REVIEW OF LITERATURE
4.1 Methods and Guidelines.
1. ICH, Guideline for stability testing of new drug substances and products Q1A (R2), 2003. This guideline provided the detail procedure to perform the stress stability or forced degradation study of molecules. So method will be proves that it is stability indicating and method can be used in stability study of Pharmaceutical drugs.

2. ICH, international Conference on harmonization of technical Requirements for registration of Pharmaceutical for human use, Validation of analytical procedure: Text and Methodology Q2 (R1), 2005. This guideline provided the guidance for Method validation procedure to prove that the method is fit for its use in regular analysis. On base of validation characteristics complies the method will be suitable for regular uses.

3. European Pharmacopoeia Book (Ph. Eur.). It is publishing by “EDQM, Europe. It is having the testing procedures with specifications for Pharmaceutical drug substances, products. Testing procedure is using for analysis of drugs in industries. No analytical method available for more than 3 combined drugs.

4. British Pharmacopoeia Book (BP). It is publishing by “MHRA, UK. It is having the testing procedures with specifications for Pharmaceutical drug substances, products. Testing procedure is using for analysis of drugs in industries. No analytical method available for more than 3 combined drugs.

5. United State Pharmacopoeia Book (USP). It is publishing by “USP commission, USA. It is having the testing procedures with specifications for Pharmaceutical drug substances, products. Testing procedure is using for analysis of drugs in industries. No analytical method available for more than 3 combined drugs.


12. Ion exchange chromatography and chromatofocusing-Principles and methods. Amersham biosciences. This book explains the basic principles of Ion exchange chromatographic separation and the analyte behavior.

13. K.S. Lakshmiet.al. has used Gemini column for the identification and quantification of metformin and Pioglitazone by RP-HPLC method. The column dimensions are 150mm*4.6 mm, 5µm. The Buffer used in mobile phase is ammonium acetate, pH 3.0. The mobile phase composition was Buffer: Acetonitrile::58:42. The Metformin retention time was found to be at 5.17 min and Pioglitazone retention time was found to be 8.10 min. The flow rate was kept at 0.3milliliter/minute. The Retention of Metformin was found to be very near or on the void volume for the column. The author didn’t perform the stress study/Forced degradation study to
establish the stability indicating nature of the method. The behavior of pioglitazone can be identified from the research author as performed. As the capacity factor for pioglitazone is 0.02, it can be confirmed that it is more non-polar in nature. It requires more organic phase for the elution.

14. Adukondalu D. et.al. has developed and validated the HPLC method for the simultaneous estimation of Pioglitazone hydrochloride in a pharmaceutical dosage form. The method is developed only for the identification of Pioglitazone hydrochloride. The Author did not perform the forced degradation study. This method cannot be commercially used for the quantification of Active drug from its pharmaceutical dosage form.

15. Zarghi A. et.al. has used phenyl column for the retention of metformin on the reversed phase. The author has used C18 stationary phase. The column used was 150mm*4.6mm, 5µm. The mobile phase buffer contains sodium dodecyl sulphate and sodium dihydrogen phosphate of molarity 10mM. pH of the buffer was 5.1. The mobile phase contains 40% Acetonitrile. Flow rate was kept at 1.5milliliter/minute. The retention time was found to be very good. All the validation parameters were covered during the study.

16. Vasudevan M. et.al. has performed using estimation of metformin in multicomponent dosage form. They has used C18 (Inertsil) as a stationary phase. The mobile phase buffer contains 75mM camphor Sulphonic acid, pH 7.0 with NaOH. The flow rate for the method was 1.0 milliliter/minute. An internal standard were used for the estimation (Tolbutamide).

17. K.S Lakshmi et.al. has used a Luna C18 Column, having dimension 250mm*4.6mm, 5µm. The metformin peaks comes in void volume. Author did not perform the forced degradation study. Very limited information and the scope of the study have been observed.

18. Wei Zeng et.al. has developed and RP-HPLC method for the identification of Sitagliptin from rat plasma. The chromatographic parameters used for the estimation and quantification of sitagliptin are column was Zorbax extend, C18, 250mm*4.6mm,
The mobile phase buffer contains, 10mM tris, 10mM Triethylamine, pH 9.0. The flow rate was 1.0 milliliter/minute. The study is very useful commercially and can be used in the identification of Sitagliptin in Human Blood plasma.

19. Cristina georgiţă et.al. has performed a comparison of LC/MS and LC/UV method for the identification of metformin in plasma sample. This research gives information regarding the method and the retention of metformin in reversed phase chromatography. For the retention of metformin, the author used Cyano column of dimension 150mm*4.6mm, 5µm. In the method of LC/MS, the sensitivity is very high. The sample is as low as nanogram/milliliter. Whereas in case of LC/UV method the sensitivity is not as good as LC/MS method. The cyano columns are infamous for their non-reproducibility. The lot to lot variability is very high. Normally people avoid using Cyano columns.

