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 stability indicating HPTLC method for the determination of alogliptin benzoate in tablet dosage form. The chromatographic separation was achieved by using Chloroform: Methanol 3:7 v/v as mobile phase and UV detection at 275 nm. 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 acid hydrolysis, alkali hydrolysis, photolysis, thermal degradation. Results found to be linear in concentration range of 500-2500 ng/band. The developed method can be used for the quantification of bulk drug as well as tablet dosage form

Keywords: Alogliptin, HPTLC, Degradation Studies, 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.

Alogliptin benzoate belongs to the class of Dipeptidyl peptidase-4 (DPP-4) inhibitors, a new class of anti-diabetic drugs which act by increasing glucose dependent insulin release \(^1\). Therapeutically DPP-4 inhibitors are used to treat type 2 diabetes alone or combination with other drugs which increase the sensitivity of insulin at target site \(^2\)-\(^6\).

Literature survey reveals very few reported HPLC \(^7\)-\(^10\) & HPTLC \(^11\)-\(^13\) methods for estimation of Alogliptin benzoate The objective of present study was to develop and validate a new, simple, precise, accurate and selective stability indicating HPTLC method for estimation of ALG as per International Conference on Harmonization (ICH) guidelines

MATERIALS AND METHOD

Chemicals and reagents

Alogliptin benzoate was purchased from Swapnroop Drugs and Pharmaceuticals, Aurangabad, Maharashtra, India with Certificate of analysis (COA) indicating its authenticity. The pharmaceutical dosage form used in this study was NESINA tablets labeled to contain 25 mg of ALGP were procured from the market. Chloroform, Methanol (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 x10 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 Chloroform: Methanol 3:7 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 275 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 of Alogliptin was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. From the standard stock solution, working standard solution was prepared containing 100 µg/ml of Alogliptin.

**Preparation of sample solution:**
20 tablets (Label Claim: Each tablet contains 25 mg of Alogliptin) were accurately weighed and powdered. From the powder, an amount equivalent to 10 mg of Alogliptin was accurately weighed and transferred to 10 ml volumetric flask. Methanol was added, sonicated for 15 min, solution was filtered. Dilutions are made to get the final concentration 100 µg/ml. 10 µl of the resultant solution was applied on TLC plate to get concentration of 1000 ng/band. Analysis was repeated for six times. Sample solution was spotted and area was recorded. % assay was determined from linearity equation.

**Selection of analytical wavelength:**
From the standard stock solution further dilutions were done using methanol and scanned over the range of 200 – 400 nm and the spectra was obtained. It was observed that the drug showed considerable absorbance at 275 nm.

**Densitogram:**
Solution of Alogliptin (100 µg/ml) was prepared. 10 µl (1000 ng/band) of solution was applied on pre-activated TLC plate with the help of Hamilton syringe (100 µl), using Linomat V sample applicator. The development chamber was saturated with mobile phase for 15 min. The spotted plate was placed in the saturated chamber and developed up to 80 mm distance. The plate was dried and was scanned at 275 nm. The retention factor was found to be 0.54 ± 0.03 respectively.

![Figure 1 Densitogram of standard solution of Alogliptin 1000 ng/band](image_url)
RESULTS AND DISCUSSION:

**Stress degradation studies of bulk drug**

Stress degradation studies were carried under condition of acid, base, neutral hydrolysis, oxidation, dry heat and photolysis. For each study working standard solution of Alogliptin subjected to stress condition. Dry heat and photolytic degradation were carried out in solid state.

**Degradation under acid catalyzed hydrolytic condition**

To 1 ml of 1000 µg/ml solution of Alogliptin, 1 ml of 0.5 N HCl was added. The volume was made up to 10 ml with methanol. The above solution was kept for overnight at room temperature. 25 µl of the resultant solution was then applied on TLC plate and densitogram was developed. Average 79.09 % of Alogliptin was recovered with no peak of degradation.

![Figure 2 Representative Densitogram of acid induced degradation of Alogliptin (2,500 ng/band)](image)

**Degradation under alkali catalyzed hydrolytic condition:**

To 1 ml of 1000 µg/ml solution of Alogliptin, 1 ml of 1 N NaOH was added. The volume was made up to 10 ml with methanol. The above solution was kept for overnight at room temperature. 25 µl of the resultant solution was then applied on TLC plate and densitogram was developed. Average 71.45 % of Alogliptin was recovered with no peak of degradation.
Figure 3 Representative Densitogram of alkali induced degradation of Alogliptin (2,500 ng/band)

Degradation under neutral hydrolytic condition:
To 1 ml of 1000 µg/ml solution of Alogliptin, 1 ml of distilled water was added. The volume was made up to 10 ml with methanol. The above solution was kept for overnight at room temperature. 25 µl of the resultant solution was then applied on TLC plate and densitogram was developed. Average 83.15 % of Alogliptin was recovered with no peak of degradation.

