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Research Article

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND METOPROLOL FROM COMBINATION DOSAGE FORMS

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ABSTRACT

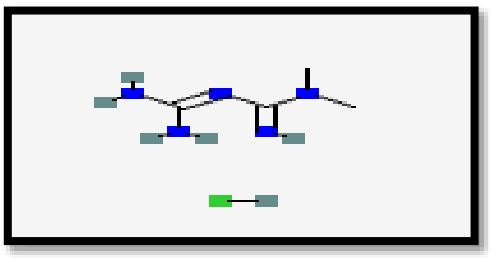
An effortless, hasty, unambiguous, robust, exact as well as precise isocratic high performance liquid chromatographic technique has been urbanized in addition to validated for the assessment of simultaneous estimation of Metformin Hydrochloride and Metoprolol Tartrate from the plasma samples. The chromatographic severance was accomplished on Phenomenex kinetex C18 (250mm×4.6mm i.d, 5µm) column by means of a mobile phase mixture containing methanol: buffer of pH 6.8: ACN in the proportion of 47:23:30 respectively at a flow rate of 1ml/min with injection volume of 20µl and recognition wavelength of 265 nm at ambient temperature. Retention time of Metformin Hydrochloride and Metoprolol Tartrate were found to be 2.312min and 3.055min. %RSD of the Metformin Hydrochloride and Metoprolol Tartrate were and found to be 0.7 and 0.3 respectively. %Recovery was obtained as 99.99% and 100.29% for Metformin Hydrochloride and Metoprolol Tartrate respectively. LOD, LOQ values obtained from regression equations of Metformin Hydrochloride and Metoprolol Tartrate were 1.05, 3.19 and 0.53, 1.61 respectively. Regression equation of Metformin Hydrochloride is y = 43247x + 27581.and Metoprolol Tartrate is y = 27862x + 5499.7. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: *Metformin Hydrochloride, Metoprolol Tartrate Assay, HPLC, Plasma*

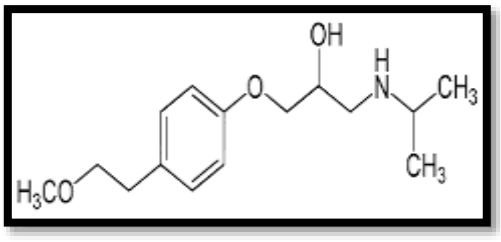
INTRODUCTION

Metformin is considered an anti-hyperglycemic drug because it lowers blood glucose concentrations in type II diabetes without causing hypoglycemia. It is commonly described as an "insulin sensitizer", leading to a decrease in insulin resistance and a clinically significant reduction of plasma fasting insulin levels.

Metoprolol is a beta-blocker used in the treatment of hypertension and angina, and used to reduce mortality due to myocardial infarction. Metoprolol is a cardio selective beta-1-adrenergic receptor inhibitor that competitively blocks beta1-receptors with minimal or no effects on beta-2 receptors at oral doses of less than 100mg in adults. It decreases cardiac output by negative inotropic and chronotropic effects.



Structure of Metformin hydrochloride



Structure of Metoprolol tartrate

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METHODOLOGY

MATERIALS AND METHODS:

Equipment: Chromatographic separation was conceded on WATERS HPLC system which is outfitted with the 515 dual head reciprocating pump & a 2489 UV Visible detector. The software used is Empower-2 software and Phenomenex kinetex C18 (250mm×4.6mm i.d, 5µm) column is used for the investigation.

Chemicals and reagents: Metformin Hydrochloride and Metoprolol Tartrate drugs were gifted by Aurobindo Pharmaceuticals, Hyderabad, and Telangana, India. Acetonitrile, methanol, HPLC grade water and mono sodium hydrogen orthophosphate and di sodium hydrogen ortho phosphate were procured from local manufacturers.

A simple, economic, accurate reverse phase isocratic HPLC method was developed for the Simultaneous Estimation of Metformin Hydrochloride and Metoprolol Tartrate from the plasma samples.

Preparation of buffer: 0.1gm of mono sodium hydrogen orthophosphate and 0.1gm of di sodium hydrogen ortho phosphate was precisely gauged and moved in to a 500ml volumetric jar, broken up by count HPLC water weakened stamp with water. Mix 51 ml of mono sodium hydrogen orthophosphate with 49 ml of di sodium hydrogen orthophosphate and adjust the pH to 6.8 with orthophosphoric acid.

Preparation of mobile phase: Methanol, Mono and disodium Hydrogen orthophosphate buffer of pH 6.8 and acetonitrile were blended in the proportion of 47:23:30 %V/V and the portable stage was then sifted through 0.45µm layer channel and sonicated for 5min in ultrasonicator shower and moved in to dissolvable repository staying away from air pockets.

