DOI 10.1007/s11094-022-02606-1
Pharmaceutical Chemistry Journal, Vol. 56, No. 1, April, 2022 (Russian Original Vol. 56, No. 1, January, 2022)

PHYSICOCHEMICAL PROPERTIES AND METHODS OF ANALYSIS OF VILDAGLIPTIN (REVIEW)

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Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 56, No. 1, pp. 44 – 51, January, 2022.

Original article submitted August 3, 2021.

Vildagliptin is an oral agent which is a member of a new class of hypoglycemic drugs, dipeptidylpeptidase-4 (DPP-4) inhibitors. This review presents the physicochemical properties of vildagliptin and assesses analysis methods for its estimation in substances, medicinal formulations, and biological media. These are chromatographic, spectrophotometric, electrochemical and other analysis methods. The material presented may be useful for developing new methods for analysis of medicinal formulations containing vildagliptin. The most widely used method for assay of vildagliptin is HPLC.

Keywords: vildagliptin, dipeptidylpeptidase-4 inhibitors, physicochemical properties, analysis methods.

Scientific studies in the search for the optimum treatment of type 2 diabetes mellitus have been directed to investigating a fundamentally novel mechanism regulating glucose homeostasis using gastrointestinal tract hormones, i.e., incretins [1]. The first evidence that the intestinal mucosa form a factor able to stimulate the endocrine part of the pancreas appeared more than 100 years ago [2]. Moore, a doctor from Liverpool, obtained a duodenal mucosal extract and used it in the treatment of "patients with glycosuria". Moore's elixir was the precursor to incretins [3]. Research in this area was reinitiated in the 1960s. Two glucagon-like peptides, GLP-1 and GLP-2, were identified in 1983. Incretins undergo rapid cleavage by the enzyme type 4 dipeptidylpeptidase (DPP-4), decreases their insulinotropic effects. However, inhibition of DPP-4 increases the level and activity of incretins. The drug DPP728 was developed and the first clinical data were presented at the annual congress of the American Diabetes Association in 2000. Studies aiming to improve the chemical stability of DPP728 substance led to creation of vildagliptin. The pioneer, Novartis, named the new class of drugs. Thus, all DPP-4 inhibitors since 2004 have been termed gliptins [4].

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The medicine Galvus (vildagliptin, Novartis Pharma) was registered in Russia in 2008. This is effective both as monotherapy and in combination with other hypoglycemics and insulin [3].

Vildagliptin is a member of the class of pancreatic islet stimulators which selectively inhibit the enzyme type 4 dipeptidylpeptidase (DPP-4) and increase the glucose sensitivity of pancreatic α and β cells. By increasing the insulin:glucagon ratio, vildagliptin decreases hepatic glucose production, which leads to a decrease in the plasma glucose level [5].

Physicochemical Properties of Vildagliptin

Vildagliptin is a member of the cyanopirrolidine-4 [1] and α-amino acid amide classes [6]. The structure and properties of vildagliptin are presented in Table 1.

The stability of vildagliptin depends on a multitude of factors, including other components in the tablets, both active and excipient. Work reported in [11] addressed the influences of several excipients on the stability of vildagliptin. It should noted that lactose proves better protection for vildagliptin in acidic conditions, while sucrose provided better protection in alkaline conditions. DPP-4 inhibitors with primary or secondary amino groups are incompatible with some excipients, such as microcrystalline cellulose, sodium starch glycolate, croscarmellose sodium, tartaric acid, citric acid, glucose, fructose, sucrose, lactose, and maltodextrin [11].

