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## Development and Validation of HPTLC Method for the Estimation of Repaglinide in Bulk and Tablet Dosage Form

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### Abstract

A simple, accurate, precise, and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of repaglinide in tablet dosage forms. The method employed TLC aluminium plates precoated with silica gel 60 F 254 as the stationary phase. The mobile phase used was a mixture of Chloroform: Methanol (9:1) v/v. The detection of spot was carried out at 254 nm. The calibration curve was found to be linear between 300 to 3000 ng mL<sup>-1</sup> Rf value 0.41±0.018 with regression coefficient of 0.9991. The proposed method can be successfully used to determine the drug content of marketed formulation. The accuracy of the proposed method was determined by recovery studies and found to be 97.98 to 98.89 %. The proposed method is applicable to routine analysis of repaglinide in bulk and pharmaceutical formulations. The proposed method was validated according to various ICH parameters like linearity, accuracy, precision, specificity, limits of detection, limits of quantification, range and solution stability.

**Key words:** Repaglinide, HPTLC, ICH guidelines

### Introduction

Repaglinide class used in the management of type 2 diabetes mellitus. Repaglinide lowers blood glucose levels by stimulating the release of insulin from the pancreas and chemically it is 2-ethoxy-4-[2-[[[(1S)-3-methyl-1-(2-piperidin-1-ylphenyl)butyl]amino]-2-oxoethyl]benzoic acid. Repaglinide closes ATP-dependent potassium channels in the  $\beta$ -cell membrane by binding at characterizable sites. This potassium channel blockade depolarizes the  $\beta$ -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. A literature survey reveals that various analytical methods like repaglinide by HPLC in pharmaceutical formulation<sup>1,4</sup>,

Simple HPLC method for the determination of repaglinide in human plasma<sup>2</sup>, Simultaneous HPLC estimation of Gliclazide and Repaglinide in Pharmaceutical Formulations<sup>3</sup> Quantitative Analysis of Repaglinide in Tablets by RP-TLC with Densitometric UV Detection<sup>5</sup>. But these methods are sophisticated, expensive and time consuming when compared to simple HPLC method. There is need for a interest to develop simple, accurate, specific, sensitive, precise and reproducible HPLC method for the estimation of rosiglitazone in bulk and its formulation.



Fig. 1: Chemical structure of Repaglinide

## Experimental Details

### Materials

Pure standard of Repaglinide (Assigned purity 99.98%) was obtained Torrent research center, Gandhinagar as a gift sample. The gift sample was used as standard without further purification. Silica gel 60 F 254 TLC plates (20x10cm) were used as stationary phase. All chemicals and reagents used were of analytical grade and obtained from Qualigens. Commercial pharmaceutical preparation (Pradin) which was claimed to contain 4mg of repaglinide is used in analysis. The chemical structure and purity of the sample obtained was confirmed by TLC, IR, Melting point studies.

### Equipment

The instrument used in the present study was Camag Linnomat V-semiautomatic sample applicator, Hamilton syringe (100 $\mu$ l), Camag TLC scanner 3, Camag Twin trough chamber of appropriate size (20X 20), Analytical weighing balance (Shimadzu AX 200), Sonicator (model SONICA 2200MH) were used throughout the experiment. Camag Wincats software was used for acquisition, evaluation and storage of chromatographic data.

### Preparation of Standard Solution

A stock solution of drug was prepared by dissolving 100 mg of Pure Repaglinide in a 100 ml volumetric flasks containing sufficient amount of methanol to dissolve the drug, sonicated for about 15 min and then made up to volume with methanol (1 mg/ml). A standard solution was prepared by dilution of the stock solution with methanol to give in concentration of 100 $\mu$ g/ml. Further dilutions were made with methanol to give a solution in concentration range of 300-3000ng/ml.

### Procedure for Sample Solution (From Formulation)

To determine the content of the drug in a solid dosage form, 20 tablet of Repaglinide Commercial pharmaceutical preparation (Pradin) which was claimed to contain 4mg were accurately weighed, their average weight was calculated. Powder equivalent to 4 mg of the drug (content of one tablet) was dissolved in sufficient amount of methanol to dissolve the drug, sonicated for about 15 min. and then filtered into a 100 ml volumetric flask through 0.45  $\mu$ m membrane filter. The residue was washed 3 times with 10 ml of methanol, and then the volume was completed to 100 ml with the same solvent. Make further dilutions with methanol to obtain a stock solution of 10 $\mu$ g/ml. An aliquot of this solution (1 ml) was transferred to a 10 ml volumetric flask and made up sufficient volume with the methanol to give an expected concentration of 1  $\mu$ g/ml.

### Prewashing of TLC plates

HPTLC was performed on 20 cm  $\times$  10 cm precoated silica gel 60 F 254 TLC plates. The adsorbent has a very large surface area; it may absorb air and other impurities from atmosphere, particularly volatile impurities, after the pack has been opened. The non-volatile impurities adsorbed by layer can lead to irregular baseline in scanning densitometry. To avoid possible interference from such impurities in quantitative analysis, plates were prewashed with methanol, dried, and activated for 30 min. at 110 C, with the plates being placed between two sheets of glass to prevent deformation of the aluminum during heating.

