

**Literature Review**

Literature review for undertaking the study was done by referring to various national and international journals, published articles in various official standard books and referring to various websites on the internet.

**Lakshmi K.S et. al.**\(^{(5)}\) has developed a method for the combination for Metformin HCl and pioglitazone. The validation was performed on the basis of ICH guidline. They haven’t performed Forced degradation study. This study will give the preliminary information on the chromatographic behaviour of the two stated compound.

**Adukondalu D et. al.**\(^{(6)}\): has developed a method for the identification of pioglitazone in solid dosage form. The validation was performed on the basis of ICH guidline. They haven’t performed Forced degradation study. This study only gives and additional information the chromatographic behaviour of compound.

**Zarghi A et. al.**\(^{(7)}\): has developed an assay method using and Ion-pair for the identification of metformin in blood plasma. The assay enables the measurement of metformin for therapeutic drug monitoring with a minimum detectable limit of 20 ng/ml. The method involves simple, one-step extraction procedure and analytical recovery was complete. The separation was performed on an analytical 150 x 4.6 mm i.d.microbondapakC (18) column. The wavelength was set at 235 nm. The mobile phase was 40% acetonitrile, 0.01 M sodium dodecyl sulphate, 0.01 M sodium dihydrogen phosphate, and distilled water to 100%, adjusted to pH 5.1 at a flow rate of 1.5 ml/min.

**Vasudevan M et. al.**\(^{(8)}\): has developed a simple, precise and accurate high performance liquid chromatography (HPLC) method for the simultaneous estimation of metformin with gliclazide and glipizide present in multicomponent dosage forms. The method was carried out on Inertsil C(18) column. A mobile phase composed of acetonitrile-water containing camphor sulphonic acid
(adjusted to pH 7 using 0.1 N sodium hydroxide; 75 mM) at a flow rate of 1 ml/min was used for the separation. They have used camphor as an ion pairing agent. They haven’t carried out forced degradation study.

**Sahoo P. K. et. al.**\(^{(9)}\): has developed high performance reverse phase liquid chromatographic procedure for simultaneous estimation of metformin hydrochloride and pioglitazone hydrochloride in combined tablet dosage form. The mobile phase used was a combination of acetonitrile:water:acetic acid (60:40:0.3) and the pH was adjusted to 5.5 by adding triethylamine. They have used different mobile phase.

**Xiang FeiJiu et. al.**\(^{(10)}\): worked on new high performance liquid chromatography (HPLC) method for the quantitative determination of sitagliptin in rat plasma was developed and validated for pharmacokinetics study. The plasma was spiked with the internal standard (hydrocortisone, IS), treated with sodium hydroxide, and extracted with ethyl acetate. The extracted analyte was injected into an Agilent Zorbax ExtendC18 column (250 mm×4.6 mm, 4 μm) maintained at 30 ºC and monitored at 267 nm. The mobile phase consisting of methanol–water (60:40, v/v, containing 10 mMTris and 10 mMtriethylamine) was titrated to pH 9.0 using 1 mol/L hydrochloric acid. The flow rate was 1.0 mL/min.

**Georgiţă1 Cristina et. al.**\(^{(11)}\): worked on LC/MS method for the analysis of the highly polar anti-diabetic drug metformin in plasma samples is compared to an ion-pair HPLC method with UV detection. Both methods showed good linearity in the concentration range of 50 to 2000 ng/mL, good precision and accuracy and similar sensitivity. The LC-MS method has the advantage of a simpler and faster preparation procedure, shorter analytical times and higher selectivity.

**Changkang Pan et. al.**\(^{(12)}\): has done study on Structure elucidation of pharmaceutical impurities in a drug product. Impurities can have unwanted
pharmacological or toxicological effects that seriously impact product quality and patient safety. This review focuses on current analytical strategies for chemical and structural identification of pharmaceutical impurities. Potential sources and mechanisms of impurity formation are discussed for both drug substance and drug product applications. The utility of liquid chromatography–mass spectrometry (LC/MS) for providing structure-rich information is highlighted throughout this review. Other hyphenated analytical techniques including LC/nuclear magnetic resonance, gas chromatography/MS, and size-exclusion chromatography/chemiluminescent nitrogen detectors are also discussed, as LC/MS alone sometimes cannot reveal or confirm the final structures as required during dosage form development.

