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Review Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF DIPEPTIDYL PEPTIDASE-4 INHIBITORS: A REVIEW

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Abstract

Dipeptidyl peptidase-4 (DPP-4) inhibitors are a new class of oral anti-diabetic agents used in the treatment of type 2 diabetes mellitus, that work to potentiate the effect of incretin hormones. This review explores the existing analytical methods for the estimation of DPP-4 inhibitors in pharmaceutical formulations and in biological matrix. There has been significant research on broad range of analytical techniques that could be useful in the estimation of DPP-4 inhibitors in formulations and in biological matrices. Analytical methods such as Ultraviolet (UV) spectrophotometry, Extractive Spectrometry, Mass spectroscopy, NMR spectroscopy, High Performance Liquid Chromatography (HPLC), High Pressure Thin Layer Chromatography (HPTLC), Ultra Performance Liquid Chromatography (UPLC), Liquid Chromatography-Mass spectrophotometry (LC-MS) and Capillary Zone Electrophoresis (CE) have been reported for the estimation of DPP-4 inhibitors in single and/or in combination. From the review it could be understood that there are a number of methods developed, but UV Spectroscopy and high performance liquid chromatography with UV detection have been popularly used in the identification and estimation of DPP-4 inhibitors.

Keywords: DPP-4 inhibitors, estimation, bulk, formulation, biological fluids.

Introduction

Type 2 diabetes is a progressive disease which may require intensification of therapy over time. Management includes a prudent diet, regular exercise and medicine to reduce blood glucose levels. Pharmacological options available in the management of type 2 diabetes include sulphonylureas, thiazolidinediones, -glucosidase inhibitors, metformin and insulin. These treatment options, although highly effective in reducing blood glucose levels, may be associated with an increased risk of hypoglycaemia, as seen with sulphonylureas and insulin; weight gain, as noted with insulin, sulphonylureas and thiazolidinediones; and gastrointestinal intolerance, as observed with metformin. These unwanted adverse effects may act as barriers to optimal glycaemic control.^[1]

Saxagliptin, Vildagliptin, Sitagliptin, Linagliptin and Alogliptin are the currently available Dipeptidyl peptidase-4 inhibitors (DPP-4 inhibitors). Sitagliptin was the first of the DPP-4 inhibitors to be approved by the US Food and Drug Administration in 2006. This was followed by the approval of vildagliptin in February 2007.

DPP-4 inhibitors are a new class of medicines that work to potentiate the effect of incretin hormones. Incretin hormones are secreted from the gastrointestinal tract (the enteroendocrine cells), into the bloodstream in response to food intake. The two most well-characterised incretin hormones are the glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide, also known as gastric inhibitory peptide

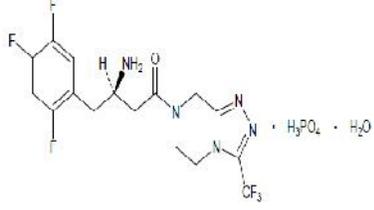
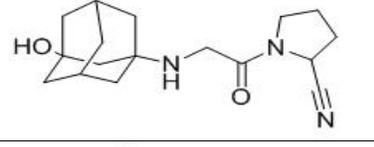
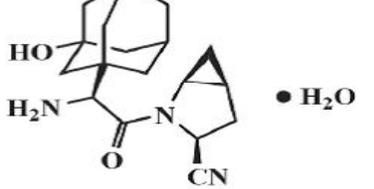
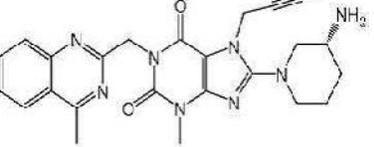
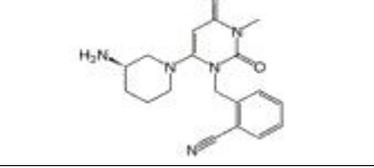
(GIP). GLP-1, in particular, appears to be responsible for the majority of the incretin effects on the pancreatic β -cell function. When blood glucose levels are elevated following a meal, GLP-1 is released from the gastrointestinal tract, and it:

- Stimulates insulin secretion from the pancreatic cells.
- Reduces glucagon secretion from the pancreatic cells.
- Improves β -cell function.

- Slows gastric emptying.^[1,2]

Circulating levels of GLP-1 are low in the fasting state, and rise quickly following a meal. However, GLP-1 has a very short half-life and is rapidly degraded by the enzyme, DPP-4. In an attempt to harness the beneficial effects of GLP-1, research has focused on interventions along the GLP-1 pathway. DPP-4 inhibitors are smaller molecules that can be absorbed intact from the gastrointestinal tract, making oral administration possible.^[2]

Table: Different DPP-4 inhibitors:

Drug	Structure	IUPAC Name
1.Sitagliptin		7 - [(3R) - 3 - amino - 1 - oxo - 4 - (2, 4, 5-trifluorophenyl) butyl] -5, 6, 7, 8 tetrahydro - 3 - (trifluoromethyl)-1, 2, 4 - triazolo [4, 3-a]pyrazine phosphate (1:1) monohydrate
2.Vildagliptin		(S)-1-[N-(3-hydroxy-1-adamantyl) glycy] pyrrolidine-2-carbonitrile
3.Saxagliptin		(1s, 3s, 5s) -2-[(2s) -2-amino-2-(3-hydroxy-1-adamantyl) acetyl] -2-azabicyclohexane-3-carbonitrile
4.Linagliptin		1HPurine-2,6-dione, 8-((3R)-3 aminopiperidin-1-yl)-7-(2-butyn-1yl)-3,7-dihydro-3-methyl-1-((4-methylquinazolin-2yl) methyl)
5.Alogliptin		2-((6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-3,4dihydropyrimidin-1(2H)yl)methyl) benzonitrile

ANALYTICAL METHODS FOR ESTIMATION OF DPP-4 INHIBITORS:

There are several simple, precise, accurate and sensitive methods reported for the estimation of DPP-4 inhibitors in either single drug, combined dosage form or in biological matrices.

A. Spectrophotometric Methods:

1.Sitagliptin: a)single :

Vinit chavhan et.al.^[3] developed and validated UV spectrophotometric method for the estimation of Sitagliptin phosphate in bulk and tablet dosage form by absorbance maxima method and area under the curve method.

Method A involves measurement at a λ_{max} of 267nm and method B in the range of 261-270nm using water as diluent which shows linearity in the range of 20-160 $\mu\text{g/ml}$.

