CHAPTER-2
LITERATURE REVIEW

2.1 LITERATURES RELATED TO HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Dey et al., (2017) revealed that a stability-indicating reverse phase–high performance liquid chromatography (RP–HPLC) method was developed and validated for the determination of atazanavir sulfate in tablet dosage forms using C18 column Phenomenix (250 mm×4.6 mm, 5 μm) with a mobile phase consisting of 900 mL of HPLC grade methanol and 100 mL of water of HPLC grade. The pH was adjusted to 3.55 with acetic acid. The mobile phase was sonicated for 10 min and filtered through a 0.45 μm membrane filter at a flow rate of 0.5 mL/min. The detection was carried out at 249 nm and retention time of atazanavir sulfate was found to be 8.323 min. Linearity was observed from 10 to 90 μg/mL (coefficient of determination R2 was 0.999) with equation, y=23.427x+37.732. Atazanavir sulfate was subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation, and the results showed that it was more sensitive towards acidic degradation. The method was validated as per ICH guidelines.

Nitin Charbe et al., (2016) revealed a new method using high-performance liquid chromatography coupled with ultra violet detection (HPLC–UV) was developed and validated for the simultaneous quantification of atazanavir, dolutegravir, darunavir, efavirenz, etravirine lopinavir, raltegravir, rilpivirine and tipranavir in human plasma. For the first time we reported here the development and validation of an HPLC–UV assay to quantify the frequently administered 9 antiretroviral compounds including dolutegravir and rilpivirine. A simple solid phase extraction procedure was applied to
500 µL aliquots of plasma. The chromatographic separation of the drugs and internal standard (quinoxaline) was achieved with a gradient of acetonitrile and sodium acetate buffer on a C18 reverse-phase analytical column with a 25 min analytical run time. Calibration curves were optimised according to the therapeutic range of drug concentrations in patients, and the coefficient of determination (r2) was higher than 0.99 for all analytes. Mean intraday and interday precisions (RSD) for all compounds were less than 15.0%, and the mean accuracy (% deviation from nominal concentration) was also found to be less than 15.0%. Extraction recovery range was between 80% and 120% for all drugs analysed. The solid phase extraction and HPLC–UV method enable a specific, sensitive, and reliable simultaneous determination of nine antiretroviral agents in plasma. Good extraction efficiency and low limit of HPLC–UV quantification make this method suitable for use in clinical trials and therapeutic drug monitoring.

2.2 LITERATURES RELATED TO ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC)

Samatha et al, (2015) UPLC technique an enhancement in speedy resolution as well as the sensitivity of analysis by using particle size less than 2 µm, operates at higher pressure, while the mobile phase runs at greater linear velocities as when compared to HPLC. It offers an advantage of time saving & reduces solvent consumption. Now a day’s substantial technological advances have been done in enhancing particle chemistry performance, improving detector design, in optimizing the system, data processors and various controls of chromatographic techniques. This review focuses on the basic principle, instrumentation of UPLC and its advantages over HPLC, furthermore emphasizes on various pharmaceutical applications of UPLC technique.
Naresh et al, (2014) revealed that Ultra Performance Liquid Chromatography (UPLC) takes the advantage of technological strides made in particle chemistry performance, system optimization, detector design and data processing and control. Using sub 2 mm particles and mobile phases at higher linear velocities and instrumentation that operates at higher pressures than those used in HPLC, dramatic increases in resolution, sensitivity and speed of analysis can be obtained. This new category of analytical separation science retains the practicality and principles of HPLC while creating a step function improvement in chromatographic performance. This review introduces the theory of UPLC and summarizes some of the most recent work in the field.

2.3 LITERATURES RELATED TO COBICISTAT AND ATAZANAVIR

Tseng Alice et al, (2017) revealed clinicians are reminded to be cognizant of the pharmacokinetic properties of drugs coformulated with cobicistat; if our patient had been switched from atazanavir/ritonavir to elvitegravir/cobicistat, it is possible that a significant change in INR may not have been observed as elvitegravir is also an inducer of CYP2C9.

Deepa et al, (2017) revealed that reverse phase high performance liquid chromatographic method has been focused with the aim of reducing analysis time and maintaining good efficiency. The present work describes a rapid, simple, precise, selective stability indicating RP-HPLC method for simultaneous estimation of Atazanavir and Cobicistat in tablet dosage forms. A Phenomenix C8 column of 250 × 4.6 mm internal diameter, 5 µm particle size with mobile phase consisting of O.1M ammonium acetate and methanol of HPLC grade in the ratio of 1:1 was used. The flow rate was maintained at 1.2 ml/min. The effluents were monitored at 268 nm.
The detector response was found to be linear over a concentration range of 30-90 mcg for atazanavir and 15-45 mcg for cobicistat. The retention times of atazanavir was found to be 3.576 min and Cobicistat 6.592 min. The correlation coefficients of atazanavir and cobicistat was 0.999 with equation y=43363x and y=59029x. Recovery of the method was 98-102%. According to ICH guidelines, the newly developed method has been validated in terms of accuracy, specificity, linearity, precision and robustness. In order to demonstrate the stability indicating power of the developed method forced degradation studies were performed for atazanavir and cobicistat by subjecting them to stress conditions including acidity, alkalinity, oxidation, photolysis and thermal degradation. The described method can be successfully employed for the quality control analysis of Atazanavir and Cobicistat in formulations.