Preparation of standard solution: 25mg of Metformin Hydrochloride and Metoprolol Tartrate (Bulk) was weighed precisely and moved in to 25ml volumetric flagon. Required amount of methanol was added to break up the medication. At that point, volume was made up check with methanol. This arrangement was set apart as standard stock arrangement (1000µg/ml). 1ml of standard stock arrangement was pipette out in to 10ml volumetric cup and volume was made sufficient with diluent. This was marked as 'working standard solution' (100µg/ml).

The amount of powder is estimated at 25mg of Metformin Hydrochloride and Metoprolol Tartrate, and it is taken into a 25ml cup containing 25ml of methanol. The flagon was then sonicated for 5 min and then fused with methanol. This leads to a stock arrangement of Metformin Hydrochloride and Metoprolol Tartrate, which contains a group of 1000µg/ml. The arrangement of the stock was then isolated with Whatman channel paper and washed with methanol, which was shed by 0.45µm layer channel. Pipette 1ml of solution into a 10ml volumetric jar and is further diluted to volume with methanol. The setting was separated as a test routine (100µg/ml).

Selection of detection wavelength: The effective standard reserved solution was organized according to the route given above and the wavelength was decided by scanning the standard solution among 200 to 400nm. The scanned outcome revealed that maximum absorbance was experimental at 265 nm.

METHOD DEVELOPMENT [4-6]

Optimized Chromatographic conditions:

Column: Phenomenex kinetex C18 (250mm×4.6mm i.d, 5µm) column

Mobile phase: Methanol: Mono and disodium Hydrogen orthophosphate buffer of pH 6.8: acetonitrile (47:23:30 %V/V)

Flow rate: *1ml/min*

Injection volume: *20µl*

Detection wavelength: *287nm*

Mode of elution: *Isocratic*

Column temperature: *Ambient*

VALIDATION OF THE METHOD [7-10]

System suitability test: Solution for system suitability test was all set by moving 1ml of standard stock arrangement (1000µg/ml) into 10ml volumetric flagon, weakening to check with diluent and sonicated. This preparation was injected six times into the HPLC system for assessing parameters like number of hypothetical plates (N), peak area and tailing factor. The results were shown in table 1 and the overlain chromatogram for system suitability was shown in figure.

System Suitability and Precision:

System Suitability and Precision was demonstrated by preparing standard solution as mentioned above and chromatographed into HPLC in six replicates. The tailing factor and number of theoretical plates were evaluated in standard solution. The peak area of analytes was recorded for these replicate injections. The precision was evaluated by computing the relative standard deviation for the peak area of analyte in the replicate injections.

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Table 1: System suitability parameters

Injection No	Peak Area of Metformin Hydrochloride (500ng/mL)	Peak Area of Metoprolol Tartrate (500ng/mL)
1	989.21	457.21
2	986.22	457.52
3	988.56	457.54
4	982.35	456.92
5	989.22	457.12
6	988.67	456.67
Average	987.37	457.16
SD	2.70	0.34
%RSD	0.27	0.07

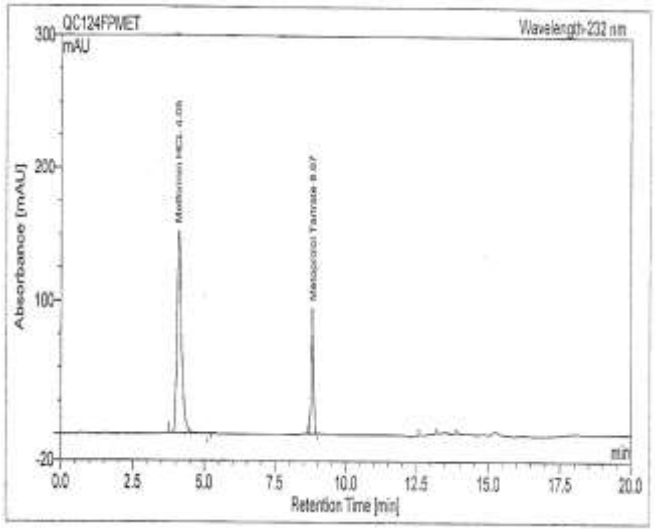

Fig 1: Overlain chromatogram for System Suitability

Linearity: The linearity of detector response was demonstrated by preparing the solutions of both the standard over the range of 10ng/mL to 1000ng/mL in the plasma sample and sample preparation as mentioned above. These solutions were injected into system and the peak area of analyte was recorded. A graph of concentration vs peak area and Y-intercept of correlation plot were evaluated. The observations are tabulated below.

