ABSTRACT:
A simple and sensitive, HPTLC method has been developed for the quantitative estimation of repaglinide in its single component tablet formulations (2 mg). Repaglinide was chromatographed on silica Gel 60 F$_{254}$ TLC plate using chloroform: methanol: ammonia (4.5:0.8:0.05 v/v) as mobile phase. Repaglinide showed Rf value 0.55±0.03 and scanned at 288 nm using Camag TLC Scanner 3. The method was validated in terms of linearity (400–2400 ng/spot), precision (intra-day variation 0.7 to 2.6%, inter-day variation 0.8 to 3.2%), accuracy (97.0 to 99.0%) and specificity. The limit of detection and limit of quantification for repaglinide were found to be 50 ng/spot and 300 ng/spot, respectively. The developed method was successfully used for the assay of repaglinide tablet formulations. The method is simple, sensitive and precise; it can be used for the routine quality control testing of marketed formulations.

Keywords: Repaglinide, Chloroform, Methanol, Ammonia, HPTLC

INTRODUCTION
Repaglinide is a meglitinide antidiabetic used in the management of type 2 diabetes mellitus, chemically $S(\pm) 2$-ethoxy-4(2((3-methyl-1-(2-(1-piperidinyl) phenyl)-butyl) amino)-2-oxoethyl) benzoic acid.1-2. It is official in USP3 which describes liquid chromatographic method for its quantitation. Literature survey reveals that one HPLC method in human plasma4, two HPLC5-6, one RPTLC7 and one spectrophotometric method8 in pharmaceutical dosage form. The purpose of this work was to develop and validate simple, specific, sensitive, accurate, precise, rapid and cost effective HPTLC method for the estimation of rosiglitazone in bulk and its formulations.

MATERIALS AND METHODS
Materials
Repaglinide working standard was procured as a gift sample from Torrent Pharma. Ltd., Ahmedabad. Silica gel 60 F$_{254}$ TLC plates (20×20 cm, layer thickness 0.2 mm, E. Merck, Germany) were used as stationary phase. Two single component tablet formulations of repaglinide (2 mg) (formulation A Eurepa, manufactured by Torrent Pharma. Ltd., Ahmedabad, formulation B- Regan, manufactured by Ranbaxy Ltd., Mumbai) were purchased from local market. Chloroform, ammonia (SD’S) and methanol (A.R., Ranbaxy Ltd., New Delhi) were used for mobile phase preparation and as solvent.

A Camag HPTLC system (Switzerland) comprising of Camag Linomat IV semiautomatic sample applicator, Camag TLC Scanner 3, Camag twin-trough chamber (10×10 cm), Camag CATS 4 software, Hamilton syringe (100 μl), Shimadzu libror AEG- 220 weighing balance, Sonicator (Frontline FS-4, Mumbai) were used during the study.

Preparation of standard solution of repaglinide
Repaglinide (10 mg) was weighed accurately and transferred in 100 ml volumetric flask. It
was dissolved in and diluted up to mark with methanol. The final solution contained 100 µg of repaglinide per ml of the solution (S1).

**Preparation of sample solution**
Twenty tablets were weighed and finely powdered. The powder equivalent to repaglinide (10 mg) was weighed accurately and mixed with methanol (30 ml) and sonicated for 10 minutes. The solution was diluted to 100 ml with methanol. The residue was washed thoroughly with methanol. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to mark with methanol.

**HPTLC method and chromatographic condition**
The chromatographic estimations were performed using following conditions; stationary phase, precoated silica gel 60 F$_{254}$ aluminum sheets (20×10 cm) (pre-washed with methanol. and dry in air); mobile phase, chloroform: methanol: ammonia (4.5:0.8:0.05 v/v); chamber saturation time, 20 min; Temperature, 29±1°C; migration distance, 45 mm; wavelength of detection, 288 nm; slit dimensions, 3×0.1 mm; scanning speed, 5 mm/s.

Following spotting parameters were used - band width, 3 mm; space between two bands, 4 mm and spraying rate, 10 sec/µl.

**Chromatographic separation**
Sixteen µl of standard or sample solution was applied on TLC plate under nitrogen stream using semiautomatic spotter. The plate was dried in air and developed up to 45 mm at constant temperature using mixture of chloroform: methanol: ammonia (4.5:0.8:0.05 v/v) as mobile phase in Camag twin-trough chamber previously saturated with mobile phase for 20 min. The plate was removed from the chamber and dried. Photometric measurements were performed at 288 nm in absorbance/reflectance mode with Camag TLC Scanner 3 with CATS4 software incorporating the track optimization option.

**Calibration curve of standard repaglinide**
Standard repaglinide solution (4, 8, 12, 16, 20 and 24 µl) was spotted on precoated TLC plate, using semiautomatic spotter under nitrogen stream. The TLC plate was developed and photometrically analyzed as described under chromatographic separation. The calibration curve was prepared by plotting peak area versus concentration (ng/spot) corresponding to each spot.

**Quantification of repaglinide in tablet formulation**
Sixteen µl of sample solution (100 µg/ml) was applied on prewashed TLC plate, developed and scanned as described in chromatographic separation. The amount of repaglinide present in sample solution was determined by fitting area values for peak corresponding to repaglinide into the equation of line representing calibration curve for repaglinide.

