WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 16, 303-314. Review Article ISSN 2277– 7105

CHROMATOGRAPHIC REVIEW: DPP-4 INHIBITORS INCLUDE: LINAGLIPTIN, SITAGLIPTIN, VIDAGLIPTIN, AND SAXAGLIPTIN

Jatin Sharad Patil*, Amitkumar R. Dhankani and Dr. Sunil P. Pawar

Department of Quality Assurance, Poojya Sane Guruji Vidya Prasarak Mandal, Shahada, Dist. Nandurbar, 425 409, (MS), India.

Article Received on
25 July 2023,
Revised on 15 August 2023,
Accepted on 04 Sept. 2023
DOI: 10. 20959/wjpr202316-29625

***Corresponding Author**
Jatin Sharad Patil
Department of Quality Assurance, Poojya Sane Guruji Vidya Prasarak Mandal, Shahada, Dist. Nandurbar, 425 409, (MS), India.

ABSTRACT

The frequency of type 2 diabetes mellitus (DM), a chronic metabolic condition, has been rising gradually worldwide. GLP-1 and GIP mediate the effect of the more recent family of oral hypoglycemic drugs known as dipeptidyl-peptidase-4 (DPP-4) inhibitors. DPP-4 inhibitors, such as linagliptin, sitagliptin, vidagliptin, and saxagliptin, treat type 2 diabetes. The analytical method for determining linagliptin, sitagliptin, vidagliptin, and saxagliptin in pharmaceutical formulations, both alone and in combination with other medications, was revealed by examining the literature. In this comprehensive study, the comparison of thirty-seven analytical methods—including HPLC and bioanalytical techniques is most clearly demonstrated. Analytical development must be tested to produce reliable results for regulatory filings. The invention of drugs ushered in a revolution in human health.

KEYWORDS: Linagliptin; Sitagliptin; Vidagliptin; Saxagliptin; Review Article.

INTRODUCTION

Globally, the prevalence of Type 2 Diabetes continues to rise, and cardiovascular illnesses continue to be the leading cause of morbidity and mortality among patients. Dipeptidyl-peptidase-4 (DPP-4) inhibitors are a more recent family of oral hypoglycemic medications whose action is mediated by the hormones GLP-1 and GIP.[1] In contrast to currently available conventional medications, DPP-4 inhibitors are weight neutral, well-tolerated, and offer better glycogenic control over a more extended period. Different reported DPP-4 inhibitors can be broadly categorized into (a) peptidomimetics, or those that imitate the penultimate dipeptide structure of DPP-4 substrates, and (b) non-peptidomimetics. These

inhibitors come in a variety of chemical categories.[2] The different DPP-4 inhibitors are used to treat type 2 diabetes, like linagliptin, sitagliptin, vidagliptin, and saxagliptin[3] In type 2 diabetic individuals, lipid profile has a significant role in predicting cardiovascular risk. Available glucose-lowering drugs may impact lipid levels. Dipeptidyl peptidase-4 (DPP-4) inhibitors have been claimed to lower total cholesterol, although the effectiveness of these claims has varied between trials.[4] In this article, we go over recent developments in HPLC methods for determining the concentrations of linagliptin, sitagliptin, vidagliptin, and saxagliptin alone or in combination with other medications in various biological media, including human plasma and rat plasma.

Chemistry of dipeptidyl-peptidase-4 (DPP-4) inhibitors

Vidagliptin (VDG) - VDG **(Fig. 1)** has an adamantine backbone with three condensed cyclohexane rings and a heterocyclic ring containing nitrogen. Its chemical name is (S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile. It ionizes in an acidic media, showing its essential character. It is a potent inhibitor of the enzyme dipeptidyl peptidase IV (dip-IV), raises the hormone glucagon-like peptide-1 (GLP-1), and controls blood sugar levels.[5]

