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Validated Chiral Ultra Fast Liquid Chromatographic Method for Quantitative Analysis of Enantiomeric Vildagliptin

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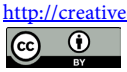

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Abstract

A rapid, accurate, and precise chiral Ultra fast liquid chromatography (UFLC) method was developed and validated for enantiomeric separation of racemic vildagliptin and *S*-vildagliptin according to the guidelines of the International Conference on Harmonization (ICH). The chiral chromatographic separation was achieved with a mobile phase consisting of 20 mM borax buffer (pH 9.0 ± 0.05), ACN, and 0.1% Triethylamine (50:50:0.1, v/v/v) at a flow rate of 1 ml/min using a chiralcel OD-RH column, tris(3,5-dimethyl phenyl carbamate) (250 mm × 4.6 mm, 5 µm) column. The UFLC analysis was monitored at 210 nm. The method showed good linearity with a regression coefficient (r^2) of 0.999 in the range of 1 - 12 µg/ml for *S*-vilda. The detection limit (LOD), quantitation limit (LOQ), and the average percentage recovery for *S*-vilda were found to be 0.024, 0.075 µg/mL, and 99.19% to 100.4%, respectively. The percentages of relative standard deviation (% RSD) for intra- and inter-day precision were found to be 0.346% and 0.364%, respectively. The developed method proved to be reproducible as % RSD was <2% and it had robustness within the acceptable limit. The percentage purity of pharmaceutical preparations of *S*-vilda was found to be 99.19 w/w. The proposed chiral method can be put in application for the enantiomeric purity determination of *S*-vilda formulations.

Keywords

Standard Vildagliptin, *S*-Vilda, Ultra Fast Liquid Chromatography, Method Development, Validation

1. Introduction

Vildagliptin (Vilda; Figure 1) chemically known as (*S*)-{[(3-hydroxy-1-adamantanyl)

amino] acetyl}-pyrrolidine-2-carbonitrile is a potent, selective, and orally bioavailable DPP-4 inhibitor. It is a potent dipeptidyl peptidase IV (DDP-IV) inhibitor, a drug for the treatment of diabetes [1]. Vildagliptin is available as Vildaprime® 50 mg, marketed formulation for monotherapy. The molecule contains one chiral center, thus giving rise to two stereoisomers *S*- and *R*-Vilda. As only the *S*-Vilda enantiomer is in clinical use, the *R*-vilda enantiomer is considered as an optical impurity [2] [3]. Chiral analysis of enantiopure drugs is essential during manufacturing, purity testing, pharmacokinetic and pharmacodynamic studies, and clinical monitoring. It is also important for impurity profiling.

UFLC is widely applied for chiral separation besides, capillary electrophoresis (CE) and Gas chromatography (GC). Major advantages of the UFLC are the high efficiency, rapid analysis time, high resolution, and small sample volume requirement. In present work, the chiralcel column (LUX-cellulose columns) was used as chiral selectors to separate the enantiomers of Vildagliptin.

Previously, vildagliptin was analyzed by only non-chiral quantitative determinations in bulk and pharmaceutical formulations [2] [3] [4] [5]. No chiral method has been reported for determination of *S*-vilda enantiomer in vildagliptin using direct chiral RP-UFLC method.

The present study describes the development of a highly sensitive and simple chiral RP-UFLC-UV method for assessment of *S*-vilda enantiomer in standard vildagliptin using chiralcel OD-RH column. The developed method was validated in accordance with the International Conference on Harmonization (ICH) [6].

2. Experimental

2.1. Chemicals

Vildagliptin (vilda) and its *S*-vildagliptin enantiomers were obtained as gift samples from Novartis, India. Vilda prime® (50 mg) tablet was purchased from Apollo pharmacy at Warangal, Telangana State, India. Disodium tetraborate decahydrate (Borax), HPLC grade methanol, acetonitrile (ACN), and triethylamine (TEA) were purchased from Merck. All analytical grade reagents were used.

