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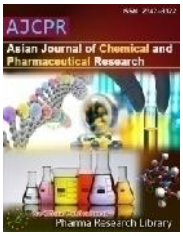

Research Article **Open Access**

Analytical Method Development and Validation for the Simultaneous Estimation of Metformin and Vildagliptin by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Metformin and Vildagliptin by using C_{18} Column (150 mm x 4.6 mm) 5 µm, flow rate was 1ml/min, mobile phase ratio was Methanol: Phosphate buffer P^H 3.0 (70:30 v/v), detection wavelength was 271 nm. The Spectroscopic method was done in solvent using methanol and the instrument lab India 3000+ with UV win software. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, UV detector, Empower-software version 2. The retention times were found to be 1.692 min and 3.344 min. The % purity of Metformin and Vildagliptin was found to be 99.24% and 100.27% respectively. The system suitability parameters for Metformin and Vildagliptin such as theoretical plates and tailing factor were found to be 2993, 1.23 and 5735, 1.12. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Metformin and Vildagliptin was found in the concentration range of 50 ppm-250 ppm and 5ppm -50 ppm and correlation coefficient (r^2) was found to be 0.999 and 0.999 respectively, % recovery was found to be 99.56% and 99.47% respectively. %RSD for repeatability and precision was found to be <2. LOD values were 2.17 and 0.0372 and LOQ value were 6.60 and 0.112 respectively for Metformin and Vildagliptin.

Keywords: Metformin, Vildagliptin, HPLC.

A R T I C L E I N F O

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1. Introduction

Analytical methods

Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available.

Description of the Various Analytical Methods

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements. Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made.

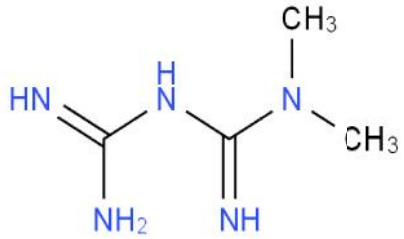

Figure 1: Metformin

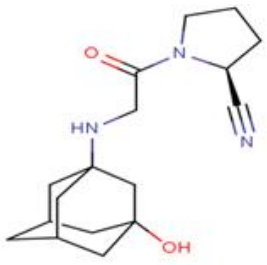

Figure 2: Vildagliptin

Chromatography:

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The Advent of high-performance liquid chromatography (HPLC) in this system pressure is applied to the column, forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC.

2. Materials and Methods

Apparatus: The instrument used for the study was Waters HPLC Auto Sampler, Separation module 2695, UV detector with Empower-software version-2.

Reagents and Materials

The solvents used were Methanol, Ortho phosphoric acid, Acetonitrile, Potassium dihydrogen ortho phosphate, Dipotassium hydrogen phosphate, Tri Ethyl Amine of HPLC Grade and HPLC Water**.**

Selection of detection wavelength:

The sensitivity of method that uses UV- Vis detector depends upon the proper selection of wavelength. An ideal wavelength is that gives maximum absorbance and good response for both the drugs to be detected. Standard solutions of Metformin and Vildagliptin were scanned in the UV range (200-400nm) and the spectrums obtained were overlaid and the overlain spectrum was recorded. From the overlain spectrum, 271 nm was selected as the detection wavelength for the present study.

Selection of mobile phase

Initially the mobile phase tried was Methanol and water, Methanol, Buffer and water in various proportions. Finally, the mobile phase was optimized to Buffer: Methanol in proportion 30:70 v/v respectively at pH 3 adjusted with Orthophosphoric Acid.

Chromatographic trials for Simultaneous Estimation of Metformin and Vildagliptin by RP- HPLC.

Trial-1 Chromatographic conditions

Column	: symmetry C18 4.6x150mm 5 µm
Mobile phase ratio	: MeOH: H_2O (50:50%v/v)
Detection wavelength:	236nm
Flow rate	: 1 ml/min
Injection volume	: 10 µl
Run time	: 10 min
Retention time	: 3.217 min & 3.831 min

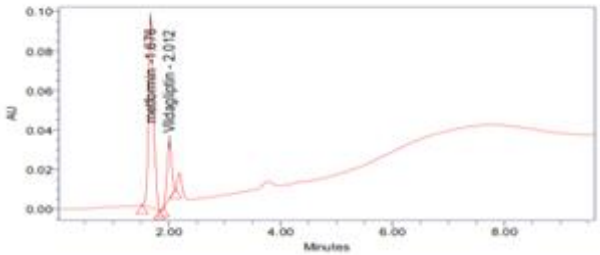

Figure 3: Chromatogram of Trial-1

Observation: The trial doesn't show any peaks in the chromatogram, so more trials were required for obtaining peaks.

