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ANALYTICAL TECHNIQUES USED FOR THE DETERMINATION OF VILDAGLIPTIN AND ITS COMBINATIONS: AN OVERVIEW

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ABSTRACT
Vildagliptin is an oral antidiabetic drug it comes under the class of dipeptidyl peptidase-4 (DPP-IV) inhibitor. An analytical technique is a method used to identify and determine the concentration of a chemical compound or element. This article reviews reported analytical methods such as spectroscopy, electrophoresis, liquid chromatography, gas chromatography and some hyphenated techniques up-to-date for the determination of vildagliptin and its combinations in bulk drugs, pharmaceutical formulations and in biological matrices such as blood, plasma, serum, oral fluids, sweat, hair, urine, etc. Based on the survey in the analytical and pharmaceutical journals we summarized the most popular analytical technique used for the determination of vildagliptin and we concluded that the high-performance liquid chromatography was the most familiar and easy method used for the determination of vildagliptin. This review may direct the research scholars by providing information regarding the analysis of vildagliptin.

KEYWORDS: Vildagliptin, anti diabetic drug, pharmaceutical formulations biological matrices, spectroscopy, chromatography.

1. INTRODUCTION
Diabetes mellitus is a category of metabolic disorder which is associated with hyperglycemia resulting from insufficient insulin secretion, defect in insulin action or both.[1] The widespread of type 2 diabetes mellitus increased with age. The morbidity and mortality also increase in elderly patients. Type 2 diabetes mellitus affecting 285 million adults worldwide.[2]

Vildagliptin is chemically (2S)-1-[2-[(3-hydroxy-1-adamantyl) amino] acetyl] pyrrolidine-2-carbonitrile). It has a molecular formula of $C_{17}H_{25}N_3O_2$. The molecular weight of vildagliptin is 303.4 g/mol. The structure of vildagliptin represented in figure1. It is available as an oral tablet known as Galvus, Xiliarx and Jalra. It is a group of cyanopyrrolidine inhibitors, in which glycyl Xaa complex is replaced. Containing nitrile group on the pyrrolidine ring has been essential for its high potency and facilitating oral administration. The adamantyl group is important to resist its catabolism, thereby prolonging its half-life.[3] It inhibits the DPP-4 (Dipeptidyl peptidase-4) by reversible competitive mechanism. This in turn inhibits the inactivation of GLP-1 (Glucagon like peptide-1) by DPP-4, allowing GLP-1 to potentiate the secretion of insulin in the beta cells. Dipeptidyl peptidase-4's role in blood glucose regulation is thought to be through degradation of GIP (Glucose dependent insulinotropic peptide) and the degradation of GLP-1.[4]

The oral route is best for vildagliptin to have a rapid absorption rate in T2DM (Type 2 diabetes mellitus) patients. It has the oral bioavailability of 85-90%. The drug absorption is unaffected by food. The volume of distribution of vildagliptin is 0.7 L/kg with a Renal clearance of 13 L/h and the systemic clearance is 41 L/h. The metabolism takes place in liver the drug is mostly catabolized by hydrolysis (69%) to an inactive metabolite known as LAY151. The drug elimination also associated with kidneys and intestinal microsomal enzymes. The metabolite LAY151 does not exhibit any toxic effect and not show any DPP-IV inhibition. A total amount of 85% of the orally administered drug or its metabolites (21% being unchanged drug) is eliminated in the urine and rest of them being eliminated via the faeces.[5]

Pharmaceutical analysis plays a very vital role in the quality assurance and quality control of bulk drug and their formulations. Pharmaceutical Analysis is specialized branch of analytical chemistry involves separating, identifying and determining the relative amounts of components in a sample of matter. It is concerned with the chemical characterization of matter

both quantitative and qualitative. Pharmaceutical analysis derives its principles from various branches of sciences like physics, microbiology, nuclear science and electronics etc.[6] In this review we focused on analytical techniques used to determine the vildagliptin such as Spectroscopy, Electrophoresis, LC (Liquid Chromatography) and hyphenated techniques such as GC-MS (Gas chromatography-Mass Spectroscopy) and LC-MS (Liquid Chromatography-Mass Spectroscopy) methods.