Gammal et al, (2016) showed the antiretroviral protease inhibitor atazanavir inhibits hepatic uridine diphosphate glucuronosyltransferase (UGT) 1A1, thereby preventing the glucuronidation and elimination of bilirubin. Resultant indirect hyperbilirubinemia with jaundice can cause premature discontinuation of atazanavir. Risk for bilirubin-related discontinuation is highest among individuals who carry two UGT1A1 decreased function alleles (UGT1A1*28 or *37). We summarize published literature that supports this association and provide recommendations for atazanavir prescribing when UGT1A1 genotype is known (updates at www.pharmgkb.org).

Uttam Prasad Panigrahy and Sunil Kumar Reddy, (2016) studied a novel approach was used to develop and validate a rapid isocratic Reversed Phase-High Performance Liquid Chromatographic method for the simultaneous estimation of Atazanavir Sulphate and Cobicistat in bulk and pharmaceutical dosage form. The separation was performed by Agilent ZORBAX eclipse plus C18 (100mm×4.6 mm, 3.5µm particle
size) column, Agilent 1220 Infinity HPLC System with VWD detector and mobile phase contained a mixture of 0.01M Potassium dihydrogen phosphate (pH adjusted to 3.5 with orthophosphoric acid) and Acetonitrile (30:70, v/v). The flow rate was set to 1ml/min with responses measured at 260nm. The retention time of Atazanavir Sulphate and Cobicistat was 2.243 min and 6.043 min respectively with resolution of 4.806. Linearity was established in the range of 15-75µg/ml for Atazanavir Sulphate and 7.5-37.5µg/ml for Cobicistat with correlation coefficients (r² =0.999). The percentage recoveries were between 99.92-100.03% and 99.91-100.05% for Atazanavir Sulphate and Cobicistat respectively. Validation parameters were evaluated according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. The developed method was successfully applied for the quantification and hyphenated instrumental analysis.

Venkata Siva Sri Nalini et al, (2016) revealed this study was to develop a stability indicating RP-HPLC method for simultaneous quantitative analysis of atazanavir and cobicistat in tablets. Atazanavir and cobicistat was eluted on the Inertsil C8, 150 mm x 4.6 mm, 5 µm analytical column with a mobile phase consisting of 0.1 M ammonium acetate and methanol (50:50 v/v), pumped at 1.2 mL/min flow rate. The column was maintained at 30°C and 10 µl of the solutions were injected. UV detection was performed at 234 nm. According to ICH guidelines, the method was validated. Under the optimized chromatographic conditions the retention times of atazanavir and cobicistat were 2.559 min and 3.576 min, respectively. Linearity was observed in the concentration range of 45-135 µg/mL for atazanavir and 22.5-67.5 µg/mL for cobicistat. The percent recovery and percent relative standard deviation for both the drugs were in the range of 99.311-100.342% and 0.290-0.401%, respectively. The results of forced degradation studies demonstrated the stability-
indicating power of the method. The proposed method was found to be appropriate for the quality control of atazanavir and cobicistat hydrochloride simultaneously in a bulk drug as well as in a pharmaceutical dosage forms.

Sindu Priya and Gowri Sankar, (2015) reported that a stability indicating reverse phase High performance liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the simultaneous determination of Atazanavir and Cobicistat in bulk and pharmaceutical formulation. Separation was achieved in isocratic mode with a Kinetex C18 100 A (250 mm x 4.6 mm, 5µ) column and mixture consisting of 0.1% OPA(pH 3) and methanol in 80:20 v/v was used as mobile phase with a flow rate of 1 ml/min, column temperature at 25°C and the run time as 10 mins. UV detection was performed at 239 nm and the sample temperature was maintained ambient. The described method for simultaneous determination of Atazanavir and Cobicistat is linear over a range of 8 µg/ml to 120 µg/ml and 5 µg/ml to 60 µg/ml respectively. The method shows good precision results which were below 2.0%RSD. Limit of Detection (LOD) and Limit of Quantification (LOQ) of Atazanavir and Cobicistat was established and found to be 1.49 and 4.97 µg/ml and 1.13 and 3.77 µg/ ml respectively. The developed method was validated according to ICH guidelines for various parameters. The method is simple, rapid, selective and stability indicating method which would be used for regular stability indicating quality control determinations.

2.4 LITERATURES RELATED TO COBICISTAT AND DARUNAVIR

using RP-HPLC. Separation was accomplished on BDS 250 x 4.6 mm, 5µm C18 column using 0.1% Perchloric acid buffer and acetonitrile (38:62 v/v) as mobile phase pumped through at a flow rate of 1 ml/min at 30°C. Optimized wavelength was 211 nm, retention time of Darunavir and Cobicistat were found to be 2.24 min and 2.63 min respectively. % RSD of the Darunavir and Cobicistat were found to be 0.3 and 0.5 respectively. Mean recovery were found to be 100.0% and 100.04% for Darunavir and Cobicistat respectively. The proposed method also proved to be suitable as a rapid and reliable quality control method.

