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SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND REPAGLINIDE IN PHARMACEUTICAL DOSAGE FORM BY UV DIFFERENCE SPECTROPHOTOMETRIC METHOD

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ABSTRACT

A simple, precise, accurate, rapid and economical Difference spectrophotometric method have been developed for simultaneous estimation of Metformin Hydrochloride and Repaglinide in pure and in combined tablet dosage form. The Metformin Hydrochloride has 234.5 nm maxima. The Repaglinide has 269 nm and 304 nm maxima and minima respectively. The proposed method depends upon measuring the absorbance of Metformin Hydrochloride at 234.5 nm on the difference spectra of Metformin Hydrochloride in 0.1 N NaOH *vs.* 0.1 N HCl. The absorbance of Repaglinide was measured at 269 nm and 304 nm on the difference spectra of Repaglinde in 0.1 N NaOH *vs.* 0.1 N HCl. Linearity was observed in the concentration range of 2-12 µg/ml for Metformin Hydrochloride and 10-50 µg/ml for Repaglinide respectively. The method was validated statistically and recovery study was performed to confirm the accuracy of the method.

INTRODUCTION

Metformin Hydrochloride (MET) is chemically *NN*-dimethylimidodicarbonimidic diamide hydrochloride. Metformin Hydrochloride decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Repaglinide (REPA) is chemically(*S*)-(+)-2-ethoxy-4-[2-(3-methyl-1-[2-(piperidin-1-yl) phenyl] butyl amino)-2-oxoethyl]benzoic acid.Repaglinide closes ATP-dependent potassium channels in the b-cell membrane by binding at characterizable sites. This potassium channel blockade depolarizes the b-cell, which leads to an opening of calcium channels. The resulting increased calcium influx induces insulin secretion. The ion channel mechanism is highly tissue selective with low affinity for heart and skeletal muscle. Combined dosage forms of MET and REPA are available in the market. Metformin Hydrochloride is official in Indian Pharmacopeia and Repaglinide is official in US Pharmacopeia and British Pharmacopeia. A survey of literature revealed that no chromatographic and Spectrophotometric methods are reported for determination Metformin Hydrochloride and Repaglinide with drug combination. The present work describe simple, precise, accurate and economical Difference spectrophotometric method have been developed for simultaneous estimation of Metformin Hydrochloride and Repaglinide form Pharmaceutical combined dosage form.

MATERIAL AND METHOD

Instrument

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions.

Reagents and Chemicals

Reference Standards of Metformin Hydrochloride and Repaglinide were obtained as gift samples from the Torrent Pharmaceutical Ltd. The drug sample (Tablets) EUREPA MF 2 manufactured by Torrent Pharmaceutical. Ltd was procured from market. All other reagents were of analytical grade for Spectrophotometric method.

Procedures

Preparation of Standard Stock Solution and Calibration curve:

Preparation of Standard Stock REPA:

Accurately weighed quantity of REPA 100mg was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with Methanol. This will give a stock solution having strength of 1000 µg/ml.

Preparation of Standard Stock Solution of MET:

Accurately weighed quantity of MET 100 mg was transferred into 100mlvolumetric flask, dissolved and diluted up to mark with Methanol. This will give a stock solution having strength of 1000 µg/ml.100 µg/ml of MET solution was prepared by diluting 10 ml of stock solution upto 100 mlwith Distilled Water.

Calibration Curve for REPA:

Calibration curve for REPA consisted of different concentrations of standard REPA solution ranging from 10-50 µg/ml. The solutions were prepared by pipetting out 1, 2, 3, 4, and 5 ml of the standard solution of REPA (1000µg/ml) into two series of 100 ml volumetric flasks and the volume was adjusted to mark with 0.1N NaOH and 0.1N HCL.

Calibration Curve for MET:

Calibration curve for MET consisted of different concentrations of standard MET solution ranging from 2-12 µg/ml. The solutions were prepared by pipetting out 2, 6, 8, 10, and 12 ml of the standard solution of MET (100 g/ml) into two series of 100 ml volumetric flasks and the volume was adjusted to mark with 0.1N NaOH and 0.1N HCL

Method (Difference Spectrophotometric Method)

Aliquots each of the stock solution were separately diluted to 100 ml with 0.1N NaOH and 0.1N HCL and the absorbance difference (ΔA) of the alkaline solutions were measured at 269 nm, 304 nm, and 234.5 nm. Using acidic solution of the corresponding drug as blank. Water as blank correction was carried out. Repaglinide was analysed by the absorbance difference (ΔA) of the alkaline solution was measured at 269 nm, 304 nm. Using acidic solution of the corresponding drug as blank, Metformin was analysed by the absorbance difference (ΔA) of the alkaline solution was measured at 234.5 nm using acidic solution of the corresponding drug as blank.