Sitagliptin (STG) - Chemically, it is Sitagliptin phosphate, chemically designed as 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. STG **(Fig. 2**) was the first DPP-4 inhibitor approved for treating type 2 diabetes in patients. As demonstrated in carefully planned clinical trials in patients with type 2 diabetes, it is used as monotherapy or in combination therapy, is typically well tolerated, and improves glycaemic control. It is available orally, has a lengthy duration of effect, and only needs to be taken once a day.[6]

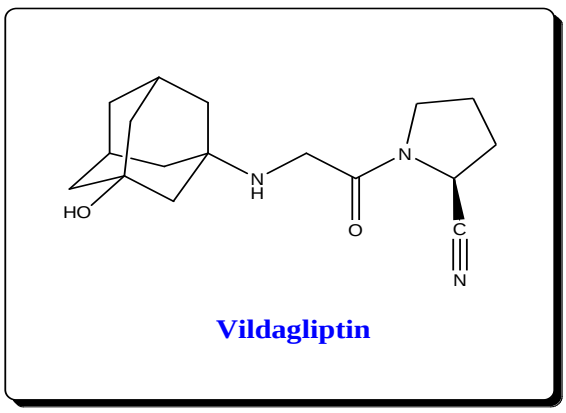

Fig. 1: Chemical Structure of Vidagliptin.

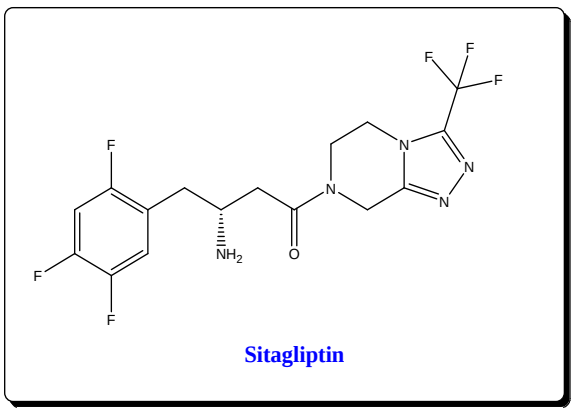

Fig. 2: Chemical Structure of Sitagliptin.

Saxagliptin (SXG) - Chemically, SXG **(Fig. 3)** is chemically known as (1S, 3 S, 5S)-2[(2S)-2- amino- 2- (3- hydroxy- 1- adamantyl) acetyl]-2azabicyclo hexane-3-carbonitrile). $C_{18}H_{25}N_3O_2$.H2O is the empirical formula, and its molecular weight is 333.43. SXG belongs to the dipeptidyl peptidase-4 (DDP-4) inhibitor class of oral hypoglycemic or anti-diabetic medications. When DPP-4 is inhibited, levels of glucagon-like peptide 1 become more active, which reduces pancreatic alpha cell synthesis of glucagon and boosts beta cell production of insulin.[7]

Linagliptin (LNG) - Chemically speaking, LNG **(Fig. 4)** is (8-(3-(R)-Aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl1 ((4-methyl-quinazoline-2-ylmethyl) dihydro-3,7 pyridine-2,6 dione. whose chemical formula is $C_{25}H_{28}N_8O_2$. One of the most essential drugs for the treatment of type-II diabetes mellitus is LNG. A novel therapeutic strategy for treating type-II diabetes is represented by the dipeptidyl peptidase-4 (DPP-4) inhibitor.[8]

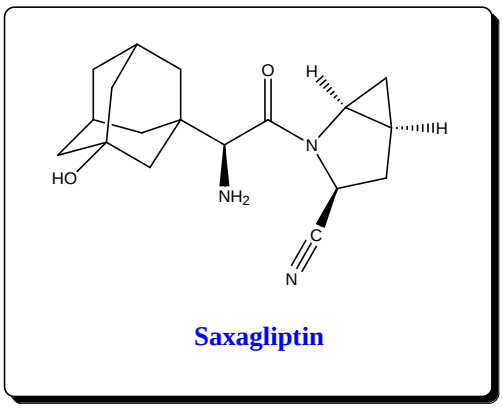

Fig. 3: Chemical Structure of Saxagliptin.