20. Rathinavel G.et.al. has worked on the simultaneous estimation of Rosiglitazone and Gliclazide in tablets. The separation has been achieved on Phenominex Gemini C18 column dimension 150mm*4.6mm, 5µm. The mobile phase contains ammonium phosphate buffer, Acetonitrile, Methanol in the proportion of 50:35:15. The retention time for Rosiglitazone and Gliclazide is 3.74 min and 8.74 min. RP-HPLC method for the simultaneous estimation of Rosiglitazone and Gliclazide in tablets. It can be inferred from the study that for the elution of Rosiglitazone, higher amount of organic is required.

21. BhamareP.Cet.al. has developed an analytical method for the estimation of metformin Hydrochloride and Finofibrate in the pure form and in the pharmaceutical tablets. The stationary phase used for the study was Inertsil ODS 3V, 250mm*4.6mm, 5µm. The mobile phase consists of water and acetonitrile in the proportion of 70:30 v/v, the pH of mobile phase was adjusted to 3.0 with orthophosphoric acid. The flow rate was 1.0 milliliter/minute and the detection was carried out at 250nm. The forced degradation study shows the stability indicating power of the analytical method. The retention time for Metformin was found to be 1.6 min and fenofibrate was 19.68 min.
The metformin peak was found to be void volume, which is not considered by regulatory authority.

22. Anand Prem D.C.et.al worked on the development of an analytical method for the simultaneous estimation of Telmisartan and Pioglitazone. They have used Phenominex C8 column dimension 250mm*4.6mm, 5µm for the separation of the two compounds. They have performed every validation parameter except specificity. The Telmisartan retention time was found to be very near to void volume.

23. Wei Zeng et.al. has developed method for identification of Sitagliptin in Human urine using Tandem mass spectroscopy. They have used narrow bore large particle column having dimension 50mm*1.0 mm, 60µm as an extraction column and Hypersil BDS 30mm *2.1mm, 3µm as a analytical column. They have performed all the parameters for validation of the given analytical method. The results found to be satisfactory.

24. Malleswararao Chellu et.al. has worked on the ultra-pressure liquid chromatography for the estimation Sitagliptin phosphate and Metformin hydrochloride in the pharmaceutical dosage form. The UPLC column used in the analytical method is UPLC, BEH C8 100mm * 2.1mm, 1.7µm. The flow used for the identification was 0.2 milliliter/minute. The mobile phase buffer contains 10mM potassium dihydrogen orthophosphate and 10 mM hexane Sulphonate. The buffer pH 5.5 was adjusted with dilute orthophosphoric acid. The elution of Sitagliptin and metformin was achieved by using acetonitrile as an organic phase. The validation was performed according to the ICH guidelines. All the parameters were covered. The specificity data gives the valuable information.

25. Al-Bagary R.I et.al. has performed the research work on the estimation of Vildagliptin in the presence of its impurity. The method for Vildagliptin contains Waters Symmetry C18 column having dimension length150 mm, internal diameter 4.6 mm and particle size is 5 µm. The buffer contains KH₂PO₄ buffer pH (4.6):ACN: MeOH (30:50:20, v/v/v) . The flow rate for the method was kept at 1.0 milliliter/minute.
The detection of degradation product and the principle peak was performed at 220 nm. Whereas, the second method for Pioglitazone contains potassium dihydrogen phosphate buffer pH (4.6) - Acetonitrile (60:40, v/v) at a flow rate of 1 milliliter/ min with UV detection at 210 nm was performed.

26. Rashmitha N. et.al. has performed the identification of pioglitazone and its impurities by HPLC. The analytical method is having stationary phase Inertsil ODS 3V column, having dimension 150mm*4.6mm, 5µm. The mobile phase contains phosphate buffer having pH 3.1 and organic phase was Acetonitrile. The degradation was observed in case of peroxide oxidation and base hydrolysis. All the other validation parameters are considered during the study.

27. AMR Lotfy Saber has performed the identification and validation of analytical method for pioglitazone using RP-HPLC. The method having column Nova pack C18 dimension for the given column are 150mm*3.9mm, 5µm. The buffer used for the solution is ammonium formate buffer, pH 3 with formic acid. The mobile phase composition is Buffer: ACN::75:25 The method has been validated according to the ICH guidelines only Forced degradation is absent.

28. Gadapa Nirupa et.al. has worked on the development and validation of an analytical method for the identification of Glimepiride, Pioglitazone and Metformin in bulk drug mixture and Pharmaceutical dosage form. For the estimation, author has used Inertsil ODS 3V column (250mm*4.6mm,5µm). The mobile phase consists of ACN, THF and buffer pH 5.0. The flow rate for the experiment was 1.7 milliliter/minute and the detection wavelength was 228nm. All the three drugs were properly resolved having run time of 5 minutes, 3.9 minutes and 1.3 minutes for glimepiride, pioglitazone, and metformin, respectively. The validated method was checked on Available pharmaceutical dosage form.

