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Research Article

Validated method for the simultaneous estimation of Metformin Hydrochloride and Vildagliptin by RP-HPLC in bulk and the pharmaceutical dosage form

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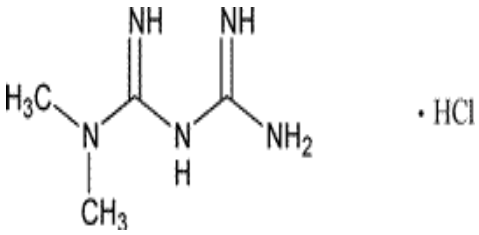
ABSTRACT: A RP-HPLC method was developed and validated for the simultaneous estimation of Metformin Hydrochloride and Vildagliptin in bulk and pharmaceutical dosage form. Chromatography was carried on Dionex C_{18} (250mm x 4.6i.d, 5µm) column with mobile phase comprising of dipotassium hydrogen phosphate(0.01M) buffer and water in the ratio 90:10 v/v. The flow rate was adjusted to 1.5ml/min with UV detection at 215nm. The retention times of Metformin Hydrochloride, Vildagliptin were found to be 2.390 min, 4.601 min respectively. The different analytical parameters such as accuracy, linearity, precision, robustness, limit of detection(LOD), limit of quantification (LOQ) were determined according to the International Conference on Harmonisation (ICH) Q2B guidelines. The detector response was linear in the range of 500-1500µg/ml, 50-150µg/ml for Metformin Hydrochloride, Vildagliptin respectively. In the linearity study, the regression equation and coefficient of correlation for Metformin Hydrochloride, Vildagliptin were found to be (y = 124986x, r^2 = 1),(y = 21377x, r^2 = 0.9999) respectively. The proposed method is highly sensitive, precise and accurate & hence was successfully applied for the reliable quantification of active pharmaceuticals present in the commercial formulations.

KEY WORDS: Metformin hydrochloride, Vildagliptin, RP-HPLC, Simultaneous estimation

INTRODUCTION

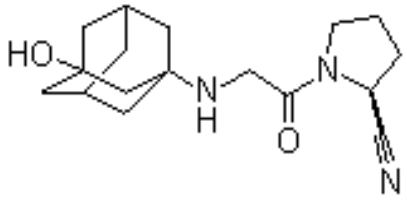
Metformin hydrochloride (MET), an oral anti-diabetic drug which is the first line of choice for the treatment of type 2 diabetes, particularly in overweight or obese peoples and those with normal kidney function. Metformin improves hyperglycemia, primarily through its suppressive action on production of hepatic glucose (hepatic gluconeogenesis). Metformin activates AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats; activation of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cells. MET HCl is known chemically as 3-(diaminomethylidene)-1,1-dimethyl guanidine.



Metformin HCl

Vildagliptin (VDG) is an oral antihyperglycemic of the dipeptidyl peptidase-4 (DPP-4) inhinitor class. It works to competitively inhibit the enzyme DPP-4. This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal. By preventing GLP-1 and GIP inactivation, they are able to increase the

secretion of insulin and suppress the release of glucagon by the pancreas. This drives blood glucose levels towards normal. It is chemically known as 1-[(3-hydroxy-adamant-1-ylamino) acetyl]-pyrrolidine-2(S)-carbonitrile.



Vildagliptin

Literature survey show that there are many methods for the quantitative estimation of Metformin separately and in combination [1-6] with other drugs.

To our knowledge simple and economical analytical method for simultaneous estimation of Metformin HCl and Vildagliptin has not been reported so far. So attempt was taken to develop and validate an economic, rapid reverse-phase high performance liquid chromatographic method for the quality control of Metformin HCl and Vildagliptin in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time. The method was validated as per ICH guidelines[7-8] and found to be accurate, precise and reproducible.

MATERIALS AND METHODS

Apparatus

Waters e2695 Alliance HPLC system connected with PDA Detector 2998 and Empower2 Software. The drug analysis data were acquired and processed using Empower2 software running under Windows XP on a Pentium PC. Pharmaceutical grade Metformin HCl and Vildagliptin were kindly supplied as a gift sample by Dr.Reddys Laboratory, Hyderabad, Andhra Pradesh, India. Methanol was of HPLC grade and collected from E.Merck, Darmstadt, Germany. Dipotassium hydrogen Phosphate were analytical reagent grade supplied by Fischer Scientific Chemicals. Water HPLC grade was obtained from a Milli-QRO purification system.