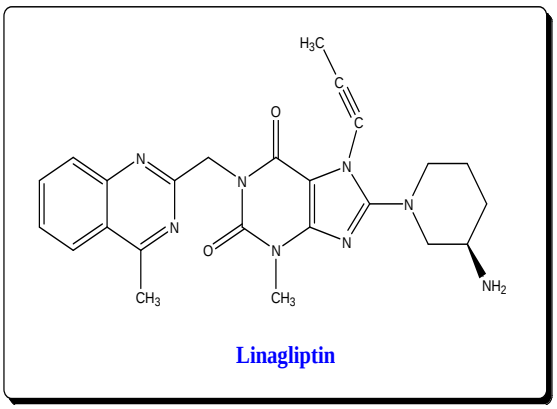

Fig. 4: Chemical Structure of Linagliptin.

HPLC/Bioanalytical methods for SXG, VDG, STG, and LNG in alone and combinations

Due to its highly effective separations and great detection sensitivity, HPLC is the most widely used separation approach in modern pharmaceutical and biological analysis. The HPLC method can analyze most medications in multi-component dosage forms because of its many advantages: quickness, specificity, accuracy, precision, and automation simplicity. Development and validation of HPLC technologies are essential for discoveries, the manufacture of pharmaceutical medications, and various other human and animal investigations. An analytical technique is developed to compare a particular property of the drug substance or drug product to the predefined acceptance criteria for that property.[9] Twenty-seven methods have been described for quantifying SXG, SXG, VDG, STG, and LNG in biological matrix and pharmaceutical dosage form utilizing human plasma. For SXG,

VDG, STG, and LNG, maximum separation was achieved utilizing the isocratic mode. The separation of SXG, VDG, STG, and LNG in varying proportions is often accomplished using a maximum RP-HPLC system with a separate C18 column as the stationary phase and polar solvents including acetonitrile, methanol, water, and buffer solutions having an acidic pH. SXG, VDG, STG, and LNG were estimated using a detection wavelength of between 210 and 270 nm. The information concerning the sample, pharmaceutical or biological matrix, chromatographic conditions (such as flow rate, mode of analysis, and wavelength detection), and linearity are all summarized in **Tables 1, 2, 3, and 4.**[10-40]

Table no 1: Pharmaceutical Analysis of VDG *via* HPLC methods in alone.

Sr. No.	Sample	Pharmaceutical or Biological Matrix	Chromatographic Conditions	Linearity µg/mL	Ref.
1.	VDG	Bulk Material &Tablet	**S.P-** C18 (250 × 4.6 mm, 5µ) Column, **M.P-** Acetonitrile: Buffer, (50:50, *v/v*), **Flow rate** -1.0 mL/min **Mode of analysis –** Isocratic **Detection –** 220 nm	10-60	[10]
2.	VDG	Bulk Material &Tablet	**S.P-** Jasco Crest Pack RP C18 (250 × 4.6 mm, 5µ) Column, **M.P-** Acetonitrile: Methanol, (70:10:20, *v/v/v*),(pH 6), **Flow rate** – 1.0 mL/min **Mode of analysis –** Isocratic **Detection –** 210 nm	5-15	[11]
3.	VDG	Bulk Material	**S.P-** RP C18 (250 × 4.6 mm, 5µ) Column, **M.P-** Phosphate Buffer : Acetonitrile (85 : 15, *v/v*), (pH 4.6) **Flow rate** - 1.0 mL/min **Mode of analysis –** Isocratic **Detection –** 210 nm	10 - 100	[13]
4.	VDG	Raw Material	**S.P-** Athena C18 -WP (250 × 4.6 mm, 5µ) Column**,** **M.P-** Ammonium Acetate Buffer :Acetonitrile (90:10, *v/v*), (Ph-7.5) **Flow rate** – 1.0 mL/min **Mode of analysis –** Isocratic **Detection –** 208 nm	0.4- 1.4 mg/mL	[12]
5.	VDG	Tablet Formulation	**S.P-** Agilent XDB C18, (150×4.6mm, 5µ) Column**,** **M.P-** Phosphate Buffer : ACN (85:15%, *v/v*), **Flow rate** - 1.0 mL/min. **Mode of analysis –** Isocratic **Detection –** 210nm	10–150	[13]
6.	VDG	Bulk Material	**S.P-** Kromasil CN (250 mm × 3.9 mm, 3.5 µm) **M.P-** Methanol:Water (55:45% *v/v*) **Flow rate** - 0.5 mL/min	-	[14]

