

## BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF VILDAGLIPTIN A NOVEL DIPEPTIDYL PEPTIDASE IV INHIBITOR BY RP-HPLC METHOD

A. B. PHARNE<sup>2</sup>, B. SANTHAKUMARI<sup>1\*</sup>, A. S. GHEMUD<sup>3</sup>, H. K. JAIN<sup>2</sup>, M. J. KULKARNI<sup>1</sup>

<sup>1</sup>Department of Center for Materials Characterization, National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411008, Maharashtra, India, <sup>2</sup>Department of Pharmaceutical Chemistry, CYMET's Siddhant College of Pharmacy, Pune- 412109, Maharashtra, India, <sup>3</sup>Department of Pharmaceutical Chemistry, Sinhgad College of Pharmacy, Vadgaon (BK) Pune, 411041, Maharashtra, India. Email: b.santhakumari@ncl.res.in

Received: 05 Nov 2011, Revised and Accepted: 25 Dec 2011

### ABSTRACT

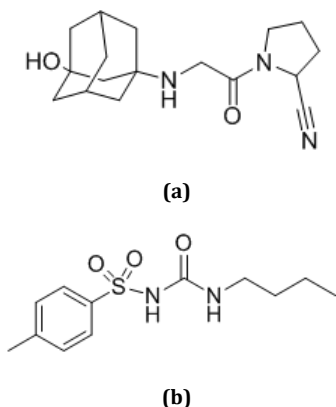
A simple, selective, rapid, precise and economical Reverse-Phase HPLC method has been developed and validated for quantitative determination of vildagliptin in plasma. Vildagliptin is a potent dipeptidyl peptidase IV inhibitor used for treatment of diabetes. Tolbutamide is used as an internal standard. The method was carried out with UV Spectrophotometric detection using a Perkin Elmer Series 200 HPLC system equipped with XBridge Shield C18 column (3.5  $\mu$ m, 4.6 $\times$ 150mm) and a guard column of the same type, at a flow rate of 1.0mL/min. Detection was carried out at 210 nm. The mobile phase consisted of 50mM ammonium bicarbonate (pH 7.8) (solvent A) and acetonitrile (solvent B). The retention times of vildagliptin and tolbutamide were 11.2 min and 13.4 min respectively. The method was developed and tested for linearity range of 10 $\mu$ g/ml to 120 $\mu$ g/ml. The developed method was validated in terms of selectivity, accuracy, precision, linearity, and stability study. The proposed method uses less biological material and the method is MS compatible also. Method can be applicable for pharmacokinetic studies using HPLC or LC-MS.

**Keywords:** HPLC, MS, Vildagliptin, Tolbutamide.

### INTRODUCTION

Methods of measuring drugs in biological media are becoming increasingly important for the study of bioavailability & bioequivalence studies, quantitative evaluation of drugs and their metabolites, new drug development, clinical pharmacokinetics, research in basic biomedical and pharmaceutical sciences and therapeutic drug monitoring <sup>1</sup>.

Vildagliptin fig.1a, chemically (S)-1-[N-(3-hydroxy-1-adamantyl) glycy] pyrrolidine-2-carbonitrile, is a potent dipeptidyl peptidase IV (dip-IV) inhibitor, a drug for the treatment of diabetes. DPP-IV inhibitors represent a new class of oral antihyperglycemic agents to treat patients with type 2 diabetes. DPP IV inhibitors improve fasting and postprandial glycemic control without hypoglycemia or weight gain. Vildagliptin inhibits the inactivation of GLP-1 and GIP by DPP IV, allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas <sup>2-5</sup>. Tolbutamide fig-1b, chemically N-[(butyl amino) carbonyl]-4-methylbenzenesulfonamide, is a first generation potassium channel blocker, sulfonylurea, oral hypoglycemic drug. It is used as an internal standard (IS) <sup>6</sup>.



**Fig. 1: Chemical structure of (a) Vildagliptin and (b) Tolbutamide**

Literature survey revealed that validated HPLC method for the quantification of vildagliptin in plasma is not reported<sup>7-9</sup>. For the

estimation of the drugs present in the biological fluid, HPLC method is considered to be more suitable since this is a powerful and rugged method. It is also extremely specific, linear, precise, accurate, sensitive and rapid. In this study we have developed a mass spectrometry compatible HPLC method with a protein precipitation extraction and improved sensitivity for the determination of vildagliptin in plasma and the developed method is validated as per regulatory requirements.