Rathinavel G et al\(^{(13)}\): has developed a reverse phase HPLC method for the determination of rosiglitazone and gliclazide in tablet dosage form. Chromatography was carried on Phenomenix Gemini C18 column using in mixture of ammonium phosphate buffer, Acetonitrile and methanol in the ratio 50: 35: 15 v/v as mobile phase at a flow rate 1 mL min\(^{-1}\) and the effluent was monitored at 254 nm. The retention time for rosiglitazone was 3.74 and gliclazide 7.84 min. The limit of detection for rosiglitazone was 4.07 μg/mL and gliclazide 1.19 μg/mL. The LOQ obtained for rosiglitazone was 12.33 μg/mL and 3.612 μg/mL. The percentage assay for rosiglitazone was 99.92% and gliclazide was 99.82%.

Bhamare P.C. et al. \(^{(14)}\): has developed selective, precise, isocratic and accurate stability indicating reverse phase high performance liquid chromatography method for the simultaneous determination of Metformin hydrochloride and Fenofibrate present in multicomponent dosage forms. The HPLC method was carried out on Inertsil octadecysilane C18(250 mm x 4.6 mm i.d., 5 μm particle size) column. A mobile phase composed of acetonitrile - water (adjusted to pH 3 using orthophosphoric acid) in proportion of 70:30 v/v, at flow rate of 1 ml/min was used for the separation. Detection was carried out at 250nm.
Anand Prem D.C. et. al.\textsuperscript{(15)}: worked on Simple, rapid, fast and precise reversed-phase high performance liquid chromatographic method development and validated for the simultaneous estimation of Telmisartan and Pioglitazone in tablet dosage form. The quantification was carried out using Phenomenex C8 (250 \times 4.6 mm, 5μ) column and mobile phase comprised of acetonitrile and ammonium di hydrogen phosphate (pH 4.5; 20mM) in proportion of 65:35 (v/v). The flow rate was 1.0 ml/min and the effluent was monitored at 210 nm. The retention time of Telmisartan and Pioglitazone were found 2.38 min and 3.16 min respectively. The validation performed according to the ICH guidelines.

Wei Zeng et. al.\textsuperscript{(16)}: worked on High turbulence liquid chromatography (HTLC, or turbulent flow online extraction) and tandem mass spectrometry (MS/MS) methods for the determination of sitagliptin in human urine and hemodialysate were developed and validated to support clinical studies. A narrow bore large particle size reversed-phase column (Cyclone, 50 mm \times 1.0 mm, 60 μm) and a BDS Hypersil C18 column (30 mm \times 2.1 mm, 3 μm) were used as extraction and analytical columns, respectively. For the urine assay, the LLOQ was 0.1 μg/ml, the linear calibration range was 0.1 to 50 μg/ml, the interday precision (R.S.D.\%, \( n = 5 \)) was 2.3–6.5\%, and the accuracy was 96.9–106\% of the nominal value. For the urine quality control samples (QCs), the intraday precision (R.S.D.\%, \( n = 5 \)) and accuracy were 1.8–2.6\% and 96.2–106\% of the nominal value, respectively.

Malleswararao Chellu S. N. et. al.\textsuperscript{(17)}: worked on novel approach used to develop and validate a rapid, specific, accurate and precise reverse phase ultra performance liquid chromatographic (UPLC) method for the simultaneous determination of Sitagliptin phosphate mono-hydrate and Metformin hydrochloride in pharmaceutical dosage forms. The chromatographic separation was achieved on Aqty UPLC BEH C8 100 x 2.1 mm, 1.7 μm, column using a buffer consisting of 10 mM potassium dihydro-gen phosphate and 2 mM hexane-1-sulfonic acid sodium salt (pH adjusted to 5.50 with diluted phosphoric acid) and
acetonitrile as organic solvent in a gradient program. The flow rate was 0.2 mL min\(^{-1}\) and the detection wavelength was 210 nm. The limit of detection (LOD) for Sitagliptin phosphate monohydrate and Metformin hydrochloride was 0.2 and 0.06 μg mL\(^{-1}\), respectively. The limit of quantification (LOQ) for Sitagliptin phosphate monohydrate and Metformin hydrochloride was 0.7 and 0.2 μg mL\(^{-1}\), respectively.