Bala sekaran C et.al.,^[4] developed and validated spectrophotometric method for the determination of DPP-4 inhibitor, Sitagliptin in its pharmaceutical dosage forms, which is based on measurement of yellow colored product at 430nm formed when primary amino group of STG is condensed with acetyl acetone and formaldehyde, the formed yellow color is stable for 1 hour, using distilled water as diluent. The method shows linearity in the range of 5-25 $\mu\text{g/ml}$.

Jeyabalan et.al.,^[5] developed and validated analytical method for Sitagliptine phosphate monohydrate in pure and tablet dosage form by derivative spectroscopy. In zero order spectrophotometry, absorbance value was measured at 267nm, first derivative spectrophotometry amplitudes at 213nm and second derivative spectrophotometry amplitudes at 276nm using methanol as diluent.

Tarkase KN et.al.,^[6] developed and validated UV spectrophotometric method for estimation of Sitagliptin phosphate, which involves measurement at 267nm using 0.1N HCl as diluent. The method was rapid, specific and linear in the range of 5-40 $\mu\text{g/ml}$.

Pathade et.al.,^[7] developed and validated Stability Indicating UV Spectrophotometric Method for the estimation of Sitagliptin Phosphate in bulk and tablet dosage form which involves measurement at 267nm. Forced degradation studies includes the effect of temperature, oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH values, were carried out according to the ICH requirements.

Arpit Patwari et.al.,^[8] developed extractive spectrophotometric method for estimation of DPP-4 inhibitors in bulk and in pharmaceutical dosage forms. Sitagliptin was reacted with Bromocresol Green (BCG) and Bromothymol Blue (BTB) in presence of acetate buffer (pH 4.1) and the coloured complex was extracted with chloroform using distilled water as diluent which was measured at 423nm. The method was economical and linear in the range of 10-50 $\mu\text{g/ml}$ (with BCG) and 5-50 $\mu\text{g/ml}$ (with BTB) for STG.

Balasekharan et.al.,^[9] developed extractive spectrometric method for determination of Sitagliptin phosphate in bulk drugs in which STG was treated with Erichrome Black T and 0.1N HCl and the resulting ion-pair complex was extracted into chloroform which was measured at 500nm.

Monila et.al.,^[10] developed two extractive spectrometric method for estimation of Sitagliptin in bulk and unit dosage forms. In method A, drug was complexed with Bromo thymol blue and in method B, with Bromo cresol green and extracted with chloroform at pH 2.4 (adjusted with phthalate buffer) and the absorbance of yellow coloured chromogen was measured at 412nm (method A), at 419nm (method B). The methods was linear in the range of 25-125 $\mu\text{g/ml}$ (for method A) and 10-50 $\mu\text{g/ml}$ (for method B).

b. Combinations: Chandanam sreedhar et.al.,^[11] developed and validated new analytical method for sitagliptin and pioglitazone which involves measurement at 267nm (STG) and 225nm (PIO), using methanol as diluent. The method was linear in the range of 20-60 $\mu\text{g/ml}$ (STG) and 6-14 $\mu\text{g/ml}$ (PIO).

Safaa M Riad et.al.,^[12] developed Spectrophotometric method for determination of Sitagliptin and Metformin in formulation. The first method involves measurement at 268nm and second method was the isosbestic point method which involves measurement at 268nm (λ_{max} of STG) and 257nm (isosbestic point of two drugs), using distilled water as diluent.

ChavhanVD et.al.,^[13] developed UV spectrophotometric method for simultaneous estimation of Sitagliptin phosphate and Simvastatin in bulk and tablet dosage form by dual wavelength method, which involves measurement at two wavelengths in which the difference in absorbance is zero for second drug. For STG, 224 and 244nm and for simvastatin 254 and 274nm were selected, using methanol : water (50:50%) as diluent and both drugs were found to be stable in this diluent for 30hrs during stability studies. The principle involved in this method is that the absorbance difference at two points on the spectra is directly proportional to the one component, independent of second component.

D Phaneendra et.al.,^[14] developed simultaneous estimation of Simvastatin and Sitagliptin by using different analytical methods. Method A (simultaneous equation method) at 238 nm (SIM) and 267nm (STG). Method B (first order derivative) at 230 nm (zero crossing point of STG) for SIM and 275nm (zero crossing point of SIM) for STG. Method C (Q-absorption) at λ_{max} of STG 267nm and 250nm (isosbestic point of 2 drugs), using distilled water as diluent. The three methods were found to be linear in the range of 3-15 $\mu\text{g/ml}$ (SIM) and 50-150 $\mu\text{g/ml}$ (STG).

Ghazala Khan et.al.,^[15] developed simultaneous estimation of Metformin and Sitagliptin in tablet dosage form which was carried out by multi-

component mode at 232nm (MET) and 267nm (STG) using distilled water as diluent.

Sujani PV et.al.,^[16] developed and validated simultaneous equation method for the estimation of Metformin and Sitagliptin by UV spectroscopy which involves measurement at 232nm (MET) and 266nm (STG), using distilled water as solvent. The developed method was linear in the range of 10-50µg/ml (MET) and 20-80µg/ml (STG).

Sheetal Sharma et.al.,^[17] developed and validated UV spectrophotometry for simultaneous estimation of Sitagliptin phosphate and Simvastatin in marketed formulation at 267nm (STG) and 238nm (SIM) using methanol: water [90:10(v/v)] as diluent. The method was linear in the range of 10-50µg/ml (STG) and 5-25µg/ml (SIM).

Narendra Nyola et.al.,^[18] developed simultaneous estimation of Sitagliptin and Metformin Hydrochloride in pure and tablet dosage form by UV spectroscopy at 231nm and 267nm (λ_{max} of MET and STG) using distilled water as diluent and shows linearity in the range of 2-10µg/ml (MET) and 20-60µg/ml (STG).

Amruta B. Loni et.al.,^[19] developed Simultaneous UV Spectrophotometric Method for Estimation of Sitagliptin phosphate and Metformin Hydrochloride in Bulk and Tablet Dosage Form. Method A (Absorbance maxima method) at 266nm (STG) and 232nm (MET). Method B (AUC method), which involves measurement in the range of 244-279nm (STG) and 222-240nm (MET) using distilled water as diluent. Linearity was observed in the range of 25-225µg/ml (STG) and 2-12µg/ml (MET).