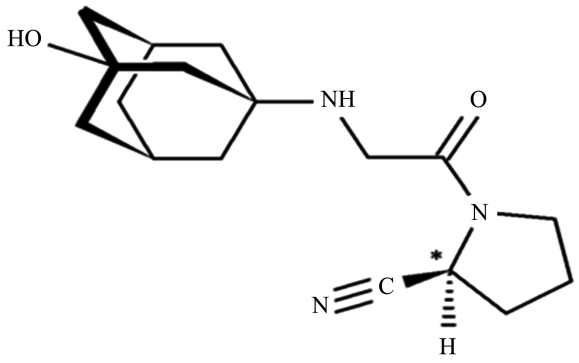

Figure 1. Chemical structure of *S*-vilda.

2.2. Instrumentation

The research work was conducted using Shimadzu LC-20 AD Ultra-fast Liquid Chromatography (UFLC) system equipped with a binary pump, and UV detector. The instrument was controlled using LC Lab solutions software. Experiments were conducted using polysaccharide-based, chiral stationary phase (CSP) (chiralcel OD-RH, chiralcel OJ-RH) columns. These columns were used for the method optimization under reverse phase mode. Ultrasonicator was used for mixing the drug in a suitable solvent. The pH was measured using a pH meter (LI 127, Elico, India) and Millipak Express Millipore (0.22 μm) was used to get ultrapure double distilled water.

2.3. Chromatographic Condition

Enantiomeric separation of Vildagliptin (Vilda) was operated on Lux cellulose-1, tris (3,5-dimethyl phenyl carbamate), Chiralcel OD-RH (250 mm × 4.6 mm, 5 μm) column using a mixture of a 20 mM borate buffer solution (pH = 9 ± 0.05), ACN in the ratio of 50:50 v/v with 0.1% TEA as mobile phase. The column temperature was maintained at 25˚C, the flow rate was 1 mL/min. The wavelength for detection of the vilda enantiomers peaks were set at 210 nm and the injection volume was 20 μL.

2.4. Preparation of 20 mM Borax Buffer Solution (pH 9.0 ± 0.005)

Accurately weighed 3.81 grams of borax was taken and transferred into a 500 ml beaker. The content was dissolved with ultrapure water and the pH of this solution was adjusted to 9 ± 0.005 with 0.1% triethylamine (TEA). The mobile phase was prepared by mixing acetonitrile and buffer solution in a specific ratio, followed by degassing on an ultrasonic bath [7].

2.5. Preparation of the Standard Solutions

50 mg of pure vildagliptin and its *S*-vilda enantiomer were weighed accurately and transferred into a separate 50 ml volumetric flask. These were dissolved by using HPLC grade methanol. After complete dissolution, the volumes were made up to the mark by using the same to give final concentration (1 mg/mL) of the drug solution. These resulting solutions were sonicated in an ultrasonic bath for 15 minutes. Dilution of these solutions with appropriate volumes of the solvent mixture was carried out to obtain solutions of concentrations of 1, 2, 4, 6, 8, 10 and 12 μg/mL. Vilda standard solution was also prepared to identify the enantiomers of vildagliptin.

2.6. Method Validation

The validation of the developed chiral RP-UFLC method for the determination of *S*-vilda enantiomer as a potential optical active eutomer in Vilda was performed following the International Conference on Harmonization (ICH) guidelines (ICH) [6].

2.6.1. Specificity

Specificity is used to analyze standard substances against potential interferences. The method was found to be specific because of the absence of any interference to the test substance. Under the optimized chromatographic conditions, the chromatograms (Figure 2) confirmed the presence of *R*- and *S*-enantiomers in vilda solution with the Rt at 5.25 ± 0.01 min and 7.24 ± 0.01 min with total run time of 12 min, respectively without any interference.

2.6.2. System Suitability

System suitability parameters like Capacity factor, Selectivity factor, Resolution, Tailing factor, and Theoretical plates were assessed to establish the optimized method.

2.6.3. Linearity

From the *S*-vilda standard solution of 100 µg/mL, S-vilda concentrations of 1, 2, 4, 6, 8, 10 and 12 µg/mL were prepared and then injected with volume of 20 µL into UFLC system and chromatogram was monitored at 210 nm by UV detector. The chromatograms were recorded and mean *S*-vilda Peak areas (Y-axis) were plotted against the corresponding concentration (X-axis) to obtain calibration graph.