Figure 4 Representative Densitogram of neutral degradation of Alogliptin (2,500 ng/band)
Degradation under oxidative condition:
To 1 ml of 1000 µg/ml solution of Alogliptin, 3 ml of 30 % H₂O₂ was added. The volume was made up to 10 ml with methanol. The above solution was kept for overnight at room temperature. 25 µl of the resultant solution was then applied on TLC plate and densitogram was developed. Average 72.56 % of Alogliptin was recovered with no peak of degradation.

Figure 5 Representative Densitogram of oxidative degradation of Alogliptin (2,500 ng/band)

Degradation under dry heat:
Dry heat studies were performed by keeping drug sample in oven (80°C) for 8 hours. Sample was withdrawn, dissolved in methanol and diluted to get 1000 µg/ml. 25 µl of the resultant solution was then applied on TLC plate and densitogram was developed. Average 91.80 % of Alogliptin was recovered with no peak of degradation.

Figure 6 Representative Densitogram of dry heat degradation of Alogliptin (2,500 ng/band)
Photo-degradation studies:
Photolytic degradation studies were carried out by exposing drug to UV light up to 200 watt hours/square meter. Sample was weighed, dissolved in methanol to get concentration of 1000 μg/ml. and further dilutions were made with methanol to get final concentration (100 μg/ml). 25 μl of the resultant solution was then applied on TLC plate and densitogram was developed. After the photo degradation study under UV light 99.68 % Alogliptin was recovered with no peak of degradation.

![Figure 7](image.png)  
**Figure 7** Representative Densitogram of Alogliptin Photolytic degradation (2,500 ng/band)

Summary of stress degradation study of Alogliptin

| Stress condition/Duration                  | % Assay of active substance | Rf values of degraded products |
|--------------------------------------------|----------------------------|--------------------------------|
| Acid (0.5 N HCl, kept for overnight hr at RT) | 79.09                      | 21.91                          |
| Base (1N NaOH, kept for overnight at RT)   | 71.45                      | 24.55                          |
| Water (kept for overnight at RT)           | 83.15                      | 10.85                          |
| 30% H₂O₂ (kept for overnight at RT)        | 72.56                      | 29.44                          |
| Heat dry (80⁰C)                             | 91.80                      | 18.2                           |
| UV light                                   | 99.68                      | -                              |

Validation of Analytical Method

Specificity
The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.999, indicating the non-interference of any other peak of degradation product or impurity.

Linearity
From the standard stock solution (1000 µg/ml) of Alogliptin, solution was prepared containing 100 µg/ml of Alogliptin. This solution was further used for spotting. Five replicates per concentration were spotted. The linearity (relationship between peak area and amount spotted) was determined by analyzing five concentrations over concentration range of 500-2500 ng/band for Alogliptin. The results obtained are shown in Table 2, the peak area were plotted against the corresponding amount spotted to obtain the calibration curve as shown in Fig. 8 for Alogliptin.

**Table 2: Linearity study of Alogliptin**

| Replicate | Amount of Alogliptin (ng/band) |
|-----------|-------------------------------|
|           | 500   | 1000  | 1500  | 2000  | 2500  |
| 1         | 1635.6 | 3409  | 5428.2| 6358.4| 8197.1|
| 2         | 1735.4 | 3365.2| 5537.4| 6402  | 8395.6|
| 3         | 1758.4 | 3296.5| 5487.8| 6014.7| 8457.6|
| 4         | 1694.3 | 3478.7| 5684.5| 6392.8| 8538.2|
| 5         | 1692.6 | 3375.2| 5543  | 6195.8| 8369.6|
| Average   | 1703.26| 3384.92| 5536.18| 6272.74| 8391.62|
| SD        | 47.02  | 66.47 | 94.93 | 166.55| 126.66|
| % RSD     | 2.76   | 1.96  | 1.71  | 2.65  | 1.50  |