Trial-2 Chromatographic condition

Column : Zodiacsil C18 4.6x150mm 5µm
Mobile phase ratio : ACN: H_2O (50:50%v/v)
Detection wavelength : 271nm
Flow rate : 1ml/min
Injection volume : 20µl
Run time : 8.0 min
Retention time : 2.35 min &4.235 mins

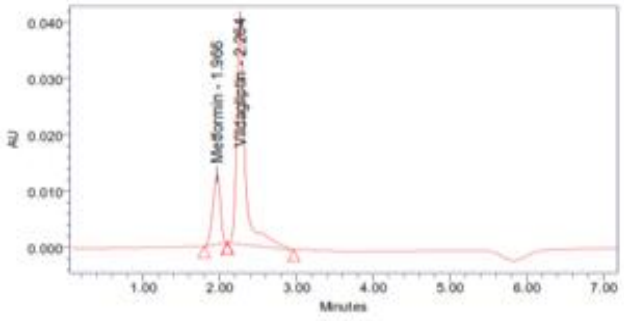

Figure 4: Chromatogram of trial-2

Observation: In this trial peaks are not eluted properly, more trials are required.

Trial-3 Chromatographic condition

Column : Hypersil RPC8 4.5×150mm 5.0 µm
Mobile phase ratio : ACN: pH 6.8 buffer (50:50 % v/v)
Detection wavelength: 271nm
Flow rate : 1.0ml/min
Injection volume : 20µl
Run time : 6.0mins
Retention time : 4.248, 4.537 mins

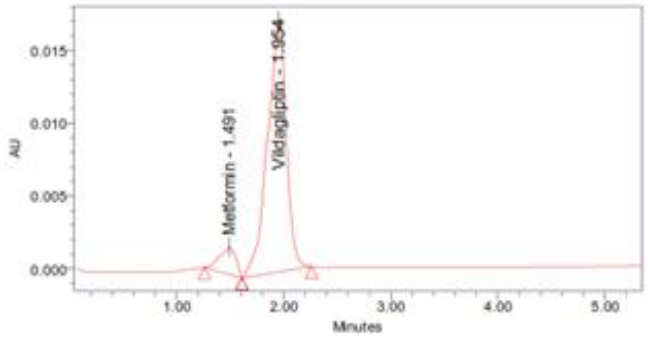

Figure 5: Chromatogram of trial-3

Observation: In this trial both Metformin and Vildagliptin were eluted but there is no proper resolution. Still more trials were required for better resolution in peaks.

Trial -4 Chromatographic conditions (Optimised Method):

Column : zodiac sil RP C18 4.6×250mm 3.0µm
Mobile phase ratio: Methanol: pH 3 buffer (70: 30 % v/v)
Detection wavelength: 271nm
Flow rate : 1.0ml/min
Injection volume : 10µl
Run time : 10min
Retention time : 1.694&3.344 mins

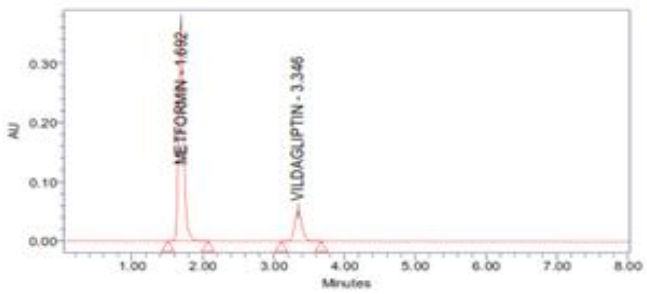

Figure 6: Chromatogram of trial-4 (Optimized Method)

Observation: The separation was good, peak shape was good, so we conclude that no trials required for separation.

Procedure

Preparation of phosphate buffer

2.95 grams of KH_2PO_4 and 5.45 grams of K_2HPO_4 was weighed and taken into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water and pH was adjusted to 3 with Orthophosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 300 ml (30%) and 700 ml of methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration. Mobile phase was used as the diluent.

Metformin and Vildagliptin standard preparations

10 mg of Metformin and 1 mg of Vildagliptin working standards was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent

Sample solutions preparation: 10 mg of Metformin and 1 mg of Vildagliptin tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2 ml of diluent and sonicated to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10 ml of the above stock solution into a 100 ml volumetric flask and was diluted up to the mark with diluent.

3. Results and discussion

Method Validation Parameters

1. Specificity: The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by Injecting blank

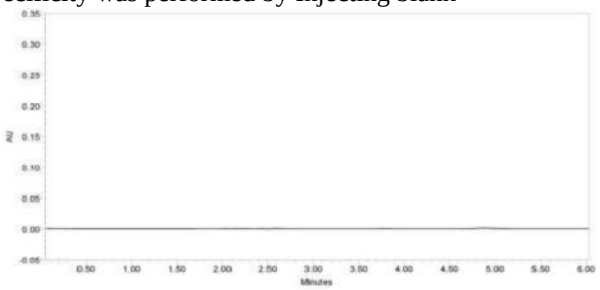

Figure 7: Chromatogram of Blank

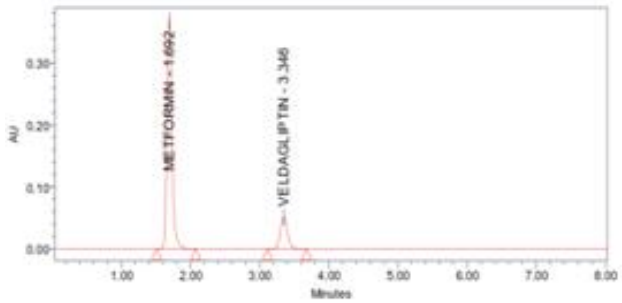

Figure 8: Chromatogram of Sample

2. Linearity: The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.Serial dilutions of Metformin and Vildagliptin (50-250 ppm and 5-25 ppm) were injected into the column and detected at a wavelength set at 271 nm. The calibration curve was obtained by plotting the concentration vs. peak area.

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 50-250 ppm and 5-25 ppm for Metformin and Vildagliptin respectively

4. Accuracy

Accuracy of the method was determined by recovery experiments. There are mainly 2 types of recovery studies are there.

a) Standard addition method: To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.
b) Percentage method: For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively.

Acceptance criteria: The mean % recovery of the Metformin and Vildagliptin at each level should be not less than 95.0% and not more than 105.0%.

Assay procedure

10µL of the standard and sample solutions of Metformin and Vildagliptin were injected into the HPLC system and the chromatograms were recorded. Amount of drug present in the Tablets were calculated using the peak areas.

5. Precision

Method precision also called as repeatability/Intra-day precision indicates whether a method gives consistent results for a single batch. Method precision was demonstrated by preparing six test solutions at 100% concentration as per the test procedure & recording the chromatograms of six test solutions. The % RSD of peak areas of six samples was calculated. The method precision was performed on Metformin and Vildagliptin formulation.

Acceptance criteria: The % RSD for the area of sample injections results should not be more than 2.

Selection of solvent

Solutions of Metformin and Vildagliptin were prepared by dissolving in mobile phase and UV spectrum of each was recorded by scanning between 200-400 nm.

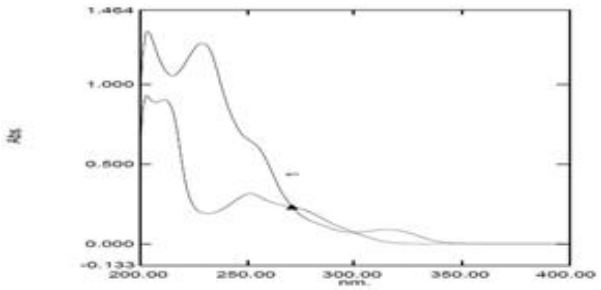

Figure 9: Overlain Spectra of Metformin and Vildagliptin

Validation of the method

Linearity

Metformin and Timolol Maleate: Serial dilutions of Metformin and Vildagliptin (50-250 ppm and 5-25 ppm) were injected into the column and detected at a wavelength set at 271 nm. The calibration curve was obtained by plotting the concentration vs. peak area and the correlation coefficient was found to be 0.999 and 0.999 respectively.