Vinit Chavhan et.al.,^[20] developed and validated economic UV spectrophotometric method for simultaneous estimation of Sitagliptin Phosphate and Simvastatin in bulk and tablet dosage form by absorption ratio method which is based on measurement at two wavelengths i.e. 250nm (isoabsorptive point of two drugs) and 237nm (λ_{max} of SIM) using methanol : water (60:40%v/v) as diluent. Linearity was observed in the range of 20-160µg/ml (STG) & 3-18µg/ml (SIM).

Patil Sachin et.al.,^[21] developed UV spectrophotometric method for simultaneous estimation of Sitagliptin and Metformin in tablet dosage form at 273nm (STG) and 237nm (MET) using methanol : water (40:60) as diluent and linear in the range of 35-85µg/ml (STG) and 10-50µg/ml (MET).

2.Saxagliptin: a) Single :

Kalaichelvi et.al.,^[22] developed and validated spectroscopic method for estimation of Saxagliptin in pure and from tablet formulation at 208nm using methanol as diluent. Linearity was observed in the range of 5-40µg/ml, LOD and LOQ were 0.0607µg/ml and 0.1821µg/ml.

Marwa S Moneeb^[23] developed spectrophotometric and spectrofluorometric methods for determination of Saxagliptin in bulk and pharmaceutical preparations. In spectrophotometric method, SAXA was derivatized with (a) 0.5% 1,2-naphthoquinone-4-sulfonic acid sodium salt (NQS) in distilled water at 25°C for 10mins at pH 10 (adjusted with borate buffer) and measured at 475nm and D₁ spectra of the resulting reaction product was recorded at 429.5–504.5nm (b) 0.2% 4-chloro-7-nitrobenzofurazan (NBD-Cl), in methanol at 70°C for 15mins at pH 9 (adjusted with borate buffer), acidified with HCl and measured at 470nm, D₁ spectra of the resulting reaction product was recorded at 441.5-489.5nm to increase the sensitivity and the fluorescence intensity of SAXA with NBD-Cl was measured at 542nm. Factors such as reagent concentration, pH, temperature, time and diluting solvent was optimized.

3.Linagliptin:a)Single:

Chandra K Sekhar et.al.,^[24] developed a new UV method for determination of Linagliptin in bulk and pharmaceutical dosage form at 241nm using methanol : water (50:50) as diluent. Linearity was observed in the range of 10-35µg/ml.

Sujan Banik et.al.,^[25] developed and validated a simple and rapid UV spectrophotometry method for Linagliptin in bulk and marketed dosage form at 294nm using methanol as diluent. Linearity was observed in the range of 5-30µg/ml, LOD and LOQ were 0.247µg/ml and 0.748µg/ml.

4.Alogliptin:Combinations:

Raval Kashyap et.al.,^[26] developed and validated first order derivative and dual wavelength spectrophotometry methods for simultaneous estimation of Alogliptin and Pioglitazone in bulk and dosage form. In first order derivative spectra, at 275.60nm (ZCP of ALO) and 268.20nm (ZCP of PIO). At the ZCP of ALO, PIO is measured and vice-versa. In Dual wavelength method, measurement at 270.20nm and 265nm (λ_1 and λ_2 for ALO), 280nm and 271nm (λ_3 and λ_4 for PIO), linear in the range of 5-30µg/ml for both drugs.

Chirag et.al.,^[27] developed and validated UV spectrophotometric method for simultaneous estimation of Metformin Hydrochloride and Alogliptin Benzoate in bulk drugs and combined dosage forms.

In simultaneous equation method, measurement at 277nm and 232nm (λ_{\max} of ALO & MET) and in Absorbance ratio method, at 250nm (iso-absorptive point of both drugs) and 277nm (λ_{\max} of ALO) using water as solvent.

5.Vildagliptin: a)Single:

Arpit Patwari et.al.,^[28] developed extractive spectrophotometric method for estimation of Vildagliptin in bulk and their pharmaceutical dosage forms. Vildagliptin was reacted with Bromocresol Green (BCG) and Bromothymol Blue (BTB) in presence of acetate buffer (p^H 4.1) and the coloured complex was extracted with chloroform using distilled water as diluent at 423nm.

Marwa S Moneeb^[29] developed spectrophotometric and spectrofluorometric methods for determination of Vildagliptin in bulk and pharmaceutical preparations. In spectrophotometric method, vildagliptin was derivatized with (a) 0.5% 1,2-naphthoquinone-4-sulfonic acid sodium salt (NQS) in distilled water at 50°C for 10mins at p^H 9.5 (adjusted with borate buffer) and measured at 470nm and D_1 spectra of the resulting reaction product was recorded at 422.5–497.5nm (b) 0.2% 4-chloro-7-nitrobenzofurazan (NBD-Cl), in methanol at 70°C for 20mins at p^H 8.5 (adjusted with borate buffer), acidified with HCl and absorbance was measured at 468nm, D_1 spectra of the resulting reaction product was recorded at 438.5-486.5nm to increase the sensitivity and the fluorescence intensity of saxagliptin with NBD-Cl was measured at 540nm.

b.Combinations:

Usharani Gundala et.al.,^[30] developed simultaneous estimation of Vildagliptin and Metformin in bulk and pharmaceutical formulations by UV spectrophotometry using multi-wavelength technique at 217nm (VIL) and 234nm (MET), using water as solvent.

Baokar Shrikrishna et.al.,^[31] developed simultaneous spectrophotometric method for estimation of Vildagliptin and Metformin in bulk and tablet dosage form at 233nm and 216nm (λ_{\max} of MET and VIL) using 0.1N NaOH as solvent, linear in the range of 30-70 μ g/ml (VIL) and 5-25 μ g/ml (MET).

B. Chromatographic Methods:

1. Sitagliptin: a)Single:

Lavanya R et.al.,^[32] developed and validated RP-HPLC method for the estimation of Sitagliptin Phosphate in Bulk and its Tablet Dosage Form which was done on Zorbax Eclipse XDB C₁₈ column

(150x4.6 mm, 5 μ m) 0.01M KH₂PO₄: Methanol (50:50 % v/v and pH 2.5 with 0.2% OPA) as mobile phase with an injection volume of 10 μ L, at a flow rate of 0.7 ml/min, an ambient column temperature and using PDA detection at 267nm. The method was cost-effective, less time consuming, linear in the range of 5-30 μ g/ml, the assay of SITA was found to be 99.89%.

b.Combinations:

Chandanam Sreedhar et.al.,^[33] developed new analytical method for some oral hypoglycemic drugs which was carried out on Agilent C₁₈ column (250 X 4.6 mm, 5 μ m) using Acetonitrile : 10mM Potassium di-Hydrogen Phosphate buffer (pH to 3.0 \pm 0.1 with OPA) (30:70 %V/V) for 8min and 28:72(v/v) for 8-15min as mobile phase in gradient mode, with an injection volume of 20 μ L at a flow rate of 1.0 ml/min, with UV detection at 270 nm using mobile phase as solvent. The retention times were 5.7 (STG) and 7.5mins (PIO). The method was economical and linear in the range of 60-90 μ g/ml (STG) & 6-14 μ g/ml (PIO).

