ISSN: 2231-3656
Print: 2231-3648

*International Journal of Pharmacy and Industrial Research* (IJPIR)

*IJPIR |Vol.13 | Issue 4 | Oct - Dec -2023*

www.ijpir.com

DOI : https://doi.org/10.61096/ijpir.v13.iss4.2023.305-312

**Research**

# Analytical method development and validation by simultaneous estimation of pioglitazone and teneligliptin in Pharmaceutical dosage form rp-hplc.

**Upparapalli Bharghavi, Dr. S. Sridhar*, Dr. C. Parthiban, Dr. M. Sudhakar.**

*Department of Pharmaceutical Chemistry, Malla Reddy College Of Pharmacy, Maisammaguda, Secunderabad 500010, Telangana.*

*Author for Correspondence: Dr. S. Sridhar
Email id: drss.chem@gmail.com

Published on: 26 Dec 2023

Published by:
DrSriram Publications



## Abstract

A simple, Accurate, precise method was developed for the simultaneous estimation of the Pioglitazone and Teneligliptin in tablet dosage form. Chromatogram was run through DiscoveyC18 (4.8 x 150mm, 5µm).Mobile phase containing Methanol: 0.01N K2HPo4 taken in the ratio 55:45 was pumped through column at a flow rate of 0.9 ml/min. Buffer used in this method Phosphate buffer and ph is adjusted to 5.4 by adding 0.1% Formic acid. Temperature was maintained at 30°C. Optimized wavelength selected was 240 nm. Retention time of Pioglitazone and Teneligliptin were found to be 2.320 min and 3.256 min. %RSD of the Pioglitazone and Teneligliptin were and found to be 0.6 and 1.5 respectively. %Recovery was obtained as 99.78% and 100.15% for Pioglitazone and Teneligliptin respectively. LOD, LOQ values obtained from regression equations of Pioglitazone and Teneligliptin were 0.02, 0.07 and 0.07, 0.21 respectively. Regression equation of Pioglitazone is y = 27728x + 4960.5. And y = 38863x + 7130.7 of Teneligliptin . Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries

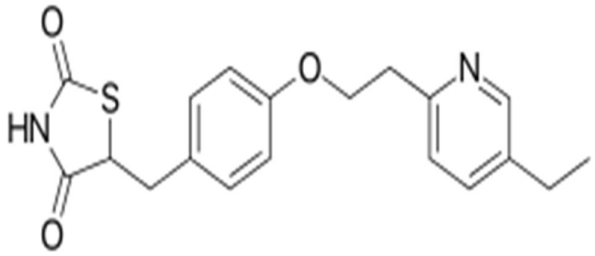
**Keywords:** Pioglitazone, Teneligliptin, RP-HPLC

## INTRODUCTION

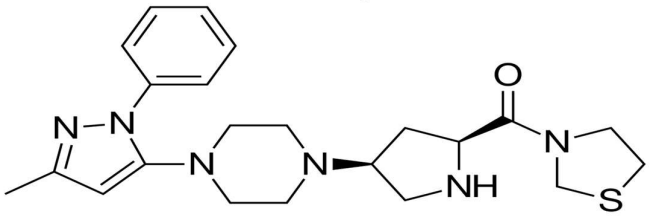
The quality of a drug plays an important role in ensuring the safety and efficacy of the drugs. Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. Hence Analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. The quality of the analytical data depends on the quality of the methods employed in generation of the data (1) . Hence, development of rugged and robust analytical methods is very important for statutory certification of drugs and their formulations with the regulatory authorities.The quality and safety of a drug is generally assured by monitoring and controlling the assay and impurities effectively. While assay determines the potency of the drug and impurities will determine the safety aspect of the drug. Assay of pharmaceutical products plays an important role in efficacy of the drug in patients. The wide variety of challenges is encountered while developing the methods for different drugs depending on its

nature and properties. This along with the importance of achieving the selectivity, speed, cost, simplicity, sensitivity, reproducibility and accuracy of results gives an opportunity for researchers to come out with solution to address the challenges in getting the new methods of analysis to be adopted by the pharmaceutical industry and chemical laboratories. Different physico-chemical methods (1) are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the physico-chemical methods, the most important are optical (refractometry, polarimetry, emission and fluorescence methods of analysis), photometry (photocolorimetry and spectrophotometry covering UV-Visible, IR Spectroscopy and nepheloturbidimetry) and chromatographic (column, paper, thin layer, gas liquid and high performance liquid chromatography) methods. Methods such as nuclear magnetic resonance (NMR) and para magnetic resonance (PMR) are becoming more and more popular. The combination of mass spectroscopy (MS) with gas chromatography is one of the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures which are based on complex formation; acid-base, precipitation and redox reactions. Titrations in non-aqueous media and complexometry have also been used in pharmaceutical analysis. The number of new drugs is constantly growing. This requires new methods for controlling their quality. Modern pharmaceutical analysis must need the following requirements.

