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Research Article

Development and Validation of RP-HPLC method for Simultaneous Determination of Vildagliptin and Metformin in Bulk and Formulation Dosage

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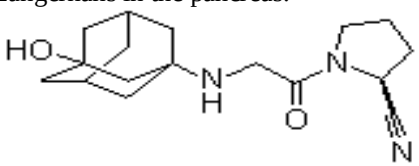
ABSTRACT
A reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of Vildagliptin and Metformin in Tablet dosage form. The determination was performed by the using of two phases one is stationary phase it's a Thermo hypersil ODS C18 column having 250 x 4.6mm 5µ, and another one is mobile phase containing 0.1M Potassium hydro phosphate and Acetonitrile at the ratio (60:40%v/v) Adjust the pH:7.0 by using Ortho phosphoric acid. The flow rate was 1ml/min and effluents were monitored at 263nm. The retention time of Metformin and Vildagliptin was 2.1min and 3.5min respectively and other replicate standard system suitability parameters are within the limit and uniform. Validation parameters those are selectivity, linearity (correlation co efficient is 1.000), recovery of Vildagliptin 99.66% and metformin 101.66 as per USP accuracy acceptance criteria is 97% to 103%, precision % RSD is less than 1 and also robustness results were uniform they were no marked changes so method is highly validated it use full for pharmaceutical analysis like Quality control , Stability and other studies.
Key words: RP-HPLC, Vildagliptin , Metformin HCl.

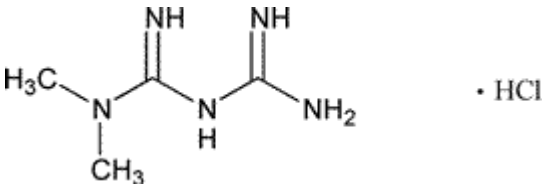
INTRODUCTION

Vildagliptin chemically (S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] 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. [1-4]



Vildagliptin

Metformin hydrochloride (MET) chemically *N*,*N*-dimethylimidodicarbonimidic diamide hydrochloride) is an orally administered biguanide widely used in the treatment of type 2 (non-insulin dependent) diabetes mellitus.[5,6] It improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis commonly found with its analogue, phenformin. MET is a hydrophilic drug with an oral bioavailability of 50–60% and a relatively short half-life of 1.5–4.5 h. [7,8]



Metformin HCl

MET has been reported to be determined by UV Spectroscopy [9], HPLC [10], HPTLC [11] and LC/MS [12] from formulations and Vildagliptin has reported UV spectroscopic [13], RP-HPLC [14], RP-LC/MS [15] from different formulation and composition.
Simultaneous determination of Vildagliptin and Metformin in pharmaceutical dosage forms was reported by HPLC. However, there is no method

available for the simultaneous determination by RP-HPLC. Therefore, an attempt was made to develop a new, rapid, and sensitive method for the simultaneous determination of Vildagliptin and Metformin. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH norm.

MATERIAL AND METHODS

Drugs and Instruments: Waters e2695 Alliance HPLC system connected with PDA Detector 2998 and Empower2 Software. A giftsample given by Novartis limited , Hyderabad, Andhra Pradesh. Formulation tablets were purchased from PIZER (USA). Potassium hydrogen phosphate and Ortho phosphoric acid those are A.R grade purchased form Fisher scientific chemicals, Mumbai. Acetonitrile and methanol were purchased form Ranbaxy Pvt. limited , Delhi, India. Water for HPLC was purchased from Fisher scientific, Mumbai.

Standard preparation: Accurately weighed 5 mg Vildagliptin and 500 mg metformin is transfer into 100 ml volumetric flask and make up with diluent sonic ate for 15 minutes and filter through the 0. 45µm filter paper.

Sample preparation: Accuratly weighed 10 tablets and calculate average weight of those tablets and crushed with motor . To take tablets powder equal to single tablet weight and transfer into 100 ml volumetric flask add 30 ml diluents and sonicaet for 15 minutes . Then filter through the 0.45µm filter paper and make up with diluent. Forther concentrations add diluents as per test method.

