Stability Indicating High Performance Thin Layer Chromatographic Determination of Alogliptin Benzoate as Bulk Drug and in Tablet Dosage Form

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ABSTRACT
Alogliptin Benzoate is a novel hypoglycemic drug that belongs to dipeptidylpeptidase-4 inhibitor class which stimulates glucose-dependent insulin release. The present work describes development and validation of a new simple, accurate, precise and selective stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of Alogliptin Benzoate in tablet dosage form. The chromatographic separation was achieved by using Benzene: Ethyl acetate: Triethylamine (7.5: 2: 0.5, v/v/v) as mobile phase and UV detection at 222 nm. The retention factor for Alogliptin Benzoate was found to be 0.62 ± 0.10. The developed method was validated with respect to linearity, accuracy, precision, limit of detection, limit of quantitation and robustness as per ICH guidelines. The drug was subjected to stress condition of hydrolysis (acid, base), oxidation, photolysis and thermal degradation. Results found to be linear in the concentration range of 250-1500 ng band-1. The method has been successfully applied for the estimation of drug in tablet dosage form. The % assay (Mean ± S.D.) was found to be 99.56 ± 1.15. The developed method can be used for the quantification of drug in the dosage form, bulk drug as well as for routine analysis in quality control laboratories.

Keywords: alogliptin, HPTLC, forced degradation, tablet dosage form

INTRODUCTION
Alogliptin (ALGP), 2-[(6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl]methyl) benzonitrile is an anti-diabetic drug in the DPP-4 inhibitor class that decreases blood sugar and stimulates glucose-dependent insulin release [1].

Extensive literature review reveals High Performance Liquid Chromatographic (HPLC) [2-13], Spectrophotometric [14-18] and High Performance Thin Layer
Chromatographic (HPTLC) [19-21] methods for determination of ALGP in human plasma and pharmaceutical formulations either as single or in combination with other drugs.

To the best of our knowledge, no reports were found for stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of ALGP in tablet dosage form. This paper describes development and validation of simple, precise, accurate stability indicating HPTLC method for determination of ALGP in accordance with International Conference on Harmonisation Guidelines [22, 23].

EXPERIMENTAL

Chemicals and reagents

Pharmaceutical grade working standard ALGP was kindly supplied by Getz Pharma Research Pvt. Ltd., Ambarnath, Thane, India. The pharmaceutical dosage form used in this study was NESINA tablets labeled to contain 25 mg of ALGP were procured from the local market. Benzene, Ethyl acetate and Triethylamine (all AR grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

Chromatographic separation of drug was performed on Merck TLC plates precoated with silica gel 60 F254 (10 cm ×10 cm with 250 µm layer thickness) from E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using Benzene: Ethyl acetate: Triethylamine (7.5: 2: 0.5, v/v/v) as mobile phase. The mobile phase was saturated in chamber for 20 min. After development, TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 222 nm for all developments operated by WINCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of Standard Stock Solution

Standard stock solution was prepared by dissolving 25 mg of drug in 10 mL of methanol to get working standard solution of concentration 2500 ng µL\(^{-1}\) from which 1 mL was further diluted to 10 mL with methanol to get solution of 250 ng µL\(^{-1}\).

Selection of Detection Wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that drug showed considerable absorbance at 222 nm. So, 222 nm was selected as the wavelength for detection.
Analysis of Tablet Formulation

Tablet sample was prepared by taking weighed quantity of powder equivalent to 25 mg of ALGP and transferred to a 100 mL volumetric flask containing 50 mL of methanol and the content was sonicated for 15 min. The solution was filtered using Whatman paper No. 41 and the volume was made up to the mark with methanol to obtain the final concentration of 250 ng band⁻¹. Two µL volume of this solution was applied on TLC plate to obtain final sample concentration of 500 ng band⁻¹. After chromatographic development peak areas of the bands were measured at 222 nm and the amount of drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies of bulk drug

The forced degradation studies were carried out on bulk drug substance in order to prove the stability-indicating property and selectivity of the developed method. The degradation was carried out under acid/base hydrolytic, oxidative, thermolytic, and photolytic stress conditions. The solutions were neutralized after subjecting to the forced degradation studies.