Table 2: Linearity table for Metformin Hydrochloride and Metoprolol Tartrate

S. No	Linearity Level (Concentration: ng/mL)	Peak Area of Metformin HCl	Peak Area of Metoprolol Tartrate
1	5	9.85	4.6
2	50	99.3	46.1
3	250	494.2	230.1
4	500	987.56	457.6
5	1000	1976.3	915.2

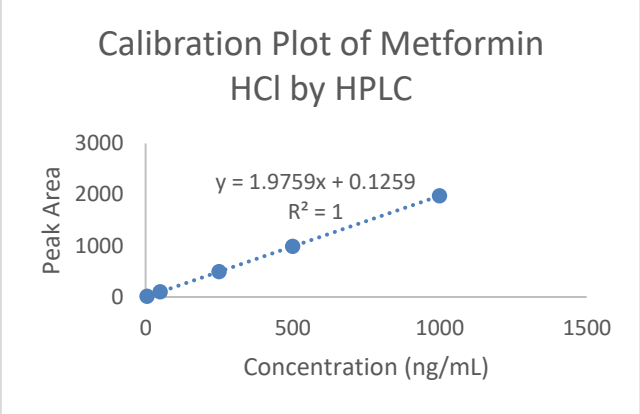

Fig 2: Calibration curve of Metformin

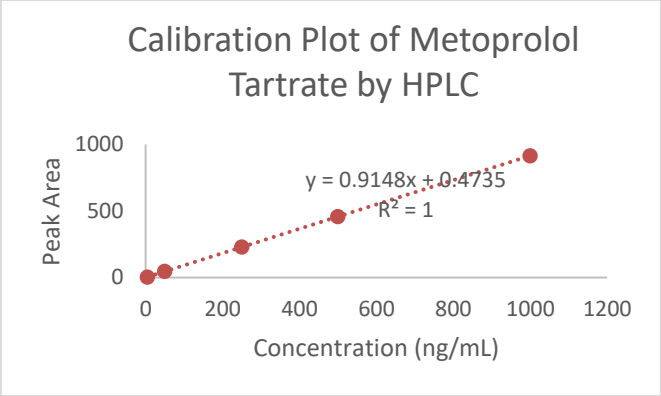

Fig 3: Calibration curve of Metoprolol

Discussion: Linear concentrations of Metformin (37.5-225µg/ml) and metoprolol (25-150µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Metformin was y = 43247x + 27581.and of metoprolol was y = 27862x + 5499.7. Correlation coefficient obtained was 1 for the two drugs.

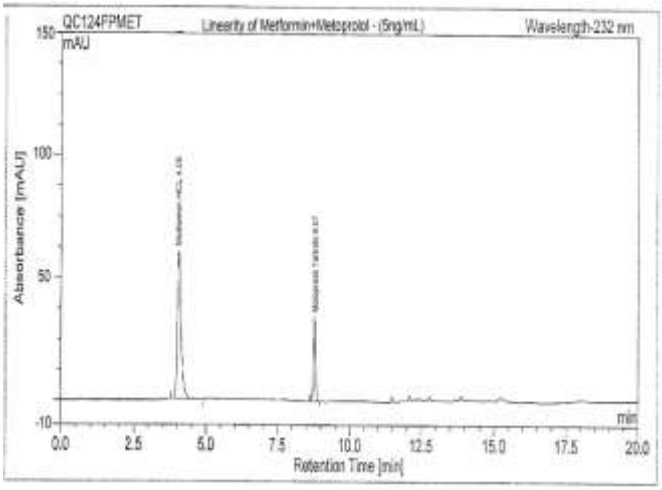

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Fig 4: Linearity curve of Metformin and Metoprolol