29. Mahendra K. Patilet.al. has conducted this study describes an isocratic RP-LC method that uses a water rich mobile phase for the estimation of rosiglitazone in the presence of its degradation products generated from forced decomposition studies. The separation was achieved with a C18 column using mobile phase comprising of
Water: methanol: ortho-phosphoric acid (80:20:0.2, v/v), the pH of which was adjusted to 4.5 with the help of liquid ammonia. The flow rate was kept at 1 milliliter/minute and analyte was screened with UV detector at 230 nm. The retention time for rosiglitazone was found to be 4.97 minutes. The degradation was observed in oxidative degradation and mild degradation in a acidic condition.

30. Jedlicka A. et.al. has developed a reversed-phase gradient HPLC method for the evaluation of Pioglitazone tablets. The developed method was on C18 column Symmetry, 250mm*4.6mm, 5µm. The mobile phase was a mixture of ammonium formate buffer adjusted with formic acid to pH 4.1 and acetonitrile. Method is a validated method.

31. A K et.al. has developed a simple, fast, and precise reverse phase, isocratic HPLC method for the separation and quantification of pioglitazone and glimepiride in bulk drug and pharmaceutical dosage form. The column used for the quantification and identification was Inertsil ODS 3V, 250mm*4.6mm, 5µm. The mobile phase contains ammonium acetate buffer having pH 4.50 with glacial acetic acid. The flow rate and detection was 1.0milliliter/minute and 230nm. The retention time for Pioglitazone and Glimepiride was 7.0 min and 10.2 min, respectively. The method validation was done according to the ICH guidelines.

32. Gebremriam Ketema et.al. has developed a simple and rapid reverse phase high performance liquid chromatography (RP-HPLC) for the simultaneous determination of Sitagliptin and simvastatin in bulk drug samples and formulations. The quantitative determination was carried out by using Luna C-18 (250mm x 4.6mm, 5µm) column with a mobile phase consisting of a mixture of buffer: acetonitrile: methanol (40:35:25v/v), pH adjusted to 3.5 with orthophosphoric acid and Triethylamine. The mobile phase was filtered through a 0.45µ nylon filter, sonicated for 15 min and delivered at a flow rate of 1.0 milliliter/minute. Analysis was performed at ambient temperature with detection at 254 nm.

33. A. S. K. Sankar et.al. has developed a simple, accurate, specific and reliable RP-HPLC method for the simultaneous estimation of Sitagliptin phosphate and Metformin hydrochloride in
pharmaceuticals. The system used for analytical method development was Shimadzu with UV detector. The analytical column used for the method was Phenomenex (C18) 100RP, 250mm*4.6mm, 5µm. The mobile phase buffer contains phosphate buffer (20mm) of pH 4.0 in combination to acetonitrile (60:40). The flow rate used for the analysis was 1.0 milliliter/minute. The retention time for Sitagliptin phosphate and metformin HCl was 2.718 min and 1.925 min.

34. Vincent S.H. et.al. has performed the identification of Sitagliptin in human body. The sample, which were collected for the study was from Urine, Feces and Blood plasma. The investigation shows that the primary route of excretion was through kidney (87%). The time interval for the sample collection was 7 days. The secondary route of excretion was from the feces (13%). From the total drug administered into the system only 16% got metabolized (13% in urine and 3% in feces. The conclusion of this study was that out of total drug excreted from the system, majority is renal excretion.

35. Rajendra A Maske et.al. has developed a simple precise and stability indicating HPLC method for the identification of Amlodipine Besylate, Valsartan, Telmisartan, Hydrochlorothiazide and Chlorthalidone. The elution was obtained on Cosmosil PAQ (150mm*4.6mm, 5 µm) column with gradient flow. The flow rate for the elution was set at 1.0 milliliter/minute. Mobile phase consist of 0.05M sodium dihydrogen phosphate buffer. The wavelength used for the identification was 220nm. The method was validated according to the ICH guidelines and the USP general chapter 1225.

36. Rajendra A Maske et.al. has developed a simple precise and stability indicating HPLC method for the identification of Irbesartan, Losartan, hydrochlorothiazide and Chlorthalidone. The method was developed on Hypersil BDS (250mm*4.6mm, 5µm). The HPLC system was used in Gradient flow. The mobile phase flow rate was 1.0 milliliter/minute. The buffer used was sodium dihydrogen orthophosphate. The Detection was carried out at 220nm. The method was validated according to the ICH guidelines and the USP general chapter 1225.

37. Blessen Philip and et.al. has developed a simple, precise and rapid method for the accurately identification of Atenolol and Amlodipine Besylate in bulk as well as tablets dosage form. The Separation has been archived on Inertsil ODS 3V(250mm*4.6mm, 5µm). The mobile phase
buffer contains Triethylamine and orthophosphoric acid, pH 3.0. The detection was carried out on 225nm. The flow rate for the experiment was set on 1.0 milliliter/minute. The elution was isocratic and for that the mobile phase composition was Buffer: Acetonitrile: Methanol::40:35:25. The retention time for Amlodipine was 5.97 min and Atenolol was 2.23 min. The specificity parameter of validation was performed on Placebo. All the other validation parameters were performed according to the ICH guidelines.