2. ANALYTICAL TECHNIQUES USED FOR THE DETERMINATION OF VILDAGLIPTIN IN PHARMACEUTICAL AND BIOLOGICAL PREPARATIONS

2.1. Spectroscopy

Spectroscopic method is the widespread technique which deals with the absorption of electromagnetic radiation by the substance probe certain features of a sample to learn about its consistency or structure and plays essential role in the qualitative and quantitative measurement of drugs.[7]

The Vildagliptin contains poorly absorbing chromophores, which determines the direct measurement of their absorption for Ultra Violet radiation is less sensitive in dosage forms. To overcome this Moneeb M. S. derivatised the Vildagliptin and Saxagliptin using 0.5 % (w/v) 1,2-naphthoquinone-4-sulfonic acid (NQS) and 0.2 % (w/v) 4-chloro-7-nitrobenzofurazan (NBD- Cl) reagents to develop a spectrophotometric method. NQS method in water with VGT (Vildagliptin) gives lambda max of 470 nm which shows LOD and LOQ of 1.03 µg/mL and 1.84 µg/mL. Whereas NBD-Cl in methanol with VGT gives lambda max of 468 nm which shows LOD and LOQ of 0.32 µg/mL and 1.04 µg/mL.[8]

The new simple spectroscopic method was developed by Housheh S. et al. for the determination of Vildagliptin in bulk using UV detection. The solvent used is 0.5 M HCl which shows the wavelength maxima of 202.5 nm. The developed method was validated according to ICH guidelines. The linearity range is 10-40 µg/mL with correlation coefficient of 0.999. The proposed method was precise with RSD less than 2% and accuracy was 100.17%. The spike recovery of vildagliptin in tablet was 99.96%, so this method has been successfully applied on pharmaceutical dosage form.[9]

Tekkeli S. E. K. et al. developed a validated spectrophotometric method for the determination and spectroscopic characterization of vildagliptin using π--acceptors in pharmaceutical preparations. The drug reacts with the following reagents like chloranilic acid, p-chloranil and TCNQ (Tetracyanoquinodimethane) to form coloured products. The coloured products were analysed at visible region and shows maximum absorbance at 520 nm, 535 nm and 842 nm for chloranilic acid, p-chloranil and TCNQ respectively. In comparison with other reported UV methods this method shows good linearity with the LOD of 0.12 µg/mL in acetonitrile, 0.28 µg/mL in acetonitrile: acetone (1:1) mixture and 0.43 µg/mL in acetonitrile respectively.[10]

Naveed S. et al.[11] and five more authors[12-15] have reported spectroscopic determination of vildagliptin and its combinations. The validation results were presented in table1.

Sherif M. E. et al. described an ATR-FTIR (Fourier transform infrared spectroscopy) method coupled with chemometrics for quantification of vildagliptin and metformin in pharmaceutical combinations having different concentration ranges (50/500, 50/850 and 50/1000 mg). They established FTIR spectroscopy in combination with PLSR multivariate analysis for determination of drugs in combinations with high ratios (1:10, 1:17 and 1:20 %) using metformin and vildagliptin pharmaceutical combination as a model. the reported method was successfully applied for the determination of both drugs with high accuracy and recovery (99 – 100 and 98 – 101% were obtained for MET and VLG, respectively).[16]

2.2. Electrophoresis

Capillary zone electrophoresis (CE) is the separation technique in which migration of charged ions takes place in an electric field. This is also the most reported analytical method used for the determination of Vildagliptin.[17]

Attimarad M. et al. proposed a multivariate optimization of a capillary zone electrophoresis assay method for simultaneous quantification of metformin and vildagliptin from a formulation. The extraction of drug from biological sample was done by protein precipitation method using acetonitrile. The experiment is carried out in untreated bonded silica capillary with a background at pH 7.5 electrolyte comprising 25 mM borate buffer and detected at 207 nm. The limit of detections for MFM and VGT were 0.22 µg/mL and 0.40 µg/mL respectively. This method was considered to be simple and effective.[18]

Attimarad M. et al. published the determination of vildagliptin in rat plasma by capillary electrophoresis tandem mass spectrometry: its application to pharmacokinetic study. This method is very useful for the determination of vildagliptin from biological matrix. It shows good linearity range 91.0-500 ng/mL) with the limit of detection of 0.31 ng/mL. This method was found to be most sensitive method.[19]

Kazsoki A. et al.[20] and two more authors[21, 22] reported some of the capillary zone electrophoresis methods. The parameters and results are mentioned in the table 2.

2.3. Liquid Chromatography (LC)

Liquid Chromatography is the most powerful analytical technique employed for the qualitative and quantitative

analysis of substance. The basic principle involved in liquid chromatography is adsorption.[23]

2.3.1. *Estimation of Vildagliptin in Pharmaceutical Formulations by LC*

Dayyih W.A et al. developed a validated reverse phase–high performance liquid chromatography (RP-HPLC) method for the determination of vildagliptin and metformin HCl in pharmaceutical dosage form. The chromatographic separation was achieved on X terra C_{18} column (250 mm × 4.6 mm × 5 µm) using acetonitrile: phosphate buffer (pH 6.0): water (65: 20:15 v/v/v) as a mobile phase with the flow rate of 1.0 mL/min. the limit of detection for VGT and MTF (Metformin) were 0.0040 µg/mL 0.025 µg/mL respectively. Hence this method was found to be more sensitive and eco friendly.[24]