### MATERIALS AND METHODS

#### Chemicals

Vildagliptin was gifted by Emcure pharmaceuticals Pvt. Ltd. and tolbutamide (IS) was purchased from Sigma Aldrich. HPLC Grade solvents (Acetonitrile, Methanol) were obtained from Merck and milli-Q water was from SG Series Compact Pretreatment Module. AR Grade ammonium bicarbonate was purchased from Sigma Aldrich.

#### Instrument

The Perkin Elmer Series 200 LC system was equipped with a UV detector and autosampler. Chromatographic separations were performed using the XBridge Shield C18 (3.5  $\mu$ m, 4.6 $\times$ 150mm) column and analyzed by LC software Turbochrome Work station.

#### Preparation of solutions

50mM ammonium bicarbonate buffer was prepared by dissolving approximately 1.96 gm of ammonium bicarbonate in 500 ml water and the pH was adjusted to 7.8 with acetic acid.

#### Preparation of standard

Vildagliptin and tolbutamide stock solutions were prepared at a concentration of 1 mg/ml by dissolving in methanol and the stock solutions were stored in the refrigerator. Spiking solutions of vildagliptin for the preparation of calibration standards and quality control samples were prepared in methanol and spiked in to the plasma at the ratio of 1:8. The calibration curve from 10.0 $\mu$ g/ml to 120.0  $\mu$ g/ml was generated using seven calibration standards at the concentrations of 10.0  $\mu$ g/ml (STD 1), 20.0  $\mu$ g/ml (STD 2), 40.0  $\mu$ g/ml (STD 3), 60.0  $\mu$ g/ml (STD 4), 80.0  $\mu$ g/ml (STD 5), 100.0  $\mu$ g/ml (STD 6) and 120.0  $\mu$ g/ml (STD 7). The Quality Control samples were prepared at the concentrations of 10.0  $\mu$ g/ml (LQC), 60.0  $\mu$ g/ml (MQC) and 120.0  $\mu$ g/ml (HQC). The bulk spiked calibration

standards and quality control samples were stored in the freezer. Internal standard dilution was prepared.

### Sample preparation and extraction

Vildagliptin from the plasma was extracted using protein precipitation extraction technique. Blood samples were collected in heparinised tubes and immediately placed on ice and taken to the lab where they were centrifuged at 5000rpm for 5 min at room temperature. The resulting plasma samples were stored at -75°C until analysis. Aliquot of 160µl plasma was taken into eppendorf tubes and added 20µl of internal standard dilution and vortexed to mix the contents. 20µl of above sample is added and vildagliptin is extracted by using methanol as a precipitating solvent. Vortexed for 30sec then the solution was centrifuged at 4°C, 7000rpm for 10 min. The supernatant is taken and transferred to HPLC vials.

### HPLC method

The mobile phase used was 50 mM ammonium bicarbonate (pH 7.8) (phase A) and 100% acetonitrile (Phase B). A gradient method is developed table-1. Before analyses, the mobile phase was filtered through 0.45 µm filter and then degassed ultrasonically for 15 min. For analysis the mobile phase was initially composed of 95% solvent A and held for 1 min. The mobile phase composition was then linearly programmed to 95% solvent B in 20 min and held for 1 min. The mobile phase condition was returned to the starting solvent mixture in 1 min. The system was allowed to equilibrate for 10 min before the next injection. The analyses were conducted at a flow rate of 1.0 ml/min. The eluent was monitored at a wavelength of 210nm for vildagliptin. The total run time was 20 min and injection volume was 20µl.

**Table 1: Optimized chromatographic conditions for analysis of Vildagliptin by RP-HPLC**

Drug	Mobile Phase			Flow Rate (ml/min)	Detection Wavelength (nm)	Injection Volume (µl)	Retention Time (min.)
	Time (min)	Buffer %A	ACN %B				
Vildagliptin	0	95	5	1	210	20	11.2
	5	80	20				
	10	50	50				
	15	20	80				
	17	5	95				
	19	95	5				
	20	95	5				

### Method validation

The method performance was evaluated for selectivity, accuracy, precision, linearity, and robustness, stability during various stress conditions including bench top stability, freeze thaw stability, autosampler stability, stability of stock solutions etc. and recovery<sup>10-13</sup>.

### Linearity

Calibration curves were constructed using linear regression (with weighting of 1/x<sup>2</sup>) within the range of 10 -120µg/ml of vildagliptin.

### Recovery

Recovery of analyte was evaluated by comparing response of vildagliptin in three quality control samples (LQC, MQC and HQC) with the response of vildagliptin in equivalent aqueous solutions.

### Precision and Accuracy

For precision and accuracy studies, samples of three concentration levels were prepared as low (LQC), medium (MQC) and high (HQC) quality controls, corresponding to 10, 60 and 120µg/ml respectively with six replicates each. Precision was evaluated with inter and intra batches.