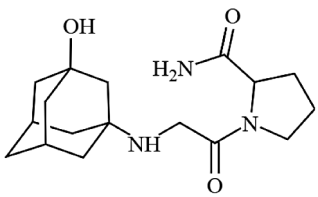


Fig. 1. Vildagliptin amide – (S)-21-[2-)3-hydroxytricyclo-[3,3,12,1*,3,7*]decan-1-ylamino)acetyl]pyrrolidin-2-carboxylic

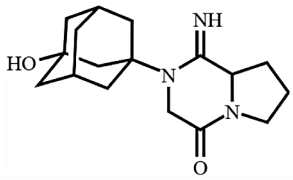


Fig. 2. Vildagliptin cyclic amidine – (2-(3-hydroxycyclo-[3,3,1,1*,3,7*]decan-1-yl)-1-iminohexahydropyrrolo[1,2-α]pyrazin-4-one)

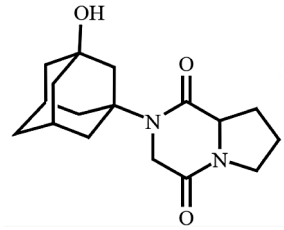


Fig. 3. Vildagliptin diketopiperazine – (2-(3-hydroxytricyclo [3,3,1,1*,3,7*]decan-1-yl)-1-hexahydropyrrolo[1,2-α]-pyrazin-1,4-dione).

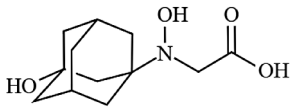


Fig. 4. N-Hydroxy derivative hydrolysis product of vildagliptin [13].

Looking at the structure of vildagliptin (Table 1) allows us to identify "weak" sites in the molecule. Thus, the nitrile group can undergo hydrolysis to form the amide and then the carboxylic acid [12, 13]. The vildagliptin molecule also contains a secondary amine group, an alcohol hydroxyl in the adamantane fragment, and an amide group bound to the pyrrolidine ring. Degradation of vildagliptin forms a multitude of products. Identified related contaminants of vildagliptin are controlled – the amide, the cyclic amidine, and the diketopiperazine (Figs. 1 – 3) [9].

Analysis of the literature [14, 15] and our own stress studies showed that the diketopiperazine forms on acid degradation. Alkaline degradation forms several products, including the amide (Fig. 1). Oxidative destruction forms the products shown in Figs. 1, 4, 5. Stress testing therefore requires the strengths and durations of the actions of the degrading factors to be considered. Thus, in alkaline conditions, vildagliptin can undergo complete conversion to the amide and then the carboxylic acid sodium salt; the sodium hydroxide concentration must be 0.01 M and the duration of exposure short (5 min) at room temperature. Peroxide decomposition is carried out with 0.1% hydrogen peroxide, also at room temperature, for 2 min [9]. The presence of lactose – a reducing sugar – as excipient promotes formation of the amide and the diketopiperazine (Figs. 1, 3). And metformin hydrochloride in combined formulations in a moist medium can lead to formation of the cyclic amidine (Fig. 2).

The authors of [13, 16] reported unstable contaminant E forming during synthesis of vildagliptin substance due to aerobic oxidation, and in water – acetonitrile (90:10) this contaminant is converted to contaminant F (Fig. 7), whose structure was determined by spectral analysis methods. Report [13] described six identified decomposition products by HPLC-MS, in some cases also by NMR. This presents *m*/*z* values and relative retention times, along with the mechanisms of formation of degradation products.

Methods for Analysis of Vildagliptin

The Russian literature contains a number of publications on vildagliptin, though all are mainly on pharmacological and medical themes. In the present article we are guided by information in non-Russian scientific journals on methods for analysis of vildagliptin, both in pure form and in mixtures with other antidiabetic components. Vildagliptin is not yet included in any of the world's pharmacopeias. Below we present possible methods for analysis of vildagliptin.

Titrimetric Methods

Report [17] presented data from studies of the potential use of conductometric titration of vildagliptin and its analogs. This method is based on the ability of vildagliptin and its analogs to form complexes with copper (II) ions in the conductometer cell, which leads to a change in the conductivity of the solution. This method is both simple, rapid, and economical.