Shaikh N. K. et al. developed a stability indicating RP-HPLC and First-order derivative UV spectrophotometric method for the simultaneous estimation of Vildagliptin and Nateglinide. The chromatographic separation was achieved using Kromstar C18 (250 × 4.6 mm, 5 µm) column. The mobile phase, Acetonitrile: Phosphate buffer (70:30; % v/v; pH 3.2) isocratically. For UV spectrophotometric method, methanol was used as a solvent, the spectrum was recorded between 200-400 nm wavelengths. Using delta lambda 2.0 all the zero-order spectrum (D0) were converted to first-order derivative spectrum (D1) and scaling factor 253 nm (zero crossing point of Nateglinide) and 270 nm (zero crossing point of Vildagliptin), respectively. Both methods were validated and showed the linearity range of 5-25 µg/mL for Vildagliptin and 9-45 µg/mL for Nateglinide. And also stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation were performed.[25]

Arar S. et al. proposed a simple RP-HPLC-UV method for separation of vildagliptin raw material and its degradation products at different conditions. The mobile phase and stationary phase used are ammonium acetate buffer (pH 7.5): methanol and Athena C18 -WP (250 mm) column. The degradation shows that six degradants have been identified using LC–MS technique, in addition to the NMR (Nuclear magnetic resonance) approach in some cases. One degradant at relative retention time (RRT) 1.3 was formed under acidic condition at m/z 304. Three degradants were formed basic hydrolysis at RRTs 1.2, 0.6 and 0.4 at m/z 337.2, 321.1 and 322. Another three degradants were also formed under oxidative oxidations of vildagliptin with RRT at 0.38, 0.6 (identical to one of basic hydrolysis) and 0.8 with m/z 241.1, 321.1 and 183.1. Formation mechanisms and names for the degraded products were described.[26]

Sultana S. et al. proposed a QbD approach for the development and validation of RP-UHPLC method for quantization of vildagliptin. The separation carried out using X-bridge C_{18} column using mobile phase combination of phosphate buffer (pH 6.8) acetonitrile (67:33 v/v) with the flow rate of 1.0 mL/min. the detection was carried out using PDA (Photo diode array) detector at 239 nm. The retention time for vildagliptin was 2.8 min. Limit of detection and quantification was 0.01 µg/mL and 0.05 µg/mL respectively.[27]

Boovizhikannan T. et al., performed the RP-HPLC determination of vildagliptin in pure and in tablet formulation. The column used for the chromatographic separation was Agilent XDB C_{18}, (150 × 4.6 mm, 5 µm) with the mobile phase of 0.1 M phosphate buffer and acetonitrile (85:15 v/v). The UV detection carried at 210 nm. The run time was 3.04 min. The limit of detection and quantification was 0.033 µg/mL and 0.999 µg/mL respectively. Hence the proposed method was more sensitive and suitable for routine analysis.[28]

Inamdar H. P. et al.[29] and more authors[30-41] have been reported various HPLC methods for the analysis of vildagliptin were summarized in table 3.

2.3.2. *Estimation of Vildagliptin in Biological Matrices by LC*

Pharne A. B. et al. proposed an economical RP-HPLC method for the estimation of vildagliptin in plasma using Tolbutamide as an internal standard. The protien precipitation extraction method was used to extract drug molecules from plasma. The method was carried out with the mobile phase of 50mM ammonium bicarbonate (pH 7.8) (solvent A) and acetonitrile (solvent B) at a flow rate of 1.0 mL/min using X Bridge Shield C18 column (3.5 µm, 4.6×150 mm). The detection wavelength was set to be 210 nm. The retention times of vildagliptin and tolbutamide were 11.2 min and 13.4 min respectively. The developed method was validated and the linearity range shows from 10µg/ml-120 µg/ml. The proposed method can be compatible with MS detection and consumption of biological material is less.[42]

Shakoor A. et al. presented a rapid method for simultaneous determination of metformin hydrochloride (MTF) and vildagliptin (VLD) in rabbit plasma and tablet dosage form. The sample extracted by protein precipitation using acetonitrile and the analytes were separated in Thermo Hypersil ODS C18 column (5 µm, 4.6 × 250 mm). They used mobile phase as methanol, acetonitrile and phosphate buffer (5:30:65, v/v, pH 3.5) with isocratic flow at 0.8 mL/min and wavelength was selected at 212 nm. The retention time of 3.36 and 5.41 min for MTF and VLD with run time was set to be 7.0 min. The method was validated according to ICH guidelines and the linearity shows from 10-140 µg/mL for MTF and 1-14 µg/mL for VLD while % RSD was less than 1.13 and 0.97 %, respectively, for repeatability and reproducibility. In addition to pharmacokinetic study in plasma after oral administration of both drugs to healthy and diabetic induced rabbits was determined by this method.[43]

