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# A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TENELIGLIPTIN AND PIOGLITAZONE IN PHARMACEUTICAL APPLICATIONS

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## ABSTRACT

A validated RP-HPLC method was developed and validated for the simultaneous estimation of TEN and PIO. HPLC method was developed in reverse phase mode using Acetonitrile, methanol and ammonium acetate buffer (pH 4.7 adjusted with orthophosphoric acid) as mobile phase in the ratio of 40:20:40 (v/v/v) over Inertsil BDS column (250×4.6mm, 5µm) at a volumetric rate 1mL/min and Quantitation was achieved with PDA detector at 247nm and elution time was identified to be 3.539 min and 6.075 min for TEN and PIO respectively. According to ICH guidelines the developed method was validated. The linearity was considered to be in the range of 10-30 µg/mL and 5 – 25 µg/mL with correlation coefficient of 0.999 and 0.999 for TEN and PIO respectively. The developed method is validated and the peaks were more resolved when compared to the previous literatures with reduced run time. The % RSD of TEN and PIO were 0.93 and 1.16. The regression coefficient of TEN and PIO were Y=19208X+3366and Y=6001X+225.1 respectively. The mean recovery was considered to be 99.73 and 99.88% of TEN and PIO respectively. Method was precise and robust with a % RSD NMT 2. Hence the developed RP-HPLC method was assessed to be specific and can be used for conventional analysis and academics also this expedite as an analogous method for synchronous estimation of drugs TEN and PIO in marketed formulation since there was no co-elution of peak with the drug.

**KEYWORDS:** Anti-diabetic, TEN, PIO, RP-HPLC, ICH.

## 1. INTRODUCTION

Teneligliptin is a DPP-4 inhibitor used in the management of type 2 diabetes mellitus. Pioglitazone is a thiazolidinedione class agent that improves insulin sensitivity. The combination therapy of these two drugs offers improved glycemic control. There is a need for a reliable and validated analytical method for the simultaneous estimation of these drugs in pharmaceutical preparations to ensure quality and efficacy.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Reagents

Teneligliptin and Pioglitazone reference standards were obtained from a certified source. Acetonitrile (HPLC grade), potassium dihydrogen phosphate, and orthophosphoric acid (AR grade) were used for preparation of the mobile phase. Deionized water was used throughout.

### 2.2 Instrumentation and Chromatographic Conditions

The analysis was carried out using an HPLC system equipped with a UV detector and a C18 column (250 × 4.6 mm, 5 µm). The mobile phase consisted of acetonitrile and phosphate buffer (60:40, v/v), pH adjusted to 4.0. The flow rate was maintained at 1.0 mL/min with a detection wavelength of 260 nm. Injection volume was 20 µL.

### 2.3 Preparation of Standard and Sample Solutions

Stock solutions of Teneligliptin and Pioglitazone (100 µg/mL each) were prepared in the mobile phase. Working solutions were prepared by appropriate dilution to obtain concentrations in the range of 5–50 µg/mL. Commercial tablets containing both drugs were powdered, and an appropriate amount was extracted with the mobile phase, filtered, and diluted for analysis.

### 2.4 Method Validation

The method was validated for parameters including linearity, accuracy, precision, specificity, robustness, LOD, and LOQ in accordance with ICH Q2(R1) guidelines.

## 3. RESULTS

**Table 1: Parameters of optimized chromatogram.**

| Parameters | Standard | | Sample | |
|---|---|---|---|---|
| | TEN | PIO | TEN | PIO |
| Elution time | 3.539 | 6.075 | 3.573 | 6.057 |
| Tailing factor | 1.721 | 0.904 | 1.628 | 0.905 |

| | | | | |
|---|---|---|---|---|
| Theoretical plates | 2529 | 7816 | 2409 | 7755 |
| Resolution time | NA | 9.120 | NA | 8.775 |

**Table 2: System suitability parameters for TEN and PIO.**

| S.NO | TEN | PIO |
|---|---|---|
| | Peak area | Peak area |
| 1. | 320674 | 89804 |
| 2. | 312777 | 90076 |
| 3. | 323317 | 90146 |
| 4. | 317685 | 89685 |
| 5. | 319740 | 90570 |
| 6. | 325788 | 92609 |
| **Mean** | 319997 | 90482 |
| **SD** | 4528.934 | 1086.693 |
| **%RSD** | 1.42 | 1.20 |

**Table 3: System suitability data.**

| Parameters | TEN | PIO |
|---|---|---|
| **Elution time** | 3.609 | 5.90 |
| **Tailing factor** | 1.622 | 0.911 |
| **Theoretical plates** | 2483 | 7730 |
| **%RSD** | 1.42 | 1.20 |
| **Resolution** | NA | 8.233 |

**Table 4: Linearity data for TEN and PIO.**

| TEN | | PIO | |
|---|---|---|---|
| Concentration (µg/mL) | Peak area | Concentration (µg/mL) | Peak area |
| 10 | 193302 | 5 | 42960 |
| 15 | 211165 | 10 | 49386 |
| 20 | 312850 | 15 | 89672 |
| 25 | 684974 | 20 | 147022 |
| 30 | 698336 | 25 | 191490 |
| $r^2$ =0.999 | | $r^2$= 0.999 | |