Metformin was analysised at 234.5 nm by subtracting absorbance from the mixture in the formula below,

- Corrected Abs of Metformin = $\Delta A_{234.5} - \Delta A_{r234.5}$
- $\Delta A_{234.5}$ = Total absorbance
- $\Delta A_{r\,234.5}$ = Absorbance of Repa at 234.5 nm

Procedure for Sample Preparation:

Weigh 20 tablets and triturate it. Weigh accurately a quantity of the powder containing about 500 mg of metformin hydrochloride and 2 mg of repaglinide in 50 ml volumetric flask in methanol. sonicated for 20 min, was then filtered through Whatman filter. The Aliquot appropiate volume to get required concentration with in calibration curve. Dilute separately in

0.1N NaOH and 0.1N HCL. The absorbance of sample solution was measured at 234.5 nm, 269nm and 304nm in 1cm cell with 0.1N NaOH against 0.1N HCL. The content of Metformin Hydrochloride and Repaglinide in a tablet was calculated by the UV Difference Spectrophotometric Method.

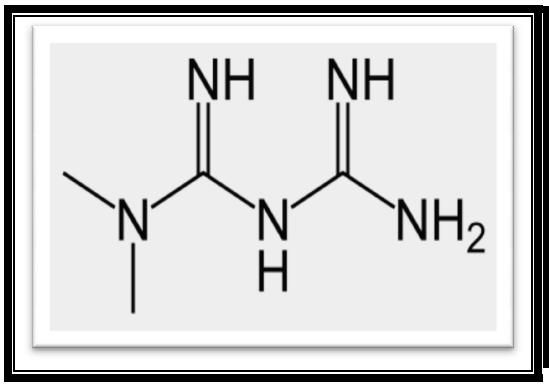

Figure-1 Metformin Hydrochloride

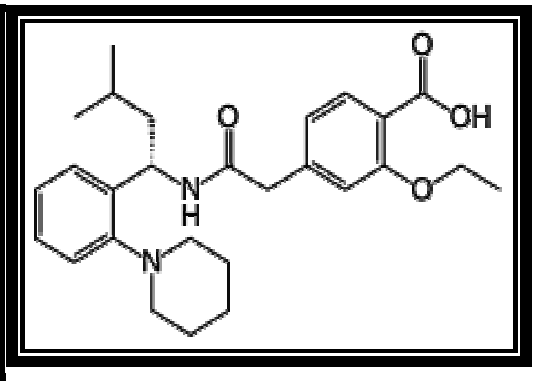

Figure-2 Repaglinide

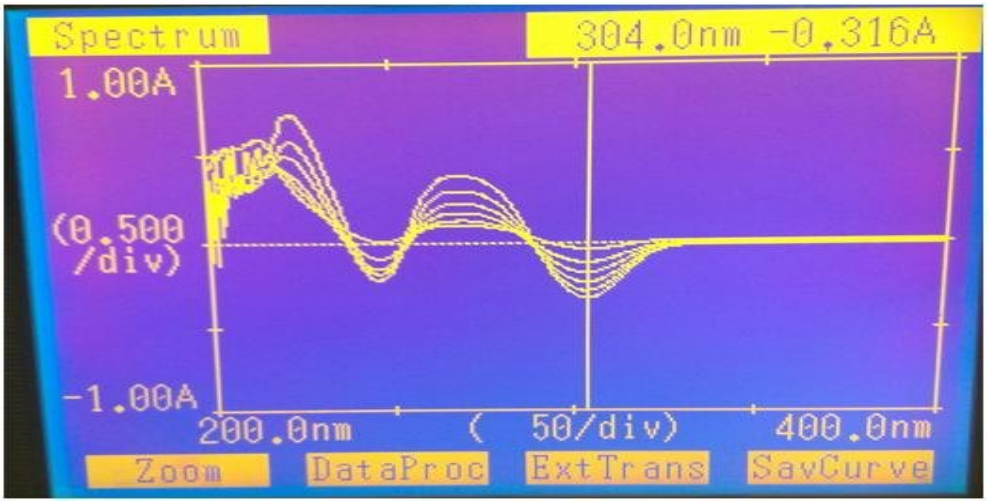

Figure-3 Overlain UV spectra of Repaglinide (10-50 µg/ml)

