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Analytical Method Development and Validation by RP-HPLC for Simultaneous Estimation of Metformin Hcl and Vidagliptine in Combined Tablet Dosage Form

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A B S T R A C T

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous estimation of vildagliptin and metformin in combination. The Chromatography was carried out on column inertsil ODS C_{18}(4.6mm x 250mm, 5µm) column using Methanol & water in the ratio of 60:40 as the mobile phase at the flow rate of 1.0 ml/min with UV detection at 263nm. The retention time of metformin & vildagliptin is 2.869mins & 3.942mins respectively. The method was validated for linearity, accuracy, precision, specificity, limt of detection, limt of quantification and robustness. The LOD for metformin and vildagliptin is 0.33mg/ml & 0.32mg/ml and LOQ for metformin & vildagliptin is 1.01 mg/ml and 0.98 mg/ml respectively. And percentage recovery of vildagliptin and metfomin Hcl was found to be 99.9% & 99.8%. The proposed method can be used for determination of these drugs in combined dosage forms.

Keywords: Reverse phase High performance liquid chromatography, vildagliptin, metformin Hcl.

A R T I C L E I N F O

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1. Introduction

Metformin Hcl is a chemically named as N, N-Dimethyl imidodicarbonimic diamide. Metformin is a biguanide agent used for treating non-insulin-dependent diabetes mellitus [1, 2]. It improves glycemic control by decreasing

hepatic glucose production, decreasing glucose absorption and increasing insulin mediated glucose uptake [3]. Metformin is the only oral antihypoglycemic that is not associates with weight gain. Metformin may induce weight loss and is the drug of choice for obese non insulin dependent diabetes mellitus patients [4]. When used alone metformin does not cause hypoglycemia, however, it may potentiate the hypoglycemic effects [5].

Vidagliptin is chemically named as (S)-1-[N-3-hydroxy-1-adamantyl)glycyl]pyrrrolidine. Vildagliptin belongs to a class of orally active antidiabetic drugs (DPP-IV inhibitors) that appear to have multiple functional benefits beyond simple blood-glucose control [6,7]. One of these is a potential protective effect on pancreatic beta cells, which deteriorate in diabetes. Vildagliptin appears to be safe, very well tolerated, and efficacious [8]. Following a meal, gut incretin hormones are released. The most important incretin hormones are GLP-1 and glucose-dependent insulinotropic polypeptide (GIP). These hormones, secreted in the human small intestine, are responsible for insulin release due to increased glucose levels. In contrast to agents that promote insulin secretion via glucose-independent mechanisms, GLP-1's dependence on glucose concentration is considered beneficial due to a lower risk of hypoglycemia [9].

GLP-1 also inhibits glucagon secretion and increases beta cell mass by stimulating proliferation and neogenesis [10,11]. However, the clinical utility of GLP-1 is limited by its short half-life (2 minutes). GLP-1 is rapidly degraded by the proteolytic enzyme DPP-IV. To enhance GLP-1 activity, inhibition of the DPP-IV enzyme is emerging as a novel therapeutic approach in the treatment of diabetes [12,13]. Administration of vildagliptin enhances GLP-1's ability to produce insulin in response to elevated concentrations of blood glucose, inhibit the release of glucagon following meals, slow the rate of nutrient absorption into the bloodstream, slow the rate of gastric emptying, and reduce food intake [14].

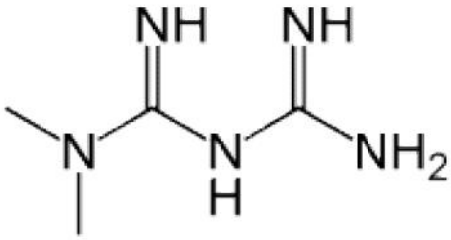

Figure 1: Structure of Metformin Hcl

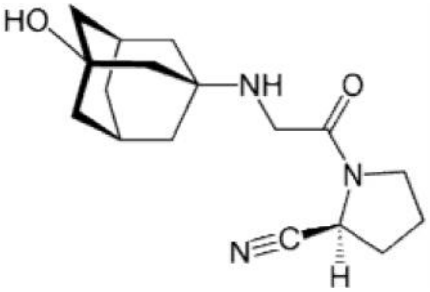

Figure 2: Structure of Vidagliptin

2. Materials and Methods

Instruments:

HPLC –Waters Model NO.2690/5 series Compact System Consisting of

- Inertsil-C18 ODS column.
- Electronic balance (SARTORIOUS)
- Sonicator (FAST CLEAN)

Chemicals:

- Methanol HPLC Grade.
- Water HPLC Grade.