Mahesh Kumar Mone et.al.,^[34] isolated and characterized degradation products of Sitagliptin and developed validated stability indicating HPLC method for Sitagliptin API and tablets. By Stress studies, the degradation pathway of STG in bulk and tablets was studied and degradation products were isolated and characterized by mass and NMR spectroscopy. 3-(trifluoromethyl)-6, 7-dihydro[1,2,4]triazolo[4, 3-a]pyrazin-8(5H)-one, (2E)-1-[3-(trifluoromethyl)-5, 6-dihydro[1, 2, 4]triazolo[4, 3-a]pyrazin- 7(8H)-yl]-4-(2, 4, 5-trifluorophenyl)but-2-en-1-one and (3E)-1-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4, 3-a]pyrazin-7(8H)-yl]-4- (2, 4, 5-trifluorophenyl)but-3-en-1-one were the unreported impurities. The estimation was done on Poroshell 120 EC-C₁₈ column (150x3.0mm, 2.7 μ m) and 5Mm ammonium acetate : acetonitrile as mobile phase by gradient elution mode in presence of spiked degradation products and impurity at a flow rate of 0.5mL/min and column temperature of 25°C and detected at 210nm, using water : methanol (9:1) as diluent. The degradation products of sitagliptin were isolated, purified and characterized by NMR, IR and mass spectroscopy.

Govindasamy Jeyabalan et.al.,^[35] developed RP-HPLC method for simultaneous estimation of Sitagliptin Phosphate Monohydrate and Metformin Hydrochloride in bulk and pharmaceutical formulation. The estimation was done on Phenomenex C₁₈ column (250x4.6mm, 5 μ m),0.02M Potassium dihydrogen phosphate (p^H 4.3) : acetonitrile (55:45v/v) as mobile phase, by isocratic elution mode, at a flow rate of 1mL/min and injection volume of 10 μ L and detected at 252nm using mobile phase as diluent. Linearity in the range of 4-20 μ g/mL (STG) and 10-50 μ g/mL (MET).

Rani Sirisha et.al.,^[36] developed and validated RP-HPLC method for simultaneous estimation of Metformin and Sitagliptin in tablet dosage forms which was done on Hypersil BDS C₁₈ (150x 4.6 mm, 5µm) with a mobile phase of Potassium dihydrogen orthophosphate buffer : Methanol (50:50 and to p^H 4 by OPA) in isocratic mode, with an injection volume of 20µL, at a flow rate of 1ml/min at 260nm using mobile phase as diluent. The run time was 6mins and the retention time was 1.773min (MET) and 3.696 min(STG).

Kavitha KY et.al.,^[37] developed and validated stability indicating RP-HPLC method for simultaneous estimation of Sitagliptin and Simvastatin from its pharmaceutical dosage form, which was done on Enable C₁₈ G column(250x4.6mm, 5µm) with a mobile phase of water : ACN (30:70%v/v) with an injection volume of 20mL at a flow rate of 1mL/min and detected at 236nm using mobile phase as diluent. The retention times were 3.46 (STG)and 4.75mins (SIM) and linear in the range of 40-400µg/ml(STG) and 10-50µg/ml (SIM).

Sachin L Patil et.al.,^[38] developed and validated Stability Indicating RP-HPLC Method for Simultaneous Determination of Sitagliptin and Metformin in Tablet Dosage Form, which was done on Supelco Column (25cmx4.6mm,5µm) with a mobile phase of ACN: ammonium acetate buffer (70:30 v/v)at a flow rate of 0.8 ml/min and detected at 245nm using mobile phase as diluent.The retention time were 6.54 (STG) and 4.24mins (MET), and linear in the range of 10-50µg/ml(MET) and 1-5µg/ml (STG).

Raja T et.al.,^[39] developed and validated RP-HPLC method for simultaneous estimation of metformin hydrochloride and sitagliptin phosphate in bulk and pharmaceutical formulation, which was done on Xterra Symmetry C₈ column (100x4.6 mm, 5µm) with mobile phase of methanol : ACN : phosphate buffer (20:35:45 v/v/v and Phosphate buffer to pH 8 with NaOH) at a flow rate of 1.0 ml/min and detected at 254nm using mobile phase as diluent. The retention times were 3.69(MET) &4.90min (STG) and linearity was 100-500µg/mL (MET) and 10-50µg/mL (STG).

Sumithra M et.al.,^[40] developed and validated RP-HPLC method for simultaneous estimation of sitagliptin and metformin, which was done on BDS hypersil C₁₈ column (250x4.0mm,5µ) with a mobile phase of phosphate buffer (p^H-4) : ACN (60:40) at a flow rate of 1.0ml/min and detected at 260nm, using mobile phase as diluent. The retention times were 2.8min(STG) and 2.0min(MET) and linearity in the range of 2-12µg/mL(STG) and 20-120µg/ML (MET).

Hitesh P Inamdar et.al.,^[41] developed RP-HPLC method for simultaneous determination of Metformin Hydrochloride, Rosiglitazone and Sitagliptin and its application to commercially available drug products, which was done on ACE 3 (150X 4.6mm, 3.5µm) column with a mobile phase of 10 mM sodium hexane sulphonate monohydrate and 10 mM Potassium dihydrogen phosphate buffer : acetonitrile : methanol in gradient ratio at a flow rate of 1.5 mL/min with an injection volume of 20µL and UV detection at 210 nm. The retention times were 1.445(MET), 7.270(RST) and 8.154 mins(STG) and the forced degradation studies were performed under Acid, Base, Oxidative, Photolytic and Thermal stress conditions, linear in the range of 12-100µg/mL for all the three drugs.