			Mode of analysis – Isocratic **Detection –**		
7.	VDG	Tablet Formulation	**S.P-** Chiralcel OD-RH (250 mm × 4.6 mm, 5 µm) Column, **M.P-** Borate Buffer Solution: ACN (50:50%, *v/v*), (pH = 9 ± 0.05) **Flow rate** - 1.0 mL/min. **Mode of analysis –** Isocratic **Detection –** 210nm	1 - 12	[15]
8.	VDG	Bulk Material	**S.P-** Qualisil BDS C18 (250 mm x 4.6 mm ,5 µm) Column, **M.P-** Phosphate Buffer : ACN (70:30% ,*v/v*), (Ph-7) **Flow rate** – 0.5 mL/min. **Mode of analysis –** Isocratic **Detection –** 263 nm	10- 60	[16]
9.	VDG	Tablet Dosage Form	**S.P-** Shimadzu (150mm x 4.6mm, 5µm) Column**,** **M.P-** Phosphate Buffer : ACN (80:20%, *v/v*), (pH 4.6) **Flow rate** - 0.7 ml/min **Mode of analysis –** Isocratic **Detection –** 210nm	40% to 140%	[17]

Table no 2: Pharmaceutical Analysis of STG *via* HPLC methods in alone.

Sr. No.	Sample	Pharmaceutical or Biological Matrix	Chromatographic Conditions	Linearity µg/mL	Ref.
1.	STG	Bulk Material& Tablet	**S.P-** C18 (250 mm × 4.6 mm, 5 µm) Column, **M.P-** Methanol : Phosphate potassium Buffer (40:60%, *v/v*), (pH-6.8), **Flow rate** - 1mL/min. **Mode of analysis –** Isocratic **Detection –** 260 nm	-	[18]
2	STG	Human plasma	**S.P-** C18 (250 mm, 4.6 mm,5 µm) Column, **M.P-** Phosphate Buffer: Acetonitrile (73:27%, *v/v*), (pH-4.5), **Flow rate** - 1mL/min. **Mode of analysis –** Isocratic **Detection –** 260 nm	0.1-3	[19]
3	STG	Tablet	**S.P-** C18 (150mm x 4.6mm I.D., 5µm) Column, **M.P-** Phosphate Buffer : ACN (40:60%, *v/v*), (pH-6.8), **Flow rate** - 0.8 mL/min. **Mode of analysis –** Isocratic **Detection –** 267 nm	25-75	[20]
4	STG	Bulk Material	**S.P-** Chiralpak AD-H (250mm×4.6mm, 5µm) Column, **M.P-** n-heptane: Ethanol: Diethylamine (35:65:0.1, *v/v/v*), (pH-6.8), **Flow rate** - 1.0 mL/min.	400 - 2250 ng/ml	[21]

			Mode of analysis – Isocratic **Detection –** 265 nm		
5	STG	Bulk and its Tablet Dosage Form	**S.P-** Zorbax Eclipse XDB C18 (150×4.6 mm, 5µm) Column, **M.P-** KH2PO4: Methano, (50:50 %, *v/v*), (pH-2.5), **Flow rate** - 0.7 ml/min. **Mode of analysis –** Isocratic **Detection –** 267 nm	5-30	[22]
6	STG	Bulk Material & Tablet	**S.P-** Poroshell 120 EC-C18 (3X150mm, 2.7µm) Column, **M.P-** Ammonium acetate and Acetonitrile , **Flow rate** - 0.5ml/min. **Mode of analysis –** Gradient **Detection –** 210 nm	10-500	[23]
7	STG	Bulk powder	**S.P-** Poroshell 120 EC-C18(100 × 4.6 mm, 2.7 µm) Column, **M.P-** Methanol:Water:Triethylamine:Acetic acid (60:40:0.1:0.1,*v/v/v/v*), **Flow rate** - 0.5 mL/min. **Mode of analysis –** Isocratic **Detection –** 268 nm	100-1000	[24]
8	STG	Plasma & Tablet Dosage Form	**S.P-** C18 ODS Hypersil (150 × 4.6 mm,5 µm) Column, **M.P-** Acetonitrile: Potassium dihydrogen phosphate (70:30, *v/v*) , (pH-5), **Flow rate** - 1.0 mL/min **Mode of analysis –** Isocratic **Detection –** 269 nm	0.19-400	[25]