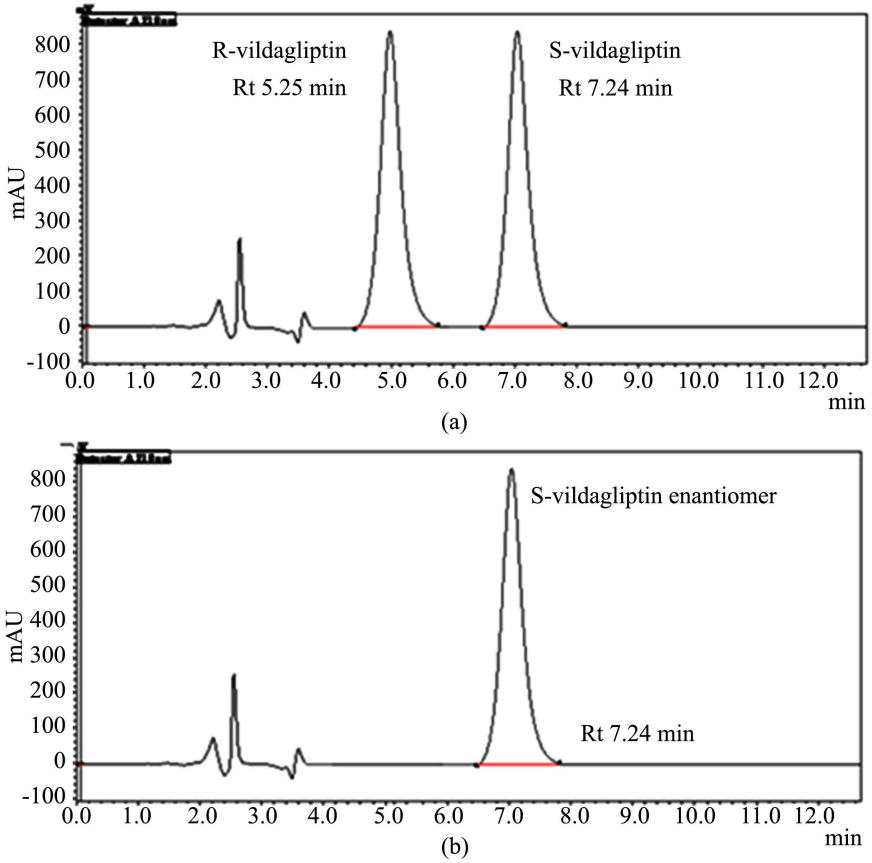

Figure 2. The representative chromatograms of (a) Racemic Vildagliptin, and (b) *S*-vilda enantiomer.

2.6.4. Precision

The standard *S*-vilda enantiomer at three concentrations levels (6, 8 and 10 μg/mL) were selected to study the precision of the developed chromatographic procedure. Intra-day precision and Inter-day precision analyses were performed to check the repeatability and reproducibility. Intra-day & inter-day precision of selected concentration were analysed in sextuplet, and in triplicate injection (on three consecutive days), respectively. These were examined by determining % RSD using following equation:

$$\%\text{RSD} = \text{SD}/\text{Mean} \times 100$$

where, SD = Standard deviation.

2.6.5. Limit of Detection and Quantification (LOD, LOQ)

The LOD and LOQ were evaluated from the linearity equation *i.e.* slope of the calibration curve and the standard deviation (SD) of the peak areas using the following equations:

$$\text{LOD} = (\text{SD}/\text{Slope}) \times 3.3 \quad \text{and} \quad \text{LOQ} = (\text{SD}/\text{Slope}) \times 10\,.$$

2.6.6. Accuracy

Percentage recovery was used to assess the accuracy of the developed method. To determine the accuracy of the method, pre-analysed sample solutions (4 μg/mL) were spiked with 50%, 100% and 150% levels of *S*-vilda enantiomer. The spiked samples were analyzed by proposed method for three times.

