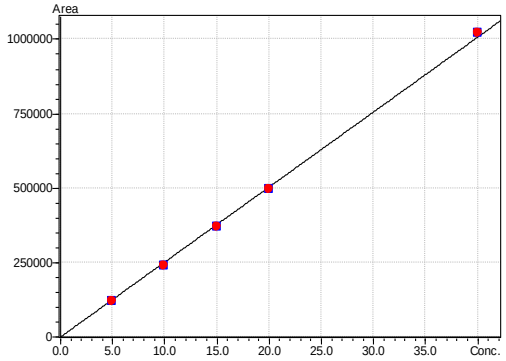

Figure 2: The calibration curve of metformin hydrochloride.

- X axis represent concentration and Y axis represent peak area
- R-squired ( )=0.9996
- Slope coefficient (B)=25987.02
- Intercept (constant) coefficient=14196.06
- Regression equation y=259187.02 x=14196.06

Specificity

The specificity test was determined by injecting placebo to test the presence of any peaks. No interferents peaks were shown in the obtained chromatogram.

Accuracy

The accuracy of the method was assessed by the recovered amount of metformin hydrochloride analyzed at four different concentration levels 50%, 80%, 100% and 120% of stock standard solution. The overall mean of recovery was 99.3% (ICH acceptance criteria for average recovery from 98.0 to 102%) (Tables 2 and 3).

Conc.	50%			80%		
Analysis No.	1	2	3	1	2	3
Avg. area	253399	253412	253595	414103	413655	413494
Assay %	48.4	48.41	48.44	79.11	78.96	78.99
Avg. assay		48.41			79.02	
Found amount	9.68	9.68	9.68	15.82	15.79	15.79
Recovery %	96.8	96.8	96.8	98.88	98.7	98.74
Average recovery		96.8			98.77	

Table 2: Showing the results of accuracy test for concentration of 50% and 80% of test solution concentration solution.

Conc.	100%			120%		
Analysis No.	1	2	3	1	2	3
Avg. area	525289	525090	524743	637548	637409	637146
Assay %	100.35	100.31	100.24	121.72	121.72	121.71
Avg. assay		100.3			121.7	
Found amount	20.07	20.062	20.049	24.35	24.35	24.34
Recovery %	100.35	100.31	100.24	101.4	101.4	101.4
Average recovery		100.3			101.4	
Over All average			99.31			
RSD%			2.008			

Table 3: Showing the results of accuracy for concentration of 100 and 120% of test solution concentration.

Precision

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by six determinations at 100% of the test solution during the same day. Results are shown in Tables 4 and 5.

Replicate	Assay-1	Assay-2	Assay-3	Assay-4	Assay-5	Assay-6
Avg. Area	525289	525090	524743	525052	525088	525064
% Assay	100.3	100.3	100.2	100.3	100.3	100.3
Average			100.28			
STD			0.0406			
RSD%			0.0408			

Table 4: Showing the results of intraday precision.

	Overall avg. assay
Day-1	100.28
Day-2	100.88
Day-3	97.8

Average	99.6
STDEV	1.63
RSD	*1.63*

Table 5: Overall intermediate precision results.

Robustness

Robustness of the method was investigated by varying flow rate (± 0.1 ml/min), column oven temperature (± 1.0°C) and wavelength of detection (± 1.0 nm) and the mobile phase pH (± 0.1).The results are shown in Tables 5-9. Clearly, the minor changes in these parameters do not affect the peak quality (Tables 6-9).

Default condition	**Flow rate 1.1 ml/min**	**Flow rate 0.9 ml/min**
Average area	479276	582798
Retention time	1.559	1.898
Tailing factor	1.2	1.16
Theoretical plate	1880	2420
Assay	96.4	117.3
RSD%	0.058	0.047

Table 6: Effect of flow rate variation.

Default condition	**PH 3.1**	**PH 2.9**
Average area	512181	512665
Retention time	1.699	1.701
Tailing factor	1.08	1.2
Theoretical plate	2043	2044
Assay	97.47	97.93
RSD%	0.042	0.032
Default condition	**PH 3.1**	**PH 2.9**
Average area	512181	512665
Retention time	1.699	1.701
Tailing factor	1.08	1.2
Theoretical plate	2043	2044
Assay	97.47	97.93
RSD%	0.042	0.032
Default condition	**PH 3.1**	**PH 2.9**
Average area	512181	512665
Retention time	1.699	1.701
Tailing factor	1.08	1.2
Theoretical plate	2043	2044
Assay	97.47	97.93
RSD%	0.042	0.032

Table 7: Effect of pH variation.

Default condition	Temp 33°C	Temp 35°C
Average area	512838	512675
Retention time	1.701	1.699
Tailing factor	1.083	1.05
Theoretical plate	2056	2071
Assay	103.2	103.2
RSD%	0.034	0.047

Table 8: Effect of temperature variation.

Default condition	Wave length 230 nm	Wave length 231 nm
Average area	511697	523282
Retention time	1.714	1.713
Tailing factor	0.967	0.9375
Theoretical plate	2206	2177
Assay	103	105.3
RSD%	0.011	0.011

Table 9: Effect of wavelength variation.

Range

It is the concentrations of analyte or assay values between the low and high limits of quantitation. Within the assay range, linearity, accuracy and precision are acceptable.

Ruggedness

It is the reproducibility of the assay under a variety of normal, but variable, test conditions. Variable conditions might include different machines, operators, and reagent lots. Ruggedness provides an estimate of experimental reproducibility with unavoidable error.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The method based on the residual standard deviation of a regression line and slope. The limit of detection (LOD) and the limit of quantification (LOQ) of the drugs were calculated using the following equations according to ICH guidelines:

- LOD=3.3(SD/S).
- LOQ=10(SD/S).
- (SD)=the standard deviation of the Root mean squired error MSE
- (S)=the slope of the calibration curve
- (SD)=the standard deviation of the root MSE=10941.25
- (S)=the slope of the calibration curve=25987.02
- LOD=3.3(SD/S)=3.3*(10941.25/25987.02)=1.389 µg/ml.
- LOQ=10(SD/S)=10*(10941.25/25987.02)=4.2102 µg/ml.

CONCLUSION

The proposed new RP-HPLC method provide simple, fast, accurate, precise, reproducible and economical approach for the identification and quantification of metformin hydrochloride and can be used for the routine quality control laboratories and the method was validated as per ICH guidelines.

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