The same researcher Shakoor A. et al. developed and validated a stability indicating RP-HPLC method for simultaneous estimation of Vildagliptin and Metformin hydrochloride in human plasma and tablet dosage form. Comparatively both the conditions and outcomes are same as mentioned above. Additionally, in this method human plasma is used as biological matrix and also undergone forced degradation studies includes acidic, basic, oxidative, photolytic, and thermal conditions which indicates a complete separation of the analytes in the presence of their degradation products providing a high degree of method specificity.[44]

2.4. Liquid Chromatography- Mass Spectroscopy (LC-MS)

LC-MS technique is the powerful analytical tool associated with bio-analysis. This hyphenated method used to determine the drug substance in biological samples like urine, serum, plasma, saliva etc.[45] There are number of LC-MS methods developed for the quantification of vildagliptin which includes samples of river water[46], rat plasma[47], rat liver and muscles[48], human plasma.[49,50]

Kai S. et al. proposed the simultaneous analysis of oral antidiabetic drug by LC-MS/MS. The drug from river water was extracted by solid phase extraction (SPE) method using methanol. The separation of analyte and IS was carried out by Hitachi Lachrom Ultra C18, 2 µm (50 × 2 mm, 2 µm) using 10mM ammonium formate: acetonitrile: formic acid (900:100:1, v/v/v) - gradient elution with the flow rate of 0.2 mL/min. The mass to charge (m/z) value was found to be 304.3 to 154.2, 97.1 and the limit of detection was 0.001 µg/mL for vildagliptin hence this method was more sensitive and used for the river water analysis.[46]

Manigandan K. S. et al. developed a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the determination of vildagliptin in rat plasma. The drug from rat plasma sample was extracted by liquid- liquid extraction (LLE) method using suitable solvent. The separation of analyte and IS was carried out in Betasil C18 (50mm × 4.6 mm ID, 5 µ) column using acetonitrile: 2 mM ammonium acetate (90:10 v/v) as a mobile phase. The electro spray ionisation (ESI) method was used as an ion source and the ions was analysed. The mass to charge (m/z) value was found to be 304.2→154.0 (for analyte) and 453.3→230.3 (for IS). The method was validated and the limit of detection was found to be 0.001 µg/mL. This method was useful for bioequivalence studies.[47]

Pontaralo R. et al.[48] and two more authors[49, 50] have been reported LC-MS method for the quantification of vildagliptin in biological samples. The parameters were given in table 4.

2.5. Gas Chromatography (GC):

Gas chromatography is the separating technique involving gaseous mobile phase and stationary phase. The principle of separation is adsorption and partition.[51]

Ucakturk E. developed and validated a selective gas chromatography-mass spectrometry (GC-MS) for the determination of Vildagliptin using Nandrolone as an internal standard in pharmaceutical formulation. Before analysis the VGT was derivatised with N-methyl-N-(trimethyl silyl) trifluoro acetamide at 60 ^{o}C for 30 min to obtain volatility. The derivatised VGT was detected by selected ion monitoring mode at *m/z* 223 and 252.This method was fully validated, in which LOD and LOQ were found to be 1.5 and 3.5 ngmL^{-1}, respectively. The GC-MS method is linear in the range of 3.5–300 ngmL−1. The intra- and interday precision values were less than ≤ 3.62%. Moreover, the method was successfully applied in pharmaceutical tablet formulation.[52]

7. Abbreviations

DPP-IV - Dipeptidyl peptidase- 4
T2DM - Type 2 diabetes mellitus
GLP - Glucagon like peptide
GIP - Glucose dependent insulinotropic peptide
TCNQ - Tetracyanoquinodimethane
FTIR - Fourier transform infrared spectroscopy
REF - Reference
PDA - Photo diode array
NMR - Nuclear magnetic resonance
GC - Gas chromatography
VGT - Vildagliptin
MTF -Metformin

TABLES

Table 1: Spectroscopic methods for the determination of Vildagliptin.