38. Anil Dubala et.al. has developed an HPLC method for the identification of Sitagliptin Phosphate in human plasma by protein precipitation technique. Stationary phase used for the identification was Phenomenex C18 (250mm*4.6mm, 5µm). The mobile phase is a mixture of 0.5% v/v of Triethylamine solution in Acetonitrile (77:23 v/v). The mobile phase buffer was Triethylamine was adjusted to pH 6.8 with Ortho-phosphoric acid. The mobile phase flow rate was 1.0 milliliter/minute. The detection was carried out at 267 nm. The retention time for sitagliptin Phosphate and internal Standard (Rosiglitazone) was 6.1 min and 7.7 min, respectively. The validation of the method was carried out in linearity, Precision and Accuracy.

39. Venkatesh P. et.al. has developed an analytical method for the simultaneous estimation of six Anti-diabetic drugs. The drugs which are used for the study were Glibenclamide, Gliclazide, Pioglitazone, Repaglinide and Rosiglitazone. The present method is also extended for the estimation of these six drugs in human plasma. The Gradient elution was obtained at a flow rate of 1.0 milliliter/minute. The column used for elution was Inertsil ODS 3V (250mm*4.6mm, 5µm). The Buffer in mobile phase was 0.01M Formic acid. The organic phase used was acetonitrile, and Methanol. Celecoxib was used as an internal standard. The detection wavelength was 260 nm.

40. Khohinur Hossain et.al. has developed a Simple, Fast and Economic method for the simultaneous estimation of Pioglitazone and Glimepiride. The method contains buffer having potassium dihydrogen phosphate, pH 3.4 with orthophosphoric acid. The isocratic mobile phase having composition has buffer: Acetonitrile::60:40 v/v. The stationary phase used
for the isolation of the given drugs is Phenomenex, (250mm*4.6mm, 5µm). The flow rate was at 0.8 milliliter/minute. For detection, the sample was scanned at 235nm. The validation was carried out on the basis of ICH, USP guidelines.
4.2. Review on drug molecules.
4.2.1 Metformin Hydrochloride

Introduction:

It is an anti-diabetic drug of biguanide class. It is the most favored first line drug for the treatment of Diabetes. It can also be used for the treatment of Polycystic Ovary Syndrome. It works by suppressing the glucose, which takes place in liver.

Structure:

![Chemical Structure of Metformin Hydrochloride]

Chemical Name: N,N-Dimethylimidodicarbonimidicdiamide.

IUPAC Name: 3-(diaminomethylene)-1,1-dimethyl -guanidine.

Chemical Properties:
Molecular Weight: 129.16364 [g/mol].
Molecular Formula: C_{14}H_{11}N_{5}
Melting point: 223°C-226°C.
pKa: 12.4

Maximum Daily Dose: 1000 mg per day.

Available marketed Formulation:
1. Ancalzide-M (Metformin/Gliclazide (80mg/500mg). A. N Pharmaceutical Pvt. Ltd.

**Mechanism of Action:**

It increases hyperglycemia primarily due to suppressing the glucose production by the liver. It also increases the insulin sensitivity of the cells by enhancing the peripheral glucose intake. It also increases fatty acid oxidation and decreases the absorption of glucose from the GIT.

**Adverse Effect:**

The most common side effects of the Metformin intake is diarrhea, nausea, and Cramps. It also decreases the level of thyroid stimulating hormones in the blood. This results into hypothyroidism in men.

**Pharmacopoeial Status:**

**USP:** Metformin Hydrochloride, Drug substance and Drug product Available.

**EP/BP:** Metformin Hydrochloride, Drug Substance and Drug product available.
Drug Substances/Drug product and its impurities:

1. **Metformin**:
   Chemical name: N,N-Dimethylimidodicarbonimidicdiamide
   Molecular weight: 129.16 g/mol
   Structure:

   ![Structure of Metformin](image)

2. **Melamine**:
   Chemical name: 2,4,6-Triamino-1,3,5-triazine,
   Molecular weight: 
   Structure:

   ![Structure of Melamine](image)
1. Cyanoguanidine:
   Chemical name:
   Molecular weight:
   Structure:
Compendial method for the identification and Quantification of metformin in a drug Substance.

1. **Analytical method for the estimation of related substances:**

   **Procedure:**
   Mobile phase: 17 g/L of monobasic ammonium phosphate in water, adjusted with phosphoric acid to a pH of 3.0

   **System suitability stock solution:** Prepare a mixture of Metformin 250ppm and 100 ppm Melamine in water.

   **System suitability solution:** Transfer 1.0 mL of the System suitability stock solution to a 50-mL volumetric flask, and dilute with Mobile phase to volume. (5 ppm Metformin and 2 ppm Melamine)

   **Standard stock solution:** Prepare a solution of 200 ppm of Metformin Related compound A RS in water.

   **Standard solution:** Prepare the solution of 1 ppm of USP Metformin Related Compound A RS in Mobile phase from the Standard stock solution.