2.6.7. Robustness

Robustness was evaluated by analyzing the sample solutions at altered conditions like small change in flow rate ±0.1 mL, (*i.e.* 0.9 mL/min to 1.1 mL/min) and wavelength ±1 nm, (*i.e.* 209 nm to 211 nm. It was calculated by estimating % RSD.

2.7. Stability

The solution stability of standards and samples were established under normal bench top conditions, normal storage conditions, and sometimes in the instrument to determine whether special storage conditions were required or not. *S*-vilda standard solution with concentration of 4 μg/ml was kept in a tightly capped volumetric flask at room temperature (25˚C) on the laboratory bench and at 4˚C in a refrigerator for 3 days and its stability was tested.

2.8. Estimation of *S*-Vilda in Vilda Prime®

Applying the optimized and validated method, the enantiomeric purity of the synthesized *S*-vilda was checked by analyzing methanolic solution (1 mg/mL) of bulk *S*-vilda. Moreover, the commercially available Vildaprime® (50 mg) tablets were chosen to demonstrate the broad applicability of the developed method to estimate the amount of chiral purity (*S*-vilda) in tablet formulation.

The present method provides a platform for chiral separation of Vilda, which

can be applied to test drug formulations as well as to bulk API powders. So, appropriate validated method is needed for each and every purpose it may be applicable.

Twenty tablets were weighed and powdered, and an accurately weighed portion of this powder equivalent to 25 mg, 50 mg, and 100 mg of Vilda were dissolved in 25, 50, and 100 mL calibrated standard flasks separately with methanol. The samples were sonicated for 15 min and centrifuged for 10 min at 4000 rpm. The clear supernatants were filtered through a 0.22 μm pore size filter. Subsequent dilutions of these solutions were made with mobile phase to get concentrations of 4 μg/mL each. These solutions (about 20 μL) were injected six times into the UFLC system.

The average values of peak areas from six injections were calculated, and the drug content in the tablet was quantified. The amount of optical active enantiomer of *S*-vilda was calculated using the corresponding regression equations.

3. Results

3.1. Method Development & Optimization

The objective of this research was to achieve chiral separation between Vildagliptin and its *S*-vilda enantiomer for accurate quantification using Ultra-Fast Liquid chromatography [8], in reverse phase mode. For the method development exercise, a system suitability solution was used. Two polysaccharide chiral stationary phases (CSPs) were used in the present method, namely, chiralcel OD-RH and chiralcel OJ-RH (from Daicel Chemical Industries, Ltd., Tokyo, Japan). Various combinations and compositions of solvents were examined to achieve optimum resolution (Rs) and selectivity for two enantiomers in vildagliptin. Initial development began with a mobile phase consisting of an equal mixture of 20 mM borax buffer solution and ACN (50:50). There was no separation on chiralcel OJ-RH (Lux Cellulose-3, Phenomenex). Insignificant separation was obtained on chiralcel OD-RH (Lux Cellulose-1, Phenomenex) *i.e.* separation with resolution 0.5. In order to improve the resolution, the concentration of buffer strength in the mobile phase was increased by a fraction of 10%. This resulted in an improvement in resolution (greater than 3.05).

To improve peak symmetry and column efficiency, TEA was introduced as a basic modifier at concentrations ranging from 0.1% - 0.4%. The addition of TEA improved the peak symmetry and resolution increased significantly with a higher percentage of TEA. Hence, a mobile phase containing 20 mM Borax buffer solution (pH 9 ± 0.05), ACN with TEA in the ratio of (60:40:0.1, v/v/v) under RP UFLC method using chiralcel OD-RH [tris (3,5-dimethyl phenyl carbamate)] column at a flow rate of 1 ml/min at ambient temperature was considered as the most suitable to achieve the optimum separation between enantiomers and also for better specificity. In all cases, the enantiomer migration order was the same i, e. the R-isomer was followed by the *S*-vilda enantiomer. The separation of enantiomers takes place because of interaction between the solute and the polar carbamate group on the Chiral Stationary Phase [9]. The *S*-enantiomer peak was

identified by spiking the sample solution with the stock solution of the pure *S*-vilda enantiomer Figure 2. The method development was tabulated in Table 1.

3.2. Method Validation

All parameters of system suitability are represented in Table 1. All parameters were within specified acceptable limits.

A series of seven prepared concentration of *S*-vilda enantiomer were applied in the concentration range of 1 to 12 µg/mL. The chromatogram was recorded at 210 nm. The linearity was performed in triplicate and the correlation coefficient was found to be 0.999 for *S*-vilda Figure 3. The data pertaining to linear regression is depicted in Table 2.

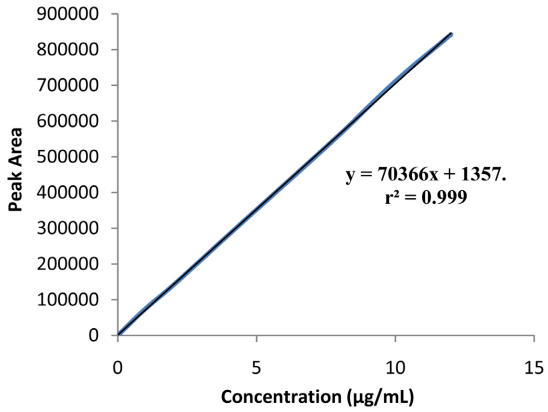

Figure 3. Linearity of *S*-vilda enantiomer.

Table 1. Results of system suitability parameters.

Parameters	(*R*)-vilda	(*S*)-vilda
Theoretical plates (≥2000) (n = 5)	7049	67,306
Tailing factor (≤2) (n = 5)	1.098	1.084
Relative retention (k_1 and k_2) (n = 5)	3.15	1.16
Selectivity (α) (n = 5)	-	3.209
Resolution (≥2) (n = 5)	-	4.66

n = number of determinations.

Table 2. Linearity data of *S*-vilda.

Parameters	**(*S*)-vilda enantiomer**
Linearity range (µg/ml)	1 - 12 µg/ml
Regression equation	$y = 70{,}366x + 1357$
Correlation equation ($r^2 \pm$ SD)	0.999 ± 0.00015
Slope ± SD	70,366 ± 89.9
Intercept ± SD	1357 ± 10

Intra-day and inter-day precision results of the method are shown in Table 3. The precision of the method was expressed in percentage relative standard deviation (% RSD). For the developed method Intra-day and inter-day precision values were found to be 0.98 and 1.07, respectively. As per ICH guide lines % RSD values limit for precision should be less than 2%, Hence, the developed method was proved to be precise.

The LOD and LOQ of *S*-vilda enantiomer were found to be 0.024 and 0.075 µg/mL, respectively.

Accuracy of the method was determined and the results are shown in Table 4. The % recovery of *S*-vilda enantiomer at test levels was found to be in the range of 99.19% - 100.4%, which was in accordance with acceptable limits. The robustness result of the proposed method is presented in Table 5. For each altered parameter, % RSD values were found to be less than 2%, hence the method was found to be robust.

Table 3. Precision data of *S*-vilda.

Concentration(µg/mL)	Intraday Precision	% RSD	Interday Precision	
	Mean ± SD		Mean ± SD	% RSD
6	430,955 ± 4262	0.98	429,288 ± 4622	1.07
8	555,967 ± 7226	1.29	554,300 ± 6441	1.16
10	713,507 ± 1671	0.23	71,184 ± 5268	0.74

Table 4. Accuracy of *S*-vilda.

Added amount (µg/ml)	Recovered concentration (µg/ml)	% Recovery
2 (50%)	1.983	99.19%
4 (100%)	4.032	100.08%
6 (150%)	6.029	100.4%

Table 5. Robustness studies for *S*-vilda.

Change in flow rate (mL/min)	Average Rt of (*S*)-vilda ± SD	% RSD
0.9	7.155 ± 0.01	0.17
1	7.241 ± 0.06	0.89
1.1	7.251 ± 0.05	0.71
Change in wavelength (nm)	Average area of (*S*)-vilda ± SD	% RSD
211	284,782 ± 513.68	0.180
210	282,096 ± 508.8	0.35
209	277,409 ± 634.219	0.22

3.3. Stability

Solution stability study was carried out to calculate % RSD of peak area of drug solution on three consecutive days at 25˚C and 4˚C. At 25˚C and 4˚C the % RSD was found to be 0.45 for both, which demonstrated that the drug was fairly stable at normal and freezing temperatures.