Acid treatment

To 1 ml of stock solution, 1ml of 2 N Hydrochloric acid was added and the volume was made with methanol to obtain the concentration 250 ng µL⁻¹. The resulting solution was refluxed at 60°C for 30 min and 3 µL volume of solution was applied on TLC plate and developed under optimized chromatographic conditions to check the stability. The densitogram of the acid degraded sample showed additional peaks at R_f values of 0.32 and 0.74. The representative densitogram of acid treated sample is shown in Figure 1.

![Figure 1](image)

**Figure 1.** Representative densitogram of acid treated ALGP with degradation products D1 (R_f = 0.32) and D2 (R_f = 0.74)
Alkali treatment

1 mL of stock solution was mixed with 1 ml of 2 N Sodium hydroxide and the volume was made with methanol to obtain the concentration 250 ng µL⁻¹. The resulting solution was refluxed at 60°C for 30 min and 3 µL volume of solution was applied on TLC plate and developed under optimized chromatographic conditions. The densitogram had degradation product peak at $R_f$ of 0.48. The representative densitogram of base treated sample is shown in Figure 2.

Neutral Hydrolysis

1 mL of working standard solution was mixed with 1 mL of water and 8 mL methanol. The solution was refluxed for 72 h. 3 µL volume of resulting solution was applied on TLC plate and developed under optimized chromatographic condition. The drug was found to be stable in neutral hydrolytic condition.

Oxidative degradation

To 1 mL working standard solution, 1 mL of 20 % solution of $\text{H}_2\text{O}_2$ was added and the volume was made with methanol. The resulting solution was refluxed at 60°C for 30 min and 3 µL volume of solution was applied on TLC plate and developed under optimized chromatographic conditions. 15.57 % degradation was observed with single degradation product at $R_f$ 0.42. The representative densitogram of $\text{H}_2\text{O}_2$ treated sample is shown in Figure 3.
Photolytic studies were carried out by exposure of drug to UV light up to 200 watt h square meter\(^{-1}\) for 7 days and subsequently to visible light illumination not less than 1.2 million lux h. Sample was weighed, dissolved in methanol to get concentration of 250 ng µL\(^{-1}\). 3 µL of the resulting solution was applied to HPTLC. No degradation was observed for the drug under photolytic degradation when the drug was exposed to above conditions. The representative densitogram of drug exposed to visible light is shown in Figure 4.

**Photo-degradation**

Photolytic studies were carried out by exposure of drug to UV light up to 200 watt h square meter\(^{-1}\) for 7 days and subsequently to visible light illumination not less than 1.2 million lux h. Sample was weighed, dissolved in methanol to get concentration of 250 ng µL\(^{-1}\). 3 µL of the resulting solution was applied to HPTLC. No degradation was observed for the drug under photolytic degradation when the drug was exposed to above conditions. The representative densitogram of drug exposed to visible light is shown in Figure 4.
Degradation under dry heat

Dry heat study was performed by keeping drug in oven at 105°C for period of 8 h. A sample was withdrawn at appropriate times, weighed and dissolved in methanol to get solution of 250 ng µL⁻¹. 3 µL of the resulting solution was applied to HPTLC. The degradation was observed with reduction in the peak area as compared to the initial area. Representative densitogram obtained for sample subjected to dry heat is shown in Figure 5.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The primary objective in developing this stability indicating HPTLC method is to achieve the resolution of drug and its degradation products. Initially, many method trials were performed using different mobile phases in order to obtain better separation. Finally the mobile phase comprising Benzene: Ethyl acetate: Triethylamine (7.5: 2: 0.5, v/v/v) was selected as optimal for obtaining well defined and resolved peak. Densitometric evaluation was carried out at 222 nm. The retention factor for ALGP was found to be 0.62 ± 0.10. Representative densitogram of standard solution of ALGP is shown in Figure 6.
Stress degradation studies

The drug was found to be susceptible to acid and base catalysed hydrolysis, thermal stress as well as oxidation and was found to be stable in neutral hydrolytic and photolytic condition. The results obtained showed no interference or merging with any drug peak indicating the specificity of the method. The results of forced degradation studies are given in Table 1.