   **Sample solution:** Prepare the solution of 5000 ppm of Metformin Hydrochloride in Mobile phase

   **Diluted sample solution:** Prepare the solution of 5 ppm of Metformin Hydrochloride in Mobile phase from the Sample solution

   **Chromatographic system**

   **Detector:** UV 218 nm

   **Column:** 4.6-mm × 250mm; Packing, Strong acidic Cation Exchange Column

   **Flow rate:** 1.0–1.7 mL/min

   **Run time:** NLT twice the retention time of metformin

   **Injection volume:** 20 µL

   Suitability requirements
Resolution: NLT 10 between melamine and metformin

2. Analytical method for the estimation of Assay

Sample Size: 60 mg of Metformin Hydrochloride

Analysis: Dissolve the Sample in 4 mL of anhydrous formic acid, and add 50 mL of acetic anhydride. Titrate with 0.1N Perchloric acid volumetric solution, determine the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Every mL of 0.1N Perchloric acid is equivalent to 8.28 mg of metformin hydrochloride.

[Note - To avoid overheating of the reaction medium, mix thoroughly throughout the titration, and stop the titration immediately after the endpoint has been reached.]
Compendial method for the estimation of Metformin hydrochloride in Metformin hydrochloride Tablets.

1. Analytical method for the estimation of related substances:

   **Procedure:**
   Mobile phase: 17 g/L of monobasic ammonium phosphate in water, adjusted with phosphoric acid to a pH of 3.0

   **System suitability stock solution:** Prepare a mixture of Metformin 250ppm and 100 ppm Melamine in water.

   **System suitability solution:** Transfer 1.0 mL of the System suitability stock solution to a 50-mL volumetric flask, and dilute with Mobile phase to volume. (5 ppm Metformin and 2 ppm Melamine)

   **Standard stock solution:** Prepare a solution of 200 ppm of Metformin Related compound A RS in water.

   **Standard solution:** 1 ppm of USP Metformin Related Compound A RS in Mobile phase from the Standard stock solution.

   **Sample solution:** 5000 ppm of Metformin Hydrochloride in Mobile phase

   **Diluted sample solution:** 5 ppm of Metformin Hydrochloride in Mobile phase from the Sample solution

   **Chromatographic system**
   **Detector:** UV 218 nm
   **Column:** 4.6-mm × 25-cm; Packing, Strong acidic Cation Exchange Column
   **Flow rate:** 1.0–1.7 mL/min
   **Run time:** NLT twice the retention time of metformin
   **Injection volume:** 20 µL

   Suitability requirements
Resolution: NLT 10 between melamine and metformin

2. Analytical method for the estimation of Assay:

Standard solution: Prepare 10ppm of Metformin Hydrochloride solution in water.

Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer the amount of powder, equivalent to 100 mg of metformin hydrochloride, to a 100-mL volumetric flask. Add 70 mL of water, shake by mechanical means for 15 min, dilute with water to volume, and filter, discarding the first 20 mL of the filtrate. Dilute 10.0 mL of the filtrate with water to 100.0 mL, and dilute 10.0 mL of the resulting solution with water to 100.0 mL. The nominal concentration of this solution is 10 ppm.

Instrumental conditions:

Mode: UV.

Analytical wavelength: Wavelength of maximum absorbance at about 232 nm.

Cell: 1 cm.

Blank: Water.
Compendial method for the estimation of Metformin hydrochloride in Metformin hydrochloride ER Tablets.

1. Analytical method for the estimation of Assay and Related Substance:

**Buffer solution:** 0.5 g/L of sodium heptanes sulfonate and 0.5 g/L of sodium chloride in water. Before final dilution, adjust with 0.06 M phosphoric acid to a pH of 3.85.

**Mobile phase:** Acetonitrile and Buffer solution (1:9).

[Note-To improve the separation, the composition of acetonitrile and Buffer solution may be changed to 1:19, if necessary.]

**Diluent:** 1.25% solution of acetonitrile in water

**Standard solution:** \((L/4000)\) mg/mL of USP Metformin Hydrochloride RS in Diluent, where \(L\) is the labelled quantity, in mg of metformin hydrochloride in each Tablet.

**System suitability stock solution:** 12.5 ppm each of USP Metformin Related Compound B RS and USP Metformin Related Compound C RS in Diluent

**System suitability solution:** Dilute 0.5 mL of the System suitability stock solution with the Standard solution to 50 mL.

**Sample stock solution:** Finely powder NLT 10 Tablets. Transfer powder, equivalent to the average Tablet weight, to a homogenization vessel, and add 500 mL of a 10% acetonitrile solution. Alternately, homogenize and allow it to soak until the sample is fully homogenized.

