ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF GENOTOXIC IMPURITY p-ANISALDEHYDE IN TENELIGLIPTIN USING GC-MS

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ABSTRACT
This research aimed to develop a sensitive GC–MS (Gas Chromatography-Mass Spectrometry) technique for the estimation of genotoxic impurity p-anisaldehyde in Teneligliptin drug to meet the current regulatory requirements. A simple and specific method was developed and validated according to ICH (International Council for Harmonization) guidelines. The LOQ (limit of quantitation) and LOD (limit of detection) values were determined as 0.38 and 0.12 ppm, respectively. The approach was linear in the range of 0.38–56 ppm with a correlation coefficient of 0.9997. The average recovery of Teneligliptin was 93%. The validation parameters strongly support that the developed technique is suitable to determine the p-anisaldehyde in Teneligliptin hydrobromide hydrate.

Keywords: p-Anisaldehyde, Teneligliptin, GC-MS, Method Development, Method Validation.

INTRODUCTION
The antidiabetic therapeutic category drug Teneligliptin hydrobromide hydrate is a widely recommended medicine for the treatment of type 2 diabetes. The residual solvents and catalysts may exist in pharmaceuticals through the manufacturing process and it is witnessed in Teneligliptin as well1-5; they should be either controlled during the process or removed from the drug to the possible amount as they do not provide any therapeutic benefits6. Indeed, the production processes do not completely eliminate these contaminants6, and hence, it is essential to evaluate the residual solvents and/or catalysts leached into the drug. p-Anisaldehyde is used as a phase transfer catalyst7 in the synthesis of Teneligliptin, which may result in the presence of p-anisaldehyde as a low-level residual contaminant in the final product (Teneligliptin drug Fig.-1).

Numerous efforts were made during the synthetic process-related modifications to eliminate such potential organic ionic impurities, but it is practically impossible to eliminate all the impurities completely from the final drug substance. Hence, some analytical methods were developed and reported for the estimation of impurities in gliptins and other pharmaceuticals8-15 but to our best, no report was available for p-anisaldehyde. Hence, we were motivated to develop an efficient robustic method for the estimation of p-anisaldehyde in Teneligliptin.

EXPERIMENTAL
Materials
All solvents and reagents used in this experiment were of analytical grade. Acetonitrile and p-anisaldehyde were obtained from Sigma-Aldrich Chemicals Pvt. Ltd., New Delhi, India.

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GC-MS Conditions Optimization
As a part of the technology development process, several chromatographic parameters were evaluated in order to get an acceptable peak shape with acceptable recoveries, compatible with the GC system. The other optimized factors are flow rate (1.0–1.5 ml at constant flow), starting column temperature (80–150 °C), and injector temperature (+10 °C above the column temperature). Particularly, during the optimization process, different capillary GC columns (HP-5, DB-1, and SPB-624) with varying film thicknesses were also tested. Among them, the HP-5 (30 m length × 0.32 mm × 1 µm film thickness) and DB-1 (30 m × 0.32 mm × 1µm) columns showed credible retention times even at low temperatures. However, only on DB-1 capillary column (60 m × 0.32 mm × 0.25 µm) with Helium carrier gas (injection temperature: 260 °C; initial temperature: 120 °C, hold 1 min, ramping: 15 °C/min up to 280 °C, held 1 min; flow rate: 2mL/min; split ratio 10:1; source and auxiliary temperatures 280 °C; MS quad temperature 150 °C) provided a satisfactory degree of selectivity, chromatographic separation, resolution, as well as sensitivity with a stable baseline. The selection of SIM ion is the process, in which p-anisaldehyde was scanned through scan mode in the range of 29 to 150 Da and selected the major as well as more stable ion at m/z 135 (Fig.-2).

![Fig.-2: GC-MS of p-anisaldehyde Standard](image)

Diluent
Acetonitrile was used as the diluent and the blank chromatogram is reproduced in Fig.-3.

![Fig.-3: Typical Blank Chromatogram](image)

Standard Stock Preparation
p-Anisaldehyde standard stock solution was prepared by transferring 75 mg of p-anisaldehyde into a 50 mL volumetric flask.

Standard Solution Preparation
2.5 ml of standard stock solution was diluted to 50 ml to prepare the standard solution and its chromatogram is reproduced in Fig.-4.

![Fig.-4: Typical Standard Chromatogram](image)

Sample Preparation
1 g of the sample was transferred into a 5 ml volumetric flask, and then diluent was added up to the mark. The sample chromatogram is shown in Fig.-5.

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RESULTS AND DISCUSSION

Method Development
Initially, the experiments were performed using a DB-5 column (5% phenyl–95% dimethylpolysiloxane) but the observed peaks were not good. Thereafter, the DB-1 column (100% dimethylpolysiloxane) was used and thus sharp peaks were achieved. The impact of injection volume on determination and separation was examined by injecting a 1 µL solution. Based on the detector response, the split ratio was set as 10:1. The influence of the initial column temperature on the separation of \( p \)-anisaldehyde was examined at 120 °C. The system was operated by the following injection sequence with the number of injections as mentioned in the brackets: blank (1 injection), calibration standard solution (6 injections), blank (1 injection), sample solution (2 injections), and standard solution (1 injection).

Method Validation
The current technique was verified in accordance with the requirements for analytical method validation established by the ICH\(^{16} \) and USFDA\(^{17} \).