Chromatographic Conditions: Mobile phase ratio 60:40, coumn c18 thermo hypercil ods, flow rate 1ml/min, sample temperature 25ºc, column temperature 45ºc and wave length 263 nm.

System Suitability: System suitability is performed by six replicate standards inject into HPLC. It can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The USP defines parameters that can be used to determine the system suitability prior to analysis. These parameters are retention time, plate count, resolution, tailing and %RSD.

Selectivity : Selectivity of the method was carried out by standards of Vildagliptin and metformin were inject into HPLC after that commercial product and placebo, excipients are one after one . It determines interference excipients peaks with analyte peaks.

Linearity : Method linearity was deter mind by prepare five replicate standard solutions of those drugs in different (50%,100%,150%)concentration levels were inject in to the HPLC . plot the graph standard area verses concentration levels .

Accuracy (Recovery Studies): Recovery studies were carried out by prepare triplicate standard solutions in 50%, 100%, 150% concentration levels and pre analyse the known amount of samples.

Precision : Method precision was performed by prepare six replicate samples from single formulation and inject into HPLC at the same manner after 24 hours or day to day variation prepare six replicate samples from same formulation and inject into HPLC observe uniformity of test result and calculate the %RSD.

Robustness: Method robustness was determined by the small changes in chromatographic conditions like as 0.2ml flow rate and ±5ºc temperature and inject the sample observe the result there were no marked changes compare to other analysis.

RESULT AND DISCUSSION

System suitability parameters of standard 1 and standard 2 five replicate injection results are given below table1 and 2 also chromatogram figure 1. Those result all are within the limit and also uniform % RSD is 0.5 so it proves method is suitable for analysis.

Result of selectivity was proved by the figure 1 and 2. These figures are standard chromatogram of Vildagliptin and Metformin second one is market formulation of Vildagliptin and Metformin they were not observed excipients and placebo peaks interference with analyte peaks so method is highly selective. Linearity of the results were given tables 3,4 and calibration curves are shown figures 3,4. Three different concentration levels of six replicate samples area was very linear and correlation coefficient was 1.000 it proves method is linear. Method accuracy results of Vildagliptin and metformin are given table 5 and 6. Three spiked level (50%,100%,150%) known amount of drugs were compare to recovery amount. %

recovery was Vildagliptin 99.66% and metformin 101.66% as per ICH acceptance criteria of accuracy was 97%TO103% so it proves method is highly accurate. Intermediate precision of Vildagliptin and metformin results were presented in table7. Inter day and intraday of those runs parameters like retention time , tailing , resolution and plate count all are uniform and area %RSD was less than 1. Robustness results were given table 8.They were no significant changes observed at deliberate changes in temperature and flow rate trails then method is robust .

CONCLUSION

We had run various trail runs at different chromatographic conditions finally we founded the above conditions are suitable for development and validation for simultaneous estimation of Vildagliptin and metformin in bulk and formulation dosage forms. This HPLC new method was very simple and accurate and also we observed validation parameters all are within the limit and % RSD is very low so it will be use full for routine analysis of quality control , stability and further studies.