Nancy Veronica B et.al.,^[42] developed and validated a new simple RP-HPLC method for simultaneous estimation of Metformin HCl and Sitagliptin phosphate in bulk and dosage forms, which was done on Inertsil ODS C₁₈ column (250x4.6 mm, 5µm) with mobile phase of ammonium di-hydrogen phosphate buffer (p^H 4.3): acetonitrile (74:26 v/v) with an injection volume of 20µL, at a flow rate of 1.0 ml/min and detected at 246nm using mobile phase as diluent. The retention times were 2.443(MET) and 4.293mins(STG), linear in the range of 75-175µg/mL (MET) and 7.5-17.5µg/mL (STG).

Ashutosh Kumar et.al.,^[43] developed stability indicating RP- HPLC method for simultaneous estimation of metformin hydrochloride and sitagliptin phosphate monohydrate in bulk as well as in formulation, which was done on Symmetry C₁₈ column (4.6 x 150mm, 3.5mm, XTerra) with mobile phase of Potassium Dihydrogen Phosphate : ACN(65:35%v/v) at a flow rate of 0.9mL/min with an injection volume of 20µL, at and detected at 254nm, using mobile phase as diluent. The run time was 7mins, and retention times were 2.592(MET) and 4.307mins(STG), and linear in the range of 100-300ppm(MET) and 10-30ppm(STG).

Ramanjaneyulu Juvvignunta et.al.,^[44] developed and validated a new method for simultaneous estimation of Sitagliptin and Metformin Hydrochloride in tablet dosage form by RP-HPLC, which was done on symmetry C₁₈ column (250x4.6 mm, 5µ), Methanol : Phosphate buffer (60 : 40%v/v) as mobile phase, at a flow rate of 1.0 ml/min and detected at 258nm, using methanol as diluent. The retention times were 2.344(STG) and 3.296 mins(MET). The method was fast and linear in the range of 200-600µg/mL(MET) and 20-60µg/mL(STG).

Swati D Bhende et.al.,^[45] developed RP-HPLC method for the simultaneous estimation of Sitagliptin Phosphate and Metformin Hydrochloride in combined tablet dosage forms, which was done on XTerra C₈ (4.6 x 100 mm, 3µm) column, phosphate buffer solution (p^H9) : acetonitrile : methanol (35:45:20%v/v/v) as mobile phase, with an injection volume of 20µL, at a flow rate of 0.6 ml/min using UV detector at 260 nm. The retention times were 3.056(STG) and 2.420mins(MET), linear in the range of 50-150ppm for both drugs.

Sankar et.al.,^[46] developed and validated simultaneous estimation of Sitagliptin and Metformin in pharmaceutical dosage form using RP-HPLC method, which was done on Phenomenex Luna C₁₈ column (250x 4.6 mmx5µm) 0.02M Potassium dihydrogen phosphate pH(4.0) : ACN (60:40%v/v) as mobile phase, at a flow rate of 1.0ml/min with an injection volume of 20µL and detected at 252nm using water as diluent. STG and MET were eluted at 2.718 and 1.925 min and linear in the range of 2-12µg/mL(STG) and 20-120µg/mL (MET).

Vani R et.al.,^[47] developed and validated analytical method for simultaneous estimation of Metformin and Sitagliptin in bulk and pharmaceutical dosage forms by RP-HPLC, which was done on Waters C₁₈ column (250x4.6mm, 5µm), 0.1M Dipotassium phosphate buffer (p^H 7) : acetonitrile (70:30%v/v) as mobile phase with an injection volume of 10µL, at a flow rate of 1mL/min and detected at 223nm(STG) and 316nm(MET), using mobile phase as diluent. The runtime was 9mins and retention times were 2.85(STG) and 6.21mins(MET) and linear in the range of 100-300µg/mL(STG) and 200-600µg/mL(MET). Because of low solvent consumption and short analytical time, it is considered as environment friendly chromatographic procedure.

Chirag B Patel et.al.,^[48] developed and validated HPTLC method for the simultaneous determination of Metformin Hydrochloride and Sitagliptin Phosphate in marketed formulation. Sample and standard solutions were applied as spots on HPTLC plates and separated using mobile phase consisting of 1%w/v ammonium acetate in methanol and developed using ascending development technique in twin trough chamber at room temperature and developed spots were detected densitometrically at 257 nm using methanol as diluent. The distance travelled by solvent front is 80mm and R_f values for MET and STG was 0.43±0.009 and 0.60±0.013 and linear in the range of 1000-7000ng/spot(STG) and 600-2000ng/spot(MET).

Dharshana K Modi et.al.,^[49] developed a simple and sensitive HPTLC method for simultaneous

determination of Metformin Hydrochloride and Sitagliptin Phosphate in tablet dosage form, which was done on silica gel 60 f₂₅₄ plates with butanol : water : glacial acetic acid (6: 2: 2% v/v/v) as mobile phase, in ascending development mode detected at a wavelength of 227nm using methanol as diluent and linear in the range of 500-1000ng/band and 50-1000ng/band for MET and STG.

Kavitha KY et.al.,^[50] developed and validated UPLC method for simultaneous estimation of Sitagliptin and Simvastatin from its pharmaceutical dosage form. The separation was carried out on Waters Acquity BEH C₁₈ (50x2.1mm,1.7µm) UPLC column, water : ACN (30:70%v/v,p^H 4 with OPA) as mobile phase with an injection volume of 5µL, at a flow rate of 0.35mL/min and detected at 236nm, using mobile phase as diluent. The run time was 5mins and retention times were 0.82(STG) and 1.80mins(SIM) and linear in the range of 40-400µg/ml(STG) and 10-50µg/ml(SIM).

Chellu SN Malleswararao et.al.,^[51] developed Simultaneous determination of Sitagliptin Phosphate Monohydrate and Metformin Hydrochloride in tablets by a validated UPLC method which was done on Aquity UPLC BEH C₈ (100 x 2.1mm, 1.7µm) column, buffer [consisting of 10 mM potassium dihydrogen phosphate and 2 mM hexane-1-sulfonic acid sodium salt pH5.5 with diluted phosphoric acid] : acetonitrile as mobile phase in gradient mode, with an injection volume of 0.5µL, at a flow rate of 0.2 mL/min and detected at 210nm using water as diluent. The runtime was 10mins, retention times were 2.2mins(MET) and 7mins(STG).