IR Spectroscopy

The IR spectrum of vildagliptin obtained in potassium bromide tablets has characteristic absorption bands at 3294 cm^{-1} (n, stretch vibrations of -O-H and -N-H), 2992, 2915, 2849 cm^{-1} (stretch vibrations of -CH in the aliphatic chain), 2238 cm^{-1} (stretch vibrations of the nitrile group -CN), 1658 cm^{-1} (stretch vibrations -C=O), 1405, 1354 cm^{-1} (δ, deformational vibrations (-CH in the aliphatic chain), 1254 cm^{-1} (ν, C-N), 1120, 1103 cm^{-1} (í C-O(H)), 1054, and

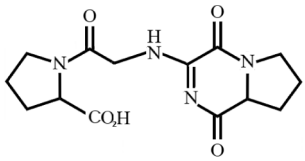


Fig. 5. Probable product of secondary oxidation reaction of vildagliptin formed as a result of hydrolysis with subsequent condensation of the reaction products [13].

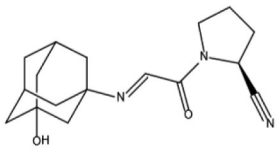


Fig. 6. Contaminant E [13, 16].

1035 cm^{-1} (ν, C-O(H)) (cycloalkane in the 3-hydroxyadamantane fragment) [9].

The literature contain descriptions of a rapid and simple method of near-infrared reflection spectroscopy for quality control of vildagliptin and other antidiabetic drugs [17, 18].

Spectrophotometry in the Visual and UV Ranges

The vildagliptin molecule does not contain a conjugated double-bond system, so its ultraviolet spectrum has no specific and marked absorption peaks. Figure 8 shows that the spectrum has a peak at around 200 nm. Despite this, work reported in [10] proposed a validated spectrophotometric method for assay of vildagliptin at an analytical wavelength of 266 nm, with a test solution concentration of 200 μg/ml and water as solvent. Report [19] proposed an analytical wavelength of 244 nm. A wavelength of 202.5 nm in 0.5 M HCl at a vildagliptin test solution concentration of 25 μg/ml has been reported [20]. A spectrophotometric method for assay of vildagliptin with metformin hydrochloride using first derivative spectra has been described [8], as has assay of vildagliptin using the second derivative spectrum [21].

Furthermore, spectrophotometric and spectrofluorimetric methods for vildagliptin and saxagliptin assay after derivatization with 4-chloro-7-nitrobenzofurazan and the sodium salt of 1,2-naphthoquinone-4-sulfonic acid based on formation of colored and fluorescent products [22]. A method for extraction spectrophotometry for assay of vildagliptin by interaction with bromocresol green and bromothymol glue in

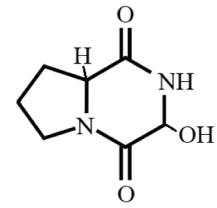
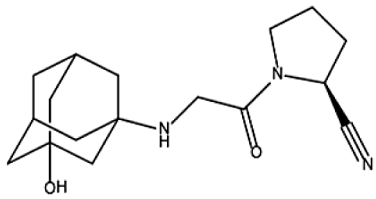


Fig. 7. Contaminant F [13, 16].

TABLE 1. Physicochemical Properties of Vildagliptin

Structural formula	
IUPAC chemical name	(S)-1-[N-(3-Hydroxy-1-adamantyl)glycyl[pyrrolidine-2-carbonitrile)
Relative molecular weight	303.4
Description	White or almost white crystalline powder [2, 7]. Crystalline powder from white to slightly yellowish or slightly grayish color [1, 8]
Melting temperature	148 – 152 °C
Solubility	Easily soluble in DMF, methanol, and 0.1 m HCl, moderately soluble in ethanol and acetonitrile, soluble in water and 0.1 M NaOH [1, 7]
pK_a	pK_a 14.71 [1, 2, 8] pK_a 9.03 pK_a 7.6 [9]
LogP (octanol:water)	0.9 [10]
λ_{max}	194 nm, no specific absorption [9]
Specific rotation	-78.3° (*c* = 9.73 in methanol) [9]
Optical isomerism	One chiral center, S isomer [9]