Table no. 3: Pharmaceutical Analysis of SXG *via* HPLC methods in alone and combinations.

Sr. No.	Sample	Pharmaceutical or Biological Matrix	Chromatographic Conditions	Linearity µg/mL	Ref.
1.	SXG	Bulk Drug	**S.P-** Grace C18 (250mm x 4.6mm,5 µm) Column, **M.P-** Methanol : Buffer (80:20, *v/v*), **Flow rate** -0.8 mL/min. **Mode of analysis –** Isocratic **Detection –** 212 nm	-	[26]
2	SXG	Bulk Drug & tablet Formulation	**S.P-** Waters X Bridge C18 (250 mm x 4.6 mm, 5µm) Column, **M.P-** 0.1% Phosphoric acid: Methanol (70: 30, *v/v*),(pH 3.0) **Flow rate** - 1mL/min. **Mode of analysis –** Isocratic **Detection –** 225 nm	15-100	[27]
3	SXG + MET	Bulk Drug & tablet Formulation	**S.P-** outon C18 (25 cm x 4.6 mm,5 µm) Column, **M.P-** G Phosphate Buffer :Acetonitrile : Methanol (75:15:10,*v/v/v*), (pH 5.0),	-	[28]

			Flow rate – 1.5 ml/min. **Mode of analysis –** Isocratic **Detection – 225** nm		
4	SXG + DPG	Bulk Drug & tablet Formulation	**S.P-** Eclipse XDB C18 (150 × 4.6 mm × 5 µm) Column, **M.P-** 0.1% Orthophosphoric acid : Acetonitrile (50:50, *v/v*), (Ph 4.5), **Flow rate** - 1 mL/min **Mode of analysis –** Isocratic **Detection** – 254 nm	SXG- 0.01- 0.5 DPG- 0.05- 2	[29]
5	SXG + DPG	Bulk Drug & tablet Formulation	**S.P-** Thermo RP C18 (250 × 4.6 mm × 5 µm) Column, **M.P-** Methanol : o-Phosphoric acid(60:40, *v/v*), **Flow rate** - 1 mL/min. **Mode of analysis –** Isocratic **Detection** – 220 nm	SXG- 10-50 DPG- 20-100	[30]

Table no 4: Pharmaceutical Analysis of LNG *via* HPLC methods in alone.