Sridhar Siddiraju, (2016) reported darunavir is a protease inhibitor medication used to treat HIV infection. Cobicistat is a pharmacokinetic enhancer used along with anti HIV agents. The US Food and Drug Administration has approved a fixed-dose combination of Darunavir (800 mg) and Cobicistat (150 mg) for HIV treatment in 2015. In the present work, we planned to develop a simple, fast, accurate and precise RPHPLC method for the simultaneous estimation of Darunavir and Cobicistat in pharmaceutical dosage form. The chromatographic method was developed by using Kromosil C18 column (250mm x 4.6mm, 5µm) and the mobile phase was pumped with acetonitrile and water (pH was adjusted to 3.2 by using ortho phosphoric acid) in the ratio of 70:30 v/v. The mobile phase was pumped at 1ml/min flow rate and the temperature was maintained at 30°C. The retention times of Darunavir and Cobicistat were found to be 3.5 min and 2.7 min, respectively. The method developed was validated in accordance with ICH guidelines with respect to the stability indicating capacity of the method including system suitability, accuracy, precision, linearity, range, limit of detection, limit of quantification, solution stability and robustness. Darunavir and Cobicistat were found to be linear in the range of 40-240 µg/ml and 7.5-45 µg/ml, respectively. The assay results of Darunavir and Cobicistat in
PREZCOBIX were found to be 100.4% and 100.9%, respectively. The sample solution was tested for degradation studies in acidic, basic, neutral, peroxide, heat, photolytic conditions and the percentage degradation was found to be within the limits. The developed methods can be used for the routine analysis of the drugs in pharmaceutical dosage forms.

Venkata Siva Sri Nalini et al, (2016) revealed a simple, sensitive and precise stability indicating reverse phase high performance liquid chromatographic method has been developed and validated for the estimation of darunavir and cobicistat simultaneously in combined dosage form. The stationary phase used was Phenomenex C18 (150 x 4.6 mm, 5 μm particle size). The mobile phase used was a mixture of 0.1 M NaH₂PO₄ and methanol (70:30 v/v). Quantification was done with photodiode array detection at 260 nm over the concentration range of 80-240 μg/mL and 15-45 μg/mL for darunavir and cobicistat, respectively. The method had accuracy in the range of 100.11-100.31% for darunavir and 99.87-99.89% for cobicistat. Darunavir and cobicistat were also subjected to acid, base, oxidation, heat, photo and UV degradation. The degradation products obtained were well resolved from the darunavir and cobicistat with different retention times. Since the method can effectively separate the darunavir and cobicistat from its degradation products, it can be used as stability indicating method.

Sigamala S. Kumar et al, (2016) revealed a simple, accurate, precise method was developed for the simultaneous estimation of the Cobicistat and Darunavir in Tablet dosage form. Chromatogram was run through BDS (250mm 4.6mm, 5μ). Mobile phase containing Buffer and Acetonitrile in the ratio of 40:50A:10M was pumped through column at a flow rate of 1ml/min. Temperature was maintained at 30°C. Optimized wavelength for Cobicistat and Darunavir was 210nm. Retention time of
Cobicistat and Darunavir were found to be 3.170 min and 3.984 min. % RSD of the Cobicistat and Darunavir were and found to be 1.06 and 1.3 respectively. % Recover was Obtained as 100.71% and 100.2% for Cobicistat and Darunavir. LOD, LOQ values were obtained from regression equations of Cobicistat and Darunavir were 0.107 ppm, 0.326 ppm and 0.333ppm, 1.009 ppm respectively. Regression equation of Cobicistat is y = 10306x + 1346, and of Darunavir is y = 8883.x + 2152.

Sathish Kumar Reddy et al, (2016) investigation was to develop a new RP-HPLC method for simultaneous estimation of Darunavir and cobicistat as per ICH guidelines. The HPLC separation was carried out by reverse phase Chromatography was carried out on an BDSC 18column (4.6x150mm, 5µ particle size) with a isocratic mobile phase composed of ortho phosphoric acid buffer, Acetonitrile, (50:50v/v) at a flow rate of 1mL/min. The column temperature was maintained at30°C and the detection was carried out using a PDA detector at 210nm. The retention times for Darunavir and cobicistat and were 2.018 min and 2.721 min respectively. The percentage recoveries of Darunavir and cobicistat were 99.97 % and 99.95 % respectively. The relative standard deviation for assay of tablets was found to be less than2%. The % RSD for method precision was found to be 0.9 for both Darunavir and cobicistat. The correlation coefficient for Darunavir and cobicistat was found to be 0.9994 and 0.9996 respectively. The detection limits were found to be 0.039µg/mL and 0.210µg/mL for Darunavir and cobicistat respectively. The quantitation limits were found to be 0.117µg/mL and 0.638µg/mL for Darunavir and cobicistat respectively. The proposed method was fast, accurate, precise and sensitive hence it can be employed for the simultaneous quantification of Darunavir and cobicistat in the dosage form, bulk drugs as well as for routine analysis in quality control.
Paul Richards et al, (2016) reported a simple, accurate, precise method was developed for the simultaneous estimation of the Cobicistat and Darunavir in Tablet dosage form. Chromatogram was run through BDS (250mm 4.6mm, 5µ). Mobile phase containing Buffer and Acetonitrile in the ratio of 40:50A:10M was pumped through column at a flow rate of 1ml/min. Temperature was maintained at 30°C. Optimized wavelength for Cobicistat and Darunavir was 210nm. Retention time of Cobicistat and Darunavir were found to be 3.170 min and 3.984 min. % RSD of the Cobicistat and Darunavir were found to be 1.06 and 1.3 respectively. % Recover was obtained as 100.71% and 100.2% for Cobicistat and Darunavir. LOD, LOQ values were obtained from regression equations of Cobicistat and Darunavir were 0.107 ppm, 0.326 ppm and 0.333ppm, 1.009 ppm respectively. Regression equation of Cobicistat is $y = 10306x + 1346$ and of Darunavir is $y = 8883.x + 2152$.