1. The analysis should take a minimal time.
2. The accuracy of the analysis should meet the demands of Pharmacopoeia.
3. The analysis should be economical.
4. The selected method should be precise and selective.




Structure of Pioglitazone




Structure of Teneligliptin

## MATERIALS AND METHODS

Instruments used

- Electronics Balance-Denver
- $p^H$ meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.
- UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Pioglitazone and Teneligliptin solutions.

**Materials used**

- Pioglitazone and Teneligliptin pure drugs (API) received from Akrivis Pharma labs.
- Combination Pioglitazone and Teneligliptin tablets (Zeta PLUS_PIo) received from local market
- Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

**Methods**

**Preparation of Standard stock solutions:** Accurately weighed 15mg of Pioglitazone, 20mg of Teneligliptin and transferred to 50ml and 50ml volumetric flasks separately. 3/4 Th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (300µg/ml of Pioglitazone and 400µg/ml of Teneligliptin)

**Preparation of Standard working solutions (100% solution):** 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (30 µg/ml of Pioglitazone and 40µg/ml of Teneligliptin)

**Preparation of Sample stock solutions:** 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters.(150µg/ml of Pioglitazone and 200µg/ml of Teneligliptin)

**Preparation of Sample working solutions (100% solution):** 2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (30µg/ml of Pioglitazone and 40µg/ml of Teneligliptin)

**Preparation of buffer**

**0.01N $KH_2PO_4$ Buffer:** Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 5.4 with dil. Formic acid .

**0.1% Formic acid Buffer:** 1ml of Conc. Formic acid was diluted to 1000ml with water.

## RESULTS AND DISCUSSION

**System suitability:** All the system suitability parameters were within the range and satisfactory as per ICH guidelines

**Table 1: System suitability parameters for Pioglitazone and Teneligliptin**

| S no | Pioglitazone | | | Teneligliptin | | | |
|---|---|---|---|---|---|---|---|
| Inj | RT(min) | USP Plate Count | Tailing | RT(min) | USP Plate Count | Tailing | Resolution |
| 1 | 2.254 | 7498 | 1.58 | 3.208 | 9719 | 1.30 | 7.7 |
| 2 | 2.254 | 7755 | 1.55 | 3.217 | 9311 | 1.31 | 7.7 |
| 3 | 2.254 | 7481 | 1.53 | 3.219 | 9397 | 1.32 | 7.7 |
| 4 | 2.255 | 7899 | 1.60 | 3.22 | 9360 | 1.31 | 7.8 |
| 5 | 2.255 | 7669 | 1.56 | 3.223 | 10112 | 1.30 | 8.0 |
| 6 | 2.266 | 7274 | 1.52 | 3.234 | 9887 | 1.30 | 8.0 |

**DISCUSSION:** According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

**Linearity:**

**Table 2: Linearity table for Pioglitazone and Teneligliptin**

| Pioglitazone | | Teneligliptin | |
|---|---|---|---|
| **Conc (µg/mL)** | **Peak area** | **Conc (µg/mL)** | **Peak area** |
| 0 | 0 | 0 | 0 |
| 7.5 | 208864 | 10 | 392052 |

| | | | |
|---|---|---|---|
| 15 | 417035 | 20 | 774725 |
| 22.5 | 649148 | 30 | 1188460 |
| 30 | 838832 | 40 | 1591385 |
| 37.5 | 1045335 | 50 | 1944350 |
| 45 | 1242747 | 60 | 2320092 |