S.NO	Compounds	Reagents	Solvents	λ max	Linearity range	LOD	Ref
1.	Vildagliptin and Saxagliptin	1) NQS 2) 4-chloro-7-nitro benzofurazan	1) Water 2) Methanol	1) 470 nm 2) 468 nm	1) 5-50 µg/mL 2) 2.5–40 µg/mL	1) 1.03µg/mL 2) 0.32 µg/mL	[8]
2.	Vildagliptin	NA	0.5 M HCl	202.5 nm	10-40 µg/mL	0.055 µg/mL	[9]
3.	Vildagliptin	1) chloranilic acid 2) p-chloranil 3) TCNQ	1) Acetonitrile 2) acetonitrile: acetone mixture (1:1) 3) Acetonitrile	1) 520 nm 2) 535 nm 3) 842 nm	1) 20-250 µg/mL 2) 25-400 µg/mL 3) 20-500 µg/mL	1) 0.12 µg/mL 2) 0.28 µg/mL 3) 0.43 µg/mL	[10]
4.	Vildagliptin	NA	Water	244 nm	12.5–200 µg/mL	NA	[11]
5.	Vildagliptin and Metformin	NA	Distilled water	217 nm	0.35–1.05 µg/mL	0.44 µg/mL	[12]
6.	Vildagliptin and Sitagliptin	1)DDQ 2)TCNQ 3)p-chloranil	1) Acetonitrile 2) Methanol 3) Dimethyl form amide	1) 461 nm 2) 837 nm 3) 555 nm	1) 50-300 µg/mL 2) 10-85 µg/mL 3) 50-350 µg/mL	1) 5.1 µg/mL 2) 0.88 µg/mL 3) 5.17 µg/mL	[13]
7.	Vildagliptin and Metformin	NA	0.1N Sodium hydroxide	233 nm	30–70 µg/mL	NA	[14]
8.	Vildagliptin and Linagliptin	NA	1) Water 2) Methanol	1)197 nm 2) 294 nm	1)8-32 µg/mL 2)5-25 µg/mL	1)0.247 µg/mL 2)0.734 µg/mL	[15]

Ref- Reference, TCNQ-7,7,8,8-tetracyanoquinodimethane, DDQ-2,3-dichloro-5,6-dicyano 1,4-benzoquinone, NQS-1,2-naphthoquinone-4-sulfonic acid sodium salt, LOD-Limit of Detection.

Table 2: Electrophoresis methods for the determination of Vildagliptin

S. No.	Compounds	Applied Voltage	Buffer solution	Detection	Linearity Range	LOD	LOQ	Ref
1.	Vildagliptin and Metformin	25 kV	25 mM Borate (pH 7.5)	UV at 207 nm	5–100 µg/mL and 5–500 µg/mL	0.40 µg/mL and 0.22 µg/mL	1.24 µg/mL and 0.71 µg/mL	[18]
2.	Vildagliptin	25 kV	0.25 mM Ammonium formate	MS/MS at MRM	1.0-500 ng/mL	0.31 ng/mL	0.87 ng/mL	[19]
3.	Vildagliptin	25 kV	75 mM Acetate-Tris buffer (pH 4.75)	UV at 200 nm	7.5-180 µg/mL	2.5 µg/mL	7.5 µg/mL	[20]
4.	Vildagliptin and Metformin hydro Chloride	25 kV	25 mM Sodium tetra borate	UV at 207 nm and 250 nm	30–60 µg/mL and 300–600 µg/mL	2.82 µg/mL and 0.83 µg/mL	8.55µg/mL and 2.50 µg/mL	[21]
5.	Vildagliptin	25 kV	25mM Potassium phosphate (pH 8.0)	UV at 207 nm	50–200 µg/mL	3.24 µg/mL	9.82 µg/mL	[22]

Ref- Reference, UV- Ultra-violet, MS-Mass Spectroscopy, MRM-Multiple Reaction Mode.

Table 3: LC methods for quantification of vildagliptin in pharmaceutical formulations.