**Ramzia I. et al.**\(^{(18)}\): has developed Two reversed-phase liquid chromatographic (RP-LC) methods are described for the determination of two binary mixtures of hypoglycemic agents. In the first method, vildagliptin (VDG) was determined in the presence of 3-amino-1-adamantanol (AAD), a synthetic intermediate and impurity of VDG. In the second method, pioglitazone hydrochloride (PGZ) and metformin hydrochloride (MET) were simultaneously determined in their binary mixture. Chromatographic separation in the two methods was achieved on a Symmetry® Waters C18 column (150mm×4.6mm, 5μm). In the first mixture, isocratic elution using a mobile phase of potassium dihydrogen phosphate buffer pH (4.6) -acetonitrile-methanol(30:50:20, v/v/v) at a flow rate of 1 mL min\(^{-1}\) with UV detection at 220 nm was performed. In the second method, isocratic elution based on potassium dihydrogen phosphate buffer pH (4.6)-acetonitrile (60:40, v/v) at a flow rate of 1 mL min\(^{-1}\) with UV detection at 210 nm was performed. Linearity, accuracy and precision were found to be acceptable over the concentration ranges of 5-200 μg mL\(^{-1}\), 0.5-3 μg mL\(^{-1}\) and 10-150 μg mL\(^{-1}\) for VDG, PGZ and MET, respectively. They performed work was according to ICH guidelines but the Force degradation study found to be absent.

**Rashmitha N. et al.**\(^{(19)}\): present paper describes the development of a reversed-phase high performance liquid chromatographic (RP-HPLC) method for Pioglitazone hydrochloride in the presence of its impurities and degradation products, generated from forced degradation studies. The drug substance was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. The degradation of Pioglitazone hydrochloride was observed under...
base and oxidative stress conditions. The drug was found to be stable in other stress conditions studied. Successful separation of the drug from the process related impurities and degradation products formed under stress conditions were achieved on an Inertsil ODS-3V (150 x 4.6 mm), 5 μm column. The gradient LC method employs solution A and solution B as mobile phase. The solution A contains phosphate buffer pH 3.1 and acetonitrile as Solution B. The developed RPLC method was validated with respect to specificity, linearity, accuracy, precision and high sensitivity with detection limits and quantification limits ranging from 0.033 mg/ml to 0.049 mg/ml and 0.100 mg/ml to 0.150 mg/ml respectively.

**AMR Lotfy Saber** (20): has developed rapid and accurate HPLC method for the determination of pioglitazone hydrochloride in tablets. Chromatographic analysis was performed on a Nova-Pak® C18 column (3.9mm x 150mm, 5µm) with a mixture of ammonium formate buffer adjusted with formic acid to pH 3 and acetonitrile (75:25, v/v) as mobile phase, at flow rate of 1.0 mL min⁻¹, and UV detection at 225 nm. The determination was completed in less than 12 min. Linearity ≥ 0.5 μg/mL, accuracy ≥ 99.14 %, and precision n ≤ 0.6 % were found to be acceptable over the range 0.5 –20 μg/mL. The forced degradation study was found to be absent

**Srinivasulu D. et. al.** (21): worked on the development and validation of Simple and precise RP-HPLC method for the determination of Pioglitazone hydrochloride in pharmaceutical dosage forms. Chromatography was carried out using C18 column (250x4.6mm), mixture of Buffer: acetonitrile (55:45%v/v) as the mobile phase at a flow rate 1.0 ml/min. The analyte was monitored using UV detector at 254 nm. The Retention time of the drug was 9.738min for Pioglitazone HCl. The proposed method was found to have linearity in the concentration range of 0.1-0.6μg/ml with correlation coefficient of r²=0.9999. The developed method has been statistically validated and found simple and accurate. The mean recoveries obtained for Pioglitazone HCL were in the range 100.09-103.11%.
E. Fedoroy et. al.\textsuperscript{(22)}: has used Perkin-Elmer series 200 pump and autosampler for injection and chromatographic separation. API-III+ triple quadrupole mass spectrometer (PE Sciex) with a Heated Nebulizer (APCI) source was used as an MS-MS detector. The plasma samples were allowed to thaw and reach room temperature. An aliquot of 200μl was taken and transferred to a screw-cap tube and 25μl of a solution of internal standard phenformin (500 ng/ml in purified water) was added. The extraction/derivatization step was performed with 0.5 ml of a solution of 4-nitrobenzoyl chloride in dichloromethane (10mg/ml) after addition of 0.5 ml of 10% aqueous sodium hydroxide. After 1 hour on a reciprocating shaker at room temperature, the phases were inversed with 4ml of ethyl acetate and separated by flash-freezing in an ethanol-dry ice bath. The organic extract was evaporated at 40°C under a gentle stream of nitrogen and the sample was reconstituted with 0.25 ml of mobile phase. The chromatographic separation was achieved using isocratic conditions on a Luna-C18 (2) 3μ 50×3mm HPLC column (Phenomenex) with a corresponding Security Guard 4×2mm pre-column and a 0.5μ pre-filter (Upchurch) at room temperature. The mobile phase consisted of methanol, acetonitrile and water (6:1:3, v/v) with 10mM of ammonium bicarbonate at a flow rate of 0.8 ml/min. The injection volume was 20μl.

