International Journal of Advances in Pharmaceutical Analysis
IJAPA Vol. 3 Issue 2 (2013) 37-41
Journal Home Page http://www.ijapa.ssjournals.com

A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF METFORMIN HCL AND VILDAGLIPTIN IN PHARMACEUTICAL FORMULATION

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Abstract

A selective and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the separation and quantification of metformin HCl (MET) and vildagliptin (VILD) in tablet dosage form. The determination was carried out using phenomenax C18 column (4.6×150 mm) as a stationary phase and mobile phase comprised of phosphate buffer (pH6.0): methanol (65:35v/v). The pH of phosphate buffer is adjusted to 6.0 by using orthophosphoric acid. The flow rate was maintained at 1.0ml/min and the eluent was monitored at 255nm.The retention time of MET and VILD were 1.503 min and 5.530 min respectively. The method was validated in terms of linearity, precision, accuracy, ruggedness, specificity and robustness. The method was linear over the range 50-150 µg/ml for both MET (r = 0.999) and VILD (0.998). For precision studies; RSD for MET and VILD were 0.24 and 0.14 respectively. The percentage recoveries for both drugs from their tablets were 100.16 and 99.98 respectively. Inter-day; intra-day RSD for both drugs were found be 0.27 and 0.26, 0.13 and 0.29 respectively.

***Keywords*:** Metformin HCL; Vildagliptin; RP-HPLC; Tablets

1. Introduction

MET is chemically,1-carbamimidamido-N, N dimethyl methanimidamide Hydrochloride an anti-diabetic drug given by oral route in the treatment of type II diabetes. It is official in Indian Pharmacopoeia, British Pharmacopoeia, European Pharmacopoeia and United States Pharmacopeia BP. All the pharmacopoeias describe HPLC method for estimation of MET. VILD is chemically(S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine 2-carbonitrile used as oral hypoglycemic agent. It is not official in any Pharmacopoeia. A literature survey revealed spectrophotometry[1], HPLC[2,3], LC-MS/MS[4] and LC-electrospray tandem mass spectrometry[5-9], methods for simultaneous estimation of MET in pharmaceutical formulation and Few UV-Spectrophotometric methods[10,11],HPLC[12-15], and ion-pair HPLC[16],other[17,18]. MET 500mg and VILD 50mg in combination are available in market by brand name Galvus Met™ which is an anti-diabetic agent. The structure of a) Metformin HCL and b) Vildagliptin is shown in figure 1.

A literature survey reveals that there are no analytical methods reported for the simultaneous determination of these drugs in combined dosage form. The present study reports a simple, accurate and precise RP-HPLC method for simultaneous determination of Metformin HCl and Vildagliptin in combined tablet dosage form.

2. Material and Methods

2.1 Instrumentation

The separation of MET and VILD was achieved by using HPLC pump Spectra System 600E with Phenomenax C18 {4.6 x 150 mm} column and PDA-996 detector connected to EMPOWER Software was used for the study.

2.2 Reagents and Chemicals

MET and VILD were kindly obtained from Piramal Healthcare limited and Novartis limited respectively. Galvus Met™ tablets 500mg were purchased from the local market. HPLC grade methanol and phosphate buffer were used. Deionized was used throughout the experiment.

2.3 Optimized Chromatographic Conditions

A phenomenax C18 (4.6×150 mm), column was used for the separation of drugs. The mobile phase comprised of methanol and phosphate buffer and in proportion of 35:65(v/v) with pH of phosphate buffer adjusted to (6.0) using orthophosphoric acid. Injection volume was 20µl and run time was 15min and flow rate1.0ml/min. The column was maintained at ambient temperature and the eluent was detected at 288nm. The separation of MET and VILD under optimized condition is shown in Figure 2.

2.4 Preparation of Standard solution:

Standard stock solution (1000µg/ml) of MET and VILD were prepared by mixing in mobile phase comprised of methanol: phosphate buffer 35:65 (v/v). The working standard solutions were prepared and further diluted with mobile phase to contain a mixture of MET and VILD over the linearity range from 10-50 µg/ml and 50-125 µg/ml respectively.

2.5 Preparation of Sample solution:

Twenty tablets were weighed and finely powdered .A quantity of powder equivalent to 500 mg of MET and 50 mg of VILD was weighed and transferred to a 100 ml volumetric standard flask and

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mixed with 50 ml of mobile phase. The sample was kept in an ultrasonic bath for 20 min and volume was made to 100 ml by using mobile phase. Then it was filtered through membrane filter; 10 ml of this solution further diluted to 50 ml with same solvent to get 80µg/ml of MET and 200µg/ml of VILD.