2.5 LITERATURES RELATED TO AMLODIPINE AND PERINDOPRIL

Naser F. Al-Tannak, (2018) revealed that the Perindopril arginine and Indapamid hemihydrate in combination were proven to have a synergistic antihypertensive impact when compared with the use of each component alone. Therefore, a new Ultra-High Performance Liquid Chromatography coupled with Ultraviolet detector (UHPLC-UV) method has been developed and subsequently validated for simultaneous determination of the anti-hypertensive combination of Perindopril arginine and Indapamid hemihydrate. The separation of Perindopril arginine and Indapamid hemihydrate was achieved using a BEH C18 (1.7 µm, 2.1 × 50 mm) analytical column (Waters® Acquity UPLC) and a mobile phase composed of 0.01% v/v formic acid in water adjusted to pH 4 with acetic acid and acetonitrile (40:60 v/v). The method was able to separate Perindopril arginine and Indapamid hemihydrates within less than 4.5 min with high accuracy, precision, resolution, and
sensitivity. The content of Perindopril arginine and Indapamide hemihydrate present in the dosage form Coversyl Plus® (5000 µg of Perindopril arginine/1250 µg of Indapamide hemihydrate) was determined in triplicate to give a concentration of 4991 µg and 1247 µg, respectively, from the manufacturer’s stated amounts with Relative Standard Deviation (%RSD) of ±0.63% for Perindopril arginine and ±0.84% for Indapamide hemihydrate. Moreover, the degradation products of the combination were elucidated by UHPLC-Quadrupole Time of Flight-Mass spectrometry (UHPLC-QToF-MS) under acidic, basic, and thermal conditions. The developed UHPLC-UV method was sensitive, rapid, and precise. Furthermore, forced degradation studies were performed and the degradants were identified by UHPLC-Electro-Spray Ionization-QToF (UHPLC-ESI-QToF).

Kalaiyarasi et al, (2018) reported the main objective of this study is to develop and validate a simple mass compatible method or quantification of both perindopril and amlodipine in human plasma. A UPLC/ESIMS/MS method for the determination of combined dosage form of perindopril and amlodipine in human plasma sample was developed. The gradient elution with flow rate at 0.3 mL per min of mobile phase was kept and 10 µL of sample was injected in each run. The total chromatographic run time was 5.5 min. Argon was used as the collision gas at the pressure of 3.5X10⁻⁵ Torr. In this developed method, a high recovery of perindopril and amlodipine in plasma samples was proved with improved quality data in terms of increased detection limits and chromatographic resolution with greater sensitivity. As per ICH guidelines, the main characteristics of a bioanalytical method validation constraints that are essential to confirm the suitability and reliability of analytical results were evaluated. Quantification of perindopril and amlodipine dosage forms by this method is time saving, cost effective and it can be used in clinical studies from PK studies or
clinical trials using LC-MS/MS to quantify the drug content in human plasma samples.

Gunasekar Manoharan, (2016) revealed that a sensitive, feasible RP-HPLC method has developed and validated for the analysis of Amlodipine with Perindopril arginine in raw and tablet formulation. Successful separation of drugs products is developed on a C(18) column reversed-phase using and using mobile phase composition of Methanol: Phosphate buffer (27:73v/v). The flow rate was adjusted to 1.1 mL/minute and the absorption maxima were observed at 270 nm utilizing Shimadzu SPD-20A Prominence UV-Vis detector. Good linearity was obtained in the range of 2-10 μg/ml, 3-15 μg/ml, for Amlodipine, Perindopril arginine respectively. The HPLC, tablet formulation assay shows percentage purity ranging from 99.16 to 100.18% for Amlodipine, 99.58 to 100.23% for Perindopril arginine. The mean percentage purity is 100.01% and 100.08% for Amlodipine and Perindopril arginine respectively. The chromatographic retention time of Amlodipine and Perindopril arginine was found to be 4.2 and 7.3 minutes respectively. The tailing factor was 0.769 and 0.780 for Amlodipine and Perindopril arginine respectively. The developed method validated according to the ICH guidelines. The method was found to be applicable for determination and validation of Amlodipine and Perindopril arginine in combined tablet form.