Patil Mahendra K. et. al.\textsuperscript{(23)}: has developed a method which describes an isocratic RP-LC method that uses a water rich mobile phase for the estimation of rosiglitazone in 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 ml/min and analyte was screened with UV detector at 230 nm. The retention time for rosiglitazone was found to be 4.97 minutes. The drug was found to degrade extensively in oxidative degradation and mild degradation in acidic degradation while it was found stable in alkaline stress. The developed
method was precise and sensitive and could be applied successfully to determine rosiglitazone in the presence of its degradation products.

**Patrícia Gomes et. al.** (24): worked on Micellar electrokinetic chromatographic (MEKC) and high-performance liquid chromatographic HPLC methods for the development and subsequently validated for the determination of Rosiglialzone (RSG) in coated tablet, a potent new oral antihyperglicemic agent. The electrophoretic separation was performed in a fused-silica capillary of total length 48.0 cm (effective length 39.5 cm, 75 μm i.d.) using 10 mM sodium tetraborate buffer (pH 9.0) containing 30 mM sodium dodecyl sulfate (SDS) as the background electrolyte (BGE). The separating voltage used was of 20 kV at 25 °C and the diode array detector was set at 247 nm. The MEKC method was compared with HPLC method using a RP-18 column (125 × 4.0 mm i.d.) eluted with a mobile phase consisting of mixture of 25 mM potassium dihydrogen phosphate buffer and acetonitrile (55:45, v/v), adjusting the pH to 6.2 with dilute potassium hydroxide. Statistical analysis by Student's t-test showed no significant differences between the results obtained by two Methods. The results indicated that MEKC can be used an alternative method to HPLC for the determination of Rosiglitazone in pharmaceutical dosage form.

**Ravikanth C. H. et. al.** (25): has developed a sensitive, accurate and rapid high-performance liquid chromatography with UV-visible detection (HPLC-UV) method for the determination of Pioglitazone in rat serum has been developed. Rosiglitazone was used as internal standard. Pioglitazone and Rosiglitazone are extracted from serum using a liquid–liquid extraction procedure using ethyl acetate. Isocratic separation of Pioglitazone and Rosiglitazone is carried out using a reversed-phase phenomenex C18 (250 mm × 4.6 mm, 5μm) column with mobile phase consisting of methanol and 30 mM ammonium acetate buffer (pH adjusted to 5 with ortho-phosphoric acid) in the ratio 60:40 (v/v) and quantified by UV detection at 269 nm. Analytical run time was less than 10 min. Mean recovery was 97.12% for 0.1-10μg/ml concentrations. The assay exhibited good linear
relationship. Quantification limit was at 50ng/ml of Pioglitazone and accuracy and precision were over the concentration range of 0.1- 10μg/ml. This method can be used for routine clinical monitoring of Pioglitazone.

**Jedlicka A et. al.** (26): has developed a reversed-phase gradient HPLC method was developed for the evaluation of pioglitazone hydrochloride (PG-HCl) in tablets. Limit of detection for PG-HCl was found to be 42 ng/ml. Analyses were performed on a C18 column (Symmetry C18, 5 microm, 250 x 4.6 mm), mobile phase was a mixture of ammonium formate buffer adjusted with formic acid to pH 4.1 and acetonitrile. Shortened purity method was used as the assay method. Methods were validated.

**Pattana Sripalakit et. al.** (27): has developed an analytical method based on high-performance liquid chromatography (HPLC) with ultraviolet detection (269 nm) was developed for the determination of Pioglitazone in human plasma. Rosiglitazone was used as an internal standard. Chromatographic separation was achieved with a reversed-phase Apollo C18 column and a mobile phase of methanol–acetonitrile-mixed phosphate buffer (pH 2.6; 10 mM) (40:12:48, v/v/v) with a flow rate of 1.2 ml/min. The calibration curve was linear over the range of 50–2000 ng/ml ($r^2 > 0.9987$) and the lower limit of quantification was 50 ng/ml. The method was validated with excellent sensitivity, accuracy, precision, recovery and stability. The assay has been applied successfully to a pharmacokinetic study with human volunteers.