2.6 System suitability

The column efficiency, resolution and peak symmetry were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of this drug combination and the system suitability parameters fall within ±3% standard deviation range during performance of the method. Here tailing factor for peaks of MET and VILD was less than 2% and resolution was satisfactory. The peaks obtained for MET HCl and VILD were sharp and have clear base line separation. The results of system suitability tests are shown in Table 1.

3. Results and Discussion

The chromatographic conditions were optimized to develop RP-HPLC method for simultaneous determination of MET and VILD with adequate resolution and rapid analysis time.

3.1 Method Validation

The developed chromatographic method for simultaneous estimation of MET and VILD was validated according ICH guidelines for linearity, accuracy, precision, specificity, robustness and ruggedness.

3.1.1 Linearity

The response for the detector was determined to be linear over the range of 50-150µg/ml (50, 75, 100, 125, 150) of MET and VILD. Each of this concentration was injected to get reproducible response. The calibration curve was plotted as concentration of the respective drug versus the response at each level. The proposed method was evaluated by its correlation coefficient and intercept value calculated in the statistical study. The results of the linearity studies are shown in Table 2.

3.1.2 Recovery

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out using standard addition method at 50, 100 and 150 % level; known amount of standards was added to reanalyzed sample and subjected them to the proposed HPLC method. Percentage recovery was calculated from the amount found and actual amount added. The mean recovery is within acceptable limits which indicate that the method is accurate .The results of recovery studies are shown in Table 3.

3.1.3 Precision

The precision of an analytical method is expressed in terms of SD or RSD of series of measurements. It was ascertained by replicate estimation of MET and VILD by proposed method. Percentage relative standard deviation (%RSD) was found to be less than 2% which proves that method is precise. The results of precision study are shown in Table 4.

3.1.4 Specificity of the method

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 20 µg/ml was injected into the column, under optimized chromatographic conditions to demonstrate the separation of both MET and VILD from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of tablet formulation. The mean retention time for MET and VILD was found to be 1.503 and 5.530 min respectively.

3.1.5 Ruggedness

The ruggedness studies were performed under different conditions, Inter-day and Intra-day. Inter-day studies were performed on different days, day1; day2 and day3 whereas intra-day study was performed at 3 hrs interval within a day. The results of ruggedness study are shown in Table 5.

3.1.6 Robustness

Robustness of the method was determined by making slight changes in the experimental conditions such as the, pH of the mobile phase, and flow rate of the mobile phase and the chromatographic characteristics were evaluated. It was observed that there were no marked changes in the chromatograms, which demonstrated that, the RP-HPLC method developed, are rugged and robust. The results of robustness study are shown in Table 6.

3.2 Application of the method in tablets

Equal volume (20µL) of working standard solution of MET and VILD and sample solution were injected and chromatograms were recorded and evaluated. The procedure was repeated three times, individually weighing the tablet powder each time. The responses from the standard and sample were used to calculate the amounts of the drug in the tablet. Results obtained are shown in Table 7. The chromatogram showing separation of MET and VILD in tablet formulation is shown in figure 3.

4. Conclusion

The developed RP-HPLC method for simultaneous estimation of MET and VILD in combined tablet dosage forms is simple, sensitive and reproducible. Therefore it can be used in routine for simultaneous estimation of MET and VILD in bulk as well as in pharmaceutical preparations. The various validation characteristics for developed method were studied to find out the suitability of the method. It was found to be valid and suitable for its intended purpose.

Acknowledgment

The Authors extends their thanks to P. Wadhwani College of Pharmacy, Yavatmal for providing necessary facilities for successful completion of this work.

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Table 1. System suitability test results

Sr. No.	Parameters	MET	VILD
1.	Peak area	6722	6109
2.	No. of theoretical plates	3079.01	12034.26
3.	Retention time (min)	1.503	5.530
4.	Asymmetry	1.89	1.95

Table 2. Linearity study data

Sr. No.	Drugs	Slope	Intercept	Correlation Coefficient (R^2)
1	MET	67.025	37.786	0.999
2	VILD	61.847	12.656	0.998

Table 3. Recovery study data

Sr. No.	Drug	% Recovery	%RSD
1	Metformin HCl	100.17	1.20
2	Vildagliptin	99.98	0.19