System Suitability
As a part of the system suitability study, \( p \)-anisaldehyde standard solution (37.5 ppm) was injected into the GC-MS and the results are summarized in Table-1.

| Preparation | Response of \( p \)-anisaldehyde |
|-------------|---------------------------------|
| Std-1       | 253874.92                       |
| Std-2       | 243089.04                       |
| Std-3       | 241523.23                       |
| Std-4       | 251994.42                       |
| Std-5       | 257339.45                       |
| Std-6       | 257207.27                       |
| Average     | 250838.06                       |
| SD          | 6931.20                         |
| % RSD       | 2.76                            |

The % relative standard deviation (% RSD) area of the \( p \)-anisaldehyde peak obtained from six preparations of the standard solution was found within the acceptance criteria and hence, the system is suitable for estimation of \( p \)-anisaldehyde content in Teneligliptin.

Specificity
The specificity investigation was carried out by infusing the solvents that were employed during the production process of Teneligliptin. According to the findings of the investigation, there was no interference in the retention time of analyte components during the analysis.

LOD and LOQ
The calibration curve approach was utilized for the estimation of LOD and LOQ values according to the ICH guidelines of analytical methods validation. For \( p \)-anisaldehyde, the LOD and LOQ values are 0.38 and 56 ppm, respectively. The % RSD peak area of obtained from six LOQ preparations is 2.24. All the data are summarized in Table-2.
Table-2: Precision at LOQ Level

| Concentration of p-anisaldehyde (200 ppm) | Response of p-anisaldehyde |
|------------------------------------------|----------------------------|
| LOQ-1                                    | 829.00                     |
| LOQ-2                                    | 850.00                     |
| LOQ-3                                    | 833.73                     |
| LOQ-4                                    | 827.49                     |
| LOQ-5                                    | 848.86                     |
| LOQ-6                                    | 799.00                     |
| Average                                  | 831.35                     |
| SD                                       | 18.59                      |
| % RSD                                    | 2.24                       |

Linearity
System and column were conditioned to have a stable baseline. The injection sequence was followed as mentioned in the protocol and the observations were recorded. Linearity was performed in the range of LOQ to 150% and 0.9995 was the correlation coefficient (Table-3).

Table-3: Linearity of p-anisaldehyde

| Linearity level | Concentration (ppm) | Area of injection-1 | Area of injection-2 | Average response |
|-----------------|---------------------|---------------------|--------------------|-----------------|
| LOQ Level       | 0.38                | 1940.00             | 1983.00            | 1961.50         |
| 50% Level       | 18.75               | 116138.92           | 114132.38          | 115135.65       |
| 100% level      | 28.13               | 171842.00           | 166348.00          | 169095.00       |
| 120% Level      | 37.50               | 231545.00           | 234393.00          | 232969.00       |
| 150% Level      | 46.88               | 290535.00           | 300350.00          | 295442.50       |

Correlation coefficient 0.9997
y-intercept 1896.51
%y-intercept 0.81
Slope 6246.73

Precision and Accuracy
The accuracy of the approach was determined by injecting the standard solution containing 37.5 ppm of p-anisaldehyde into six replicate preparations. p-Anisaldehyde has had 1.83% RSD (within the acceptance range) for the six replicates as shown in Table-4. As a result of the low % RSD values of the analyte peak area, the precision of the devised technique was confirmed as good.

Table-4: Method Precision Analysis

| Sample spiked solution | p-Anisaldehyde (ppm) |
|------------------------|-----------------------|
| 1.00                   | 34.23                 |
| 2.00                   | 33.34                 |
| 3.00                   | 33.45                 |
| 4.00                   | 32.57                 |
| 5.00                   | 33.95                 |
| 6.00                   | 32.96                 |
| Mean                   | 33.42                 |
| SD                     | 0.61                  |
| %RSD                   | 1.83                  |

Accuracy analysis was carried out by spiking the p-anisaldehyde in samples at QL levels, 50, 100, and 150%; the chromatogram (at 100%) and data are reproduced in Table-5. From the table, it is clear that the average recovery % is well within the acceptance criteria.

Table-5: Accuracy of p-anisaldehyde

| Level | % Recovery |
|-------|------------|
| QL level | 107.56    |
Robustness

Robustness was assessed by studying the impact of small variations in oven temperature, injector temperature, and detector temperature on the peak area of \( p \)-anisaldehyde at 37.5 ppm. The % RSD of \( p \)-anisaldehyde peak areas is summarized in Table-6. The results show that the RSD is well within 5.20% and thus demonstrated the robustness of the suggested method. (Acceptance criteria: RSD of peak areas should be ≤15% for six injections).

| Injection Method’s idle condition | Oven temperature (120 °C) | Injector temperature (260 °C) | Flow rate (2 mL/min) |
|----------------------------------|---------------------------|-------------------------------|---------------------|
|                                  | 108 | 132 | 234 | 286 | 1.80 | 2.20 |
| %RSD                             | 2.76 | 2.24 | 2.28 | 5.20 | 4.28 | 0.36 | 2.20 |

Based on the outcome of the method validation studies, the recommendations of the standard test procedure of \( p \)-anisaldehyde in Teneliglitin are summarized in Table-7.

**CONCLUSION**

Identification and quantification of \( p \)-anisaldehyde in Teneliglitin were successfully achieved by the direct GC-MS method with significant LOD and LOQ values of 0.12 and 0.38 ppm, respectively. The validated method is simple, rapid, precise, specific, linear, and robust. In addition, the method is very easy to adapt in any industrial analytical lab as it does not demand the need of derivatization.

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