**Figure 8 Calibration curve for Alogliptin**

**X Range**
Alogliptin = 500-2500 ng/band

**X Precision**
The precision of the method was demonstrated by Intra-day and Inter-day variation studies. In the Intra-day studies, 3 replicates of 3 concentrations were analyzed on the same day, and percentage RSD was calculated. For the Inter-day variation studies, 3 replicates of 3 concentrations were...
analyzed on 3 consecutive days and percentage RSD were calculated. For Intra-day precision % RSD found to be 1.41% and for Inter-day precision % RSD found to be 1.43%

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

LOD and LOQ are calculated from the formula:

\[ \text{LOD} = 3.3 \frac{\sigma}{S} \]
\[ \text{LOQ} = 10 \frac{\sigma}{S} \]

Where, \( \sigma \) = standard deviation of lowest response, \( \sigma = 47.02 \)
\( S \) = slope of calibration curve, \( S = 3.25 \)

**LOD of Alogliptin = 47.70 ng/band**

**LOQ of Alogliptin = 144.56 ng/band**

**Assay**

20 tablets were accurately weighed and powdered. From the powder, an amount equivalent to 10 mg of Alogliptin was accurately weighed and transferred to 10 ml volumetric flask. Methanol was added, sonicated for 15 min, solution was filtered. Dilutions are made to get the final concentration 100 \( \mu \)g/ml. Analysis was repeated for six times. Sample solution was spotted and area was recorded. % assay was determined from linearity equation. The results obtained are shown in Table 3

| Sr. No. | Peak area of Alogliptin (1000 ng/band) | Amount Recovered (ng/band) | % Recovery |
|---------|--------------------------------------|-----------------------------|------------|
| 1       | 3451.5                               | 1007.114                    | 100.711    |
| 2       | 3394.2                               | 989.483                     | 98.948     |
| 3       | 3407.4                               | 993.545                     | 99.354     |
| 4       | 3397.8                               | 990.591                     | 99.059     |
| 5       | 3431.7                               | 1001.022                    | 100.102    |
| 6       | 3421.6                               | 997.914                     | 99.791     |
| Mean    | 3417.37                              | 996.611                     | 99.661     |
| % RSD   | 0.642                                | 0.677                       | 0.677      |
Figure 9 Densitogram of sample solution of Alogliptin (1000 ng/band)

Accuracy

Recovery studies were carried out by addition of standard drug to pre-analysed sample solution at three different levels 80, 100 and 120 % to check the accuracy of the method. Basic concentration of sample chosen was 1000 ng/band. % recovery was determined from linearity equation. The results obtained are shown in Table 4

| Level | Amount Taken (ng/band) | Amount added (ng/band) | Area     | % Recovery | % RSD |
|-------|------------------------|------------------------|----------|------------|-------|
| 80    | 1000                   | 800                    | 5987.5   | 99.301     | 0.86  |
|       |                        |                        | 5904.6   | 97.884     |       |
|       |                        |                        | 5897.7   | 97.766     |       |
| 100   | 1000                   | 1000                   | 6513.4   | 97.462     | 1.95  |
|       |                        |                        | 6587.9   | 98.608     |       |
|       |                        |                        | 6758.5   | 101.233    |       |
| 120   | 1000                   | 1200                   | 7354.2   | 100.361    | 0.93  |
|       |                        |                        | 7419.7   | 101.277    |       |
|       |                        |                        | 7285.6   | 99.402     |       |

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which chamber saturation time and time was also changed from spotting to development and development to scanning and the effects on the peak area was noted. It was found that method was robust.
Summary of validation study

The summary of validation parameters are summarized in Table 5

Table 5. Summary of validation parameters

| Sr. No. | Validation parameters | Alogliptin |
|---------|-----------------------|------------|
| 1.      | Linearity Equation    | y = 3.252 x + 178.3 |
|         | (R²)                  | R² = 0.984 |
|         | Range                 | 500-2500 ng/band |
| 2.      | Precision (% RSD)     | Intraday: 1.41 |
|         |                       | Inter-day: 1.43 |
| 3.      | Assay                 | 99.661% |
| 4.      | Accuracy              | 80 %: 98.31 |
|         |                       | 100 %: 99.10 |
|         |                       | 120 %: 100.34 |
| 5.      | LOD                   | 47.70 ng/band |
| 6.      | LOQ                   | 144.56 ng/band |
| 7.      | Specificity           | Specific |
| 8.      | Robustness            | Robust |

CONCLUSION:

A simple, precise, accurate, reproducible, and stability-indicating HPTLC method 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.

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