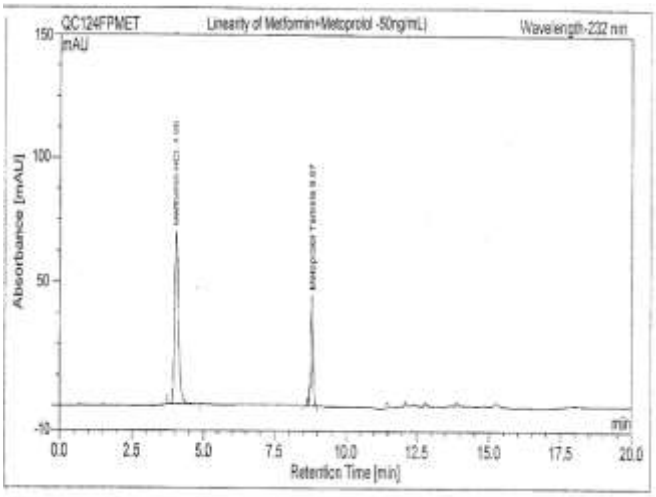

Fig 5: Linearity curve of Metformin and Metoprolol

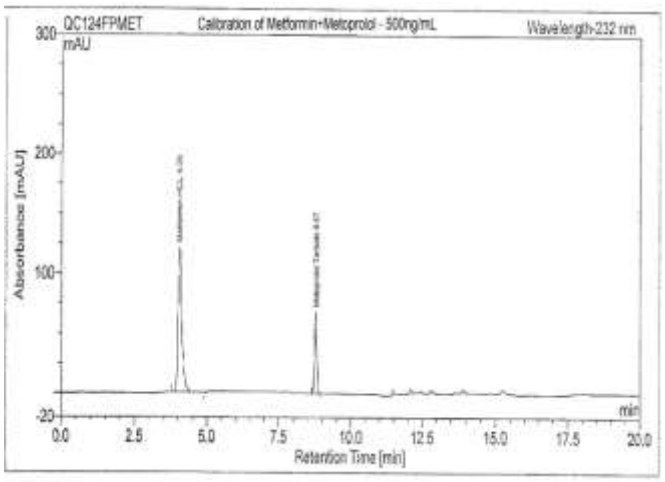

Fig 6: Linearity curve of Metformin and Metoprolol

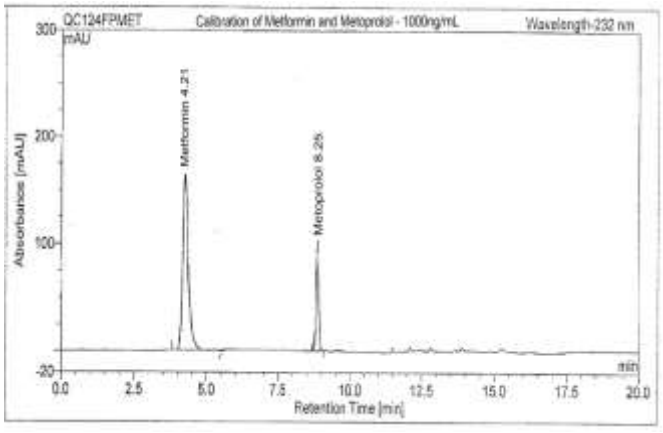

Figure 7: Linearity curve of Metformin and Metoprolol

Accuracy:

Accuracy of the test method was demonstrated by preparing the recovery samples (spiking the known amount of drug to the placebo) at the level of 50%, 100% and 150% of target concentration (500ng/mL). The recovery samples were prepared in triplicate at each level. The above samples were chromatographed and the percentage recovery for the amount added was estimated. The precision of the recovery at each level was determined by computing the relative standard deviation of triplicate recovery results. The observations are tabulated below.

Table 3: Recovery at 50% Level:

Sample No.	Amount Spiked Metformin	Amount Spiked Metoprolol	% Recovery Metformin	% Recovery Metoprolol
1	50.44	50.37	99.9	99.9
2	51.54	51.51	100.1	99.9
3	50.39	50.13	100.0	99.5
Average			100.0	99.8
%RSD			0.10	0.25

Table 4: Recovery at 100% Level:

Sample No.	Amount Spiked Metformin	Amount Spiked Metoprolol	% Recovery Metformin	% Recovery Metoprolol
1	100.45	100.41	99.8	100.1
2	100.43	100.39	99.9	100.0
3	99.89	99.85	100.1	99.9
Average			99.9	100
%RSD			0.15	0.0

Table 5: Recovery at 150% Level

Sample No.	Amount Spiked Metformin	Amount Spiked Metoprolol	% Recovery Metformin	% Recovery Metoprolol
1	150.51	150.51	100.2	100.0
2	150.67	150.57	99.8	99.9
3	150.64	150.35	99.8	99.7
Average			99.9	99.9
%RSD			0.23	0.14

Inference: As the recovery results at each level are ranged from 98.0-102.0% with RSD at each level is less than 2.0%, the study proves that the method is accurate for the estimation of metformin and metoprolol from plasma.

Range:

The specified range covered in the validation study is 5ng/mL to 1000ng/mL wherein the desired accuracy, precision and linearity has been demonstrated for the estimation of both analytes.

Precision:

System Precision:

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Table 6: System precision table of Metformin and Metoprolol

S. No	Area of Metformin	Area of Metoprolol
1.	6519161	2769123
2.	6490233	2803334
3.	6404399	2812972
4.	6506465	2749462
5.	6499301	2817563
6.	6515380	2822187
Mean	6489157	2795774
S.D	42837.9	29598.7
%RSD	0.7	1.1

System precision chromatogram

Discussion: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.7% and 1.1% respectively for Metformin and Metoprolol was passed. As the limit of Precision was less than "2" the system precision was passed in this method.

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Metformin and Metoprololfrom plasma samples. Retention time of Metformin and Metoprolol were found to be 2.312min and 3.055min. LOD, LOQ values obtained from regression equations of Metformin and Metoprololwere 1.05, 3.19 and 0.53, 1.61 respectively. Regression equation of Metformin is y = 43247x + 27581.and y = 27862x + 5499.7of Metoprolo.Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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