**N. Rashmitha et. al.** (28): his work describes the development of a reversed-phase high performance liquid chromatographic (RP-HPLC) method for Pioglitazone hydrochloride in the presence of its impurities and degradation products, generated from forced degradation studies. The drug substance was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. The degradation of Pioglitazone hydrochloride was observed under base and oxidative stress conditions. The drug was found to be stable in other stress
conditions studied. Successful separation of the drug from the process related impurities and degradation products formed under stress conditions were achieved on an Inertsil ODS-3V (150 x 4.6 mm), 5 µm column. The gradient LC method employs solution A and solution B as mobile phase. The solution A contains phosphate buffer pH 3.1 and acetonitrile as Solution B. The developed RP-LC method was validated with respect to specificity, linearity, accuracy, precision and high sensitivity with detection limits and quantification limits ranging from 0.033 mg/ml to 0.049 mg/ml and 0.100 mg/ml to 0.150 mg/ml respectively. To the best of our knowledge, a rapid LC method, which separates all the impurities, disclosed in this investigation was not published elsewhere.

**Gebremriam Ketema et al.** (29): has developed a simple and rapid reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous determination of sitagliptin and simvastatin in bulk drug samples and formulations. The quantitative determination was carried out by using Luna C-18 (250 x 4.6mm, 5µ) 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 ml/min. Analysis was performed at ambient temperature with detection at 254 nm. The calibration curves were linear (r2=0.998) over a concentration range from 50-500µg/ml for sitagliptin and (r2=0.999) over a concentration range from 20 -200 µg/ml for simvastatin. Limit of detection (LOD) and Limit of quantitation (LOQ) were 0.26 µg/ml and 0.77 µg/ml for sitagliptin and 0.06 µg/ml and 0.49 µg/ml for simvastatin respectively. The developed method was fast, accurate, precise and successfully applied to estimate the amount of sitagliptin and simvastatin in bulk sample and tablet dosage forms so it can be used for regular quality control of the drug.

**Shyamala.M et al.** (30): has developed a Rapid and accurate High performance liquid chromatography method is described for Simultaneous estimation of
Metformin Hydrochloride and Sitagliptin Phosphate from the combination tablet dosage form. The separation of two drugs was achieved on HYPERSIL (250 x 4mm i.d) 5μ column. The mobile phase consists of Acetonitrile and phosphate buffer in the ratio of 45:55. The detection was carried out at a wavelength 260nm. The method was validated for system suitability, linearity, accuracy, precision, robustness and stability of sample solution. The linear ranges for Metformin Hydrochloride and Sitagliptin Phosphate were 20-120μg/mL, 2-12μg/mL respectively with good recoveries i.e. 99.16% to 99.89%.

Stella H. et. al. (31): has developed a method for the determination of metabolism and excretion of [14C] sitagliptin, an orally active, potent and selective dipeptidyl peptidase 4 inhibitor, were investigated in humans after a single oral dose of 83 mg/193 μCi. Urine, feces, and plasma were collected at regular intervals for up to 7 days. The primary route of excretion of radioactivity was via the kidneys, with a mean value of 87% of the administered dose recovered in urine. Mean fecal excretion was 13% of the administered dose. Parent drug was the major radioactive component in plasma, urine, and feces, with only 16% of the dose excreted as metabolites (13% in urine and 3% in feces), indicating that sitagliptin was eliminated primarily by renal excretion. Approximately 74% of plasma AUC of total radioactivity was accounted for by parent drug. Six metabolites were detected at trace levels, each representing <1 to 7% of the radioactivity in plasma. These metabolites were the N-sulfate and N-carbamoyl glucuronic acid conjugates of parent drug, a mixture of hydroxylated derivatives, an ether glucuronide of a hydroxylated metabolite, and two metabolites formed by oxidative desaturation of the piperazine ring followed by cyclization. These metabolites were detected also in urine, at low levels. Metabolite profiles in feces were similar to those in urine and plasma, except that the glucuronides were not detected in feces. CYP3A4 was the major cytochrome P450 isozyme responsible for the limited oxidative metabolism of sitagliptin, with some minor contribution from CYP2C8.