Mastannamma et al, (2016) focused on to develop and validate an accurate, simple, precise, rapid, and stability-indicating reversed phase high performance liquid chromatography (RP-HPLC) method for simultaneous estimation of Perindopril Erbumine (PDE) and Amlodipine Besylate (AMD) in bulk drugs and their combined commercial tablets. The chromatographic separation was performed on a YMC C18 (250mm×4.6mm I.D; 5 μm) column, with a mobile phase comprising of a mixer of
Orthophosphoric acid (0.1%) pH 2.1 and Methanol (40:60 ), at a flow rate of 1 ml/min, with detection at 262nm. Retention times of Perindopril Erbumine and Amlodipine Besylate were found to be 2.2 min and 3.1 min respectively. The developed method was validated according to ICH guidelines. Linearity of Perindopril Erbumine was found to be in the range of 4-12μg/ml and that for Amlodipine Besylate was found to be in the range of 5-15μg/ml. The percentage recoveries for both drugs were found in the range of 97-102%. The limits of detection values were found to be 2.96μg/ml and 2.72μg/ml for Perindopril Erbumine and Amlodipine Besylate respectively. The proposed RP-HPLC method is simple, rapid, isocratic, specific, accurate and precise. Hence, this method can be recommended for the estimation of Perindopril Erbumine (PDE) and Amlodipine Besylate (AMD) in pharmaceutical dosage form.

Kalaiyarasi Duraisamy, (2015) validated UPLC/ESI-MS/MS method for the determination of combined dosage form of perindopril and amlodipine in human plasma sample was optimized. This method was responsive and an adequate amount to observe the low-dosage PK studies of perindopril and amlodipine in human plasma. Methods: Chromatographic separation was achieved on a Waters ACQUITY UPLC™ BEH C18 (100.0 mmX2.1 mm; 1.7 μm) column. UPLC analysis consisted of mobile phase A for 0.1% formic acid in MilliQ water and mobile phase B for 0.1% formic acid in acetonitrile, which was degassed. The gradient elution with flow rate at 0.3 mL.min⁻¹ of mobile phase was kept and 10 μL of sample was injected in each run. The total chromatographic run time was 5.5 min. Mass spectrometric detection was carried out in Multiple Reaction Monitoring (MRM) mode using electrospray ion source in positive ion polarity to profile the abundances using the transitions m/z 369 → m/z 172, and m/z 409 → m/z 238 for perindopril and
amlodipine, respectively, and the transitions m/z 612.75 → m/z 280.30 for lercanidipine as internal standard. Argon was used as the collision gas at the pressure of 3.5X10^{-5} Torr. In this developed method, a high recovery of perindopril and amlodipine in plasma samples was proved with improved quality data in terms of increased detection limits and chromatographic resolution with greater sensitivity. UPLC with MS/MS has the advantage over problems of poor chromatography, wearisome extraction steps, uncertain characterized peak and high injection load. As per FDA guidelines, the method is validated for its accuracy, robustness and reproducibility. Quantification of perindopril and amlodipine dosage forms by this method is time saving, cost effective and it can be used in clinical studies to quantify the drug content in human plasma samples.

Nouruddin W. Ali et al, (2012) reported two accurate, selective and precise chromatographic methods, namely TLC-densitometric method & RP-HPLC method & were developed and validated for the simultaneous determination of Amlodipine besylate and Perindopril Arginine in their binary mixtures. The developed TLC-densitometric method depends on separation and quantitation of the studied drugs on silica gel 60F254 TLC plates. Chloroform: methanol: deionized water: glacial acetic acid: triethylamine (10:7:5:0.3:0.2, by volume) was used as developing system and the separated bands were scanned at 208 nm. Linear relationship was obtained in the range 1-10 µg for both drugs. The developed RP-HPLC depends on quantitative chromatographic separation of the studied drugs on a C18 column using phosphate Buffer: acetonitrile (60:40, v/v), pH=4.6 as a mobile phase delivered at constant flow rate of 1 mL.min^{-1} with UV detection at 210 nm. Calibration curves for Amlodipine besylate and Perindopril Arginine were constructed over the concentration range of 1-50 µg.mL^{-1} for both drugs. The proposed methods were successfully applied for
determination of the studied drugs in their bulk powder and in pharmaceutical formulations. The proposed methods were statistically compared to each other using student’s t-test and F-test and no significant difference was found between them.

2.6 LITERATURES RELATED TO METFORMIN AND LINAGLIPTIN

Ralph A. DeFronzo et al, (2015) evaluated the efficacy and safety of combinations of empagliflozin/linagliptin as second-line therapy in subjects with type 2 diabetes inadequately controlled on metformin. Subjects were randomized to a combination of empagliflozin 25 mg/linagliptin 5 mg (n = 137), empagliflozin 10 mg/linagliptin 5 mg (n = 136), empagliflozin 25 mg (n = 141), empagliflozin 10 mg (n = 140), or linagliptin 5 mg (n = 132) as add-on to metformin for 52 weeks. The primary end point was change from baseline in HbA1c at week 24. At week 24, reductions in HbA1c (mean baseline 7.90–8.02% [62.8–64.1 mmol/mol]) with empagliflozin/linagliptin were superior to those with empagliflozin or linagliptin alone as add-on to metformin; adjusted mean (SE) changes from baseline were −1.19% (0.06) (−13.1 mmol/mol [0.7]) with empagliflozin 25 mg/linagliptin 5 mg, −1.08% (0.06) (−11.8 mmol/mol [0.7]) with empagliflozin 10 mg/linagliptin 5 mg, −0.62% (0.06) (−6.8 mmol/mol [0.7]) with empagliflozin 25 mg, −0.66% (0.06) (−7.2 mmol/mol [0.7]) with empagliflozin 10 mg, and −0.70% (0.06) (−7.6 mmol/mol [0.7]) with linagliptin 5 mg (P < 0.001 for all comparisons). In these groups, respectively, 61.8, 57.8, 32.6, 28.0, and 36.1% of subjects with baseline HbA1c ≥7% (≥53 mmol/mol) had HbA1c <7% (<53 mmol/mol) at week 24. Efficacy was maintained at week 52. The proportion of subjects with adverse events (AEs) over 52 weeks was similar across treatment arms (68.6–73.0%), with no hypoglycemic AEs requiring assistance. Combinations of empagliflozin/linagliptin as
second-line therapy for 52 weeks significantly reduced HbA1c compared with the individual components and were well tolerated.