Sr. No.	Sample	Pharmaceutical or Biological Matrix	Chromatographic Conditions	Linearity µg/mL	Ref.
1.	LNG	Bulk Drug	**S.P-** KROMASIL CN (250 mm × 3.9 mm, 3.5 µm) Column, **M.P-** Acetonitrile: Water (75:25, *v/v*), **Flow rate** -1.0 mL/min. **Mode of analysis –** Isocratic **Detection** – 254 nm	0.9 - 450 ppm	[31]
2	LNG	Bulk Drug & tablet Formulation	**S.P-** symmetry C18 (250 mm x 4.6 mm, 5µm) Column, **M.P-** Methanol: Water (83:17, *v/v*),(pH 4.1), **Flow rate** - 1mL/min. **Mode of analysis –** Isocratic **Detection** – 241 nm	5-30 ppm	[32]
3	LNG	Bulk Drug & Rat Plasma	**S.P-** Zorbax Eclipse XDB C18 (250cm x 4.6 mm, 5µm) Column, **M.P-** Methanol: Formic acid (75:25, *v/v*,),(pH 4.1)**,** **Flow rate** – 1.0 ml/min. **Mode of analysis –** Isocratic **Detection** – 254 nm	-	[33]
4	LNG	Bulk Drug & tablet Formulation	**S.P-** zorbax Eclipse XDB-C18 (4.6 × 150 mm, 5 µm) Column, **M.P-** Methanol:Water (40:60, *v/v*), (pH 4.5), **Flow rate** - 1 mL/min. **Mode of analysis –** Isocratic **Detection** – 225 nm	1–50	[34]
5	LNG	Bulk Drug & tablet Formulation	**S.P-** C18 (250 × 4.6 mm × 5 µm) Column, **M.P-** Phosphate Buffer : Acetonitrile(70:30, *v/v*), (pH 6.8±0.2), **Flow rate** - 1 mL/min **Mode of analysis –** Isocratic **Detection** – 239 nm	40 - 60	[35]

DISCUSSION

The SXG, VDG, STG, and LNG in pharmaceutical formulations and bulk drugs can be determined using various HPLC techniques. Only a few analytical methods, such as simple HPLC, bioanalytical, and stability-indicating, can be used to estimate the concentrations of pharmaceuticals like SXG, VDG, STG, and LNG combined with other drugs like MET and DPG. Additionally, it has been said that 27 HPLC techniques have been developed and are being used to measure.

CONCLUSION

The examination of several analytic methods for detecting SXG, VDG, STG, and LNG in pharmaceutical formulations, human plasma, and bulk form utilising HPLC. Among the most often used solvents for sample processing is the acetonitrile, water, and methanol mixture. Some of the solvents used to separate SXG, VDG, STG, and LNG include acetonitrile, methanol, and different buffer solutions with acidic pH levels. Isocratic mode is used for most HPLC operations for reverse phase chromatography analysis. The current review study contains essential information the researcher may find helpful regarding the many SXG, VDG, STG, and LNG analysis methods. likewise, it can get knowledge of the numerous possibilities for SXG, VDG, STG, and LNG**.**

ACKNOWLEDGEMENT

The principal of the Poojya Sane Guruji Vidya Prasarak Mandal, Shahada, Dist. Nandurbar (MS) 425 409, is gratefully acknowledged by the authors for providing the essential library resources.

Abbreviations Used

- DPG - Dapagliflozin
- DPP-4dipeptidyl-peptidase-4
- HPLC- High performance liquid chromatography
- LC- Liquid chromatography
- LNG - Linagliptin
- M.P – Mobile Phase
- MET - Metformin
- pH- Power of hydrogen
- S.P- Stationary phase

- STG - Sitagliptin
- SXG - Saxagliptin

REFERENCE

1. Jose, T. and Inzucchi, S.E., Cardiovascular effects of the DPP-4 inhibitors. *Diabetes and Vascular Disease Research*, 2012; *9*(2): 109-116.
2. Patel, B.D. and Ghate, M.D., Recent approaches to medicinal chemistry and therapeutic potential of dipeptidyl peptidase-4 (DPP-4) inhibitors. *European journal of medicinal chemistry*, 2014; *74*: 574-605.
3. Dicker, D., DPP-4 inhibitors: impact on glycemic control and cardiovascular risk factors. *Diabetes care*, 2011; *34*(2): S276-S278.
4. Monami, M., Lamanna, C., Desideri, C.M. and Mannucci, E., DPP-4 inhibitors and lipids: systematic review and meta-analysis. *Advances in therapy*, 2012; *29*: 14-25.
5. Housheh, S., Mohammad, H. and Alahmad, Y., Spectrophotometric method for the determination of vildagliptin in bulk and pharmaceutical dosage forms. *Int J Pharm Sci Rev Res.*, 2019; *17*: 117-120.
6. Ravanello, A., Schreiner Delgado, L., Isa Pedroso Marcolino, A., Franco Codevilla, C., Ines Horn Adams, A. and Madalena Bueno Rolim, C., A simple stability-indicating LC-UV method to assay sitagliptin phosphate in tablets. *Current Analytical Chemistry*, 2012; *8*(4): 569-573.
7. Usman, M.R.M., Ahmad, S., Deore, C., Shaikh, T., Jain, B. and Imran, M., Stability indicating RP-HPLC method for determination of saxagliptin and dapagliflozin in bulk and tablet dosage forms. *Journal of Pharmaceutical Sciences and Research*, 2020; *12*(4): 499-506.
8. Balasubramanian, V., Venkatachalam, S., Venu, K. and Palanisamy, N.K., Spectroscopic characterization and electronic structure analysis of Linagliptin by DFT method, 2021.
9. Bhardwaj, S.K., Dwivedia, K. and Agarwala, D.D., A review: HPLC method development and validation. *International Journal of Analytical and Bioanalytical Chemistry*, 2015; *5*(4): 76-81.
10. Sultana, R., Bachar, S.C. and Rahman, F., Development and validation of stability indicating assay method of vildagliptin in bulk and tablet dosage form by RP-HPLC. *International journal of pharmacy & life sciences*, 2013; *4*(4): 2530-2534.