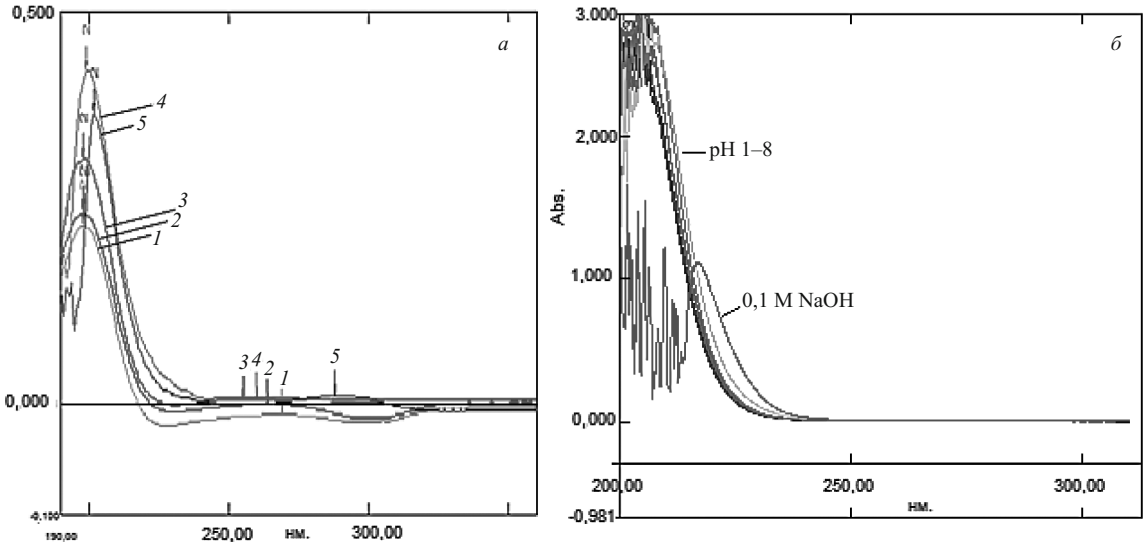


Fig. 8. *a*) UV spectrum of vildagliptin (10 μg/ml) obtained on a Shimadzu UV-1800 spectrometer (Laboratory for Physicochemical Analysis Methods, OOO Parma Clinical) in phosphate buffer solution: *1*) pH 3.0; *2*) pH 4.5; *3*) pH 6.0; *4*) pH 7.0; *5*) pH 8.0; *b*) UV spectra of vildagliptin (200 μg/ml).

acetate buffer solution pH 4.1 was described in chapter 10 of [23] and in [24]. The secondary amino group of vildagliptin is protonated at pH 4.1 and interacts with the sulfo group of the dye. The colored complex is extracted with chloroform and the optical density of the solution is measured at 423 and 514 nm. Vildagliptin assays using reactions with 2,3-dichloro-5,6-dicyano-1,4-dibenzoquinone (DDO), 7,7,8,8-tetracyanoquinodimethane (TCNQ), and tetrachloro-1,4-benzoquinone (*p*-chloranil) in acetonitrile, methanol, and DMF solutions at 486, 838, and 555 nM respectively have been proposed [25]. An assay method based on reaction of the secondary amino group of vildagliptin (a Lewis base) with picric acid (a Lewis acid) forming a charge transfer complex with a yellow color, with measurement of the optical density of the resulting solution in chloroform at 410 nm, was described in [26, 27]. Data have also been obtained on the reaction of vildagliptin with *p*-dimethylaminobenzaldehyde in acidic ethanol based on formation of a Schiff base and measurement of optical density at 446 nm [28].

High-Performance Liquid Chromatography with UV Detection

Studies [29 – 35] used analytical wavelengths of 239, 260, and 290 nm. We obtained UV spectra in buffer solutions with different pH at concentrations of 10 and 200 μg/ml (Fig. 8). As shown in Figs. 8 and 9, the expected second absorption bands at around 239, 260, or 290 nm proposed as analytical wavelengths, were not seen.

Vildagliptin is basic in nature (it ionizes in acidic media) [36]. Selection of mobile phase buffer solution pH usually depends on the pKa of the substance being assayed. Vildagliptin has to be in the ionized state. Publications therefore generally use a mobile phase pH of 7.0 [9, 16, 21, 30, 32, 34]. As shown in Table 2, some authors use buffer solutions with other pH values, as guided by the task being addressed and the properties of substances accompanying vildagliptin.