**RESULTS AND DISCUSSION**
In present work HPTLC method was developed for estimation of repaglinide pure powder and its pharmaceutical formulation. HPTLC method is cost effective and less time consuming. Repaglinide is soluble in methanol; therefore methanol was selected as solvent. The formulation was dissolved in methanol with sonication for 10 min to assure complete release of drug from the formulation matrix.

**Method optimization**
For optimization, different mobile phases and composition were employed to achieve the good separation. The method development was initiated with using a mobile phase of toluene–methanol in various proportions. In the above conditions elution was very broad...
for repaglinide. Early elution with a broad separation was observed with the mobile phase consisting of toluene: acetone: methanol: ammonia (2:3:1:0.05 v/v). In the same mobile phase change proportion of toluene: acetone: methanol not gave reasonable Rf and not sharp band. Therefore need further optimization with using mobile phase consisting of chloroform: methanol: ammonia (4.5:0.5:0.05 v/v) helped in sharpening of the peak but early elution (Rf =0.27). In the same mobile phase slightly increase the methanol helped in better elution. Finally, the mobile phase consisting of the mixture of chloroform: methanol: ammonia (4:0.8:0.05 v/v) could resolve repaglinide spot with better peak shape. Combination of chloroform and methanol offered optimum migration (Rf= 0.55±0.03) and resolution of repaglinide from other components of formulation matrix. Even saturation of TLC chamber with mobile phase for 20 min assured better reproducibility and better resolution. Repaglinide shows significant UV absorbance at wavelength 288 nm. Hence this wavelength has been chosen for detection in the analysis of repaglinide.

The method was validated in terms of linearity, inter-day and intra-day precision, repeatability of measurement of peak area as well as repeatability of sample application, accuracy and specificity. The limit of detection and limit of quantification were also determined.

A representative calibration curve of repaglinide was obtained by plotting the mean peak area of repaglinide against the concentration over the range of 400 - 2400 ng/spot. A correlation coefficient was found to be 0.998 and RSD was ranging from 0.8 - 3.2. The average linear regression equation was represented as $Y=2.608X+729.4$, where $X=$concentration of repaglinide and $Y=$peak area. The limit of detection and limit of quantification for repaglinide were found to be 50 ng/spot and 300 ng/spot, respectively. Inter-day and Intra-day variation range for repaglinide was found to be 0.7 - 2.6 and 0.8 - 3.2 respectively. Precision of the instrument was checked by repeated scanning of the same spot (1600 ng/spot) of seven times without changing position of the plate and % CV for measurement of peak area was found to be 0.5%. Repeatability of the method was checked by spotting 16 µl of standard solution seven times on TLC plate (n=7) and % CV for peak area was found to be 1.9%. Both the % CV, for measurement of peak area and sample applications (less than 1% and 3%, respectively), ensuring proper functioning of HPTLC system.

Accuracy of method was evaluated by calculating recovery of drug by standard addition method at 3 levels of the calibration curve (n=3). The percentage recovery was found to be 97.0 to 99.0% ensuring that the method is accurate.

The method is found to be specific for repaglinide. The purity of the peak was determined by comparing the spectra at three different levels i.e. at peak start(S), peak apex (M) and peak end (E). Correlation between these three spectra indicated the purity of peak (correlation, r(S,M)=0.9998, r(M,E)=0.9998). The spectrum of extracted from tablet was also compared with spectrum of standard, which showed correlation 0.9978. It was observed that the excipients present in formulation did not interfere with the peak of repaglinide.
Table 1: Summary of validation parameters

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameters</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Linearity range (ng/spot)</td>
<td>400-2400 ng/spot</td>
</tr>
<tr>
<td>2</td>
<td>Correlation co-efficient</td>
<td>0.998</td>
</tr>
<tr>
<td>3</td>
<td>Precision</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intra-day % CV (n = 3)</td>
<td>0.7 – 2.6</td>
</tr>
<tr>
<td></td>
<td>Inter-day % CV (n =3)</td>
<td>0.8 – 3.2</td>
</tr>
<tr>
<td></td>
<td>Repeatability of sample application (n = 7)</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Repeatability of peak area ( n = 7)</td>
<td>1.9</td>
</tr>
<tr>
<td>4</td>
<td>% Recovery</td>
<td>97.0 – 99.0</td>
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<tr>
<td>5</td>
<td>Limit of detection</td>
<td>50 ng/spot</td>
</tr>
<tr>
<td>6</td>
<td>Limit of quantification</td>
<td>300 ng/spot</td>
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<tr>
<td>7</td>
<td>Specific</td>
<td></td>
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</tbody>
</table>

Different validation parameters for the proposed HPTLC method for determining repaglinide content are summarized in Table 1. This method was applied to determine the content of repaglinide in two different market samples of single component repaglinide tablets. The content and percentage of repaglinide in two different market samples were found to be 2.0 mg, 100.1±2.9% and 1.99 mg, 99.4±1.5%, respectively (n=3). The results indicate that the proposed HPTLC method was found to be simple, specific, rapid, precise and accurate for estimation of repaglinide in its formulations.

CONCLUSION:
The results indicate that the proposed method is simple, accurate, precise and specific, for estimation of repaglinide in bulk and its formulations.

REFERENCES: