QUANTIFICATION OF VILDAGLIPTIN IN TABLETS USING HIGH LIQUID-PERFORMANCE CHROMATOGRAPHY

Duong Dinh Chung*, Truong Thi Thu Thao, Tran Thi Trang, Vo Thi Nhan, Tran Le Nhat Vy, Chau Khoi Nguyen, Dinh Minh Nguyen, Le Thi Yen Chi, Nguyen Huu Khanh Quan, Nguyen Thi Ngoc Yen
Nguyen Tat Thanh University

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ABSTRACT

The study aimed to establish a fast, simple and economical determination procedure for vildagliptin content in tablets by reverse phase high-performance liquid chromatography (Rp-HPLC). Research method: Chromatographic conditions affecting vildagliptin analysis, such as solvent system, sample processing and optimization of chromatographic conditions, and mobile phase ratio... were investigated. After determining the appropriate chromatographic parameters, the analytical process was validated according to Circular No. 32/2018/TT-BYT on regulations for marketing authorization of drugs and medicinal ingredients, including a survey of systemic suitability, specificity, the limit of quantification, limit of detection, linearity, accuracy, and precision. The analytical method showed a linear range from 12.5 to 100 µg/mL, and the limit of quantification was 1.21 µg/mL. The results of this study can be used as a routine procedure to analyze, control and test vildagliptin stability in tablet formulations.

ĐỊNH LƯỢNG VILDAGLIPTIN TRONG VIÊN NÉN BẰNG PHƯƠNG PHÁP SẮC KÝ LỎNG HIỆU NĂNG CAO

Dương Đình Chung*, Trương Thị Thu Thảo, Trần Thị Trang, Võ Thị Nhàn, Trần Lê Nhật Vy, Châu Khôi Nguyên, Đinh Minh Nguyên, Lê Thị Yến Chi, Nguyễn Hữu Khánh Quan, Nguyễn Thị Ngọc Yến
Trường Đại học Nguyễn Tất Thành

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TỪ KHÓA

DPP4
Định lượng
Rp-HPLC
Vildagliptin
Thẩm định

TÓM TẮT

Nghiên cứu được thực hiện với mục tiêu xây dựng quy trình xác định hàm lượng vildagliptin trong viên nén nhanh chóng, đơn giản, ít tiêu tốn dung môi bằng phương pháp sắc ký lỏng hiệu năng cao pha đảo (Rp-HPLC). Phương pháp nghiên cứu: Khảo sát một số điều kiện sắc ký ảnh hưởng đến quá trình phân tích vildagliptin như hệ dung môi, quy trình xử lý mẫu và tối ưu hóa các điều kiện sắc ký, tỷ lệ pha động... Sau khi xác định điều kiện sắc ký thích hợp, tiến hành thẩm định quy trình phân tích theo Thông tư số 32/2018/TT-BYT quy định về lưu hành thuốc, nguyên liệu làm thuốc bao gồm khảo sát tính phù hợp hệ thống, tính đặc hiệu, giới hạn định lượng, giới hạn phát hiện, tính tuyến tính, độ chính xác và độ đúng. Phương pháp phân tích được thiết lập khoảng tuyến tính từ 12,5 – 100 µg/mL và giới hạn định lượng là 1,21 µg/mL. Kết quả nghiên cứu này có thể được sử dụng như một quy trình thường quy để phân tích, kiểm soát hàm lượng và thử nghiệm độ ổn định của vildagliptin trong các công thức viên nén.

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* Corresponding author. *Email: ddchung@ntt.edu.vn*

1. Introduction

Vildagliptin is a dipeptidyl-peptidase-4 (DPP-4) inhibitor that prevents the breakdown of glucagon-like peptide 1 (GPL-1) and gastric inhibitory polypeptide (GIP) (endogenous incretin hormones), thereby increasing insulin secretion and decreasing glucagon production by the pancreas [1]-[4]. The result is an increase in glucose uptake into the muscles and a decrease in glucose production by the liver leading to a reduction in blood sugar levels [4].

Vildagliptin was developed by Novartis under the brand name Galvus and is used alone or in combination with metformin in the treatment of type 2 diabetes [5]-[7].

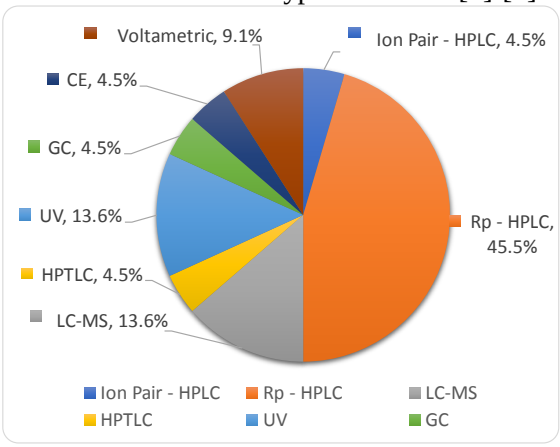


Figure 1. *Proportion (%) of vildagliptin quantification methods*

Analytical methods such as molecular absorption spectroscopy (UV) [8], gas chromatography (GC) [9], voltammetric titration [10], [11], high-performance thin layer chromatography (HPTLC) [10], HPLC-UV/PDA [12]-[19], and LC-MS [20] have been developed for the determination of vildagliptin in the wide range of formulations. Figure 1 depicts a comparison of previously reported vildagliptin quantification methods.

High-performance liquid chromatography is the analytical technique for quantifying vildagliptin in dosage forms among quantitative methods. Nevertheless, there are fewer studies in Vietnam on the quantification of vildagliptin by this method. In addition, Vietnam Pharmacopoeia V does not have a particular treatise for the 50 mg vildagliptin tablet dosage form. Therefore, to supplement data for routine vildagliptin determination, the research team in this study developed a chromatographic method for identifying and quantifying vildagliptin in tablet formulations.

2. Materials and methods

2.1. Raw materials, chemicals, and reagents

Test sample: tablets containing 50 mg of vildagliptin.

Chemicals: Methanol, acetonitrile (chromatographic standards provided by Merck, Germany), water reached the quality used for chromatography from Pall ultrapure water purification system (USA) and other chemicals meeting the analytical standard (Merck, Germany).

Reference: Vildagliptin (97% purity) provided by Toronto Research Chemicals (Canada).

Equipment: Agilent 1260 chromatography system with PDA probe and the ChemStation software version B.04.32 (USA).

2.2. Research methods

2.2.1. Investigation of chromatographic conditions

A reverse phase HPLC was developed for the quantification of vildagliptin in tablets with initial chromatographic conditions including Luna C8 chromatographic column (150 mm x 4.6

mm; 5 µm), column oven temperature at 40 ºC, flow rate 1 mL/min and sample injection volume 10 µL. The vildagliptin signal was recorded at the wavelength of 210 nm on chromatograms. Chromatographic conditions were surveyed and chosen for vildagliptin chromatographic peak signal to achieve the requirements of N > 3000; 1 < k' < 5; 0.8 < Symm < 1.5. Specific experiments are as follows: pH, mobile phase, and concentration of KH_2PO_4.

Mobile solvent ratio: The proportions (5, 10, 15, 20, 25%) of acetonitrile solvent in the aqueous phase were investigated and selected based on the capacity coefficient k'.

Buffer concentration: The concentrations of KH_2PO_4 buffer solution (5, 10, 15, 20, 25 and 50 mM) were optimized using the asymmetric coefficient of vildagliptin peak.

The mobile phase pH: The pH value was optimized following experimental tests at different pH ranges from 3.5 to 7.5 (pH was adjusted using KOH or H_3PO_4).

2.2.2. Process of sample treatment

Sample preparation: 20 tablets were determined as the mean weight, then ground finely in a porcelain mortar. A quantity of powder corresponding to 50 mg vildagliptin was transferred into a 100-mL volumetric flask. About 80 mL of solvent mixtures, including methanol, acetonitrile, and water, with different ratios, were added. Experimental runs at the various solvent combination and ultrasonic times (0-40 min) were performed at ambient temperature. The samples were diluted to a concentration of 50 µg/mL, then filtered through a 0.45 µm nylon filter, and injected into a chromatographical system. The appropriate sample processing conditions were selected based on the peak area of the vildagliptin signal.

2.2.3. Method validation

The analytical method was evaluated for the following criteria: Precision, accuracy, linearity, system compatibility and quantitative limit according to the guidelines of Circular No. 32/2018/TT-BYT dated November 12, 2018, about marketing authorization of drugs and medicinal ingredients Viet Nam Ministry of Health promulgated.

2.2.4. Statistical method

Experimental data were entered and processed by ANOVA statistical algorithm on Microsoft Excel software. The t-test was calculated to test the statistically significant differences between variables ($p \leq 0.05$).

3. Results and discussion

3.1. Investigation of chromatographic conditions

3.1.1. Effect of pH on the k' coefficient

As the pH of the mobile phase increases, the capacity factor k' increases. The pH levels from 2.5 to 5.5 show k' value that less than 1 is unsuitable. The ideal pH range of the mobile phase suitable for conducting quantitative analysis of vildagliptin is between 6.5 and 7.5. The results are shown in Figure 2a.

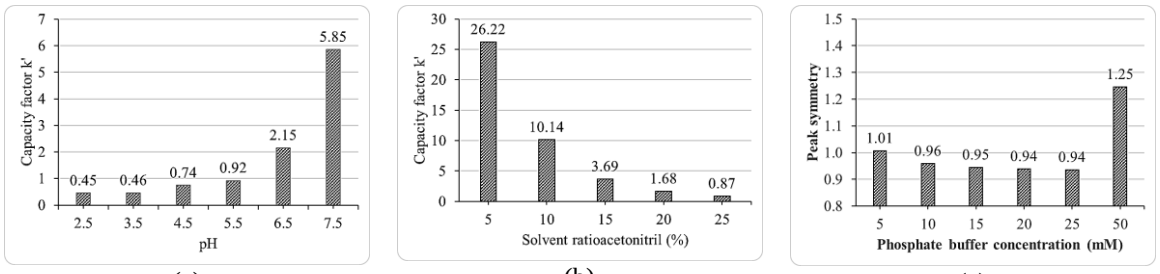
3.1.2. Effect of acetonitrile on the k' coefficient

The results in Figure 2b range from 0.87 to 26.22, with a decrease in the acetonitrile ratio, accompanies by an increase in retention time during the analysis. The percentage of 15% acetonitrile, giving a suitable k' coefficient (1 < k' < 5), was selected for further investigations.

3.1.3. Effect of KH_2PO_4 concentration on the symmetry coefficient

Figure 2c shows that the KH_2PO_4 buffer concentration did not significantly affect to the asymmetry coefficient (Symm.) of the vildagliptin peak (0.8 < Symm. < 1.5). However, a larger

concentration of buffer causes time-consuming to equilibrate and wash the column after the analysis procedure. Therefore, the concentration value of KH_2PO_4 buffer of 5 mM was chosen for further studies.



(a) (b) (c)

Figure 2. *Effects of factors including (a) pH factor; (b) mobile solvents; and (c) phosphate buffer concentration on capacity coefficient (k')*

3.2. Sample treatment method

In the study, powder samples containing vildagliptin ingredients were extracted using methanol, acetonitrile, and distilled water at different ratios. As a result of the experiments, the distilled water was selected by high extraction yield of vildagliptin.

The parameters, such as frequency level 40 KHz and no heating mode, were fixed when ultrasound time was surveyed. The results presented in Figure 3 shows that the concentration of vildagliptin did not change significantly after 10 minutes of sonication (%RSD = 0.62). Thus, the optimal ultrasonic time was 10 minutes.

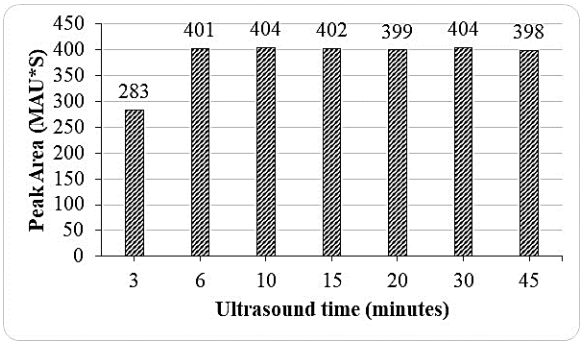


Figure 3. *Effect of ultrasound time*

3.3. Method validation

3.3.1. System compatibility

The standard solution at the quantitative concentration was injected six times repeatedly. The chromatographic parameters consisting of retention time, peak area, and the number of theoretical plates were recorded. Table 1 shows the %RSD values of retention time, peak area < 2%, symmetry factor 0.8 < Symm < 1.5 and the number of theoretical plates larger than the requirement (N > 3000), which proves that the chromatographic system is suitable for quantification of vildagliptin in tablets.