### Stability studies

The stability of vildagliptin in solutions and plasma samples were evaluated during method validation. vildagliptin stability was evaluated using two concentration levels (low and high quality control, corresponding to 10 and 120µg/ml respectively). The stability of vildagliptin was also evaluated in post extracted samples kept in the autosampler at 4°C 60 hours, as well as in plasma samples kept at freezer and after being stressed to 3 freeze-thawing cycles (24 hours each cycle). All samples described above were compared to freshly prepared vildagliptin samples at the same concentration level.

### Ruggedness

The ruggedness of the method was studied by changing the experimental condition such as,

Changing to another instrument (waters HPLC → Shimadzu HPLC)

Different operation in the same laboratory.

## RESULTS AND DISCUSSION

### Chromatographic Optimization

A RP-HPLC method was developed for vildagliptin, which can be conveniently employed for routine analysis in biological fluids. The chromatographic conditions were optimized in order to provide a good performance of the assay. The mobile phase for drug was selected based on its polarity. Different trials were taken and the final working mobile phases are listed in table 1. The retention times of vildagliptin and tolbutamide were 11.2 and 13.4 min, respectively.

The chromatograms of vildagliptin and vildagliptin with IS have been shown in fig. 2 and 3. The method is validated as per regulatory guidelines.

### Selectivity

The described method used reversed-phase HPLC for separation of vildagliptin from tolbutamide (IS) and was shown to be selective for the analyte and its IS (retention times for vildagliptin and Tolbutamide were 11.2 and 13.4 minutes respectively). No interfering peaks were observed with the same retention time of the analyte when different plasma samples were analysed. Fig. 4 and fig.5 represent the chromatograms of blank plasma sample and plasma sample spiked with drugs respectively.

### Linearity

Linearity was demonstrated from 10.0-120.0µg/ml. fig. 6 shows calibration curve of vildagliptin. The calibration curve includes 7 calibration standards which are distributed throughout the calibration range. Correlation coefficient was demonstrated for the evaluation of goodness fit. The average correlation coefficient was found to be 0.997 with goodness of fit.

### Accuracy and Precision

Accuracy and precision was evaluated by analysing 3 batches. Each batch consists of six replicates of LQC, MQC and HQC. Precision was evaluated both interday and intraday batches. The interday and intraday precision and accuracy of the method for each vildagliptin concentration levels (10.0, 60.0 and 120.0µg/ml) are represented in Table 2. The mean accuracy for each concentration level ranged from 91.79 to 98.83 and the mean precision for each concentration level ranged from 2.91 to 9.69.

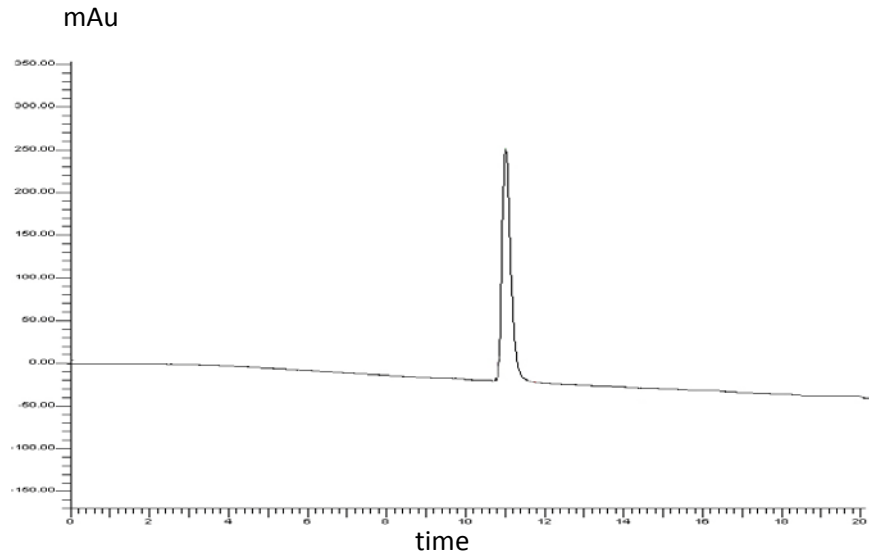


Fig. 2: Typical chromatogram of Vildagliptin (RT-11.2min)