In vildagliptin substance, content and related contaminants are determined using a column filled with a C18 sorbent and a mobile phase consisting of acetonitrile and buffer solution pH 7.0 with gradient elution. Detection is at 210 nm [9]. Data have been obtained on detection of vildagliptin at 203 – 215 nm (see Table 2), and, as noted above, at 250 nm [33], 263 nm [21, 31, 37], 260 nm [32], and 293 nm [38], though these sources contain no indication of any transformation of vildagliptin itself or its UV spectrum.

Chapter 9 of [23] describes a method using derivatization of vildagliptin with benzoyl chloride, which attaches via the

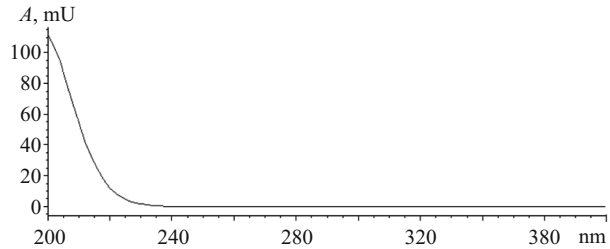


Fig. 9. UV spectrum of vildagliptin in phosphate buffer solution pH 7.0 obtained by chromatography on an Agilent 1260 Infinity chromatograph with an Agilent ChemStation B.04.03 SP1 on an Inertsil ODS-3 C18, 250 × 4.6 mm, 5 μm column, GL Sciences, at the Laboratory for Physicochemical Analysis Methods, OOO Parma Clinical.