3.4. Estimation of *S*-Vilda in Marketed Formulation

This analytical method was applied to quantitate the content of *S*-vilda enantiomer in samples and as well as to calculate the % purity. The average content of *S*-vilda was found to vary from 100.02% to 100.025% in the formulations while the average content of *R*-vilda found as impurity varied from 0.02% to 0.025% which was in accordance to ICH guidelines of being less than 0.05%. The results are presented in Table 6.

4. Discussion

Authors are aimed at development of sensitive, accurate chiral RP-UFLC method for detection and quantification of *S*-vilda enantiomer using chiralcel OD-RH column. A number of analytical methods have been reported for vildagliptin by using achiral methods [2]. The developed method optimized the ideal retention times *i.e.* Rt 5.2 min (*R*-vilda) and 7.2 min (*S*-vilda) for vildagliptin enantiomers indicating the method to be cost effective and efficient whereas [9], reported a chiral isocratic method with retention times of 9.9 and 11.8 min for *R* and *S* enantiomers, respectively.

The percentage recovery for the developed method was found to be greater than 99.19% which is comparable to indirect achiral method developed by [10] in which they validated the method using cyclodextrin-modified capillary electrophoresis. In contrast, the present optimized method was simple and direct chiral method (using chiralcel OD-RH column).

The sensitivity of the method is indicated in the LOD and LOQ values which were found to be 0.024 and 0.075 µg/ml, respectively. One of the achiral RP-HPLC method [11] reported values to be 0.0329 and 0.0998 µg/ml. The correlation coefficient value was found to be 0.999 juxtaposition to an UV method [12] with r^2 value of 0.985. The present optimized method was improvised and perked up than the achiral methods.

Table 6. Assay of *S*-vildagliptin marketed sample (Vilda prime® (50 mg) tablets).

Vilda marketed Sample (Equivalent amount)	Concentration prepared (µg/mL)	Concentration found (mg/mL)	(%) Label claim Purity of *S*-vilda	*R*-vilda as impurity
25 mg	(4 µg/mL)	25.005	100.02%	0.02%
50 mg		50.005	100.01%	0.01%
100 mg		100.025	100.025%	0.025%

The developed chiral RP-UFLC method established its sensitivity, precision, accuracy, LOD and LOQ which were in accordance with ICH guide lines. The resolution between the enantiomers in reverse phase mode was exemplary, as it was above the accepted limit of >1.5 (*i.e.* 4.66). The stability indicated sturdy nature of enantiomer.

In collation to the reported methods, present method used 0.1% TEA, a basic additive in the mobile phase favorable for the separation & quantification of enantiomers in vildagliptin. The distomer *R*-vilda was also resolved from the active enantiomer (*S*-vilda). This kind of profiling is an important need for chiral drug analysis.

The concentration of organic modifier (ACN), 0.1% TEA and buffer strength (Borax) played an important role to achieve better retention and resolution between the enantiomers of vilda. The system suitability parameters like separation factor (α), capacity factor (k) were found to be better than the reported methods. The sensitivity of the method was found to be extremely low compared with reported methods. The method developed in reverse phase mode generated results which are more reproducible compared with the existing method. Similarly, the percentage recovery was found to be better in contrast to reported work. In addition to aforementioned observation, chiral RP-UFLC method is proved to be cost effective also. The developed method was further applied for the determination of the optical purity (*S*-vilda enantiomer) in commercially available Vilda prime® (50 mg tablets).