Commercial Formulation

Metformin HCl and Vildagliptin tablets available in the market as Galvus Met in composition of Metformin HCL (500mg), Vildagliptin (50mg). The samples were properly checked for their manufacturing license numbers, batch numbers, production, expiry dates and stored properly.

Preparation and Selection of Mobile phase

The preliminary isocratic studies on a reverse phase C_{18} column with different mobile phase combination of Dipotassium hydrogen phosphate buffer and methanol were studied for simultaneous estimation of both drugs. The optimal composition of mobile phase determined to be Buffer: Methanol (90:10 v/v) and filtered through 0.45µ membrane filter.

Preparation of standard solution

1000mg Metformin HCl and 100mg Vildagliptin was dissolved in 100ml of Diluent (distilled water) and was further diluted to get stock solution of Metformin HCl (1000µg/ml) and Vildagliptin (100µg/ml). This was taken as a 100% concentration. Solution containing mixture of Metformin HCl and Vildagliptin of five different concentrations (50%, 75%, 100%, 125% and 150% of target concentration) were prepared in the same way.

Preparation of Sample solution

Sample solution containing both the drugs was prepared by dissolving tablet powder into Diluent (Distilled water). Ten tablets were weighed separately. Their average weights were determined. Powder of tablets equivalent to two tablets weight were weighed and taken in a 100ml volumetric flask, dissolved in diluents and shaken and sonicated for about 10 minutes, then filtered through 0.45µ membrane filter. The filtererd solution was further diluted in the diluents to make the final concentration of working sample equivalent to 100% of target concentration.

Chromatographic Conditions

The mobile phase, a mixture of Dipotassium hydrogen buffer and methanol (90:10 v/v) pumped at a flow rate of 1.5ml/min through the column (C_{18} ; 5µ, 4.6 x 250 mm, Dionex ODS) at 40^{o} C. The mobile phase was degassed prior to use under vacuum by

filtration through a 0.45µ membrane filter. Both drugs showed good absorbance at 215 nm, which was selected as wavelength for further analysis.

Development and validation of HPLC method

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of Metformin HCl and Vildagliptin in tablet dosage form. The experiment was carried out according to the official specifications of USP-30, ICH-1996 and Global Quality Guidelines-2002. The method was validated for the parameters like system suitability, specificity, linearity, precision, accuracy, LOD, LOQ and robustness.

System Suitability

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of Metformin HCL and Vildagliptin. Various chromatographic parameters such as retention time, peak area, tailing factor, theoretical plates of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

Specificity

Specificity test determines the effect of excipients on the assay result. To determine the specificity of the method, standard sample of Metformin HCl and Vildagliptin were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another.

Linearity

Linearity of the method was determined by constructing calibration curves.

Standard solutions of Metformin HCl and Vildagliptin of different concentrations level (50%, 75%, 100%, 125% and 150%) were used for this purpose. Each measurement was carried out in 6 replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients.

Accuracy (Recovery Studies)

Accuracy is the percentage of analyte recovered by assay from a known added amount. To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard Metformin HCl and Vildagliptin were added to pre- analyzed samples and were subjected to the proposed HPLC method.

Precision

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (% RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were calculated for the sensitivity of the method. They were quantified based on the signal to noise ratio. LOD is lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated according to ICH guidelines.

$$LOD = 3.3 \times SD/SLOPE$$

$$LOQ = 10 \times SD/SLOPE$$

Robustness of Method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, temperature, on the retention time and tailing factor were studied. The method was found to be unaffected by small changes ± 0.2 change in flow rate and ± 5ºC change in temperature.

RESULTS AND DISCUSSION

Results of system suitability are summarized in Table 1. Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis.