TABLE 2. Chromatographic Conditions For Vildagliptin Assay

Name	Technique	Column	Mobile phase	Detection, nm	Flow rate, ml/min	RT, min	Ref.
Vildagliptin (V), substance, contaminants: cyclic amidine (CA), amide (A), diketopiperazine (DKP)	RP-HPLC	Inertsil® ODS-3 250 × 4.6 mm, 5μ	Phosphate buffer pH 7.0 - ACN (90:10), gradient	21 – 0	12.0	V 22.1 CA 6.54 A 9.36 DKP 23.5	[7, 39]
Vildagliptin (V), substance, enantiomeric purity	NP-HPLC	CHIRAL-PACK D-H, Daicel 250 × 4.6 mm, 5μ	*n*-Hexane - ethanol - DEA - TFA (80:8:12:0.05:0.05)	210	0.5	V (R) 9.46 V (S) 10.41	[7]
Vildagliptin (V), substance, tablets	Ion pair HPLC	Zorbax SB-C18 250 × 4.6 mm, 5μ	Phosphate buffer with ammonium hexafluorophosphate pH 3.0 - ACN (75:25)	210	1.0	V 7.00	[7, 39]
Vildagliptin derivatized with benzoyl chloride	RP-HPLC	BDS C18 150 × 4.6 mm, 5μ	Water - ACN (50:50)	220	‘0.5	VVV4.70	[9], chapter 9
Vildagliptin (V), 3-amino-adamantol (3-AA), metformin (M), and pioglitazone (P) (	RP-HPLC	Symry® Waters C18 150 × 4.6 mm, 5μ	0.05 M KH_2PO_4, pH 4.6 - ACN - MeOH (50:30:20)	210	1.0	V 6.30 3-AA 8.50 M 4.07 P 5.80	[29]
Vildagliptin (V), metformin (M)	RP-HPLC	Xterra C18 250 × 4.6 mm, 5μ	Ph buffer pH 6.0 - ACN - water (65:20:15)	239	1.0	M 2.35 V 4.37	[37]
Vildagliptin (V), metformin (M), in tablets and plasma	RP-HPLC	Kromosil C18 4.6 × 250 mm, 5μ	0.05 M KH_2PO_4 pH 5.8 - ACN (80:20)	215	1.0	M 2.59 V 4.30	[38]
Vildagliptin (V), metformin (M)	RP-HPLC	Sunfire BDS C8 250 × 4.6 mm, 5μ	0.1 M phosphate buffer pH 7.0 - ACN (60:40)	263	1.0	M 2.07 V 3.52	[30]
Vildagliptin (V), metformin (M), gatifloxacin (GGG) as internal standard	RP-HPLC	Phenomenex C18, 250 × 4.6 mm, 5μ	2 mM phosphate buffer pH 3.5 - ACN (70:30)	293	1.0	C 2.07 G 2.55 M 5.63	[31]
Vildagliptin (V), metformin (M)	NP HPLC	Cosmosil CV18, 250 × 4.6 mm, 5μ	Phosphate buffer pH 2.5 - ACN (70:30)	227	1.0	M 2.08 V 6.50	[40]
Vildagliptin, metformin	NP HPLC	Thermo hypersil ODS C18 250 × 4.6 mm, 5μ	0.1 M KH_2PO_4 pH 7.0 - ACN (60:40)	263	1.0	M 2.10 V 3.50	[32]
Vildagliptin, metformin	NP HPLC	Lichrocart C18 250 × 4.6 mm, 5μ	0.05 M KH_2PO_4 pH 3.5 - ACN (70:30)	215	1.0	M 5.19 V 6.64	[41]
Vildagliptin, metformin	NP HPLC	Phenomenex Kromosil C18, 250 × 4.6 mm, 5μ	0.1 M KH_2PO_4 pH 6.8 - ACN (75:25)	260	1.0	M 2.40 V 3.40	[33]
Vildagliptin, metformin	NP HPLC	Hypercil VBDS C18	0.1 M KH_2PO_4 pH 6.8 - ACN (60:40)	210	1.0	M 3.29 V 4.64	[42]
Vildagliptin, metformin, degradation	NP HPLC	Shimadzu Primesil C18 150 × 4.6 mm, 5μ	Buffer solution KH_2PO_4 pH 3.2 - ACN (60:40)	203, 225	0.7	M 3.40 V 7.60	[43]
Vildagliptin, metformin	NP HPLC	Kromacil C18 150 × 4.6 mm, 5μ	0.1 M KH_2PO_4 pH 7.0 - ACN (70:30)	250	1.0	M 2.58 V 11.69	[34]
Vildagliptin	NP HPLC	XBridge C8	0.3% TEA pH 7.0 - ACN (85:15)	207	1.90	V 6.10	[44]
Vildagliptin	NP HPLC	Xterra® Waters C18 150 × 4.6 mm, 5μ	25% NH_4OH 1 ml/liter pH 9.5 - ACN (60:40)	210	1.0	V 6.30	[45]