Haak et al, (2013) determined the efficacy and safety of linagliptin in initial combination with metformin in patients with type 2 diabetes. This 1-year randomised, double-blind study was an extension of a 6-month randomised controlled trial, in which adults with type 2 diabetes received one of six treatment regimens (linagliptin 2.5 mg plus metformin 500 mg bid, linagliptin 2.5 mg plus metformin mg 1000 bid, metformin 1000 mg bid, metformin 500 mg bid, linagliptin 5 mg qd or placebo). In the extension, patients in the first three treatment groups continued their regimen (non-switched group, n = 333) while the metformin 500 mg bid, linagliptin 5 mg qd and placebo groups were re-randomised to one of the three continuing regimens (switched group, n = 233). All three non-switched groups maintained reductions in glycosylated haemoglobin (HbA1c; mean ± standard deviation reductions across the 1.5-year period: linagliptin 2.5 plus metformin 1000 bid, −1.63 ± 1.05%; linagliptin 2.5 plus metformin 500 bid, −1.32 ± 1.06%; metformin 1000 bid, −1.25 ± 0.91%) while the switched groups showed additional HbA1c reductions. During the extension, there were no clinically meaningful changes in body weight in any group. Adverse event rates were similar between groups, with most events being mild or moderate, and the incidence of investigator-defined hypoglycaemia was low, with no severe events. Initial combination of linagliptin and metformin was well tolerated over the 1-year extension period, with low risk of hypoglycaemia, and improved glycaemic control vs. metformin alone. The initial combination of linagliptin and metformin appears to provide a useful treatment option in patients whose blood glucose levels are
increased to an extent that metformin monotherapy may not achieve treatment targets.

Barnett et al, (2012) investigated the efficacy and safety of linagliptin, a dipeptidyl peptidase-4 inhibitor, in type 2 diabetes mellitus (T2DM) patients for whom metformin was inappropriate. This 1-year double-blind study (ClinicalTrials.gov, NCT00740051) enrolled T2DM patients with inadequate glycaemic control, treatment-naïve [glycated haemoglobin (HbA1c) 7.0–10.0%] or previously treated with one oral antidiabetes drug (HbA1c 6.5–9.0% before washout), ineligible for metformin because of contraindications (e.g. renal impairment) or previous intolerable side effects. Patients were randomized to monotherapy with linagliptin 5 mg once daily (n = 151) or placebo (n = 76) for 18 weeks, after which placebo patients switched to glimepiride 1–4 mg once daily and treatments continued for another 34 weeks. The primary endpoint was change from baseline in HbA1c after 18 weeks (full-analysis set, last observation carried forward). At week 18, adjusted mean difference in change from baseline HbA1c (8.1%) was −0.60% (95% confidence interval −0.88, −0.32; p < 0.0001) (−0.39% with linagliptin, +0.21% with placebo). At week 52, mean HbA1c was decreased from baseline in both groups [linagliptin: −0.44%; placebo/glimepiride: −0.72% (observed cases)]. Adverse events occurred in 40.4 and 48.7% of linagliptin and placebo patients, respectively, during the initial 18 weeks. During the 34-week extension, patients receiving linagliptin experienced less hypoglycaemia (2.2% vs. 7.8%) and no weight gain (mean change from baseline of −0.2 and +1.3 kg, respectively) compared with glimepiride patients. In T2DM patients for whom metformin was inappropriate, linagliptin improved glycaemic control and was well tolerated, with less hypoglycaemia and relative weight loss compared with glimepiride.
Baptist Gallwitz et al, (2012) revealed that the addition of a sulphonylurea to metformin improves glycaemic control in type 2 diabetes, but is associated with hypoglycaemia and weight gain. We aimed to compare a dipeptidyl peptidase-4 inhibitor (linagliptin) against a commonly used sulphonylurea (glimepiride). In this 2-year, parallel-group, non-inferiority double-blind trial, outpatients with type 2 diabetes and glycated haemoglobin A1c (HbA1c) 6.5–10.0% on stable metformin alone or with one additional oral antidiabetic drug (washed out during screening) were randomly assigned (1:1) by computer-generated random sequence via a voice or web response system to linagliptin (5 mg) or glimepiride (1–4 mg) orally once daily. Study investigators and participants were masked to treatment assignment. The primary endpoint was change in HbA1c from baseline to week 104. Analyses included all patients randomly assigned to treatment groups who received at least one dose of treatment, had a baseline HbA1c measurement, and had at least one on-treatment HbA1c measurement. This trial is registered at ClinicalTrials.gov, number NCT00622284. 777 patients were randomly assigned to linagliptin and 775 to glimepiride; 764 and 755 were included in analysis of the primary endpoint. Reductions in adjusted mean HbA1c (baseline 7.69% [SE 0.03] in both groups) were similar in the linagliptin (−0.16% [SE 0.03]) and glimepiride groups (−0.36% [0.03]; difference 0.20%, 97.5% CI 0.09–0.30), meeting the predefined non-inferiority criterion of 0.35%. Fewer participants had hypoglycaemia (58 [7%] of 776 vs 280 [36%] of 775 patients, p<0.0001) or severe hypoglycaemia (1 [<1%] vs 12 [2%]) with linagliptin compared with glimepiride. Linagliptin was associated with significantly fewer cardiovascular events (12 vs 26 patients; relative risk 0.46, 95% CI 0.23–0.91, p=0.0213). The results of this long-term randomised active-controlled trial advance the clinical evidence and comparative effectiveness bases for treatment
options available to patients with type 2 diabetes mellitus. The findings could improve decision making for clinical treatment when metformin alone is insufficient.