**Sample solution:** Pass a portion of the Sample stock solution through a suitable filter of 0.45-µm pore size, discarding the first 3 mL of filtrate. Transfer 25 mL of the filtrate to a 200-mL volumetric flask, and dilute with water to volume.
Chromatographic system:
Mode: LC
Detector: UV 218 nm
Column: C18, 3.9-mm × 300 mm; 10-µm packing
Column temperature: 30°C
Flow rate: 1 mL/min
Injection volume: 10 µL
Run time: Until after the elution locus of Metformin Related Compound C

System suitability:
Sample: System suitability solution
[Note-The relative retention time for Metformin Related Compound B, Metformin, and Metformin Related Compound C is 0.86, 1.0, and 2.1-2.3, respectively. Metformin related compound C can have a variable retention time. The composition of the Mobile phase may be changed to 1:19, if it elutes at a relative retention time of less than 2.1. ]

Suitability requirements:
Resolution: Not less than 1.5 for Metformin B.

Tailing factor: It should not be less than 0.8 and should not be more than 2.0 for the Principle peak

Relative standard deviation: For principle peak the %RSD should be less than 1.5% for the metformin peak and 10% for Related Compound B and Related compound C.
4.2.2 Vildagliptin

Introduction:
Vildagliptin is a novel oral anti-hyperglycemic agent. It belongs to the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. It reduces hyperglycemia in Type-2 diabetes. The EMEA has also proved a new oral treatment released by Novartis, called Eucreas, a combination of Vildagliptin and Metformin.

Structure:

![Structure of Vildagliptin](image)

**IUPAC name:** (S)-1-[N-(3-hydroxy-1-adamantyl)glycyl] pyrrolidine-2-carbonitrile

**Chemical Properties:**
Molecular Weight: 303.399[g/mol].
molecular formula: C₁₇H₂₅N₃O₂
Melting point: 153°C-155°C.
pKa: 9.1

**Maximum daily Dose:** 100 mg per day

**Mechanism of Action:**
Vildagliptin inhibits dipeptidyl peptidase-4 (DPP-4) enzyme. This is turn inhibits the inactivation of GLP-1 by DPP-4, allowing GLP-1 to potentiate the secretion of insulin in the beta cells.

**Adverse effect:**

The chronic exposure can result into generation of malignancies in the tissues. Inhibiting the DPP-4 enzyme may allow some cancerous growth.

**Available marketed Formulation:**

1. Galvus-Novartis. 25mg, 50mg and 75 mg

**Pharmacopoeial Status:**

USP: Not Available.


**Note:** As Valsartan is non-compendial, so no compendial method is available.
4.2.3 Pioglitazone

Introduction:

It is the prominent drug of thiazolidinedione of Pharmacological class. It is used to treat Type-2 diabetes. The used of Pioglitazone is not only limited to single drug therapy but also can be used in combination. The combination therapy includes Sulfonylurea, Metformin and Insulin. It is also found that pioglitazone reduces the risk of conversion of pre-diabetes to Type-2 Diabetes by 77%.

Structure:

![Structure of Pioglitazone](image)

IUPAC Name: (RS)-5-(4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl) thiazolidine-2,4-dione

Chemical Properties:
Molecular Weight: 356.44[g/mol].
Molecular Formula: C_{19}H_{20}N_{2}O_{3}S
Melting point: 183°C-184°C
pKa: 5.5 and 6.7

Maximum Daily Dose:

It is supplied in oral tablets containing 15mg, 30mg or 45 mg of pioglitazone base. It is also available in combination with metformin as tablets containing 15mg pioglitazone and either 500mg or 850 mg of metformin.
**Mechanism of Action:**

Pioglitazone selectively stimulates the nuclear receptor peroxisome proliferators-activated receptor gamma (PPAR-1) and to lesser extent PPAR-1. Although not clinically significant, pioglitazone decreases the level of triglycerides and increases that of high density lipoprotein without changing low-density lipoproteins and total cholesterol in patients with disorder of lipid metabolism, although statins are the drugs of choice for these side effects.

**Adverse Effects:**

Pioglitazone cannot be used in patients with a known hypersensitivity to pioglitazone, other thiazolidinedione or any of components of its pharmaceutical form.

French agency for safety has withdrawn the drug from the market due to high risk of bladder cancer

USFDA has announced that a person associated with pioglitazone for more than one year can be induced with increased risk of bladder cancer.

**Available marketed Formulation:**

1. Asoformin-P (Pioglitazone, metformin 30mg/500mg). AS Pharma Pvt. Ltd.
2. K Pio-M (Blue cross Laboratories Ltd.)

**Pharmacopoeial Status:**

USP: Pioglitazone Hydrochloride, Drug Substances and Drug product available.


**Pioglitazone and its Impurities:**

**Pioglitazone:**

Chemical name: (RS)-5-(4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl) thiazolidine-2,4-dione

Molecular weight: 356.44[g/mol].

Structure:
Hydroxyl pioglitazone:
Molecular Weight: 372.45

Chemical name: (Z)-5-{4-[2-(5-Ethylpyridin-2-yl) ethoxy] benzylidene} thiazolidine-2,4-dione.
Chemical name: (±)-5-{4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl}-3-[2-(5-ethylpyridin-2-yl)ethyl]thiazolidine-2,4-dione.
Compendial method for the identification and Quantification of Pioglitazone HCL in a drug Substance.