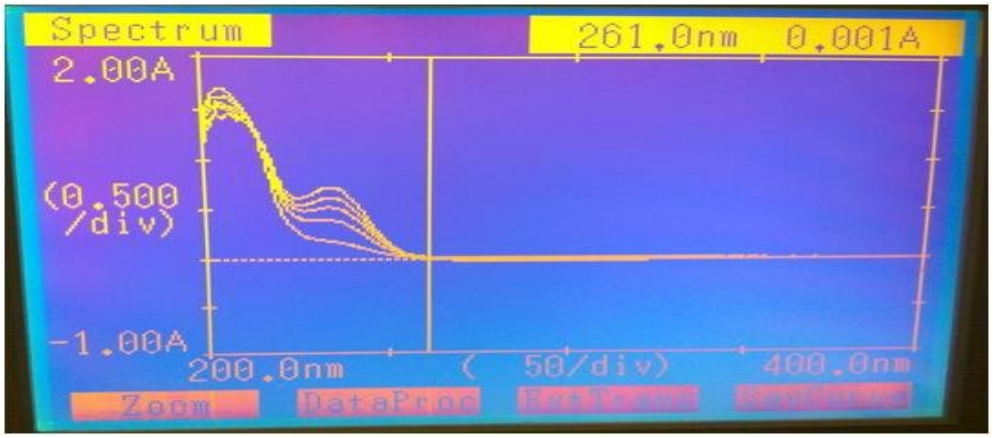

Figure-4 Overlain UV spectra of Metformin Hydrochloride (2-12 µg/ml)

Table-1 Optical Characteristic:

Parameters	Difference Spectrophotometric Method		
	Repaglinide		Metformin Hydrochloride
Wavelength (nm)	269-304	234.5	234.5
Beer's law limit (µg /ml)	10-50	10-50	2-12
Regression equation (y = a + bc) Slope (b) Intercept (a)	y = 0.0117x + 0.0064 0.0117 0.0064	y = 0.0046x + 0.0152 0.0046 0.0152	y = 0.0548x + 0.0618 0.0548 0.0618
Correlation coefficient (r^2)	0.9970	0.9968	0.9965
LOD (µg/ml)	0.58	1.21	0.14
LOQ(µg/ml)	1.77	3.68	0.44
Precision(% RSD) Interday (n=9) Intraday (n=3)	 0.84 0.57	 1.55 1.28	 0.77 0.72

Table-2 Results of the recovery studies:

Method	Recovery Level	% Recovery	% Recovery ± RSD	% Recovery	% Recovery ± RSD
		Repaglinide		Metformin Hydrochloride	
Difference Spectroscopy method	80%	99.43	101.01 ± 1.35	101.85	101.45 ± 0.53
	100%	100.78		100.84	
	120%	101.82		101.66	

* RSD=Relative Standard deviation

Table-3 Results of analysis of Tablet formulation:

Method	Tablet sample	Label claim (mg/tablet)	% ASSAY(n=6) ± RSD
Difference Spectroscopy method	Repaglinide	2 mg	99.05± 0.95
	Metformin Hydrochloride	500 mg	99.81± 0.69

Validation of the Method according to ICH Guidelines

Validation of method was done according to ICH guidelines for Simultaneous Equation method.

Linearity

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in the samples. MET was linear with the concentration range of 2-12 µg/ml at 234.5. REPA showed the linearity in range of 10–50 µg/ml at 269nm and 304 nm.

Precision (repeatability)

The repeatability of the method was confirmed by the analysis of formulation was repeated for 6 times with the same concentration.

Intermediate precision (reproducibility):

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days 3 different concentrations of standard solutions of MET and REPA.

Accuracy (recovery study):

To check the accuracy of the proposed methods, recovery studies carried out at 80%, 100%, and 120% of the test concentration as per ICH Guideline. The recovery study was performed three times at each level.

Limit of detection and Limit of quantification:

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$, $LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

RESULTS AND DISCUSSION

A simple, precise, accurate, rapid and economical Difference spectrophotometric method have been developed for simultaneous estimation of Metformin Hydrochloride and Repaglinide in pure and in combined tablet dosage form. The Metformin Hydrochloride has 234.5 nm maxima.The Repaglinide has 269 nm and 304 nm maxima and minima respectively. The proposed method depends upon measuring the absorbance of Metformin Hydrochloride at 234.5 nm on the difference spectra of Metformin Hydrochloride in 0.1 N NaOH *vs.* 0.1 N HCl. The absorbance of Repaglinide was measured at 269 nm and 304 nm on the difference spectra of Repaglinde in 0.1 N NaOH *vs.* 0.1 N HCl.Linearity was observed in the concentration range of 2-12 µg/ml for Metformin Hydrochloride and 10-50 µg/ml for Repaglinide respectively.The

linearity of the calibration curve was validated by the high values of correlation coefficient of regression. LOD and LOQ values for MET were found to be 0.14 and 0.44 µg/ml at 234.5 nm. LOD and LOQ values for REPA were found to be 0.58 and 1.77 µg/ml and 1.21 and 3.68 µg/ml at Difference (269 nm-304 nm) and 234.5 nm respectively.These data show that method is sensitive for the determination of MET and REPA. Both drugs showed good regression values at their respective wavelengths and at Difference, and the results of a recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed method.The proposed validated method was successfully applied to determine MET and REPA in their combined dosage form. The results obtained for MET and REPA were comparable with the corresponding labeled amounts (Table-3).

CONCLUSION

The proposed methods are simple, rapid and validated in terms of linearity, precision, accuracy, reproducibility, and can be used successfully for routine simultaneous estimation of Metformin Hydrochloride and Repaglinide in pure and tablet dosage forms.

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