## Results and Discussion

### Procedure

A methanolic solution of repaglinide (1 mg/ml) was prepared. This solution was further diluted with methanol to yield a solution containing 1 $\mu$ g/ml. Different concentrations of repaglinide in a concentration range of 300-3000ng/ml were applied on plates as 8 mm bands, 8 mm apart and 1 cm from edge of the plate, by means of Camag Linomat V automatic sample applicator fitted with 100  $\mu$ l Hamilton syringe. A methanol blank was applied to parallel track. The mobile phase, Chloroform: Methanol (9:1) v/v was poured into the twin trough glass chamber and the glass chamber left to equilibrate for 10 min at  $25 \pm 2^{\circ}$  C. After that the plate was placed in Camag twin trough glass chamber. After development, the plate was removed from the chamber, dried in current of hot air, and scanned at 254 nm, using a deuterium lamp, by means of Camag TLC scanner III densitometer. Densitograms were obtained by HPTLC of repaglinide at various concentrations. This method was followed for all quantitative analysis. The Wincats software was used for data acquisition and processing of the plate. Peak height and peak area were integrated for the entire track. The calibration curve was established by plotting the obtained peak area on ordinate against corresponding concentration on abscissa.

### Validation of Analytical Method

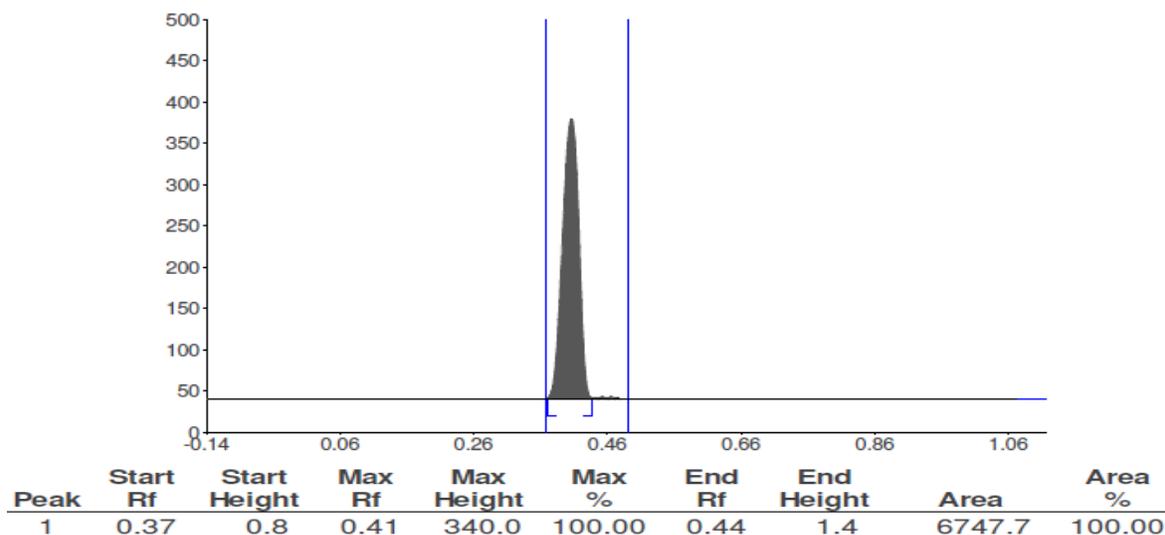
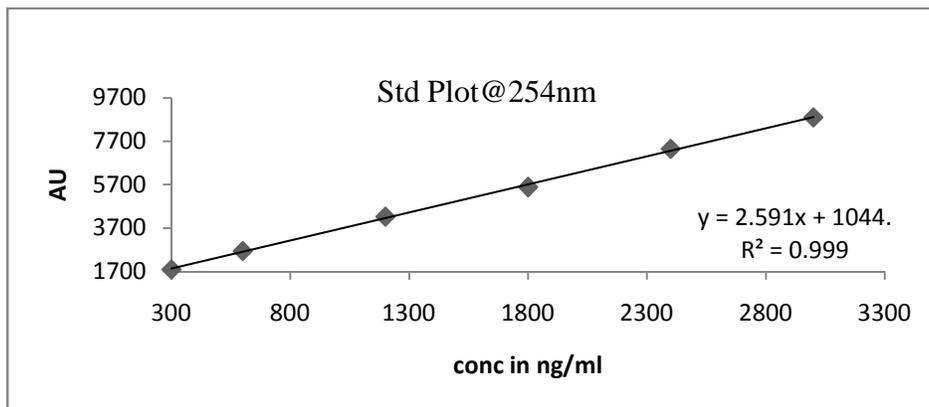
Validation of an analytical method is process to establish by laboratory studies that the performance characteristics of the method meet the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