2.Saxagliptin:a.Single:

Srikanth Inturi et.al.,^[52] developed and validated novel LC method for determination of Saxagliptin in pure bulk and pharmaceutical dosage forms which was done on Grace smart C₁₈ (150 x 4.6mm, 5µm), buffer (0.02M sodium dihydrogen phosphate, p^H 3 with OPA) : methanol : Acetonitrile (45:20:35 v/v) as mobile phase, at a flow rate of 1.0 ml/min, an injection volume of 20µL and detected at 220nm. The retention time was 8.20 min(SAXA), assay was found to be 97.8% and linear in the range of 10-100µg/ml.

b.Combinations:

NVMS Bhagavanji,^[53] developed and validated stability indicating Liquid chromatographic method for simultaneous estimation of Metformin and Saxagliptin in combined formulations which was done on Thermo Hypersil BDS C₈ (250 x 4.6mm, 5µm) column, water (p^H3 with OPA) : methanol (70:30%v/v) as mobile phase, with an injection volume of 10µL, at a flow rate

of 1.0mL/min and detected at 241nm, using water as diluent. The retention times were 4.7(MET) and 6.8mins(SAXA).

Pravin Cumar et.al.,^[54] developed and validated RP–HPLC method for simultaneous estimation of Metformin and Saxagliptin in tablets which was done on C₁₈ column (25cm x 4.6mm, 5µm) using phosphate buffer (p^H 5.0): Acetonitrile : methanol (75:15: 10v/v/v) as mobile phase, with an injection volume of 50µL, at a flow rate of 1.5mL/min and detected at 225nm with water as diluent. The retention time were 5.65(MET) and 6.20mins(SAXA).

Rambabu C et.al.,^[55] developed RP-HPLC method for simultaneous estimation of Saxagliptin and Pioglitazone in tablets which is based on separation of drugs on Inertsil C₁₈ column (150x4.6mm,5µm) with a mobile phase of Acetonitrile : phosphate buffer (60:40 v/v, p^H 7.0) at a flow rate of 0.8ml/min and detected at 260nm using mobile phase as diluent. Aceclofenac was used as internal standard. The retention times were 2.48(SAXA),4.45(PIO) and 6.34mins(ACECLO) and linear in the range of 20-80µg/ml(SAXA) & 10-70µg/ml(PIO).

Nyola Narendra et.al.,^[56] developed analytical method for simultaneous estimation of Saxagliptin Hydrochloride and Metformin Hydrochloride in bulk by RP-HPLC which was done on Phenomenex C₁₈ column(250x4.6mm,5µm) with a mobile phase of 0.02M Potassium dihydrogen phosphate : Acetonitrile : Methanol (50:25:25 v/v/v) at p^H 4.3 and detected at 240nm, linear in the range of 10-50µg/ml(SAXA) and 5-25µg/ml(MET).

3.Linagliptin: a.Single:

Sujatha K et.al.,^[57] developed a new RP-HPLC method for the estimation of Linagliptin in tablet dosage forms which was done on X Bridge C₁₈ column (100X4.6mm;5µm) with a mobile phase of phosphate buffer (p^H 3.4) : Acetonitrile (70:30 %v/v), with an injection volume of 20µL, at a flow rate of 1 ml/min and detected at 240nm, using methanol as diluent. The run time was 5min and retention time was 2.791min, linear in the range of 25-150µg/ml.

Lakshmi B et.al.,^[58] developed a novel RP–HPLC method for the quantification of Linagliptin in formulations which was done on symmetry Chromosil C₁₈ column (250x4.6mm, 5µm) with a mobile phase of Acetonitrile : Water : Methanol (25:50:25 (v/v/v))with an injection volume of 20µL, at a flow rate of 1mL/min and detected at 238nm using mobile phase as diluent. The run time was 15mins and retention time was 7min.

Archana M et.al.,^[59] developed and validated RP-HPLC method for determination of new antidiabetic agent Linagliptin in bulk and in pharmaceutical formulation which was done on Khromosil C₁₈ column (150x4.6 mm,5µm) with mobile phase 0.02 M potassium dihydrogen phosphate : acetonitrile (70:30%v/v, pH 5.0 with 1% OPA solution), at a flow rate of 1.2 mL/min and detected at 226 nm, using mobile phase as diluent. The run time was 15mins and retention time was 4.2min, linear in the range of 0-75µg/ml.

Lakshmana Raju et.al.,^[60] developed and validated RP-HPLC method for the determination of Linagliptin which was done on symmetry C₁₈ column with a mobile phase of Methanol : Water (83:17%v/v, pH4.1 with 0.1% OPA) at a flow rate of 1ml/min and detected at 241nm. The retention time was 5.85min, linear in the range of 5-30ppm.

b.Combinations:

Kavitha KY et.al.,^[61] developed and validated stability indicating RP-HPLC method for the simultaneous estimation of Linagliptin and Metformin in pure and pharmaceutical dosage form which was done on BDS Hypersil C₈ column (250 x 4.6mm, 5µm) with mobile phase of Acetonitrile : Water : Methanol (25:50:25%v/v/v, pH4.1 with 0.1% OPA),at a flow rate of 1mL/min and detected at 243nm using mobile phase as diluent. The retention times 2.9(MET) and 7.0mins(LINA), linear in the range of 5-30µg/MI(LINA) and 10-100µgmL(MET).

Janardhan Swamy et.al.,^[62] developed and validated RP-HPLC method for the simultaneous estimation of Metformin HCL and Linagliptin in bulk and tablet dosage form which was done on Hypersil C₁₈ column (250 x 4.6 mm, 5µm) with a mobile phase of phosphate buffer (pH5.6 with OPA) : methanol : ACN(40:5:55 v/v) at a flow rate of 1.0 mL/min and detected at 233nm. The runtime was 10mins and retention times were 6.6(MET) and 5.4mins(LINA), linear in the range of 125-750µg/mL(MET) and 0.625-3.75µg/MI(LINA).

Rajasekaran A et.al.,^[63] developed and validated HPTLC method for simultaneous estimation and stability indicating study of Metformin HCl and Linagliptin in pharmaceutical formulation which was done on precoated silica gel 60 GF254 plates with a mobile phase of acetone : methanol : toluene : formic acid (4:3:2:1 v/v/v/v) and detected at 259nm using methanol as diluent. The R^f values were 0.61(MET) and 0.82(LINA).