TABLE 2. Continued

Vildagliptin, metformin	Ion pair HPLC	Zorbax Eclipse Plus C8 150 × 4.6 mm, 5µ	Sodium heptanesulfonate pH 3.0 - ACN - MeOH (81:18:1)	207,250	1.0	M 2.50 V 5.80	[14]
Vildagliptin	NP HPLC	Zorbax Eclipse Plus RP-C8 150 × 4.6 mm, 5µ	Phosphate buffer pH 7.0 - ACN (15:85)	207	1.0	V 6.60	[21]
Vildagliptin, metformin	Cyano HPLC	Grace cyano column 250 × 4.6 mm, 5µ	25 mM $(NH_4)_2CO_3$ - ACN (65:35)	207	1.0	No data	[46]
Vildagliptin, related contaminants	Ion pair HPLC	Agilent Eclipse Plus C18 250 × 4.6 mm, 5µ	10 mM sodium octylsulfonate pH 2.1 - ACN	210	1.0	No data	[47].
Vildagliptin, stress test	NP HPLC	Athena C18, 250 × 4.6 mm, 5µ	$(NH_4)_2CO_3$ 2.56 g/liter pH 7.5 - MeOH (90:10) gradient	308	1.0	V 12.30	[11]
Vildagliptin, L-proline	Cyano HPLC	Cyano 250 × 4.6 mm, 5µ	0.04 M KH_2PO_4 with 0.2% TEA pH 5.5 - MeOH (78:32)	210	1.90	No data	[48]
Vildagliptin and degradation products	NP HPLC	Athena C18-WP250 × 4.6 mm , 5µ	Ammonium acetate pH 7.5 - MeOH	210	1.0	No data	[13]
Vildagliptin, metformin	NP HPLC	Symmetry C18 XterraC18 250 × 4.6 mm, 5µ	KH_2PO_4 6.75 g/liter pH 6.09 - ACN - water (65:20:15)	239	1.0	M 2.343 V 4.29	[49]
Vildagliptin	NP HPLC	Jasco Crest Pack RP C18 250 × 4.6 mm, 5µ	Buffer pH 6 - ACN - MeOH (70:10:20)	210	1.0	V 7.50	[36]
Vildagliptin, metformin, saxagliptin	Cyano HPLC	Inertsil® CN-3 column 250 × 4.6 mm, 5µ	0.1 M KH_2PO_4 pH 4.6 - ACN (85:15)	208	1.0	V 7.20 M 8.70	[35]
Vildagliptin, technical contaminants	NP HPLC	Purospher STAR-C18, 150 × 4.6 mm, 3µ	0.01 M $NaH_2PO_4.H_2O$ pH 7.0 - ACN (70:30), gradient	210	1.0	V 19.10	[16]
Vildagliptin, metformin in tablets and plasma	NP HPLC	Thermo hypersil ODS C18 250 × 4.6 mm, 5µ	0.1 M KH_2PO_4 - MeOH - ACN pH 3.5 (65:5:30)	212	0.8	M 3.34 V 5.41	[32]
Vildagliptin (V) in plasma, tolbutamide (T) as internal standard	NP HPLC	XBridge Shield COVID-19 150 × 4.6 mm, 3.5µ	50 mM $(NH_4)_2HPO_4$ pH 7.8 - ACN	210	1.0	V 11.2 T 134	[50]
Vildagliptin, metformin, dorzolamide, lornoxicam in plasma	Micellar HPLC	Chromolith® C18 monolithic column 50 × 4.6 mm, 5µ	10 mM NaH_2PO_4 with 10 mM sodium dodecylsulfate pH 4.5 - ACN (70:30)	208	2.5		[51]

Abbreviations: RP – reverse phase; NP – normal phase; ACN – acetonitrile; MeOH – methanol; DEA – diethylamine; TEA – triethylamine; TFA – trifluoroacetic acid

secondary amino group. The peak of the benzoylated derivative is at 212 nm.

We have attempted to assay vildagliptin in mixtures with metformin hydrochloride on columns with the strong cation Zorbax 300-SCX, 250 × 4.6 mm using a mobile phase consisting of 0.15 M ammonium hydrogen phosphate buffer. Good retention of metformin was seen, while vildagliptin eluted at 30 min as a wide and very protracted peak. The retention times of metformin and vildagliptin were reduced by adding acetonitrile to the mobile phase. At a ratio of 85:15 (buffer solution pH 3.0 – acetonitrile), vildagliptin eluted by 20 min as a peak with satisfactory purity and symmetry.

Table 2 presents data from a literature review on assay of vildagliptin both alone and in mixtures, and its related contaminants, by HPLC.

As shown in Table 2, use of HPLC methods for analysis of vildagliptin is very widespread. The dominant technique is reverse-phase HPLC, though, depending on the task undertaken and the concomitant components, ion-pair [7, 14, 39, 47] chromatography is also found. Cyano-phase separation is

reported in [35, 48]. Use of the micellar component of sodium dodecylsulfate in the mobile phase is described in [51]. Chiral HPLC is used for determination of enantiomeric purity [7].

High-Performance Liquid Chromatography with Mass Detection

The authors of [16] used mass spectra to propose the structure of a vildagliptin contaminant formed during synthesis. The main degradation products were confirmed and clarified in [44]. The mechanisms of formation of degradation products were described and their structures were determined in [13]. Alkaline hydrolysis produced peaks with products with relative retention times (RRT) of 1.2, 0.6, and 0.4, while acid hydrolysis generated a product with RRT 1.3; *m*/*z* values were 337.2, 321.1, and 322.6. A further three decomposition products were also observed on oxidative oxidation of vildagliptin, one with RRT 0.38 and *m*/*z* 241.1, the second identical to the product formed on alkaline hydrolysis with RRT 0.6, and a third with RRT 0.8 and *m*/*z* 183.1 [13].