Figure-1: Chromatograme of metformin and vildagliptin in market formulation

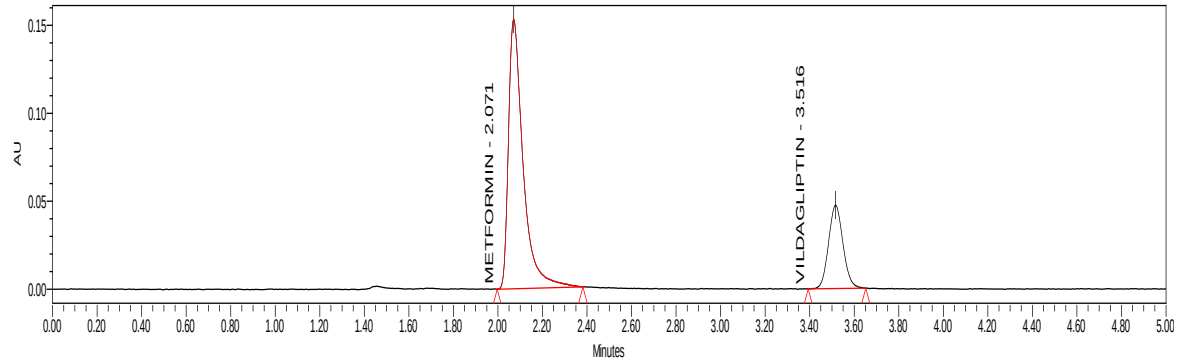


Figure-2: Standard chromatogram of Metformin and Vildagliptin

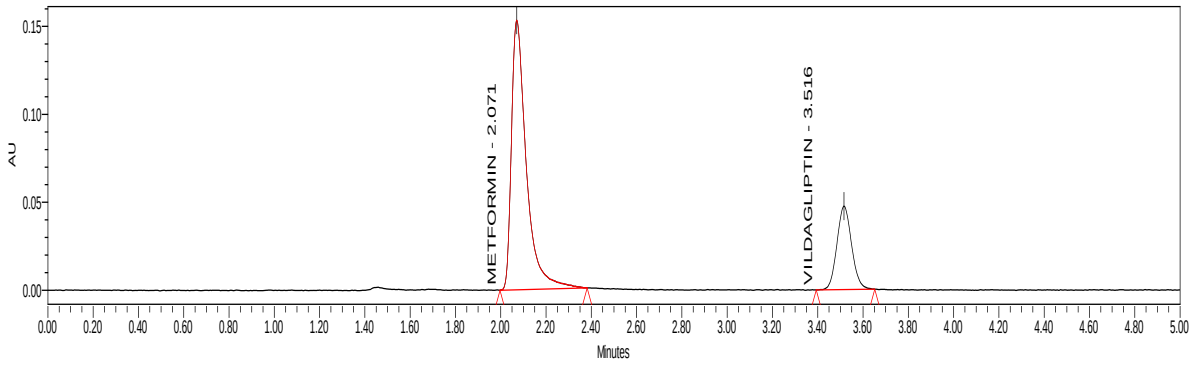


Table-1: Standard 1 results

Name	RT	Area	Resolution	Tailing	Plate count
Metformin	2.070	690089		1.814	5444
Vildagliptin	3.515	2166225	12.180	1.144	14042

Table-2 for STD2 results

S.NO	Sample name	**Name**	RT	**Area**	Resolution	**Tailing**	**Plate Count**
1	STD2	Metformin	2.071	703012		1.822	5438
2	STD2	Metformin	2.072	706467		1.849	5376
3	STD2	Metformin	2.072	708310		1.857	5395
4	STD2	Metformin	2.073	700348		1.875	5380
5	STD2	Metformin	2.074	705040		1.871	5318
Mean				705040			
Std.Dev				3277			
%RSD				0.5			

S.NO	Sample name	**Name**	RT	**Area**	**Resolution**	**Tailing**	**Plate Count**
1	STD2	Vildagliptin	3.516	217978	12.160	1.109	14013
2	STD2	Vildagliptin	3.516	218218	12.083	1.097	13784
3	STD2	Vildagliptin	3.518	218035	12.192	1.113	13974
4	STD2	Vildagliptin	3.518	216698	12.145	1.117	14074
5	STD2	Vildagliptin	3.517	218382	12.091	1.123	13956
Mean				217862			
Std.Dev				670			
%RSD				0.3			

Table-3: Linearity results of Vildagliptin

S.NO	Conc.	Area
1	0	0
2	50	106331
3	75	158637
4	100	210444
5	125	263037
6	150	315605