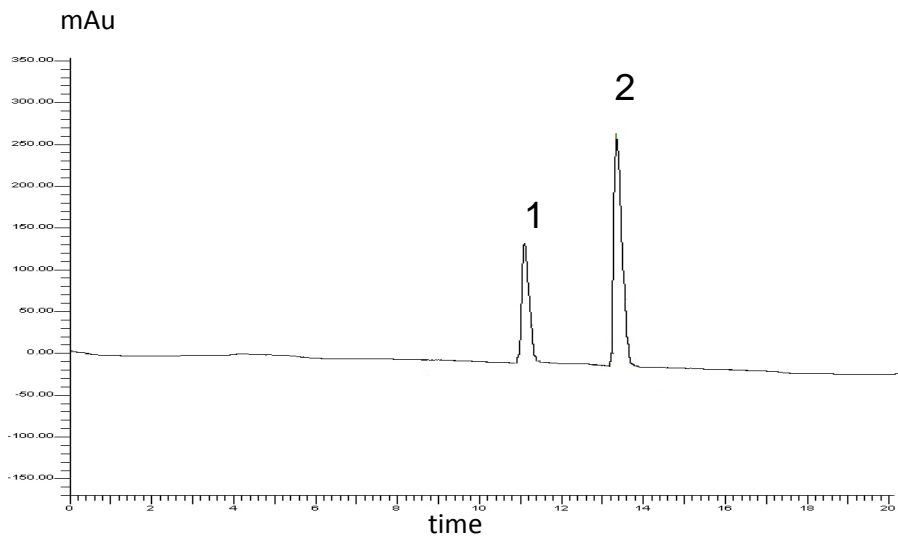


Fig. 3: Typical chromatogram of 1.Vildagliptin, 2.Tolbutamide

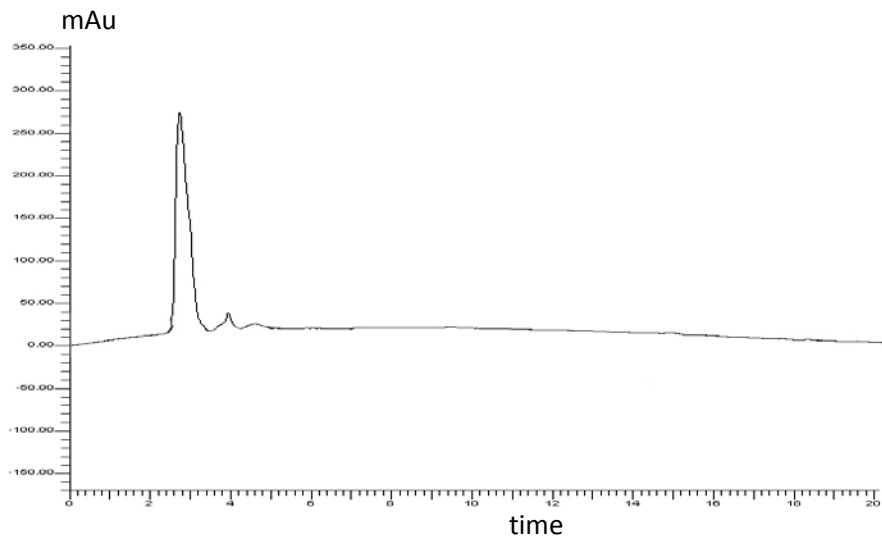


Fig. 4: Typical chromatogram of blank plasma sample

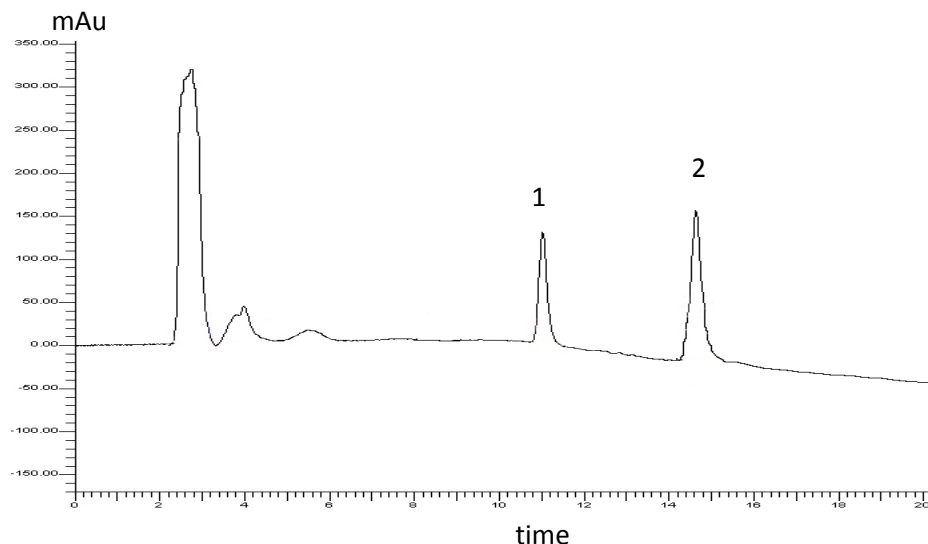


Fig. 5: Chromatogram of blank plasma spiked with (1) Vildagliptin and (2) Tolbutamide (IS)