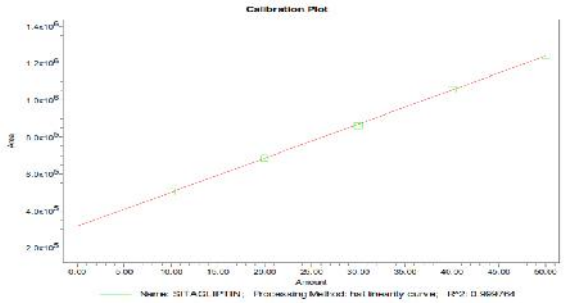

Figure 10: Calibration graph of Vildagliptin

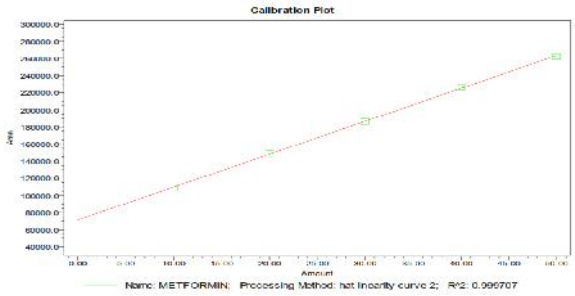

Figure 11: Calibration graph of Metformin

Table 1: Calibration data of Metformin and Vildagliptin

Peak Name: METFORMIN

	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	METFORMIN	1.689	1274954	264879	3109.6	1.2
2	METFORMIN	1.691	1548753	325904	3151.3	1.2
3	METFORMIN	1.692	1790563	381389	3111.1	1.2
4	METFORMIN	1.689	2045498	402853	3060.7	1.2
5	METFORMIN	1.688	2272948	466405	3034.3	1.3
Mean			1773747.3		3093.4	1.2
Std. Dev.			361086.8			
% RSD			21.5			

Peak Name: VILDAGLIPTIN

	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	USP Resolution
1	VILDAGLIPTIN	3.303	257309	39680	5873.9	1.1	10.9
2	VILDAGLIPTIN	3.299	321497	48431	5780.7	1.1	10.7
3	VILDAGLIPTIN	3.294	380369	57171	5696.2	1.1	10.6
4	VILDAGLIPTIN	3.290	418105	62980	5631.1	1.1	10.6
5	VILDAGLIPTIN	3.288	470352	69863	5631.3	1.1	10.7
Mean			369546.4		5823.0	1.1	10.7
Std. Dev.			82964.0				
% RSD			22.5				

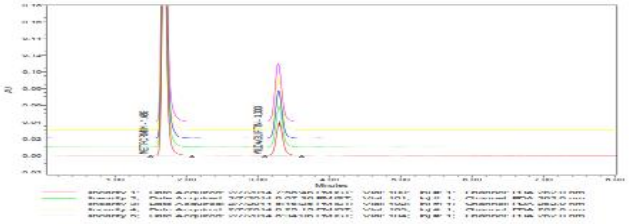

Figure 12: Overlay Chromatogram of Linearity

Recovery studies

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. To an equivalent quantity of formulation powder a known quantity of standard Metformin and Vildagliptin were added at 50%, 100% and 150% level and the contents were re-analyzed by the proposed method.

Table 2: Showing accuracy results for Metformin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	7371253	5	4.96	99.91%	99.56%
100%	14634226.7	10	9.98	99.18%	
150%	2243270.7	15	15.02	99.60%	

Table 3: Showing accuracy results for Vildagliptin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	484733	0.5	0.99	99.53%	99.47%
100%	967998	1.0	1.05	99.38%	
150%	145437	1.5	1.495	99.52%	

Table 4: System Suitability Results for Metformin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	2590	1.39
2	1	2294	1.27
3	1.2	2146	1.26

Table 5: System Suitability Results for Vildagliptin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5435	1.04
2	1	4891	1.03
3	1.2	4781	1.04

Table 6: Precision

Peak Name: metformin

	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	metformin	1.691	1819456	377420	3038.7	1.2
2	metformin	1.691	1822446	374222	3019.0	1.3
3	metformin	1.691	1824679	376000	2997.8	1.3
4	metformin	1.693	1825211	371345	2932.8	1.2
5	metformin	1.690	1826102	384153	3162.5	1.2
Mean			1823578.7		3030.2	1.3
Std. Dev.			2670.1			
% RSD			0.1			

Peak Name: vildagliptin

	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	USP Resolution
1	vildagliptin	3.308	339557	54848	6445.0	1.2	10.9
2	vildagliptin	3.319	351364	54315	6047.3	1.2	10.8
3	vildagliptin	3.314	359377	55298	5992.5	1.1	10.8
4	vildagliptin	3.328	361817	54713	5795.0	1.1	11.0
5	vildagliptin	3.335	368227	54247	5554.4	1.1	10.7
Mean			356068.6		5966.9	1.1	10.8
Std. Dev.			11029.2				
% RSD			3.1				

Table 7: LOD and LOQ

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Metformin	371827.90	563365963	2.17
Vildagliptin	5401.60	479884400	0.0372

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Metformin	371827.90	563365963	6.60
Vildagliptin	5401.60	479884400	0.112

4. Conclusion

A new method was established for simultaneous estimation of Metformin and Vildagliptin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Metformin and Vildagliptin by using Agilent C18 column (4.6×150 mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Buffer, detection wavelength was 271 nm. Precision and

recovery studies were also found to be with the range. The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Metformin and Vildagliptin in pharmaceutical dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims.

Hence, the suggested RP-HPLC method can be used for routine analysis of Metformin and Vildagliptin in API and Pharmaceutical dosage form.

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