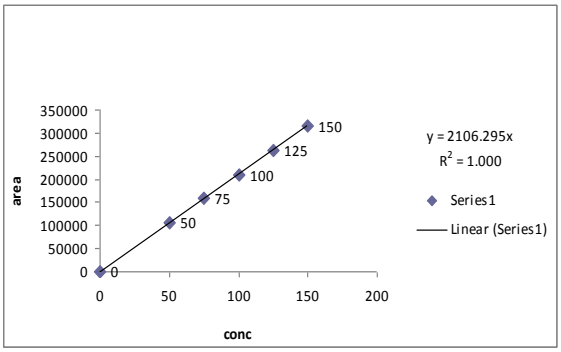


Figure-3: Calibration curve of Vildagliptin

Table-4 : Linearity results of metformin

S.NO	Conc.	Area
1	0	0
2	50	352104
3	75	526230
4	100	701594
5	125	876102
6	150	1057498

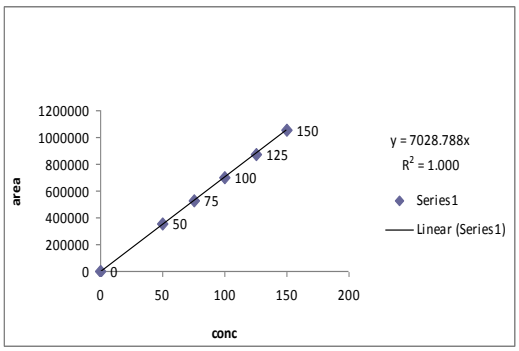


Figure-4: Calibration curve of metformin

Table-5: Accuracy result of metformin

METFORMIN						
Spiked level	Sample Weight	Sample Area	µg/mL added	µg/mL found	%Recovery	Mean
50%	331.00	396394	1076.683	1113.21	1033	103
50%	332.00	391342	1079.936	1099.03	102	
50%	331.00	394258	1076.683	1107.21	103	
50%	331.00	393006	1076.683	1103.70	103	
50%	331.00	394514	1076.683	1107.93	103	
50%	610.0	390436	1076.683	1096.48	102	
100%	610.0	702504	1984.219	1972.88	99	100
100%	610.0	708267	1084.219	1989.06	100	
100%	915.00	709264	1084.219	1991.86	100	
150%	910.00	1072677	2976.329	3012.45	101	102
150%	913.00	1070892	2960.064	3007.44	102	
150%	910.00	1072086	2969.823	3010.79	101	
150%	910.00	1079526	2960.064	3031.69	102	
150%	910.00	1079001	2960.064	3030.21	102	
150%	910.00	1079401	2960.064	3031.34	102	

Table -6: Accuracy result of Vildagliptin

VILDAGLIPTIN				
Sample AREA	µg/mL added	µg/mL found	%Recovery	Mean
113564	105.536	103.21	98	98
113123	105.855	102.81	97	
113540	105.536	103.19	98	
113148	105.536	102.83	97	
113249	105.536	102.92	98	
113589	105.536	103.23	98	
212970	194.493	193.55	100	100
213553	194.493	194.08	100	
212469	194.493	193.10	99	
323271	291.739	293.80	101	101
321060	290.145	291.79	101	
326158	291.101	296.42	102	
321513	290.145	292.20	101	
323519	290.145	294.02	10	
315379	290.145	286.63	99	

Table-7: result of Vildagliptin and metformin intermediate precision

Drug	%RSD (intra-day)	%RSD (inter-day)
METFORMIN	0.90	0.54
VILDAGLIPTIN	0.86	0.37

Table-8: result of Vildagliptin and metformin robustness

Parameters count	Changes	RT	USP Tailing	USP Plate
METFORMIN				
Flow rate(ml/min)	0.8	2.604	1.982	5182
	1.2	1.745	1.895	5149
Temperature	40	2.089	1.941	5147
	50	2.082	1.949	5064
VILDAGLIPTIN				
Flow rate(ml/min)	0.8	4.398	1.150	14868
	1.2	2.942	1.130	14345
Temperature	40	3.525	1.150	14868
	50	3.504	1.130	14778