Table 4. Precision study data

Sr. No.	Wt. of Sample (mg)	Peak area of Std.		Peak area of Std.		% Label Claim	
		MET	VILD	MET	VILD	MET	VILD
1.	635.25	6722	6109	6702	6137	99.70	100.45
2.				6739	6105	100.25	99.93
3.				6725	6123	100.04	100.22
Mean						99.99	100.2
S.D.						0.27	0.26
%RSD						0.27	0.26

Table 5. Ruggedness study data

		MET (% Recoveries)	VILD (% Recoveries)
Intra-day studies	T1	100.07	100.01
	T2	100.1	99.81
	T3	99.86	99.44
	Mean	10.03	99.75
	% RSD	0.13	0.29
		MET (% Recoveries)	VILD (% Recoveries)
Inter-day studies	D1	99.70	100.45
	D2	100.25	99.93
	D3	100.04	100.22
	Mean	99.99	100.2
	%RSD	0.27	0.26

T1,T2 and T3 are Time1,Time2 and Time3 in intra-day studies.
D1,D2 and D3 are Day1,Day2 and Day3 in inter-day studies

Table 6. Robustness study data

System suitability Parameters		% RSD of peak area response (n=3)		Mean tailing factor (n=3)		Mean retention time in min. (n=3)	
Variations		MET	VILD	MET	VILD	MET	VILD
Change in flow Rate	+10	1.74	1.63	1.66	1.43	1.58	1.64
	0	1.21	1.20	1.65	1.59	1.73	1.31
	-10	1.21	1.25	1.32	1.73	1.29	1.74
Change in % Organic phase (Methanol)	+10	1.21	1.65	1.35	1.27	1.23	1.60
	0	1.21	1.20	1.65	1.59	1.77	1.31
	-10	1.04	1.29	1.73	1.22	1.50	1.71
Change in pH	+0.2	1.56	1.78	1.76	1.26	1.80	1.30
	0	1.21	1.20	1.65	1.59	1.77	1.31
	-0.2	1.75	1.36	1.88	1.51	1.45	1.58

Table 7. Results of Tablet formulation study

Sr. No.	Weight of std. (mg)		Weight of sample (mg)	Peak area of std		Peak area of sample		% Label claim	
	MET HCl	VILD		MET HCl	VILD	MET HCl	VILD	MET HCl	VILD
1.	500	50	635.27	6722	6109	6751	6112	100.43	100.04
2.			635.19			6749	6098	100.40	99.81
3.			635.09			6734	6105	100.17	99.93
Mean								100.33	99.92
S.D.								0.14	0.11
% RSD								0.14	0.11

Figure 1. Structure of a) Metformin HCL and b) Vildagliptin

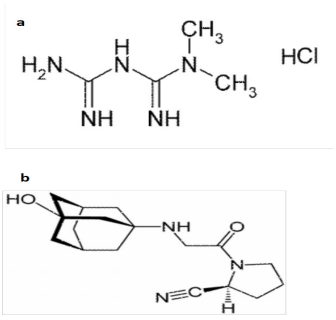

Figure 2. Typical HPLC chromatogram corresponding to mixed standard solution of VILD and MET

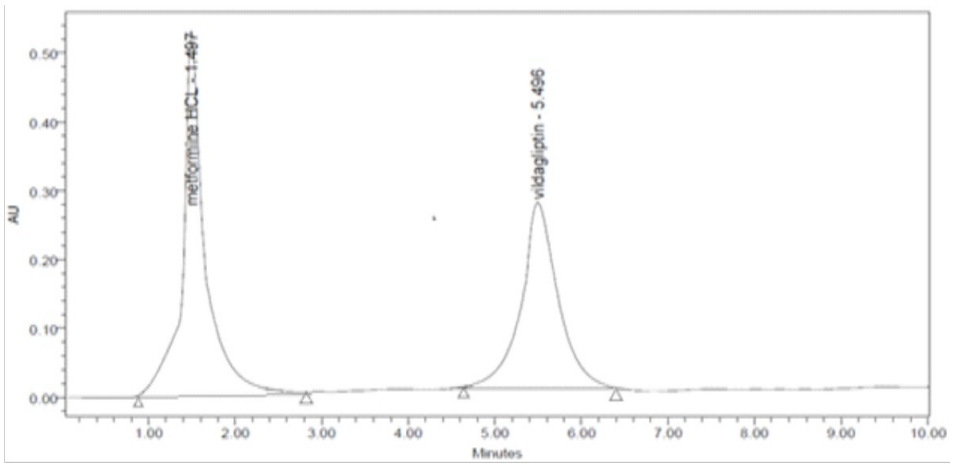

Figure 3. Typical HPLC Chromatogram corresponding to marketed formulation of VILD and MET