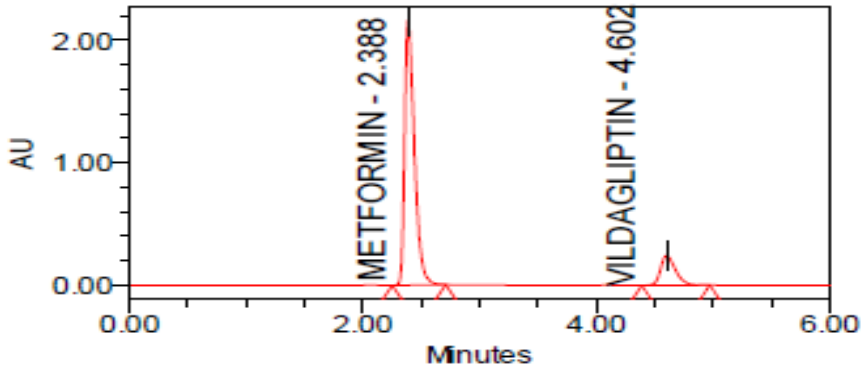


Figure 1: Typical chromatogram of Metformin HCl and Vildagliptin in marketed formulation.

Name	Retention time	Area	USP Tailing	USP Plate Count
METFORMIN HCl	2.388	12490568	1.58	3951
VILDAGLIPTIN	4.602	2137176	1.36	6506

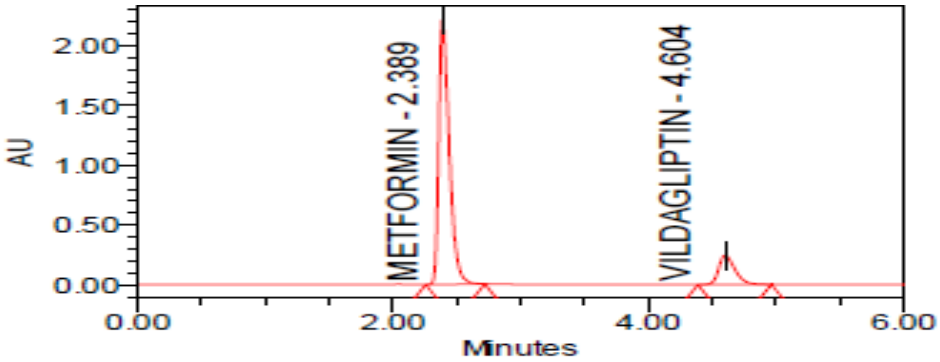


Figure 2 Typical Chromatogram of standard Metformin HCl and Vildagliptin

Name	Retention time	Area	USP Tailing	USP Count	s/n
Metformin HCl	2.390	12523307	1.56	3983	4251
Vildagliptin	4.601	2151496	1.36	6362	477

Table 1: Result of system suitability tests of Metformin HCl and Vildagliptin

Parameters	Metformin Hcl	Vildagliptin
Linearity range	500-1500 µg/ml	50-150 µg/ml
Correlation coefficient	1	0.9999
Slope	12511x-14553	21406x-3234
Retention time	2.390	4.601
USP plate count	3983	6362
Tailing factor	1.56	1.36

Limit of Detection (LOD)	0.7057 µg/ml	0.6289 µg/ml
Limit of quantification(LOQ)	2.3523 µg/ml	2.0964 µg/ml

Chromatograms shown in figure 1 and figure 2 explain that retention time for standard sample and commercial product of Metformin HCl and Vildagliptin are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective. A linear relationship between peak areas (average peak areas of six replicates) versus concentrations was observed for Metformin HCl and Vildagliptin in the range of 50% to 150% of nominal concentration. Correlation coefficient was 1 for Metformin HCl and 0.999 for Vildagliptin which prove that the method is linear. Calibration curve of Vildagliptin and Metformin HCl are shown in Fig 3 and 4.