4. Alogliptin: a. Single:

Ramzia et.al.,^[64] developed RP-HPLC method for determination of Alogliptin in bulk and formulation which was done on Symmetry cyanide column (150 mm × 4.6mm, 5µm) with mobile phase of potassium dihydrogen phosphate buffer (pH 4.6) : acetonitrile (20:80%v/v) and detected at 215nm, using methanol as diluent. The retention time was 2.8mins(ALO), linear in the range of 5-160µg/mL.

b. Combinations:

Praveen Kumar et.al.,^[65] developed and validated RP-HPLC method for Alogliptin and Metformin Hydrochloride in tablet dosage form which was done on Agilent C₁₈ (250 x 4.6 mm, 5µ) with a mobile phase of methanol : water (0.2% of TEA was added to water and pH6.0 with OPA) in the ratio of 70:30%v/v and detected at 254nm, using methanol as diluent. The run time was 10 mins and retention times were 3.30(ALG) and 6.26mins(MET), linear in the range of 25-150µg/mL for both drugs.

Satya Sri et.al.,^[66] developed a new RP-HPLC method for simultaneous estimation of Metformin and Alogliptin in bulk and formulation by using PDA detector which was done on X-Terra column (250x 4.6mm, 5µm) with mobile phase of potassium dihydrogen phosphate buffer [p^H 4.0] : ACN (70:30, v/v) and detected at 235nm using mobile phase as diluent. The run time was 8mins and retention times were 2.73(MET) and 4.45mins(ALG) and linear in the range of 300-700µg/mL(MET) and 7.5-17.5µg/mL (ALG).

Neelima B et.al.,^[67] developed and validated stability indicating RP-HPLC method for simultaneous determination of Alogliptine and Pioglitazone in bulk and formulations which was done on Hypersil BDS C₁₈ column (250 x 4.6mm, 5µm) with a mobile phase of Phosphate Buffer : ACN (45:55 % v/v) and detected at 215nm using water as diluent. The runtime was 10mins and retention times were 3.42(ALG) and 5.24mins(PIO).

5. Vildagliptin: a. Single:

Aparajita Malakar et.al.,^[68] developed and validated RP-HPLC method for estimation of Vildagliptin from tablet dosage form which was done on Xterra Waters C₁₈ column (150mm×4.6mm, 5µm) with a mobile phase of aqueous phase (mixture of 1ml of 25% ammonium hydroxide in 1000ml of water, p^H9.5 with 50% solution of phosphoric acid) : organic phase (methanol) in the ratio of 60:40 v/v, and detected at 210nm using mobile

phase as diluent. The retention time was 6.3 min, linear in the range of 5-200µg/mL.

Prem Kumar Bichala et.al.,^[69] developed and validated new RP-HPLC method for estimation of Vildagliptin in bulk and tablet dosage forms which was done on Shimpack VP-ODS, Shimadzu C₁₈ column (150x4.6mm, 5µm) with a mobile phase of 0.01M phosphate buffer (p^H5.3 with OPA) : Acetonitrile (30:70%v/v) and detected at 210nm, using mobile phase as diluent. The runtime was 10mins and retention time was 3.8mins.

Rahima Khatun et.al.,^[70] developed and validated RP-HPLC method for the determination of Vildagliptin from tablet dosage form which was done on Shimpack VP-ODS column (150x4.6mm, 5µm) with a mobile phase of 0.02M phosphate buffer (p^H4.6 with OPA) : acetonitrile (80:20%v/v) and detected at 210nm, using 20% acetonitrile in water as diluent. The runtime was 10mins and retention time was 3.6mins, linear in the range of 20-70µg/ml.

Ramzia et.al.,^[71] developed Liquid Chromatographic Methods for the Determination of Vildagliptin in the Presence of its Synthetic Intermediate which was done on Symmetry waters C₁₈ column (150mmx4.6mm, 5µm), potassium dihydrogen phosphate buffer p^H 4.6 : ACN : Methanol (30:50:20 v/v/v) as mobile phase and detected at 220nm using methanol as diluent.

Hanumantha Rao et.al.,^[72] developed and validated HPLC method for estimation of Vildagliptin in pharmaceutical dosage forms which was done on Altima C₁₈ column (150x4.6mm, 5µm), buffer (p^H2.6±0.5 with dilute OPA) : acetonitrile (72:28v/v) as mobile phase and detected at 266nm using water as diluent. The runtime was 7.0mins and retention time was 3.258mins, linear in the range of 25-150µg/mL.

b. Combinations:

Lakshman Rao et.al.,^[73] developed stability indicating RP-HPLC method for simultaneous estimation of Vildagliptin and Metformin in solid dosage form which was done on Sunfire BDS C₈ column (250x4.6mm, 5µm) disodium hydrogen phosphate p^H 7.0 buffer : ACN(60:40 v/v) as mobile phase and detected at 263nm using buffer : water (50:50%v/v) as diluent. The runtime was 5mins and retention times were 2.07(MET) and 3.52mins(VLD).

Pradeep G Shelke^[74] developed and validated RP-HPLC method for simultaneous determination of Metformin HCl and Vildagliptin in formulation which was done on Phenomenax C₁₈ column

(4.6X150mm), phosphate buffer (p^H6.0) : methanol (65:35v/v) as mobile phase at a flow rate of 1.0ml/min and detected at 255nm. The retention times were 1.503min(MET) & 5.530mins(VLD), linear in the range of 50-150µg/mL for both drugs.

Pushpa Latha K et.al.,^[75] developed and validated analytical method for simultaneous estimation of Vildagliptin and Metformin in tablet dosage form by RP-HPLC which was done on Phenomenex C₁₈ column (250 x 4.6 mm, 5µm) 2mM phosphate buffer (pH3.5 with OPA) : Acetonitrile (70: 30%v/v) as mobile phase at a flow rate of 1 ml/min and detected at 293nm, using Gatifloxacin as internal standard. The retention times were 2.1(VLD), 2.5(MET) and 5.5min(GAT).

Subhakar Nandipati et.al.,^[76] developed and validated RP-HPLC method for simultaneous estimation of Vildagliptin and Metformin in bulk and formulation dosage which was done on Thermo Hypersil ODS C₁₈ column (250x4.6mm, 5 µm) 0.1M Potassium hydrogen phosphate (p^H7.0 with OPA) : acetonitrile (60:40%v/v) as mobile phase at a flow rate of 1mL/min and detected at 263nm. The retention times were 2.1(MET) and 3.5mins(VLD).

• **For estimation from biological matrices:** Methods of measuring drugs in biological media are becoming increasingly important for the study of bioavailability & bioequivalence studies, quantitative evaluation of drugs and their metabolites, new drug development, clinical pharmacokinetics, research in basic biomedical and pharmaceutical sciences and therapeutic drug monitoring. For the estimation of the drugs present in the biological fluid, HPLC method is considered to be more suitable since this is a powerful and rugged method. It is also extremely specific, linear, precise, accurate, sensitive and rapid.