Typical analytical parameters used in validation area: Linearity, Accuracy, Precision, Specificity, Limit of detection, Limit of quantification, Range, Solution stability

### Linearity

**Acceptance criteria:** Coefficient of correlation ( $r_2$ ) should be greater than 0.998

A standard solution was prepared by dilution of the stock solution with methanol to give in concentration of 100µg/ml. Further dilutions were made with methanol to give a solution in concentration range of 300-3000ng/ml. (Graph No. 1 and chromatogram No. 1)



**Accuracy**

The accuracy is the closeness of the measured value to the true value of the sample. To evaluate the accuracy of the method, known amount of pure drug was added to the previously analyzed solution containing pharmaceutical formulation and the mixture was analyzed by the proposed method and the recoveries were calculated. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. Aliquots of 0.2 ml, 0.8ml and 1.4 ml of sample drug (Repaglinide) solution of 10µg/ml were pipetted into each of three volumetric flasks. To this 0.4 ml of standard drug (Repaglinide) solution of 10µg/ml was added to each volumetric flask respectively. The volume was made up to 10 ml with mobile phase. The range of recovery studies were found between 97.98 to 98.89 %. The values of recovery justify the accuracy of the method. The % recovery values were obtained within the standard limit which confirms that the method is accurate and free from any positive or negative interference of the excipients. (Table No. 1)

Conc. taken in ng/ml (A)	Std addition in ng/ml (B)	Total drug conc.inng/ml (A+B)	Peak Area	%Recovery ± SD	Average	%RSD
400	200	600	2581.3	97.98± 1.63	98.29	0.830
400	800	1200	4140.2	98.00±0.94		
400	1400	1800	5518.9	98.89±0.80		

### Precision

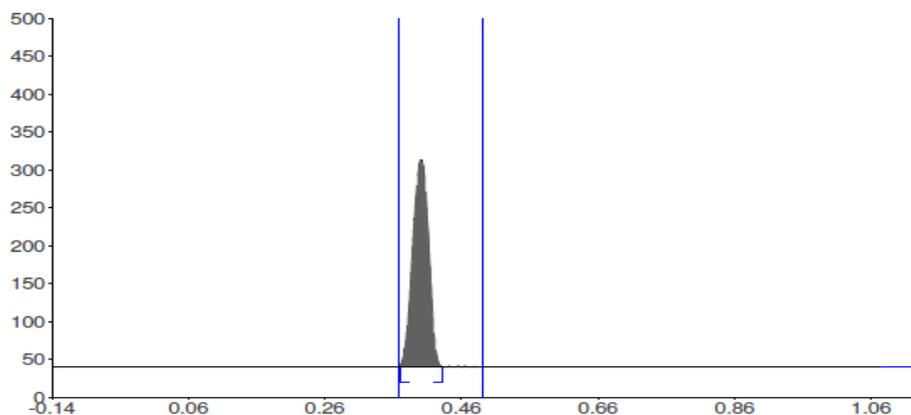
Precision is measure of repeatability or reproducibility and it was determined by injecting 5 times the expected operating range concentration. The chromatograms were recorded to determine mean standard deviation and relative standard deviation. (Table No. 2)

S.No	Area Response	Average $\pm$ SD	% RSD
1	4103.2	4200.9	1.429
2	4220.6		
3	4190.4		
4	4260.2		
5	4230.4		

**Result:** From the above analytical data it is observed that RSD for the assay is 1.429 % which indicates that the method is precise and reproducible.

### Specificity:

Specificity is the ability to assess the analyte in the presence of components that may be expected to be present in the sample matrix (USP 2004). For demonstrating the specificity of the method for drug formulation the drugs was spiked and observe the chromatogram (chromatogram No.2).



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.37	1.8	0.40	273.9	100.00	0.43	0.8	5213.5	100.00

### Result:

The excipients used in different formulation products did not interfere with the drug peak and thus, the method is specific for repaglinide.

### Limits of detection and quantification:

The detection limit (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as a concentration that gives a signal-to-noise ratio of 2:1 or 3:1. The lower limit of detection for Repaglinide is 52.91 ng/ml in reference material and formulation. Limit of Quantification (LOQ) is the lowest amount analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal-to-noise ratio of 10:1 can be taken as LOQ of the method. The LOQ values were found to be 176.39 ng/ml for raw material and formulations

### Range

The specific range derived from the linearity studies. The range was calculated from the linearity graph. From the lower to higher concentration between which the response is linear, accurate and precise.

**Acceptance criteria:** RSD < 2.0 The range for repaglinide was found to be 300-3000 ng/ml.

### Solution Stability

The solution stability of the standard and sample prepared in methanol was studied for 5 days at bench top. The solution under study was compared with freshly prepared standard solution, the samples were found to be stable for period of more than 48 hours.

### Conclusion

The proposed HPTLC method is found to be simple, accurate, precise, linear, and specific, and, for quantitative estimation of repaglinide in bulk and its formulation.

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