Haak et al, (2012) evaluated the efficacy and safety of initial combination therapy with linagliptin plus metformin versus linagliptin or metformin monotherapy in patients with type 2 diabetes. In this 24-week, double-blind, placebo-controlled, Phase III trial, 791 patients were randomized to one of six treatment arms. Two free combination therapy arms received linagliptin 2.5 mg twice daily (bid) + either low (500 mg) or high (1000 mg) dose metformin bid. Four monotherapy arms received linagliptin 5 mg once daily, metformin 500 mg or 1000 mg bid or placebo. Patients with haemoglobin A1c (HbA1c) ≥11.0% were not eligible for randomization and received open-label linagliptin+high-dose metformin. The placebo-corrected mean (95% confidence interval) change in HbA1c from baseline (8.7%) to week 24 was −1.7% (−2.0, −1.4) for linagliptin + high-dose metformin, −1.3% (−1.6, −1.1) for linagliptin + low-dose metformin, −1.2% (−1.5, −0.9) for high-dose metformin, −0.8% (−1.0, −0.5) for low-dose metformin and −0.6 (−0.9, −0.3) for linagliptin (all p < 0.0001). In the open-label arm, the mean change in HbA1c from baseline (11.8%) was −3.7%. Hypoglycaemia occurred at a similar low rate with linagliptin + metformin (1.7%) as with metformin alone (2.4%). Adverse event rates were comparable across treatment arms. No clinically significant changes in body weight were noted. Initial combination therapy with linagliptin plus metformin was superior to metformin monotherapy in improving glycaemic control, with a similar safety and tolerability profile, no weight gain and a low risk of hypoglycaemia.