3.3.2. Specificity

The chromatograms of the blank sample (Figure 4a) and the mobile phase (Figure 4b) did not appear peaks at the retention time corresponding to the retention time of the vildagliptin standard (Figure 4c). The samples (at the same concentration of 50 mM vildagliptin) were decomposed under different extreme conditions (in 2M HCl, 2M NaOH, 3% H_2O_2, UV irradiation at 254 nm for 8 hours, and water bathing at 60^{o}C for 4 hours). As a result, there are additional impurity

peaks in the chromatograms of the samples. However, under established conditions, the peak of vildagliptin completely separated the impurity peaks (Figure 4d). The vildagliptin signal was checked with a PDA probe and reached purity (Figure 5), which further confirmed that there was no co-elution of impurities (from the degradation of vildagliptin) with vildagliptin, so data confirmed the specificity of the analytical procedure.

Table 1. *System compatibility assessment*

No.	Chromatographic parameters				
	Rt	k'	mAU*S	Symm.	N
1	7.12	3.76	367.58	0.93	9846
2	7.13	3.77	366.33	0.93	9842
3	7.11	3.75	367.13	0.94	9789
4	7.11	3.75	369.42	0.94	9934
5	7.11	3.75	367.53	0.94	9710
6	7.11	3.76	367.84	0.93	9845
Mean	7.11	3.76	368	0.936	9828
RSD(%)	0.13	0.15	0.28	0.49	0.75

*Note: Rt - Retention time; k' - Capacity factor; Peak area (mAU*S); Symm. - Symmetry factor; N - Theoretical plate number*

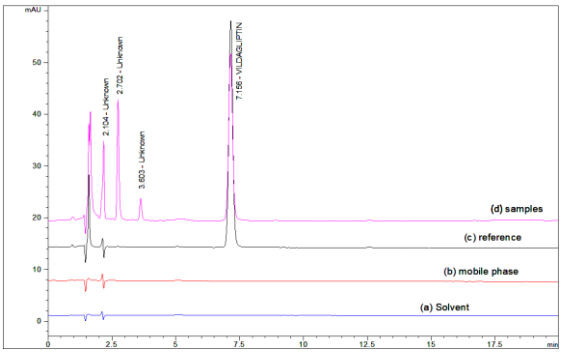


Figure 4. *The chromatogram of specificity analysis: (a) solvent; (b) mobile phase; (c) reference, and (d) samples*

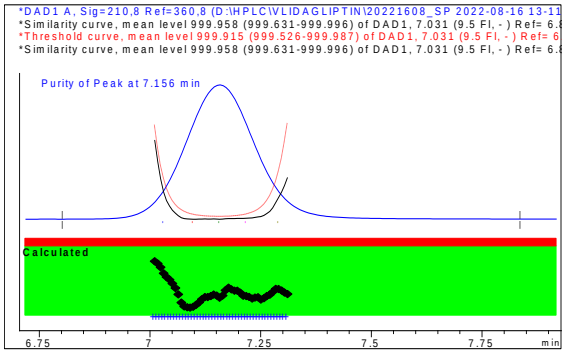


Figure 5. *Purity of the vildagliptin peak in the specificity test*

3.3.3. Linearity and limit of quantification

Under the chromatographic conditions set up as described, the linear range was built in the concentration range of 12.5-100 µg/mL. Then, the limit of quantitation (LOQ) was calculated based on the standard deviation at established concentrations. The result of the determination of the limit of quantitation was 1.21 µg/mL. Table 2 and Figures 6 and 7 present data analysis, chromatogram, and linearity for the standard solutions, respectively.

Table 2. *Linearity assessment results*

No.	Established concentration of vildagliptin (µg/mL) - x	Vildagliptin concentration calculated from the calibration curve (µg/mL)	Precision (Rev%)	Peak area (mAU*S) - y	ANOVA analysis
1	12.50	12.48	99.81	91.85	y = 7.34x + 0.17
2	25.00	25.08	100.33	184.49	r^2 = 1.0000
3	37.50	37.60	100.26	276.45	Rev = 99.68% -
4	50.00	49.94	99.89	367.17	100.33%
5	60.00	59.81	99.68	439.64	RSD = 0.24%
6	75.00	75.03	100.05	551.54	LOD = 0.37µg/mL
7	100.00	100.06	100.06	735.41	LOD = 1.21µg/mL

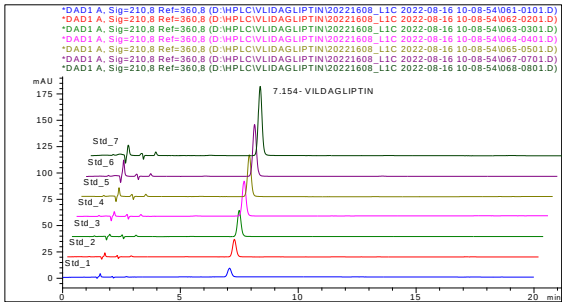


Figure 6. *Chromatography of vildagliptin standard solutions in the concentration range of 12.5-100 µg/mL*