S. NO.	Stationary phase	Mobile phase	Flow rate	Detector-Detection wave length	Retention time (Rt)	LOD	LOQ	Ref
1.	X terra C_{18} column (250 mmL × 4.6 mm × 5 µm)	Acetonitrile: phosphate buffer (pH 6.0): water (65: 20:15v/v/v)	1.0 mL/min	UV -239 nm	VGT-2.32 min MTF-4.29 min	VGT-0.040 µg/mL MTF-0.025 µg/mL	-	[24]
2.	Kromstar C18 (250 × 4.6 mm, 5 µm)	Acetonitrile: Phosphate buffer (70:30; % v/v; pH 3.2)	1.0 mL/min	UV- 222 nm	VGT- 6.0 min NTG- 3.0 min	VGT- 0.1809 µg/mL NTG- 0.1407 µg/mL	VGT- 0.603 µg/mL NTG- 0.469 µg/mL	[25]
3.	X-bridge C_{18} column	Phosphate buffer (pH 6.8): Acetonitrile (67:33 v/v)	1.0 mL/min	PDA- 239 nm	2.8 min	0.01 µg/mL	0.05 µg/mL	[27]
4.	Agilent XDB C_{18}, 150 × 4.6 mm, 5 µm, column	0.1 M Phosphate buffer and Acetonitrile (85:15 v/v)	1.0 mL/min	UV- 210 nm	3.04 min	0.033 µg/mL	0.999 µg/mL	[28]
5.	ACE 3 (150 mm × 4.6 mm, 3.5 µm)	10 mM sodium hexane sulphonate monohydrate: 10 mM KH_2PO_4 buffer with acetonitrile and methanol in gradient ratio	1.5 mL/min	UV- 210 nm	VGT- 2.58 min PT- 8.88 min	-	-	[29]
6.	Waters C_{18} column (150 mm × 4.6mm, 5 µm)	Ammonium hydroxide: methanol 60:40 v/v (pH adjusted to 9.5 using 50 % phosphoric acid	1.0 mL/min	UV- 210 nm	6.3 min	1.47 µg/ mL	4.90 µg /mL	[30]
7.	Lichrocart C_{18} column (250 x 4.60 x 5 µm)	0.05 M KH_2PO_4: Acetonitrile (70:30 v/v pH 3.5 with Ortho Phosphoric Acid)	1.0 mL/min	UV- 215 nm	VGT - 6.64 min MTF - 5.18 min	-	VGT-5 µg/mL MTF-10 µg/mL	[31]
8.	HiQsil C_{18}HS (4.6 mm × 250mm)	50 mM phosphate buffer (pH adjusted to 6 using 3M KOH): methanol: acetonitrile in the ratio of (50:30:20 v/v/v)	0.8 mL/min	UV- 220 nm	VGT-4.8 min MTF-3.7 min	VGT-1.70 µg/mL MTF-1.09 µg/mL	VGT-5.15 µg/mL MTF-3.32 µg/mL	[32]
9.	Altima C_{18} column (150 mm x 4.6 mm)	Ortho phosphoric acid solution (pH 2.6) as buffer and acetonitrile (72:28 v/v)	1.0 mL/min	UV- 266 nm	3.25 min	0.06 µg/mL	0.21 µg/mL	[33]
10.	Jasco Crest Pack RP C_{18} (250 × 4.6 mm, 5 µ)	Buffer (pH 6): Acetonitrile: Methanol (70:10:20 v/v)	1.0 mL/min	PDA- 210 nm	7.21 min	200 ng/mL	600 ng/mL	[34]
11.	C_{18} column (4.6 × 150 mm id., particle size 5 µm)	10 mM phosphate buffer (pH 4.6) and acetonitrile (85: 15, v/v)	1.0 mL/min	PDA- 210 nm	3.38 min	1 µg/mL	3.2 µg/mL	[35]
12.	Thermosil Symmetry C18 (150 mm x 4.6 mm, 5 mm)	Buffer: acetonitrile: methanol (450: 480: 70)	0.5 mL/min	UV- 254 nm	3.9 min	2.9 µg/mL	9.9 µg/mL	[36]

13.	Shimpack VP-ODS (150 × 4.6 mm, 5 µm)	0.02 M phosphate buffer (pH 4.6): acetonitrile (80:20 %, v/v)	0.7 mL/min	PDA- 210 nm	3.6 min	-	-	[37]
14.	Thermo hypersil ODS C18 (250 mm × 4.6 mm, 5 µm)	0.1 M potassium hydro phosphate (pH 7.0): Acetonitrile (60: 40 %, v/v)	1.0 mL/min	UV- 263 nm	VGT- 3.5 min MTF- 2.1 min	-	-	[38]
15.	Kromasil-C_{18} column [4.5 x 250 mm; 5 µm]	0.05 mmol Potassium dihydrogen phosphate buffer (pH 3.5): Acetonitrile (80:20 v/v)	0.9 mL/min	PDA- 263 nm	VGT- 2.60 min MTF-2.2 min	VGT-0.018 µg/mL MTF-0.055 µg/mL	VGT-0.45 µg/mL MTF-1.35 µg/mL	[39]
16.	Grace Cyano (250 × 4.6 mm × 5 µm) column	25 mM Ammonium bicarbonate buffer: Acetonitrile (65:35 v/v)	1.0 mL/min	PDA- 207 nm	VGT-5.3 min MTF-7.5 min	VGT-0.75 µg/mL MTF-0.36 µg/mL	VGT-2.51 µg/mL MTF-1.22 µg/mL	[40]
17.	Dionex C_{18} (250 mm x 4.6 i. d, 5 µm) column	Dipotassium hydrogen phosphate (0.01 M) buffer: water (90:10 v/v)	1.5 mL/min	UV- 215 nm	VGT-4.60 min MTF-2.39 min	VGT-0.706 µg/mL MTF-0.629 µg/mL	VGT-2.35 µg/mL MTF-2.09 µg/mL	[41]

NA- not applicable, Ref- Reference, HPLC- High Performance Liquid Chromatography, UV-Ultra-violet, PDA- Photo Diode Array detector, VGT- Vildagliptin, MTF-Metformin HCl, NTG-Nateglinide, PT- Poiglitazone, KH_2PO_4 - Potassium Dihydrogen Phosphate, KOH- Potassium Hydroxide, LOD- Limit of Detection and LOQ- Limit of Quantification.