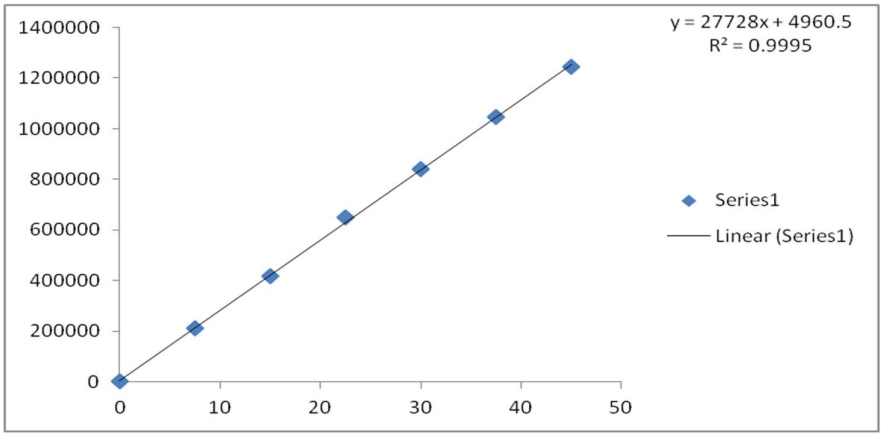



**Fig No.1 Calibration curve of Pioglitazone**

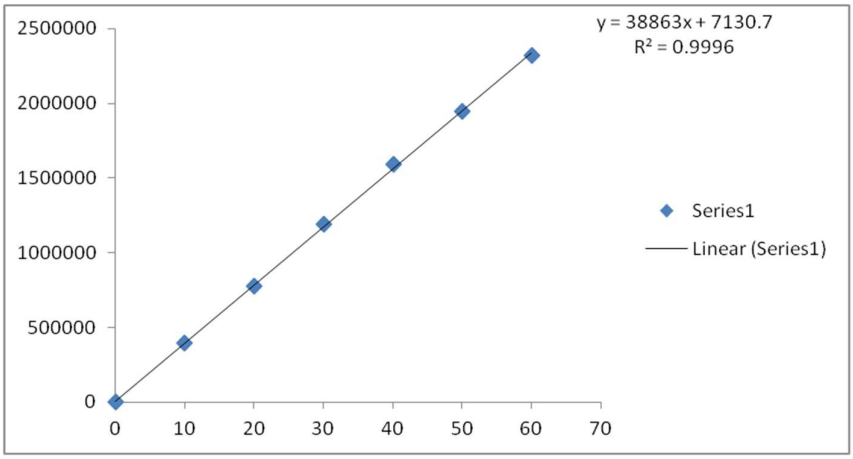



**Fig No.2 Calibration curve of Teneligliptin**

**Discussion:** Six linear concentrations of Pioglitazone (7.5-45µg/ml) and Teneligliptin (10-60µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Pioglitazone was y = 27728x + 4960.5. And of Teneligliptin was y = 38863x + 7130.7. Correlation coefficient obtained was 0.999 for the two drugs.

**Precision:**

**System Precision:**

**Table 3: System precision table of Pioglitazone and Teneligliptin**

| S. No | Area of Pioglitazone | Area of Teneligliptin |
|---|---|---|
| 1. | 831040 | 1599679 |
| 2. | 840022 | 1541136 |
| 3. | 839811 | 1538633 |
| 4. | 830212 | 1564377 |

| | | |
|---|---|---|
| 5. | 833256 | 1560780 |
| 6. | 839213 | 1542135 |
| Mean | 835592 | 1557790 |
| S.D | 4596.9 | 23222.6 |
| %RSD | 0.6 | 1.5 |

**Discussion:** From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.6% and 1.5% respectively for Pioglitazone and Teneligliptin .As the limit of Precision was less than "2" the system precision was passed in this method.

**Method precision**

**Table 4: Method precision table of Pioglitazone and Teneligliptin**

| S. No | Area of Pioglitazone | Area of Teneligliptin |
|---|---|---|
| 1. | 840755 | 1564006 |
| 2. | 839705 | 1553726 |
| 3. | 846775 | 1561221 |
| 4. | 837762 | 1553716 |
| 5. | 833344 | 1561891 |
| 6. | 839072 | 1573747 |
| Mean | 839569 | 1561385 |
| S.D | 4373.0 | 7449.1 |
| %RSD | 0.5 | 0.5 |

**Discussion:** Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.5% and 0.5% respectively for Pioglitazone and Teneligliptin. As the limit of Precision was less than "2" the system precision was passed in this method.