Gomis et al, (2011) compared the efficacy, safety and tolerability of linagliptin or placebo administered for 24 weeks in combination with pioglitazone in patients with
type 2 diabetes mellitus (T2DM) exhibiting insufficient glycaemic control (HbA1c 7.5–11.0%). Patients were randomized to receive the initial combination of 30 mg pioglitazone plus 5 mg linagliptin (n = 259) or pioglitazone plus placebo (n = 130), all once daily. The primary endpoint was change from baseline in HbA1c after 24 weeks of treatment, adjusted for baseline HbA1c and prior antidiabetes medication. After 24 weeks of treatment, the adjusted mean change (±s.e.) in HbA1c with the initial combination of linagliptin plus pioglitazone was −1.06% (±0.06), compared with −0.56% (±0.09) for placebo plus pioglitazone. The difference in adjusted mean HbA1c in the linagliptin group compared with placebo was −0.51% (95% confidence interval [CI] −0.71, −0.30; p < 0.0001). Reductions in fasting plasma glucose (FPG) were significantly greater for linagliptin plus pioglitazone than with placebo plus pioglitazone; −1.8 and −1.0 mmol/l, respectively, equating to a treatment difference of −0.8 mmol/l (95% CI −1.2, −0.4; p < 0.0001). Patients taking linagliptin plus pioglitazone, compared with those receiving placebo plus pioglitazone, were more likely to achieve HbA1c of <7.0% (42.9 vs. 30.5%, respectively; p = 0.0051) and reduction in HbA1c of ≥0.5% (75.0 vs. 50.8%, respectively; p < 0.0001). β-cell function, exemplified by the ratio of relative change in adjusted mean HOMA-IR and disposition index, improved. The proportion of patients that experienced at least one adverse event was similar for both groups. Hypoglycaemic episodes (all mild) occurred in 1.2% of the linagliptin plus pioglitazone patients and none in the placebo plus pioglitazone group. Initial combination therapy with linagliptin plus pioglitazone was well tolerated and produced significant and clinically meaningful improvements in glycaemic control. This combination may offer a valuable additive initial treatment option for T2DM, particularly where metformin either is not well tolerated or is contraindicated, such as in patients with renal impairment.
Owens et al, (2011) examined the efficacy and safety of the dipeptidyl peptidase-4 inhibitor linagliptin in persons with Type 2 diabetes mellitus inadequately controlled [HbA1c 53–86 mmol/mol (7.0–10.0%) ] by metformin and sulphonylurea combination treatment. A multi-centre, 24-week, randomized, double-blind, parallel-group study in 1058 patients comparing linagliptin (5mg once daily) and placebo when added to metformin plus sulphonylurea. The primary endpoint was the change in HbA1c after 24 weeks. At week 24, the linagliptin placebo-corrected HbA1c adjusted mean change from baseline was −7 mmol/mol (−0.62%) [95% CI −8 to −6 mmol/mol (−0.73 to −0.50%); P<0.0001]. More participants with baseline HbA1c≥53mmol/mol (≥7.0%) achieved an HbA1c<53mmol/mol (<7.0%) with linagliptin compared with placebo (29.2% vs. 8.1%, P<0.0001). Fasting plasma glucose was reduced with linagliptin relative to placebo (−0.7mmol/l, 95% CI −1.0 to −0.4; P<0.0001). Improvements in homeostasis model assessment of β-cell function were seen with linagliptin (P<0.001). The proportion of patients who reported a severe adverse event was low in both groups (linagliptin 2.4%; placebo 1.5%). Symptomatic hypoglycaemia occurred in 16.7 and 10.3% of the linagliptin and placebo groups, respectively. Hypoglycaemia was generally mild or moderate; severe hypoglycaemia was reported in 2.7 and 4.8% of the participants experiencing hypoglycaemic episodes in the linagliptin and placebo groups, respectively. No significant weight changes were noted. In patients with Type 2 diabetes, adding linagliptin to metformin given in combination with a sulphonylurea significantly improved glycaemic control and this was well tolerated. Linagliptin could provide a valuable treatment option for individuals with inadequate glycaemic control despite ongoing combination therapy with metformin and a sulphonylurea.
Taskinen et al, (2011) evaluated the efficacy and safety of the potent and selective dipeptidyl peptidase-4 (DPP-4) inhibitor linagliptin administered as add-on therapy to metformin in patients with type 2 diabetes with inadequate glycaemic control. This 24-week, randomized, placebo-controlled, double-blind, parallel-group study was carried out in 82 centres in 10 countries. Patients with HbA1c levels of 7.0–10.0% on metformin and a maximum of one additional antidiabetes medication, which was discontinued at screening, continued on metformin ≥1500 mg/day for 6 weeks, including a placebo run-in period of 2 weeks, before being randomized to linagliptin 5 mg once daily (n = 524) or placebo (n = 177) add-on. The primary outcome was the change from baseline in HbA1c after 24 weeks of treatment, evaluated with an analysis of covariance (ANCOVA). Mean baseline HbA1c and fasting plasma glucose (FPG) were 8.1% and 9.4 mmol/l, respectively. Linagliptin showed significant reductions vs. placebo in adjusted mean changes from baseline of HbA1c (−0.49 vs. 0.15%), FPG (−0.59 vs. 0.58 mmol/l) and 2hPPG (−2.7 vs. 1.0 mmol/l); all p < 0.0001. Hypoglycaemia was rare, occurring in three patients (0.6%) treated with linagliptin and five patients (2.8%) in the placebo group. Body weight did not change significantly from baseline in both groups (−0.5 kg placebo, −0.4 kg linagliptin). The addition of linagliptin 5 mg once daily in patients with type 2 diabetes inadequately controlled on metformin resulted in a significant and clinically meaningful improvement in glycaemic control without weight gain or increased risk of hypoglycaemia.

Forst et al, (2010) studied the efficacy and safety of dipeptidyl peptidase-4 inhibitor, linagliptin, added to ongoing metformin therapy, were assessed in patients with Type 2 diabetes who had inadequate glycaemic control (HbA1c≥7.5 to ≤10%; ≥58.5 to ≤85.8 mmol/mol) with metformin alone. Patients (n=333) were randomized to
receive double-blind linagliptin (1, 5 or 10mg once daily) or placebo or open-label glimepiride (1–3mg once daily). The primary outcome measure was the change from baseline in HbA1c at week 12 in patients receiving combination therapy compared with metformin alone. Twelve weeks of treatment resulted in a mean (sem) placebo-corrected lowering in HbA1c levels of 0.40% (±0.14); 4.4 mmol/mol (±1.5) for 1mg linagliptin, 0.73% (±0.14); 8.0 mmol/mol (±1.5) for 5 mg, and 0.67% (±0.14); 7.3 mmol/mol (±1.5) for 10mg. Differences between linagliptin and placebo were statistically significant for all doses (1mg, P=0.01; 5mg and 10mg, P<0.0001). The change in mean (sem) placebo-corrected HbA1c from baseline was −0.90% (±0.13); −9.8 mmol/mol (±1.4) for glimepiride. Adjusted and placebo-corrected mean changes in fasting plasma glucose were −1.1mmol/l for linagliptin 1mg (P=0.002), −1.9mmol/l for 5mg and −1.6mmol/l for 10mg (both P<0.0001). One hundred and six (43.1%) patients reported adverse events; the incidence was similar across all five groups. There were no hypoglycaemic events for linagliptin or placebo, whereas three patients (5%) receiving glimepiride experienced hypoglycaemia. The addition of linagliptin to ongoing metformin treatment in patients with Type 2 diabetes was well tolerated and resulted in significant and clinically relevant improvements in glycaemic control, with 5mg linagliptin being the most effective dose.