11. Chaphekar, M.M. and Hamrapurkar, P.D., Development and validation of RP-HPLC assay method for vildagliptin using QbD approach and its application to forced degradation studies. *Int. J. Pharm. Sci. Drug Res*, 2016; *8:* 157-165.
12. Kashid, A.M., Ghorpade, D.A., Toranmal, P.P. and Dhawale, S.C., Development and validation of reversed-phase HPLC method for determining vildagliptin using an experimental design. *Journal of Analytical Chemistry*, 2015; *70:* 510-515.
13. Arar, S., Al-Qudah, E., Alzweiri, M. and Sweidan, K., New forced degradation products of vildagliptin: Identification and structural elucidation using LC-MS, with proposed formation mechanisms. *Journal of Liquid Chromatography & Related Technologies*, 2015; *43*(15-16): 633-644.
14. Boovizhikannan, T. and Palanirajan, V.K., RP-HPLC determination of vildagliptin in pure and in tablet formulation. *Journal of pharmacy research*, 2013; *7*(1): 113-116.
15. Al-Sabti, B. and Harbali, J., Development and validation of an analytical method for quantitatively determining three potentially genotoxic impurities in vildagliptin drug material using HPLC-MS. *Journal of Separation Science*, 2021; *44*(13): 2587-2595.
16. Srinivas, C., Qureshi, H.K. and Veeresham, C., Validated Chiral Ultra Fast Liquid Chromatographic Method for Quantitative Analysis of Enantiomeric Vildagliptin. *American Journal of Analytical Chemistry*, 2021; *12*(11): 429-439.
17. Khatun, R. and Mirazunnabi, M., A validated reversed-phase HPLC method for the determination of vildagliptin from tablet dosage form. *International Journal of Pharmaceutical and Life Sciences*, 2013; *2*(3): 90-98.
18. Qassas, M.K., Ameral-mardini, M. and Ghazal, H., A validated HPLC stability indicating method for the determination of sitagliptin in bulk drug substance and tablets. *Int. J. Pharm. Sci. Rev. Res.*, 2015; *32*(1): 33-194.
19. Ahmed, R.M., Hadad, G.M., El-Gendy, A.E. and Ibrahim, A., Development of HPLC method for determination of sitagliptin in human plasma using fluorescence detector by experimental design approach. *Analytical Chemistry Letters*, 2018; *8*(6): 813-828.
20. Ravanello, A., Schreiner Delgado, L., Isa Pedroso Marcolino, A., Franco Codevilla, C., Ines Horn Adams, A. and Madalena Bueno Rolim, C., A simple stability-indicating LC-UV method to assay sitagliptin phosphate in tablets. *Current Analytical Chemistry*, 2012; *8*(4): 569-573.
21. Reddy, B.V.R., Raman, N., Kumar, B.S. and Rambabu, C., Chiral separation of sitagliptin phosphate enantiomer by HPLC using amylose based chiral stationary phase. *Journal of Pharmacy Research*, 2013; *7*(6): 546-550.