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REFERENCES

1. McIntosh CH, Demuth HU, Pospisilik JA, Pederson R., Dipeptidyl peptidase IV inhibitors. How do they work as new antidiabetic agents? *Regul Pept.* **2005**, 159–165.
2. Weber AE. Dipeptidyl peptidase IV inhibitors for the treatment of diabetes. *J Med Chem.* **2004**, 47, 4135–4141.
3. Lauster CD, McKaveney TP, Muench SV. Levalbuterol: A novel oral therapy for type 2 diabetes mellitus. *Am J Health Syst Pharm.* **2007**,64, 1265–1273.
4. Handan He, Phi Tran, Hequn Yin, Harold Smith, Yannick Batard, Lai Wang, Heidi Einolf, Helen Gu, James B. Mangold, Volker Fischer and Dan Howard. Absorption, Metabolism, and Excretion of [14C] Vildagliptin, a Novel Dipeptidyl Peptidase 4 Inhibitor, in Humans, *Drug Metabolism and Disposition,* **2009**, 37, 536-544.
5. Cai-Li Z, Jian-Jie J, Yan-Na W, Feng-Rui Y, Jun-Qui S, Jian-Shi L. Study on pharmacokinetics and bioawailability of metformin hydrochloride sustained release tablet in healthy Chinese volunteers. Asian. *J. Pharmacodyn. Pharmacokinet.* **2010**;10: 221-228.
6. Obaid A, Roohi O, and Syed WH. Hypoglycemic potential of tablet metformin 500mg (Glucophage & Metphage): A pharmacological end point evaluation. *Pak. J. Pharm*. Sci. **2003**;6: 29-41.
7. S.C. Sweetmann, Ed. Martindal: The Complete Drug Reference, 33rd Ed. The Pharmaceutical Press, London, UK, **2002**, pp. 322.
8. K.D. Tripathi. Essentials of Medical Pharmacology, 4th Ed. Jaypee Brothers Medical, New Delhi, India, **1999**, pp. 276–83.
9. Dhabale PN, Seervi CR. Simultaneous UV Spectrophotometric Method for Estimation of Gliclazide and Metformin Hydrochloride in Tablets Dosage Form. *Int. J. ChemTech Res.* **2010**; 2: 813-817.
10. Mousumi K, Choudhury PK. HPLC Method for the estimation of Metformin Hydrochloride in Formulated Microspheres and Tablet Dosage Form. *Indian. J. Pharm. Sci.* **2009**; 71: 318-320.
11. Ghassempour A, Ahmali M, Ebrahimi SN, Aboul-Enein HY. Simulataneous Determination of Metformin and Glyburide in Tablets by HPTLC. *Chromatographia* **2006**; 64: 101-104.
12. Xiaoyan C, Qi-Gu, Freng Q, Dafang Z. Rapid determination of metformin in human plasma by liquid chromatography-tandem mass spectroscopy method. *J. Chromatogr*. B **2004**; 802: 377-381.
13. Ramzia I. El-Bagary, Ehab F. Elkady, Bassam M. Ayoub, Spectrophotometric Methods for the Determination of Sitagliptin and Vildagliptin in

Bulk and Dosage Forms, *International journal of Biomedical science*, **2011**, 7(1): 55-61.

14. A. B. Pharne, B. Santhakumari, A. S. Ghemud, H. K. Jain, M. J. Kulkarni, *International Journal of Pharmacy and Pharmaceutical Sciences*, **2012**, 4(1): 119-123.
15. Amanda Thomas Barden, Bárbara Salamon, Elfrides Eva Sherman Schapoval and Martin Steppe, Stability-Indicating RP-LC Method for the Determination of Vildagliptin and Mass Spectrometry Detection for a Main Degradation Product , *J Chromatogr Sci* **2012**, 50 (5): 426-432.