• **Anil Dubala et.al.**,^[77] developed and validated bioanalytical method for estimation of Sitagliptin Phosphate by RP-HPLC and its application to pharmacokinetic study. The plasma was extracted with methanol by protein precipitation and estimation was done on Phenomenex C₁₈ column (250x4.6mm, 5µm) 0.5%v/v Triethylamine (p^H6.8 with OPA) : acetonitrile (77:23v/v) as mobile phase and detected at 267nm. The runtime was 10mins and retention times were 6.1(STG) and 7.7mins(internal standard), linear in the range of 10-1000ng/mL for STG, LOD and LOQ were 1ng/mL and 10ng/ml.

Hiren Havelikar et.al.,^[78] developed spectrofluorimetric method for determination of Sitagliptin Phosphate in bulk, formulation and spiked human urine which was done at fluorescence

wavelength of 297nm using distilled water as diluent, linear in the range of 0.6-10µg/mL for STG.

Bonde et.al.,^[79] developed a simple and sensitive method for the determination of Metformin and Sitagliptin in human plasma using Liquid chromatography and tandem mass spectrometry. Samples were extracted using Acetonitrile-induced protein precipitation and estimation was done on High purity C₁₈ column (50 x 4.6mm, 5µm) 10mM Ammonium formate (p^H 3.0 with formic acid) : Acetonitrile (40:60%v/v) as mobile phase, using methanol : water (80:20) as diluent. The most abundant ions found in the mass spectrum were m/z 71.0, 235.1, 77.0 and 239.5 for MET, STG, MET D6 AND STG D4, linear in the range of 25-3000ng/mL(MET) and 2-800ng/ml(STG) and shorter runtime (2mins).

Samanthula Gananadhamu et.al.,^[80] developed a rapid LC-ESI-MS-MS method for simultaneous determination of Sitagliptin and Pioglitazone in rat plasma and its application to pharmacokinetic study which was done on Phenomenex Synergy C₁₈ column (30x4.6mm, 4µm) methanol : 2mM ammonium acetate buffer (p^H4.5 with acetic acid) as mobile phase by gradient elution mode, using tolbutamide as internal standard, methanol as diluent and detected using LC-MS/MS in Multiple reaction mode. The runtime was 5.0mins and retention times were 2.53, 2.81 and 2.50mins for STG, PIO and TOL.

Mohamed Salim et.al.,^[81] developed Simultaneous determination of Sitagliptin and Metformin in Pharmaceutical Preparations by Capillary Zone Electrophoresis and its Application to Human Plasma Analysis which was done using fused silica capillary (50cm TL x 43cm EL x 49 µm i.d.) by applying a potential of 15 KV (positive polarity) with a buffer of 60mM phosphate buffer (pH 4.0) and detected at 203nm, using Phenformin as internal standard, linear in the range of 10-100µg/ml and 50-500µg/ml for SITA and MET.

Jing Wen Gao et.al.,^[82] developed a rapid UPLC-MS/MS method for quantification of saxagliptin in rat plasma and its application to pharmacokinetic study. Plasma samples were processed by liquid-liquid extraction with ethyl acetate and analysed on C₁₈ column (2.1x50 mm i.d., 1.7 µm), methanol : 0.1% formic acid (40:60, v/v) as mobile phase. Multiple reaction monitoring transitions were performed for detection in positive-ion mode with an electrospray ionization source.

Xu Xs et.al.,^[83] developed Liquid chromatography and tandem mass spectrometry method for the quantitative

determination of Saxagliptin and its major pharmacologically active 5-monohydroxy metabolite in human plasma : method validation and overcoming specific and non-specific binding at low concentrations. Protein precipitation with acetonitrile was used to extract the analytes from plasma matrix and estimation was done on Atlantis C₁₈ column (50 mm x 2.1 mm, 5 µm) for LC-MS/MS analysis.

Rutvik H Pandya et.al.,^[84] developed and validated RP-HPLC method for simultaneous estimation of Linagliptin and Metformin drugs in human plasma which was done on Grace vyadyec genesis CN column (150x4.6mm, 4 µm) 0.01M di-potassium hydrogen phosphate buffer(p^H 7 with OPA) : acetonitrile (25:75%v/v) as mobile phase and detected at 237nm using methanol as diluent and phenformin as internal standard. The runtime was 18.0mins and retention times were 4.95, 15.41, 11.06mins for LNG, MET and PHEN, linear in the range of 1-32ng/mL for both drugs.

Santhakumari B et.al.,^[85] developed and validated RP-HPLC method for estimation of Vildagliptin which was done on XBridge Shield C₁₈ column (3.5 µm, 4.6x150mm) and a guard column of the same type, 50mM ammonium bicarbonate (pH 7.8) : acetonitrile as mobile phase by gradient elution mode and detected at 210nm using methanol as extracting solvent. The runtime was 20mins and retention times were 11.2min(VLD) and 13.4min(TOL), linear in the range of 10-120µg/ml(VLD).

RI El Bagary et.al.,^[86] developed Simultaneous determination of Metformin, Vildagliptin, and Vildagliptin impurity in bulk, tablet, and human plasma using UPLC-MS/MS. The estimation employed electro-spray positive ionization and multiple-reaction monitoring mode. The m/z values for M, V, and VI were 130: 71/ 60; 304: 154/97; and 168: 151/93.1. Separation was done on UPLC C₁₈ column using pregapalin as an internal standard in 2mins. The retention times were 0.35, 1.51, and 0.74 min for MET, VLD, and VLD impurity, linear in the range of 20-1200ng/mL for MET and VLD and 2-120ng/mL for VLD impurity.

Conclusion

Pharmaceutical industry is in need of rapid, efficient, economic and accurately validated analytical methods to develop safe and effective drugs for use of mankind. Advancements in analytical instrumentation leads to the development of new techniques like isocratic and gradient RP-HPLC, which was evolved as the primary techniques for the analysis of nonvolatile APIs and impurities. The review highlighted

a broad range of analytical techniques used in separation, detection and estimation of DPP-4 inhibitors. Both Spectrophotometric methods and HPLC with UV detector are the most widely employed techniques for determination of DPP-4 inhibitors in single and in combined formulations. HPLC and UPLC/MS and Capillary Zone Electrophoresis is used for estimation of these drugs in biological matrices. Future trend would be to concentrate on designing of more rapid and sensitive tools for the estimation of DPP-4 inhibitors.

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