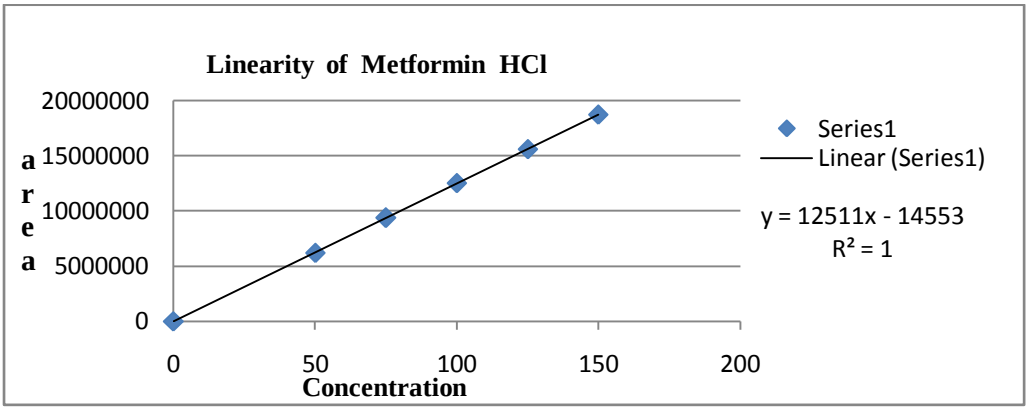


Figure 3 Linearity of Metformin HCl

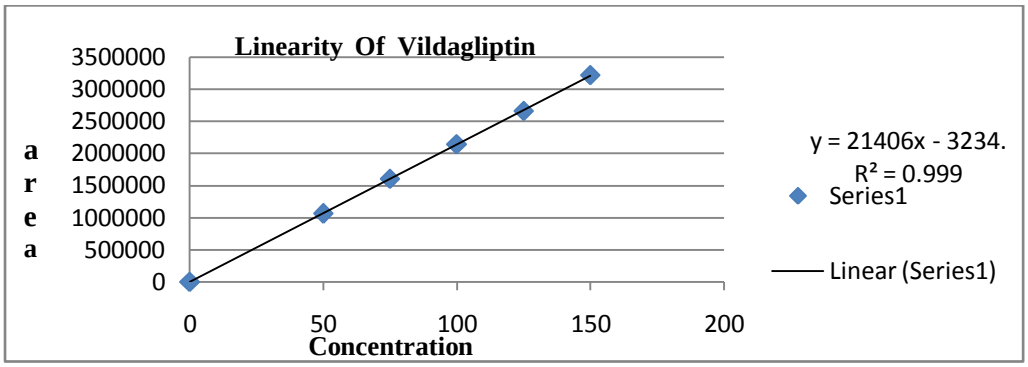


Figure 4 Linearity of Vildagliptin

Table 2: Intra day and inter day precision result of Metformin HCl and Vildagliptin

Drug	%RSD (intra day)	% RSD (inter-day)
Metformin HCl	0.6	1.05
Vildagliptin	0.6	0.99

Table 3: Accuracy (% recovery) results of Metformin HCl and Vildagliptin

Sample No	Metformin HCl			
	Spiked Amount (mg)	**Recovered Amount (mg)**	**% Recovered**	**% Average recovery**
1	25mg	24.75mg	99	98.6%
2	50 mg	49.5 mg	99	
3	75mg	73.5 mg	98	
	Vildagliptin			
1	2.5 mg	2.45 mg	98	98.6 %
2	5 mg	4.95 mg	99	
3	7.5 mg	7.425 mg	99	

Results of Intra day and inter day variability were summarized in table 2. Inta day variability was done from 9.00 am to 6.00 pm on the same day. % RSD of peak areas was calculated for various run.The method is highly precise as % RSD of peak area was less than 1% in all tests.

Results of accuracy study are presented in table 3. The measured value was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount. % Recovery was 98.6% for Metformin HCl and 98.6% for Vildagliptin. All the results indicate that the method is highly accurate.The results of robustness of the present method showed that small changes were made in the flow rate and temperature did not produce significant changes in analytical results which are not significant, we can say that the method is robust.

Table 4: Results for robustness test of Metformin HCl and Vildagliptin

Parameters count	Changes	RT	USP Tailing	USP Plate
Metformin HCl				
Flow rate (ml/min)	1.3	3.181	1.53	5275
	1.7	1.922	1.50	3811
Temperature	35^0 C	2.394	1.60	3867
	45^0 C	2.403	1.60	4195
Vildagliptin				
Flow rate (ml/min)	1.3	6.098	1.41	7434
	1.7	3.753	1.32	6236
Temperature	35^0 C	4.663	1.37	6328
	45^0 C	4.642	1.33	7353

CONCLUSION

The new HPLC method developed and validated for simultaneous estimation of Metformin HCl and Vildagliptin pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

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