Development and validation of high performance thin layer chromatographic (HPTLC) technique for quantification of Glipizide in tablet dosage forms

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ABSTRACT

A new simple, accurate and precise HPTLC method has been developed for the estimation of glipizide in tablet formulation. In this method, standard and sample solutions of glipizide were applied on precoated silica gel G 60 F254 TLC plate, and developed using ethyl acetate:formic acid: dichloromethane (1:1:2 v/v/v), as mobile phase. The drugs on plate were scanned at 275.5 nm. The dynamic linearity range was 200-800 ng/spot for glipizide. The method was validated for linearity, accuracy, limit of detection, limit of quantification, inter-day and intra-day assay precision, repeatability of measurement, and repeatability of sample application.

Key words: HPTLC, Glipizide, tablet dosage forms.
concentration range of 200-800 ng/spot for glipizide. Intra-day assay precision was found by analysis of standard drug at three times on the same day. Inter-day assay precision was carried out using the standard drug at three different days, and % relative standard deviation (RSD) was calculated. The RSD was found to be less than 2 for both inter-day and intra-day assay precision. Repeatability of sample application was assessed by spotting 10 ml of drug solution, 6 times and % RSD was determined. Repeatability of measurement was determined by spotting 10 ml of standard drug solution on TLC plate, after development spot was scanned six times without changing position. The % RSD calculated for glipizide is 0.3672 (Table 1).

Recovery studies were carried out at three levels for the accuracy parameter. To the powdered formulation, the standard drugs of glipizide were added at 50%, 100% and 150% levels, dilutions

Table 1: Validation Parameters

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rf</td>
<td>0.63±0.03</td>
</tr>
<tr>
<td>2</td>
<td>Linearity (ng/spot)</td>
<td>200-800ng</td>
</tr>
<tr>
<td>3</td>
<td>Correlation coefficients (r^2)</td>
<td>0.9995</td>
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<tr>
<td>4</td>
<td>LOD (ng /spot)</td>
<td>200ng</td>
</tr>
<tr>
<td>5</td>
<td>LOQ (µg /spot)</td>
<td>0.339</td>
</tr>
<tr>
<td>6</td>
<td>Accuracy %</td>
<td>99.38%</td>
</tr>
<tr>
<td>7</td>
<td>Precision (%RSD)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Inter Day</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>b) Intra-day</td>
<td>0.49</td>
</tr>
<tr>
<td>8</td>
<td>Repeatability of sample application</td>
<td>0.8420</td>
</tr>
<tr>
<td>9</td>
<td>Repeatability of measurements</td>
<td>0.3672</td>
</tr>
<tr>
<td>10</td>
<td>No. of theoretical plates</td>
<td>3760</td>
</tr>
</tbody>
</table>

Rf : Retention factor, RSD : Relative standard deviation
LOD: Limit of detection, LOQ: Limit of quantification

Fig. 1: Chromatographic characters of Glipizide
were made, and analyzed by the method. The %
recovery and % RSD were calculated, and found
for the method. The %

Stability studies were also carried out by
keeping the sample solution prepared at room
temperature for several hours, and was spotted
every time on a fresh plate. After development and
scanning, the plates were observed for change in
peak areas and appearance of additional peaks. It
was observed that the plates were stable up to 3 h,
after which, there was a significant change found
in peak areas and Rf values. The stability of drugs
on developed plates were also checked up to 24 h,
and were found to be stable up to 9 h. Hence, the
plates should be scanned within 9 h after
development.

The efficiency of the method is determined
by means of number of theoretical plates. It was
calculated using the formula, \( n=16 \times \frac{x}{y^2} \), where \( x \)
= Rf value of drugs and \( y \) = width of peaks.
The number of theoretical plates was 3760 for
glipizide. The complete validation parameters are
shown in Table 1.

The HPTLC method developed is simple,
accurate, and cost effective, and the statistical
analysis proved that the method is reproducible and
efficient for the analysis of glipizide, in combined
dosage form.

**EXPERIMENTAL**

The instrument used for the estimation,
was Camag Linomat V semi automatic sample
application, Camag TLC scanner 3, CATS V.4.06
software for interpretation of the data, Hamilton
syringe and Camag twin trough chamber. Standard
glipizide (98%) was obtained from SPARK
Laboratory, Baroda, India as gift sample. The pre
coated silica gel G 60 F \( \text{254} \) was used as stationary
phase, obtained from E. Merck. The mobile phase
used was ethyl acetate: formic acid: dichloromethane (1:1:2 v/v/v), chamber saturation
time 20 min, migration distance 70 mm, wavelength
scanning at 275.5 nm, band width 8 mm, slit
dimension 5±0.45 mm, scanning speed 20 nm/sec,
and the source of radiation was a deuterium lamp.
and methanol was used as solvent. All the solvents
used were of AR grade, obtained form S.D. Fine
Chemicals Ltd., Mumbai. Glipizide tablet
formulations were purchased from local market of
Raipur, C.G., India.

A standard glipizide solution was prepared
with accurately weighed 10 mg of glipizide dissolved
in methanol, into a 10 ml volumetric flask.

The sample drug solution was prepared
by taking 20 tablets, each containing 5 mg of
glipizide. The tablets were weighed and powdered.
The quantity of powder equivalent to 100 mg of
glipizide was weighed, transferred to a 100 ml of
volumetric flask, and extracted with 25 ml of
methanol thrice. The extracts were filtered through
Whatman filter paper, and the residue washed with
small amount of methanol. The filtered extract and
washings were transferred into 100 ml volumetric
flask, and volume was made up to 100 ml with
methanol.

On to a pre-washed and activated TLC
plate, 2-10 ml of standard stock solution of glipizide
was spotted with Linomat V Semi applicator. The
plates were developed and scanned. The peak
areas of each standard were obtained from the
system, and a calibration graph was plotted with
concentration vs. peak area.

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REFERENCES