Table 4: LC-MS/MS methods for the determination of Vildagliptin

S. NO.	Samples-Extraction method	Internal standard (IS)	Stationary phase	Mobile phase	Flow rate	Ion source and Detection mode	m/z value	LOD	Ref
1.	River water -SPE	-	Hitachi Lachrom Ultra C18, 2 µm (50 × 2 mm, 2 µm)	10 mM ammonium formate: acetonitrile: formic acid (900:100:1, v/v/v) - gradient elution	0.2 mL/ min	ESI and MRM	304.3 to 154.2, 97.1 (VLG)	0.001µg/mL	[45]
2.	Rat plasma -LLE	Repaglinide (RG)	Betasil (C18 50 mm 4.6 mm ID, 5 µ) column	Acetonitrile: 2 mM ammonium acetate (90:10 *v/v*)	-	ESI and MRM	304.2→154.0 (VLG) and 453.3→230.3 (RG)	0.157ng/mL	[46]
3.	Human plasma -PPT	Pyrantel Pamoate	Atlantis HILIC Silica 150 × 2.1 mm, 3 µm) column	80% acetonitrile in water (95:5, v/v) with 0.1% formic acid and 3 mM ammonium formate	400 µL/ min	ESI and MRM	304.1 to 154.1, 97.1 (VLG) and 207.2 to 150.2, 136.2 (IS)	0.75 ng/mL	[47]
4.	Human plasma -PPT	Aloglip tin	Monolithic silica column (100 × 4.6 mm, 1.15 µm)	0.01 M Ammonium formate buffer (pH 3.0): acetonitrile (80:20, v/v)	0.4 mL/ min	ESI and MRM	304.2 to 154.1, 97 (VLG) and 340 to 323.1, 116.1 (IS)	0.17 ng/mL	[48]
5.	Rat liver and muscle -NA	-	Shimadzu C18 (15 cm x 4.6 mm (5 µm)	Methanol: 5 mM ammonium acetate (95:5, v/v)	0.4 mL/ min	ESI	304.19 to 153.90 (VLG)	-	[49]

NA- not applicable, Ref- Reference, VLG-Vildagliptin, IS-Internal Standard, PPT-Protein Precipitation, SPE-Solid Phase Extraction, and ESI- Electro spray Ionization, MRM-Multiple Reaction Monitoring.

FIGURES

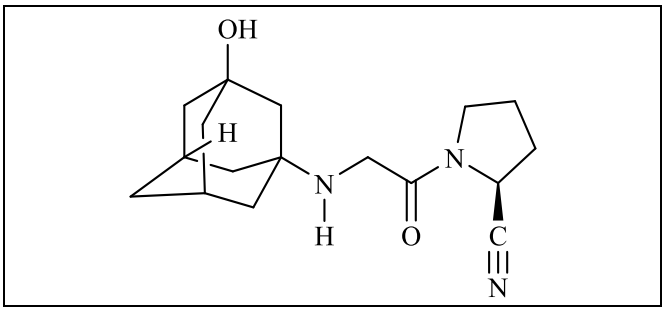

Figure 1: Structure of Vildagliptin.

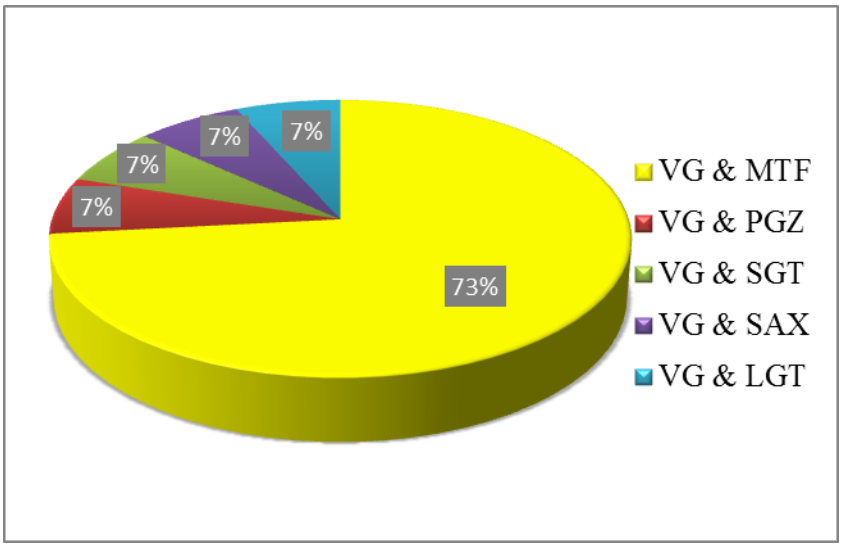

Figure 2: Availability of Analytical Methods for the Determination of Vildagliptin and its Combinational Drugs.