1. Analytical method for the estimation of Assay:

Procedure:
Mobile phase: Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)

Standard solution: Prepare a 500 ppm solution of USP Pioglitazone Hydrochloride RS in methanol, and dilute with Mobile phase to obtain a solution containing 50 ppm of pioglitazone hydrochloride.

System suitability stock solution: Prepare the solution of 500 ppm of USP Pioglitazone hydrochloride RS and 130 ppm of Benzophenone in methanol

System suitability solution: Dilute System suitability stock solution with Mobile phase to obtain a solution containing 50 ppm of Pioglitazone Hydrochloride and 13 ppm of Benzophenone.

Sample solution: Prepare a 500 ppm solution of Pioglitazone Hydrochloride in methanol, and dilute with Mobile phase to obtain a solution containing 50 ppm of Pioglitazone Hydrochloride.

Chromatographic system
Mode: LC
Detector: UV 269 nm
Column: C18, 4.6-mm × 150 mm; 5-µm.
Column temperature: 25°C ± 2.5°C
Flow rate: 0.7 mL/min
[Note-Adjust the flow rate so that the retention time of the pioglitazone peak is about 7 min]
Injection size: 20 µL
Suitability requirements

Tailing factor: NMT 1.5 for pioglitazone and Benzophenone, System suitability solution

Resolution: NLT 15 between pioglitazone and Benzophenone, System suitability solution

Relative standard deviation: NMT 2.0% for six replicate injections, Standard solution
2. Analytical method for the estimation of Related Substances:

**Procedure:**
Mobile phase and System suitability stock solution: Proceed as directed in the Assay.

**System suitability solution:** Dilute the System suitability stock solution with Mobile phase to obtain a solution containing 25 ppm of pioglitazone hydrochloride and 6.5 ppm of Benzophenone.

**Sample solution:** Prepare the solution of 200 ppm of pioglitazone hydrochloride dissolved in 20% of the final volume with methanol, then diluted with Mobile phase to final volume

**Standard solution:** Prepare the solution 1 ppm of pioglitazone hydrochloride prepared by diluting the Sample solution with Mobile phase

**Chromatographic system**

**Mode:** LC

**Detector:** UV 269 nm

**Column:** C18, 4.6-mm × 150 mm; 5-µm packing L1

**Column temperature:** 25°C ± 2.5°C

**Flow rate:** 0.7 mL/min

[Note—Adjust the flow rate so that the retention time of the pioglitazone peak is about 7 min.]

**Injection size:** 40 µL

**Run time:** At least four times the retention time of pioglitazone

**System suitability**

**Samples:** System suitability solution and Standard solution
Suitability requirements

**Tailing factor:** NMT 1.5 for pioglitazone and Benzophenone, System suitability solution

**Resolution:** NLT 15 between pioglitazone and Benzophenone, System suitability solution

**Relative standard deviation:** NMT 3.0%, Standard solution
4.2.4 Rosiglitazone

Introduction:

Rosiglitazone is an anti-diabetic drug. It belongs to the class of thiazolidinedione. It make cell more responsive by getting adhered to the PPAR receptor. These receptors which are present in the fat cell makes cell more responsive to the insulin which is naturally available in the body.

Rosiglitazone is having more adverse effects than that of benefits. The FDA authority is more concerned to the chronic use of this drug as it creates more disorder than that of benefits. Some adverse effects associated which rosiglitazone is cancer and heart attack.

The drug has itself having a record of killing about 83000 people by heart attack in United States of America alone.

Structure:

![Structure of Rosiglitazone](image.png)

IUPAC Name: (RS)-5-[4-(2-[methyl(pyridine-2-l)amino]ethoxy)benzyl]thiazolidine-2,4dione.

Chemical Properties:
Molecular Weight: 357.428 [g/mol].
Molecular Formula: C$_{18}$H$_{19}$N$_3$O$_3$S
Melting point: 122ºC-123ºC
pKa: 6.9

Maximum Daily Dose: 4mg twice a day or 8mg a day depends upon the patient’s response.
**Mechanism of Action:**
Rosiglitazone is a member of thiazolidinedione class of drugs. Thiazolidinedione act as insulin sensitizers. They reduce glucose, fatty acid, and insulin blood concentrations.

**Adverse Effect:**
Study in some universities as well as industries concluded that Rosiglitazone can increases the fatalities from Heart attack. It is also associated with other effects like Strokes, Bone fractures, Damage to eye and Hepatotoxicity.