5. Conclusion

An enhanced and honed chiral UFLC method for determination of enantiomeric purity of *S*-vilda from racemic mixture was developed. The developed method was practically suitable for detection of the eutomer and separation of *S*-vilda at 0.024 µg/mL level. The optimized method was validated according to the ICH guideline and is considered suitable for the industrial application for controlling the optical purity of *S*-vilda formulations.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] McIntosh, C.H., Demuth, H.U., Pospisilik, J.A. and Pederson, R. (2005) Dipeptidyl Peptidase IV Inhibitors: How Do They Work as New Antidiabetic Agents? *Regulatory Peptides*, **128**, 159-165. https://doi.org/10.1016/j.regpep.2004.06.001

[2] Barden, A.T., Piccoli, B.L., Volpato, N.M. and Steppe, M. (2013) A Simultaneous Assay Method Using Capillary Zone Electrophoresis for a Fixed Dose Combination of Vildagliptin and Metformin Hydrochloride in Coated Tablets. *Analytical Methods*, **5**, 5701-5708. https://doi.org/10.1039/c3ay41051j

[3] Abdel-Ghany, M.F., Abdel-Aziz, O., Ayad, M.F. and Tadros, M.M. (2014) Validation of Different Spectrophotometric Methods for Determination of Vildagliptin and Metformin in Binary Mixture. *Spectrochimica Acta Part A*: *Molecular and Biomolecular Spectroscopy*, **125**, 175-182. https://doi.org/10.1016/j.saa.2014.01.055

[4] Barden, A.T., Piccoli, B.L., Volpato, N.M., Schapoval, E.E. and Steppe M. (2014) Capillary Zone Electrophoresis for Determination of Vildagliptin (a DPP-4 Inhibitor) in Pharmaceutical Formulation and Comparative Study with HPLC. *Pharmazie*, **69**, 86-91.

[5] Pednekar, S., Lokhande, R., Sutar, R., Kolhal, S., Surve, S. and Gudekar, S. (2014) Simultaneous Determination of Metformin, Sitagliptin, Saxagliptin, Linagliptin and Vildagliptin in Multicomponent Pharmaceutical Preparations by RP-HPLC. *International Journal of Pharmaceutical Sciences Review and Research*, **28**, 128-133.

[6] ICH-Q2R1 (2005) Validation of Analytical Procedures: Test and Methodology. ICH Steering Committee, 30 rue de St-Jean, 1211 Geneva 13, Switzerland 1-13.

[7] Younes, A.A., Mangelings, D. and Heyden, Y.V. (2012) Chiral Separations in Reversed-Phase Liquid Chromatography: Evaluation of Several Polysaccharide-Based Chiral Stationary Phases for a Separation Strategy Update. *Journal of Chromatography A*, **1269**, 154-167. https://doi.org/10.1016/j.chroma.2012.07.070

[8] Palem, C.R., Goda, S., Dudhipala, N.R., Yamsani, M.R. (2016) Development of Ultra Fast Liquid Chromatography (UFLC) Method for Fluorescence Detection of Domperidone in Human Serum and Application to Pharmacokinetic Study. *American Journal of Analytical Chemistry*, **7**, 12-21. https://doi.org/10.4236/ajac.2016.71002

[9] Rao, Ch.S.N.M., Reddy, G., Mukkanti, K., Suryanarayana, M.V. and Reddy, P.P. (2009) A Validated New Chiral LC Method for the Enantiomeric Separation of Vildagliptin. *Analytical Chemistry*, **8**, 371-375.

[10] Kazsoki, A., Fejős, I., Sohajda, T., Zhou, W., Hu, W., Szente, L. and Béni S. (2016) Development and Validation of a Cyclodextrin-Modified Capillary Electrophoresis Method for the Enantiomeric Separation of Vildagliptin Enantiomers. *Electrophoresis*, **37**, 1318-1325. https://doi.org/10.1002/elps.201500442

[11] Boovizhikannan, T., Palanirajan, V.K. (2013) RP-HPLC Determination of Vildagliptin in Pure and in Tablet Formulation. *Journal of Pharmacy Research*, **7**, 113-116. https://doi.org/10.1016/j.jopr.2013.01.001

[12] Naveed, S., Rehman, H., Qamar, F. and Zainab, S. (2014) Method Development and Validation of Vildagliptin Using UV Spectrophotometer. *International Journal of Pharma Sciences and Research*, **5**, 714-717.