22. Lavanya, R., Yunoos, M. and Pradesh, A., Development and validation of RP-HPLC method for the estimation of sitagliptin phosphate in bulk and its tablet dosage form. *Journal of Advanced Pharmacy Education & Research, Oct-Dec*, 2013; *3*(4).
23. Sonune, D.P. and Mone, M.K., Isolation, characterization of degradation products of sitagliptin and development of validated stability-indicating HPLC assay method for sitagliptin api and tablets. *International Journal of Pharmaceutical Sciences and Research*, 2013; *4*(9): 3494.
24. Saleh, O.A., El-Azzouny, A.A.E.S., Aboul-Enein, H.Y. and Badawey, A.M., A validated stability indicating HPLC method for determination of sitagliptin. *European Journal of Chemistry*, 2014; *5*(3): 497-502.
25. Ashraf, M., Shahzad, M.N., Hayat, M.M., Rahman, J., Ejaz, S., Altaf, H. and Nasim, F.H., Development and Validation of an HPLC Method for the Quantification of Sitagliptin in Plasma and Tablet Dosage Form. *Lat. Am. J. Pharm*, 2015; *34*(3): 456-61.
26. Zinjad, S.S., Patel, S.G., Gaikwad, D.D. and Jadhav, S.L., Analytical method development of saxagliptin HCl by RP-HPLC. *Journal of Drug Delivery and Therapeutics*, 2019; *9*(4): 274-278.
27. Scheeren, L.E., Marcolino, A.I.P., Adams, A.I.H. and Rolim, C.M.B., Stability indicating RP-LC-PDA method for the quantitative analysis of saxagliptin in pharmaceutical dosage form. *Brazilian Journal of Pharmaceutical Sciences*, 2015; *51*: 461-466.
28. Kumar, P., Vasudevan, R. and Deecaraman, A., Validated RP-HPLC Method for Simultaneous Estimation of Metformin and Saxagliptin in Tablets. *Rasayan Journal Chemistry*, 2012; *5*: 137-141.
29. Donepudi, S. and Achanta, S., Simultaneous estimation of saxagliptin and dapagliflozin in human plasma by validated high performance liquid chromatography-ultraviolet method. *Turkish Journal of Pharmaceutical Sciences*, 2019; *16*(2): 227.
30. Usman, M.R.M., Ahmad, S., Deore, C., Shaikh, T., Jain, B. and Imran, M., Stability indicating RP-HPLC method for determination of saxagliptin and dapagliflozin in bulk and tablet dosage forms. *Journal of Pharmaceutical Sciences and Research*, 2020; *12*(4): 499-506.
31. Al-Sabti, B. and Harbali, J., Trace analysis of potential genotoxic impurity N, N-dimethylaniline in linagliptin active pharmaceutical ingredient using HPLC. *Separation Science Plus*, 2020; *3*(7): 306-312.
32. Badugu, L.R., A Validated RP-HPLC method for the determination of Linagliptin. *Am. J. PharmTech Res*, 2012; *2*(4): 463-470.

33. Hanafy, A. and Mahgoub, H., A Validated HPLC method for the determination of linagliptin in rat plasma. Application to a pharmacokinetic study. *Journal of chromatographic science*, 2016; *54*(9): 1573-1577.
34. Mourad, S.S., El-Kimary, E.I., Hamdy, D.A. and Barary, M.A., Stability-indicating HPLC-DAD method for the determination of linagliptin in tablet dosage form: application to degradation kinetics. *Journal of chromatographic science*, 2016; *54*(9): 1560-1566.
35. Rajbangshi, J.C., Alam, M.M., Hossain, M.S., Islam, M.S. and Rouf, A.S.S., Development and validation of a RP-HPLC method for quantitative analysis of Linagliptin in bulk and dosage forms. *Dhaka University Journal of Pharmaceutical Sciences*, 2018; *17*(2): 175-182.