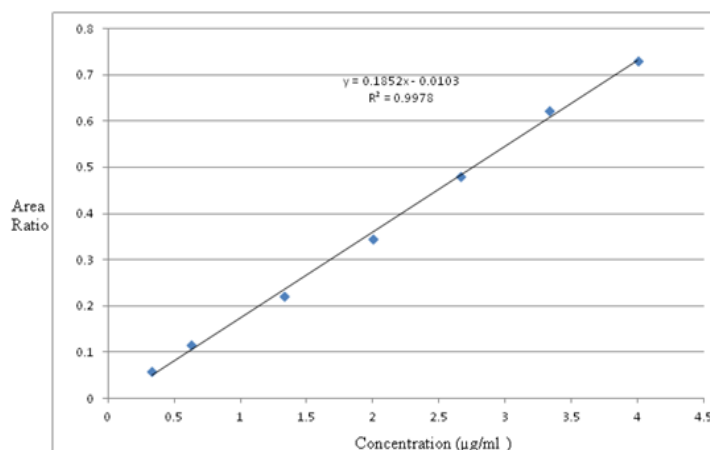


Fig. 6: Calibration Curve of Vildagliptin

Table 2: Intraday and Interday Precision and Accuracy of Vildagliptin

Concentration (µg/ml)	Accuracy (% nominal) (n=6)	Precision (%CV) (n=6)	
		Interday	Intraday
10	98.18	8.18	6.62
60	98.83	7.72	9.69
120	91.79	5.69	2.91

### Recovery

The recovery was evaluated by comparing response of extracted and unextracted samples. Extracted samples include six replicates of extracted LQC, MQC and HQC samples. Unextracted samples included the aqueous solutions equivalent to extracted samples. The average recovery for vildagliptin in plasma was ranged from 85.2 to 88.7% for the low, medium and high quality control samples with an average of 87.4% represented in table 3.

Table 3: Recovery result of Vildagliptin from plasma

Concentration (µg/ml)	Mean Relative Recovery (%) (n=6)
10	88.77
60	85.28
120	88.23

### Stability Studies

Stability studies were performed to evaluate the stability of vildagliptin both in aqueous solution and in plasma after exposing to various stress conditions. The stability studies performed include stock solution stability in stock solution, bench top stability in plasma, freeze thaw stability in plasma, long term storage stability in plasma and auto sampler stability of processed samples. All Stability evaluations were performed as per international regulatory guidelines.

Vildagliptin stock solution (1mg/ml) remained stable when stored at refrigerator conditions for 7 days including the storage at room temperature for 8 hours. Vildagliptin was stable in plasma samples when store at room temperature for 18 hours. Vildagliptin was found to be stable for three freeze and thaw cycles. Vildagliptin is stable in plasma sample for 20 days when stored at -20°C.

Vildagliptin in the processed samples was stable for 60 hours when stored in the autosampler at 4°C.

#### Ruggedness

The ruggedness of the method was carried out by changing the instrument and analysts in the same lab. The percentage CV of the HQC and LQC were found to be 5.5 and 3.3% respectively.

**Table 4: Validation Parameters of Vildagliptin by HPLC method**

Sr. No.	Parameters	Results
1.	Selectivity	Pass
2.	System suitability	Pass
3.	Accuracy and precision	Pass
4.	Linearity	R <sup>2</sup> = 0.997
5.	Recovery	Pass
6.	Short term stock stability	8 Hrs.
7.	Long term stock stability	7days
8.	Long term plasma stability	20 days
8.	Bench top stability	18 Hrs
9.	Freeze thaw stability	Pass (3cycles)
10.	Auto sampler Stability	60 Hrs
11.	Ruggedness	Pass

#### CONCLUSION

The current validated HPLC method for vildagliptin offers good accuracy and significant advantages in terms of sensitivity, selectivity and sample preparation. It can be used for the estimation of vildagliptin in biofluids. The separation method developed produce acceptable values of recovery. The chromatogram developed has well resolved peak of vildagliptin without any interference. Validation parameters of the developed method are given in table 4. From the results we conclude that the developed method can be applied in bioequivalence, pharmacokinetic and toxicokinetic studies with desired precision and accuracy along with high-throughput.

#### ACKNOWLEDGEMENT

Authors are thankful to Emcure pharmaceuticals for providing drug as gift sample.

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