**Intermediate precision (Day_ Day Precision)**

**Table 5: Intermediate precision table of Pioglitazone and Teneligliptin**

| S. No | Area of Pioglitazone | Area of Teneligliptin |
|---|---|---|
| 1. | 841275 | 1569075 |
| 2. | 835601 | 1540345 |
| 3. | 831649 | 1546152 |
| 4. | 833604 | 1554844 |
| 5. | 838020 | 1518770 |
| 6. | 838303 | 1548701 |
| Mean | 836409 | 1546315 |
| S.D | 3494.0 | 16678.4 |
| %RSD | 0.4 | 1.1 |

**Discussion:** Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.4% and 1.1% respectively for Pioglitazone and Teneligliptin . As the limit of Precision was less than "2" the system precision was passed in this method.

**Accuracy**

**Table 6: Accuracy table of Pioglitazone**

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|
| 50% | 15 | 14.88 | 99.19 | 99.78% |
| | 15 | 14.91 | 99.43 | |
| | 15 | 14.93 | 99.52 | |
| 100% | 30 | 30.23 | 100.75 | |
| | 30 | 30.08 | 100.27 | |
| | 30 | 29.84 | 99.46 | |
| 150% | 45 | 45.15 | 100.32 | |
| | 45 | 44.93 | 99.84 | |
| | 45 | 44.65 | 99.23 | |

**Table 7: Accuracy table of Teneligliptin**

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|
| 50% | 20 | 19.84 | 99.22 | 100.15% |
| | 20 | 20.18 | 100.92 | |
| | 20 | 19.92 | 99.62 | |
| 100% | 40 | 40.24 | 100.60 | |
| | 40 | 40.39 | 100.98 | |
| | 40 | 40.16 | 100.41 | |
| 150% | 60 | 60.45 | 100.76 | |
| | 60 | 59.62 | 99.37 | |
| | 60 | 59.68 | 99.47 | |

**Discussion:** Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.78% and 100.15% for Pioglitazone and Teneligliptin respectively.

**Sensitivity**

**Table 8: Sensitivity table of Pioglitazone and Teneligliptin**

| Molecule | LOD | LOQ |
|---|---|---|
| **Pioglitazone** | 0.02 | 0.05 |
| **Teneligliptin** | 0.07 | 0.21 |

**Robustness**

**Table 9: Robustness data for Pioglitazone and Teneligliptin.**

| S.no | Condition | %RSD of Pioglitazone | %RSD of Teneligliptin |
|---|---|---|---|
| 1 | Flow rate (-) 0.8ml/min | 0.5 | 0.9 |
| 2 | Flow rate (+) 1.0ml/min | 0.4 | 0.7 |
| 3 | Mobile phase (-) 50B:50A | 0.4 | 0.6 |
| 4 | Mobile phase (+) 60B:40A | 0.4 | 0.6 |
| 5 | Temperature (-) 27°C | 0.6 | 0.8 |
| 6 | Temperature (+) 33°C | 0.4 | 0.9 |

**Discussion:** Robustness conditions like Flow minus (0.8ml/min), Flow plus (1.0ml/min), mobile phase minus (50B:50A), mobile phase plus (60B:40A), temperature minus (27°C) and temperature plus (33°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

## CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Pioglitazone and Teneligliptin in tablet dosage form. Retention time of Pioglitazone and Teneligliptin were found to be 2.320 min and 3.256 min. %RSD of the Pioglitazone and Teneligliptin were and found to be 0.6 and 1.5 respectively. %Recovery was obtained as 99.78% and 100.15% for Pioglitazone and Teneligliptin respectively. LOD, LOQ values obtained from regression equations of Pioglitazone and Teneligliptin were 0.02, 0.07 and 0.07, 0.21 respectively. Regression equation of Pioglitazone is y = 27728x + 4960.5. And y = 38863x + 7130.7 of Teneligliptin . Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

### CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

## REFERENCES


1. B.k Sharma, Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23rd Edition Goel publication , Meerut, (2007)
2. Lindholm.J, Development and Validation of HPLC Method for Analytical and Preparative purpose. Acta Universitatis Upsaliensis, pg. 13-14, (2004).
3. Rashmin, An introduction to analytical Method Development for Pharmaceutical formulations. Indoglobal Journal of Pharmaceutical Sciences, Vol.2, Issue 2, Pg 191-196 (2012).
4. Malvia R, Bansal V, Pal O.P and Sharma P.K. A Review of High-Performance Liquid Chromatography. Journal of Global Pharma technology (2010)
5. Douglas A Skoog, F. James Holler, Timothy A. Niemen, Principles of Instrumental Analysis Pg 725-760.
6. Dr.S. Ravi Shankar, Text book of Pharmaceutical analysis, Fourth edition, Pg 13.1-13.2
7. David G.Watson. Pharmaceutical Analysis, A text book for Pharmacy students and Pharmaceutical Chemists. Harcourt Publishers Limited; 2nd Ed., Pg 221-232.
8. Remingtonn's The Sciences and Practise of Pharmacy, 20th Edition (2000)
9. Connors Ka. A Textbook of Pharmaceutical Analysis, Wiley intersciences Inc; Delhi, 3rd Ed, Pg 373-421, (1994)
10. Gurdeep R.Chatwal , Sham K .Anand, Instrumental Methods of Chemical Analysis , Pg 2.566-2.638 (2007)
11. David G. Watson Pharmaceutical Analysis, A text book for pharmacy students and Pharmaceutical Chemists. Harcourt Publishers Limited; 2nd Ed.,Pg- 267-311
12. Ashok Kumar, Lalith Kishore, navpreet Kaur, Anroop Nair. Method Development and Validation for Pharmaceutical Analysis. International Pharmaceutica Sciencia, Vol 2, Issue 3, Jul-Sep (2012)
13. Kaushal.C, Srivatsava.B, A Process of Method Development: A Chromatographic Approach. J Chem Pharm Res, Vol.2, Issue 2, 519-545, (2010)
14. Vibha Gupta, Ajay Deep Kumar Jain, N.S.Gill, Kapil, Development and Validation of HPLC method. International Research Journal of Pharmaeutical and Applied Sciences, Vol 2, Issue 4, Jul-Aug (2012)
15. Hokanson GC. A life cycle approach to the validation of analytical methods during Pharmaceutical Product Development. Part 1: The Initial Validation Process. Pharm Tech (1994) 92-100
16. Green JM. A Practicle guide to analytical method validation, Anal Chem (1996) 305A-309A
17. ICH, Validation of analytical procedures: Text and Methodology. International Conference on Harmonization, IFPMA , Geneva , (1996)
18. IUPAC. Compendium of Chemical Terminology, 2nd edn. (The Gold Book). PAC69, 1137 (1997). Glossary of terms used in computational drug design (IUPAC Recommendations.
19. K. D. Tripathi, Essentials of Medical Pharmacology, 6th Edition, Jaypee brother's medical publishers (P) LTD, p-254-255.
20. Indian Pharmacopoeia, Indian Pharmacopoeial Commission, Controller of Publication, Government of India, Ministry of health and Family Welfare, Ghaziabad, India, 2 (2010) 1657-1658.
21. British Pharmacopoeia, The British Pharmacopoeial Commission, the stationary office, UK,

London, 1408-1409 2 (2011).
22. https:// drugbank.com/drugs/DB01132
23. https:// drugbank.com/drugs/DB11950
24. Jinal Alkesh Gheewala et al. Development and Validation of UV Spectrophotometric Method and RP – HPLC Method for Simultaneous Estimation of Teneligliptin and Pioglitazone In Synthetic Mixture, Asian Journal of Pharmaceutical Technology & Innovation, 05 (23); 2017.
25. Meenaxi M. Maste et all.., RP-HPLC Method Development and Validation for Pioglitazone in Bulk and Marketed Formulation, Der Pharma Chemica, 2021.
26. M.Chandana et al., ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TENILIGLIPTIN IN PHRMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD, IJRDO, Issue-12 | December,2016.
27. Gopal S Irache et all.., RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF TENELIGLIPTIN AND METFORMIN IN PHARMACEUTICAL DOSAGE FORMS, September 2017International Research Journal of Pharmacy.
28. Rajani VETAPALEM et all.., Development and Validation of a Stability Indicating RP-HPLC Method for Simultaneous Estimation of Teneligliptin and Metformin, Turk J Pharm Sci. 2020 Apr.
29. Gadapa Nirupa et all.., RP-HPLC Analytical Method Development and Validation for Simultaneous Estimation of Three Drugs: Glimepiride, Pioglitazone, and Metformin and Its Pharmaceutical Dosage Forms, Journal of Chemistry,2013.
30. Pallavi S. Hade et all.., Analytical Method Development and Validation Studies of Anti Diabetic Drugs by HPLC, 2021 IJCRT.
31. Rajani Vetapalem *et al* , Development and Validation of Stability Indicating RP-HPLC Method for
32. Simultaneous Estimation of Teneligliptin and Metformin