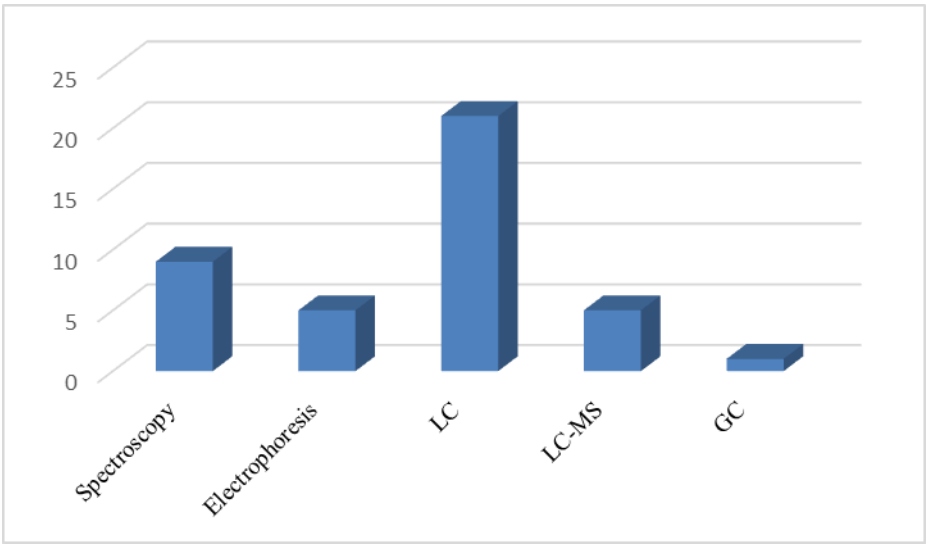

Figure 3: Various Analytical Techniques used for the Determination of Vildagliptin and its Combinations.

3. DISCUSSION

Vildagliptin is the first of DPP-IV inhibitor class of drug used for the treatment of type 2 Diabetes Mellitus. It is soluble in water and acetonitrile. Even though Vildagliptin comes under the brand name of Galvus from the year 2007, the drug in combination with other gliptins and Metformin was significantly increased due to its efficacy. Figure 2 indicates all the available analytical techniques used for the determination of Vildagliptin and its combinations in bulk, pharmaceutical formulations and in biological samples.

This review provides information about all the reported analytical methods for the quantification of vildagliptin and its combinations from the year 2012 to 2020. Figure 3 represents Various analytical techniques used for

determination of vildagliptin and its combinations. The Vildagliptin contains poorly absorbing chromophores, which is responsible for the direct measurement of their UV absorption. Vildagliptin has less ultra violet radiation absorptivity. To overcome this problem, researchers used vaccum UV region or reagents used prior to analysis to determine vildagliptin and its combinations in visible region. The Capillary Zone electrophoresis methods were also reported most commonly with UV detection at 25 kV applied voltage for the estimation of Vildagliptin and its combinations. As per literature survey, Liquid Chromatography methods were reported abundantly when compared with other analytical methods. Among LC methods, more commonly HPLC techniques with UV detection and rarely UHPLC technique were reported. The LC-MS methods reported for the simultaneous estimation of vildagliptin and its combinations in biological matrices and also in river water which is most sensitive and useful for the determination of water pollution. Only one researcher reported Gas chromatography method using MS detection for the determination of Vildagliptin. There is a smaller number of bioanalytical methods were reported for the vildagliptin quantification. This review is useful for viewers to acquire knowledge about analysis of Vildagliptin and its combinations, as well as to develop a new formulations and analytical methods for future consideration.

4. CONCLUSION

We discussed about various analytical techniques reported for the quantification of vildagliptin in bulk, pharmaceutical formulations and in biological samples. An overview of all analytical methods shows that the HPLC technique is the most sensitive, accurate and reliable method for the quantification of vildagliptin and its combinations. Hence, we concluded that the HPLC technique was the most suitable technique for routine analysis for the determination of vildagliptin. There was no Electro chemical, NMR, MS and TLC techniques were reported for the determination of vildagliptin with its combinations. So, researchers can take those techniques to develop methods for future research for determination of Vildagliptin with its combinations.

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