### Table 1. Summary of forced degradation studies of ALGP

<table>
<thead>
<tr>
<th>Stress conditions/ duration</th>
<th>% Assay of active substance</th>
<th>R_f values of degraded products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic / 2 N HCl/ Refluxed at 60°C for 30 min</td>
<td>81.24</td>
<td>0.32, 0.74</td>
</tr>
<tr>
<td>Alkaline /2 N NaOH/ Refluxed at 60°C for 30 min</td>
<td>83.46</td>
<td>0.48</td>
</tr>
<tr>
<td>oxidative /20 % H_2O_2 / Refluxed at 60°C for 30 min</td>
<td>84.43</td>
<td>0.42</td>
</tr>
<tr>
<td>Neutral/H_2O/ Reflux for 72 h</td>
<td>100.23</td>
<td>---</td>
</tr>
<tr>
<td>Photolysis: UV light 200 watt h square meter^{-1}</td>
<td>100.07</td>
<td>---</td>
</tr>
<tr>
<td>Visible light illumination not less than 1.2 million lux h</td>
<td>99.56</td>
<td>---</td>
</tr>
<tr>
<td>Dry heat/ 105°C/ 8 h</td>
<td>92.80</td>
<td>---</td>
</tr>
</tbody>
</table>

### Stress degradation studies

The method was validated with respect to linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness, in accordance with ICH guidelines [20, 21].
Preparation of Calibration Curve

For preparation of a calibration plot, 1, 2, 3, 4, 5 and 6 µL of standard solutions of ALGP (250 ng µL⁻¹) were applied to the TLC plates. Straight-line calibration graphs were obtained in the concentration range 250-1500 ng band⁻¹ with high correlation coefficient. The calibration equation and correlation coefficient was found to be \( y = 4.027x + 332.1 \) and \( R^2 = 0.999 \). The peak area of drug was plotted against the corresponding concentrations to obtain the calibration curve as shown in Figure 7.

Precision

Set of three different concentrations in three replicates of standard solutions of ALGP were prepared. All the solutions were analyzed on the same day in order to record any intra day variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.47 to 1.42. For Inter day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.64 to 1.20. The lower values of % R.S.D. (< 2) indicated that method was found to be precise. The % recoveries and % R.S.D. values obtained for Intraday and Interday variation studies are represented in Table 2.
Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ were found to be 67.14 ng band\(^{-1}\) and 203.46 ng band\(^{-1}\), respectively.

Recovery Studies

To check accuracy of the method, recovery studies were carried out by adding standard drug to pre-analysed sample at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 500 ng band\(^{-1}\) from tablet solution. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies indicated that the method is accurate for estimation of drug in tablet dosage form. The results obtained are shown in Table 3.

Robustness Studies

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition (±1% Ethyl acetate), chamber saturation time (±10%) was altered and the effect on the area of drug was noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

### Table 2. Intraday and Interday variation studies data

<table>
<thead>
<tr>
<th>Concentration (ng/band)</th>
<th>Intra-day Precision</th>
<th>Inter-day Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average area</td>
<td>Average % recovery</td>
</tr>
<tr>
<td>750</td>
<td>3338</td>
<td>99.52</td>
</tr>
<tr>
<td>1000</td>
<td>4388</td>
<td>100.71</td>
</tr>
<tr>
<td>1250</td>
<td>5383</td>
<td>100.34</td>
</tr>
</tbody>
</table>

### Table 3. Recovery Studies of ALGP

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount taken (ng band(^{-1}))</th>
<th>Amount added (ng band(^{-1}))</th>
<th>Total amount found (ng band(^{-1}))</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALGP</td>
<td>500</td>
<td>400</td>
<td>895.34</td>
<td>99.77</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>500</td>
<td>998.73</td>
<td>99.87</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>600</td>
<td>1091.35</td>
<td>99.20</td>
<td>1.39</td>
</tr>
</tbody>
</table>

*Average of three determinations*
CONCLUSION

A simple, precise, accurate, reproducible, and stability-indicating HPTLC method without interference from the excipients or from degradation products has been developed and validated for the determination of ALGP as bulk drug and in tablet dosage form. The developed method can be used for quantitative analysis of ALGP in pharmaceutical dosage form. The method was developed by using easily available and cheap solvents for analysis of drug hence can be considered as economic. As the method is stability indicating one it may be extended to study the degradation kinetics of drug.

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