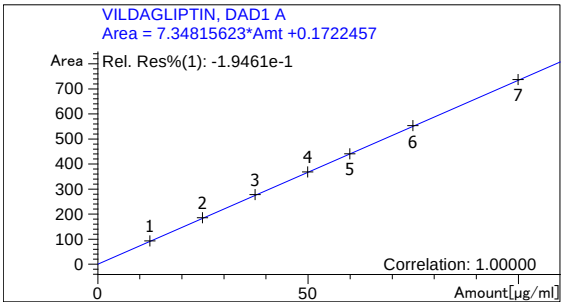


Figure 7. *The peak area response-concentration curve of vildagliptin*

3.3.4. Intra-day and inter-day accuracy

Data on the intraday and interday accuracy of vildagliptin are presented in Table 3. The t-test showed that the mean value of vildagliptin content of the two experimental days was not significantly different (t_{lt} = 2.22 > t_{tn} = 0.36). The %RSD value of the vildagliptin content in the intraday and inter-day accuracy testing is less than 2, within limits according to Circular No. 32/2018/TT-BYT guidance. Therefore, the developed method proved to achieve precision.

Table 3. *Results of accuracy validation*

No.	First day	Second day
1	100.16	99.40
2	99.15	100.17
3	99.40	99.65
4	100.15	100.41
5	99.91	99.40
6	99.65	99.92
Mean	**99.74**	**99.82**
S_i^2 =	**0.171**	**0.172**
RSD (%)	0.412	0.416

3.3.5. Accuracy

Table 4. *Result of the precision assessment*

Samples	No.	The initial concentration of vildagliptin (µg/mL)	Added concentration of the standard (µg/mL)	Peak area (mAU*S)	Recovered concentration of vildagliptin (µg/ml)	Recovery (%)
QC1	1	50.14	10.00	442.65	60.24	101.0
	2			441.12	60.03	98.9
	3			442.78	60.25	101.1
					Mean	100.3
					RSD (%)	1.25
QC2	1	50.14	20.00	512.86	69.79	98.3
	2			513.60	69.89	98.8
	3			513.43	69.87	98.6
					Mean	98.6
					RSD (%)	0.27
QC3	1	50.14	30.00	588.34	80.07	99.7
	2			592.09	80.58	101.4
	3			592.75	80.67	101.7
					Mean	101.0
					RSD (%)	1.07

The accuracy is assessed by the recovery value of the sample added with the standard. The results showed that the method's recovery is from 98.3 - 101.7%, within the 98.0 – 102.0% range. Furthermore, the relative standard deviation is below 2% (%RSD < 2%). Therefore, the vildagliptin quantification procedure meets the requirements of accuracy. Table 4 summarizes the results of the method validation.

Table 5. *Results of applying the method to quantify vildagliptin in the study sample*

No.	Weighted mean of tablets (mg)	Sample weight (mg)	Peak area (mAU*S)	Concentration of vildagliptine (µg/mL)	Percentage of labelled and measured content (%)
1	197.86	196.91	372.61	50.12	99.8
2		197.03	379.78	51.09	101.8
3		201.33	379.46	51.05	103.9
4		197.95	379.03	50.99	102.0
5		199.11	373.50	50.24	101.1
6		198.38	365.12	49.11	98.5
				Mean	101.2
				RSD (%)	1.86

The analytical procedure was used to determine the content of vildagliptin in tablets on the market

After treatment and analysis of 06 independent test samples, labelled vildagliptin content in the products meet the requirements regulated in Appendix 11, Vietnam Pharmacopoeia V. The %RSD value of measured active ingredient content ≤ 2.0 % proves that the process is stable and accurate. The results are presented in Table 5.

4. Conclusion

This study aims to develop and validate the Rp-HPLC method for the quantification of vildagliptin in tablet preparations. The results obtained from different experiments have proved that the method meets the requirements of specificity, linearity, accuracy, and correctness according to the guidance of Circular No. 32/2018/TT-BYT (Vietnam Ministry of Health) on regulations for marketing authorization of drugs and medicinal ingredients. Furthermore, the composition of the excipients presenting in the dosage form and the solubilizer of vildagliptin did not affect the effectiveness of the developed analytical method. Therefore, this method can be used routinely for quality control and/or stability testing of vildagliptin in the tablet dosage form.

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