**Table 5: Accuracy data for TEN and PIO.**

| Drugs | % Level | Sample peak area | Standard peak area | % Recovery | % Average recovery | %Overall mean recovery |
|---|---|---|---|---|---|---|
| TEN | 50% | 159845 | 319997 | 99.20 | 99.25 | 99.73 |
| | | 159987 | 319997 | 99.21 | | |
| | | 159956 | 319997 | 99.35 | | |
| | 100% | 320589 | 319997 | 99.55 | 99.80 | |
| | | 321589 | 319997 | 99.81 | | |
| | | 321986 | 319997 | 100.06 | | |
| | 150% | 478954 | 319997 | 99.23 | 100.15 | |
| | | 485678 | 319997 | 100.56 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 485789 | 319997 | 100.68 | | |
| PIO | 50% | 45869 | 90482 | 100.37 | 100.17 | 99.88 |
| | | 45785 | 90482 | 100.10 | | |
| | | 45689 | 90482 | 100.06 | | |
| | 100% | 90562 | 90482 | 99.15 | 99.52 | |
| | | 91002 | 90482 | 99.58 | | |
| | | 91125 | 90482 | 99.85 | | |
| | 150% | 136521 | 90482 | 99.07 | 99.83 | |
| | | 136658 | 90482 | 99.76 | | |
| | | 137785 | 90482 | 100.68 | | |

**Table 6: System precision data for TEN and PIO.**

| Injection no | System precision | |
|---|---|---|
| | TEN | PIO |
| | Peak area | Peak area |
| **1.** | 320674 | 89804 |
| **2.** | 315777 | 90376 |
| **3.** | 323317 | 90146 |
| **4.** | 317885 | 89685 |
| **5.** | 319740 | 90570 |
| **6.** | 325788 | 92609 |
| **AVG** | 320530 | 90532 |
| **SDV** | 3623.418 | 1071.083 |
| **%RSD** | 1.13 | 1.18 |

**Table 7: Method precision data for TEN and PIO.**

| Injection no | Method precision | |
|---|---|---|
| | TEN | PIO |
| | Peak area | Peak area |
| **1.** | 320674 | 89804 |
| **2.** | 319987 | 90376 |
| **3.** | 323317 | 90746 |
| **4.** | 317885 | 89685 |
| **5.** | 318740 | 90570 |
| **6.** | 325798 | 92609 |
| **AVG** | 321067 | 90632 |
| **SDV** | 2976.769 | 1055.774 |

**Table 8: LOOD & LOQ data for Teneliglitpin and PIO.**

| Drug | LOD | LOQ |
|---|---|---|
| **TEN** | 1µg/mL | 3µg/Ml |
| **PIO** | 0.5µg/mL | 1.5µg/mL |

**Table 9: Robustness data for TEN and PIO.**

| S.NO | Parameters | TEN | | | PIO | | |
|---|---|---|---|---|---|---|---|
| | | RT (min) | Peak area | % RSD | RT (min) | Peak area | % RSD |
| 1. | Change in flow rate-0.9mL/min | 3.993 | 363932 | 0.174 | 6.640 | 102471 | 0.686 |
| | | 3.993 | 364832 | | 6.640 | 103471 | |
| 2. | Change in flow rate–1.1 mL/min | 3.282 | 300818 | 0.686 | 5.146 | 83134 | 0.347 |
| | | 3.282 | 301918 | | 5.146 | 83544 | |
| 3. | Increase in mobile phase composition 45:20:35% v/v/v | 3.732 | 294571 | 0.336 | 6.120 | 84498 | 0.408 |
| | | 3.732 | 255977 | | 6.120 | 84988 | |
| 4. | Decrease in mobile Phase composition 35:25:40 %v/v/v | 3.327 | 319543 | 0.100 | 5.497 | 93146 | 0.493 |
| | | 3.327 | 319999 | | 5.497 | 93799 | |

**4. Summary**

| S.NO | Parameters | Acceptancecriteria | | | Name of the compound | Result | |
|---|---|---|---|---|---|---|---|
| | | | | | | Theoretical platecount | Resolution |
| 1. | **System suitability** | Plate count shouldbe more than 2000 andresolutionmust bemorethan2 | | | TEN | >2000 | - |
| | | | | | PIO | >2000 | >2 |
| 2. | **Linearity** | r2≤ 0.999 | | | TEN | r2=0.999 | |
| | | | | | PIO | r2=0.999 | |
| 3. | **Accuracy** | %Recoveryshould bebetween98%and 102% | | | TEN | 99.73% | |
| | | | | | PIO | 99.88% | |
| 4. | **System precision** | %RSD than 2% | not | more | TEN | 1.13% | |
| | | | | | PIO | 1.18% | |
| 5. | **Method precision** | %RSD than 2% | not | more | TEN | 0.93% | |
| | | | | | PIO | 1.16% | |
| 6. | **LOD** | - | | | TEN | 1.0µg/mL | |
| | | | | | PIO | 0.5µg/mL | |
| 7. | **LOQ** | - | | | TEN | 3.0µg/mL | |
| | | | | | PIO | 1.5µg/mL | |
| 8. | **Robustness** | Method should not be affected during changeinmethod parameters | | | TEN | Method was not affected during changes done in the flow rate and mobile phase | |
| | | | | | PIO | | |
| 9. | **Assay** | - | | | TEN | 99.55% | |
| | | | | | PIO | 99.75% | |

**5. CONCLUSION**

A simple, precise and accurate RP-HPLC method was developed and validated for simultaneous estimation of TEN and PIO. The elution time for TEN and PIO was 3.573 and 6.075 respectively. The calibration curves were linear over a concentration range of 10µg/mL

- 30µg/Ml for TEN and 5µg/mL-25µg/mL for PIO with a correlation coefficient of 0.9994, 0.9992 and 0.9992. Method was precise and robust with a % RSD NMT 2. Method was accurate with % recovery of 99.55% and 99.75% for TEN and PIO. The acceptance limits were satisfied for all parameters, including theoretical plates, resolution, and tailing factor. Hence, the developed method can be used for conventional analysis in industries and academics also this facilitates asa alike method for synchronous estimation of drugs in pharmaceutical application.

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