**Available marketed Formulation:**
1. Avandia, Rosiglitazone Maleate, 2mg (GlaxoSmithKline).

**Pharmacopoeial Status:**
USP: Rosiglitazone, Drug Substance and Drug Product available.
Rosiglitazone and its Impurities:
Chemical name: (RS)-5-[4-(2-[methyl(pyridine-2-l)amino]ethoxy)benzyl]thiazolidine-2,4-dione.
Molecular weight: 357.428 g/mol
Structure:

2, 4-Thiazolidinedione

![Structure of Rosiglitazone](image)
N-Desmethyl Rosiglitazone
Molecular weight: 342.428 g/mol
Compendial method for the identification and Quantification of Rosiglitazone maleate in a drug Substance.

1. **Analytical method for the estimation of Assay:**

**Procedure**

**Buffer:** Dissolve 5.75 g of phosphoric acid in 800 mL water, adjust with 4 N sodium hydroxide to a pH of 3.0 and dilute with water to 1 L.

**Mobile phase:** Acetonitrile and Buffer (25:75)

**System suitability solution:** Transfer 2.5 mg of USP Rosiglitazone Maleate RS and 1 mg of USP Rosiglitazone Related Compound A RS to a 50-mL volumetric flask, dissolve in 1 mL of stabilizer-free tetrahydrofuran, and dilute with Mobile phase to volume.

**Standard solution:** Prepare a solution of 50 ppm of USP Rosiglitazone Maleate RS in Mobile phase

**Sample solution:** Prepare a solution of 50 ppm of Rosiglitazone Maleate in Mobile phase

**Chromatographic system**

**Mode:** LC  
**Detector:** UV 235 nm  
**Column:** C18, 4.6-mm × 250mm; 5-µm packing  
**Column temperature:** 40°C  
**Flow rate:** 1 mL/min  
**Injection size:** 20 µL  
**System suitability:** Samples: System suitability solution and Standard solution
Suitability requirements

Resolution: Greater than 2.0 between rosiglitazone and rosiglitazone related compound A, System suitability solution.

Tailing factor: NMT 2.0, Standard solution, Relative standard deviation: NMT 1.0% of Standard solution.
2. Analytical method for the estimation of Related Substance:

Procedure:

**Buffer 1:** Prepare 0.05 M dibasic potassium phosphate buffer as follows. Dissolve 11.4 g of dibasic potassium phosphate trihydrate in 800 mL of water, adjust with a mixture of phosphoric acid and water (1:1) to a pH of 7.0, and dilute with water to 1000 mL.

**Solution A:** Acetonitrile and Buffer 1 (30:70)

**Solution B:** Acetonitrile and Buffer 1 (70:30)

Mobile phase: See table.

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>Solution A %</th>
<th>Solution B %</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0</td>
</tr>
<tr>
<td>25</td>
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</tr>
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</tr>
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<tr>
<td>60</td>
<td>100</td>
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</tr>
</tbody>
</table>

**Buffer 2:** Prepare 0.05 M monobasic potassium phosphate buffer by dissolving 6.8 g of monobasic potassium phosphate in 1000 mL of water.

**Diluent:** Acetonitrile and Buffer 2 (30:70)

**System suitability solution:** Prepare a solution of 500 ppm of USP Rosiglitazone Maleate RS in diluent, using sonication, if necessary, to dissolve.

**Sample solution:** Prepare a solution of 500 ppm of Rosiglitazone Maleate in Diluent, using sonication, if necessary, to dissolve.
Chromatographic system:
Mode: LC
Detector: UV 246 nm
Column: C18, 4.6-mm × 250mm; 5-μm packing
Flow rate: 1 mL/min
Injection size: 20 μL

Suitability requirements: Resolution: NLT 2.0 between Rosiglitazone and Rosiglitazone Related Compound A
4.2.5 Sitagliptin

Introduction:

Sitagliptin is an oral anti-hyperglycemic (anti-diabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitors class for the treatment of diabetes mellitus Type 2. The benefit of this medicine is its fewer side effects (e.g. Less hypoglycemia, Less weight again) in the control of blood glucose values. While safety is its advantage efficacy is often challenged as it is often recommended to be combined with other agents like Metformin.

Structure:

IUPAC Name: (R)-4-oxo-4-[3-trifluoromethyl]-5,6-dihydro[1,2,4] triazole [4,3-α]pyrazin-7 (8H)-yl]1-(2,4,5-trifuophenyl)butane-2-amine.

Chemical Properties:
Molecular Weight: 407.314 [g/mol].
Molecular Formula: C_{16}H_{15}F_{6}N_{5}O
Melting point: 198 ºC -202ºC
pKa: 7.7

Maximum Daily Dose: 100mg once a day.
Mechanism of Action:

Sitagliptin works to competitively inhibit the enzyme DPP-4. This enzyme breaks down the incretins GLP-1 and GIP, Gastrointestinal hormones release in response to meal. By preventing GLP-1 and GIP inactivation, they are able to increase the secretion of insulin and suppress the release the glucagon by the pancreas.

Adverse Effect:

During Clinical trials, The most common Adverse effects are nausea and common cold symptoms.

Available marketed Formulation:
1. Januvia- Sitagliptin Phosphate, 25 mg (MSD, Italy)

Pharmacopoeial Status:
2. USP: Not available.