Furthermore, there are data on vildagliptin assay in plasma by HPLC-MS in bioanalytical studies [50] and in mixtures with metformin hydrochloride in plasma using an Atlantis HILIC Silica 150 × 2.1 mm, 3μ column [52].

Capillary Electrophoresis

The literature contains a description of vildagliptin assay by capillary electrophoresis. The assay used fused quartz capillaries with a potential of 25 kV (positive polarity). The supporting electrolyte was 25 mM potassium phosphate pH 8.0 with detection at 207 nm. Electrophoretic separation was run in 6 min and was linear over the concentration range 50 – 200 μg/ml [53]. The authors of [54] assayed vildagliptin in rat plasma, after precipitation of plasma protein with acetonitrile, using sitagliptin as internal standard. Vildagliptin was separated from plasma components using flamed quartz capillaries with supporting electrolyte consisting of 0.25 mM ammonium formate. Detection was by mass spectrometry.

Gas-Liquid Chromatography

A method for vildagliptin assay by gas chromatography with mass detection was described in [46]. The stationary phase was a capillary column with 5% methylphenylpolysiloxane (30 m × 0.25 mm, 0.25 μ, Agilent Technologies, USA) and the derivatization agent was N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) and catalysts were β-mercaptoethanol and ammonium iodide (NH_4I). The trimethylsilyl derivative was attached at the hydroxyl group of vildagliptin. The internal standard was nandrolone.

GLC with flame ionization detection was used to determine the initial product of 3-aminoadamantol as contaminant of vildagliptin [7, 9].

Thin-Layer Chromatography

HPTLC combined with densitometric assay of vildagliptin and its degradation products was described in chapter 8 of [23]. The stationary phase was Silica gel G60 F254 on an aluminum support. Several mobile phase compositions were tested and a mobile phase containing *n*-butanol, methanol, acetone, and concentrated ammonia solution (7:1:1:1) was found to give compact vildagliptin spots and the best separation between vildagliptin and its decomposition products. The coefficient of retention (R_f) of vildagliptin was 0.68 ± 0.02. Two types of detection were assessed: Dragendorff's reagent (scanning at 480 nm) and iodine (scanning at 280 nm). The main decomposition products were identified by IR spectroscopy and mass spectroscopy. An HPTLC method for assay of vildagliptin in mixtures with linagliptin, sitagliptin, and metformin was described in [55]. The mobile phase was a mixture of methanol and 0.5% ammonium sulfate (8:2). Chromatography was by an ascending method to a height of 8 cm. Densitometric scanning was carried out at specific wavelengths. A densitometric assay of vildagliptin mixed with metformin and melamine was described in [56]. The mobile phase was a solvent system consisting of methanol, chloroform, and formic acid (7:3:0.3). The author of [49] suggested a mobile phase consisting of a mixture of chloroform, *n*-butanol, and methanol (5:2:3), with detection at 227 nm; R_f was 0.62 ± 1.92.

Voltammetry

Studies reported in [57] addressed several types of voltammetry. The working electrodes were platinum and glassy carbon and the optimum conditions for voltammetric analysis were 1 M KNO_3 solution and phosphate buffer solution pH 6.8. Results obtained by cyclic voltammetry show that vildagliptin is electrically active and demonstrates irreversible oxidative-reductive cycles, while results from linear voltammetry gave a peak oxidation current of about 1.35 V. The authors recommended linear voltammetry as a method for assay of vildagliptin with a platinum working electrode and a supporting electrolyte consisting of 1 M KNO_3 solution.

Potentiometry

Report [58] presented a novel selective electrode for assay of vildagliptin using tetrakis(*p*-chlorophenyl)ammonium borate as cationite in a polyvinylchloride polymer matrix plasticized with *ortho*-nitrophenyloctyl ether.

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