Raw Material:

Metformin and Vildagliptin Working Standards.

Method Development for HPLC:

The objective of this experiment was to optimize the assay method for simultaneous estimation of Metformin and Vildagliptin on the literature survey made. So here the trials mentioned describes how the optimization was done.

Trial: 1

Mobile Phase: Degassed Acetonitrile 100%.

Preparation of Standard Solution:

Weigh down 10mg's of Metformin and Vildagliptin drugs and dissolved in 10ml of Mobile phase taken in two 10ml of volumetric flasks seperately and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from each solution into a 10ml volumetric flask and diluted to 10 ml with mobile phase.

Chromatographic Conditions:

Flow rate : 1.0ml/min
Column : Inertsil - C18, ODS column
Detector wavelength : 263nm
Column temp : Ambient
Injection volume : 20µl
Run time : 10min
Retention time : 4.092min for Metformin and 4.550 min for Vildagliptin.

Observation:

Two peaks are merged and not separated completely. The trial 1 chromatogram result was shown in Fig:3.

Trail: 2

Mobile Phase: Degassed Acetonitrile and methanol in the ratio of 90:10 V/V.

Preparation of Standard Solution:

Weigh down 10mg's of Metformin and Vildagliptin drugs and dissolved in 10ml of Mobile phase taken in two 10ml of volumetric flasks seperately and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from each solution and diluted to 10 ml with mobile phase.

Chromatographic Conditions:

Flow rate : 1ml/min
Column : Inertsil -C18, ODS column
Detector wavelength :263nm
Column temp : Ambient
Injection volume : 20µl
Run time : 10min
Retention time : 3.757 min for Metformin and 4.0 min for Vildagliptin.

Observation: The two peaks are not separated completely but peak shapes are not good. The trial 2 chromatogram result was shown in Fig:4.

Trail: 3

Mobile Phase: Degassed Acetonitrile and Methanol in the ratio of 80:20 V/V.

Preparation of Standard Solution:

Weigh down 10mg's of Metformin and Vildagliptin drugs and dissolved in 10ml of Mobile phase taken in two 10ml of volumetric flasks separately and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from each solution and diluted to 10 ml with mobile phase.

Chromatographic Conditions:

Flow rate : 1.0ml/min
Column : Inertsil - C18, ODS column
Detector wavelength : 254 n.m
Column temp : Ambient
Injection volume : 20µl
Run time : 10min etformin and 2.821 min for Vildagliptin.

Observation: Vildagliptin got peak fronting and base line between two peaks is not straight. The trial 3chromatogram result was shown in Fig:5.

Optimized Method

Mobile Phase: Degassed Methanol and Water in the ratio of 60:40 V/V.

Preparation of stock solution:

Reference solution: The solution was prepared by dissolving 20.0 mg of accurately weighed Metformin and 25.0 mg Vildagliptin in Mobile phase, in two 100.0 mL volumetric flasks separately and sonicate for 20min. From the above solutions take 10.0 mL from each solution into a 50.0 mL volumetric flask and then makeup with mobile phase and sonicate for 10min.

Preparation of working standard solution:

The stock solutions equivalent to 20ppm to 80ppm with respect to both drugs were prepared in combination of Metformin and Vildagliptin above, sonicated and filtered through 0.45µ membrane.

Table 1: Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Inertsil -ODS C18(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol : Water (60:40)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	263nm
Drug RT (min)	2.869min for Metformin and 3.942 for Vildagliptin.

Method Validation

System Suitability:

A Standard solution was prepared by using Metformin and Vildagliptin working standards as per test method and was injected Five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Metformin & Vildagliptin retention times and peak areas.

Acceptance Criteria:

The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0%. The number of theoretical plates (N) for the Metformin and Vildagliptin peaks is NLT 3000. The Tailing factor (T) for the Metformin and Vildagliptin peaks is NMT 2.0. The %RSD for retention times and peak areas were found to be within the limit.

Specificity:

Metformin and Vildagliptin: Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

Acceptence Criteria: Chromatograms of standard and sample should be identical with near Retention time.

Observation:

The chromatograms of Standard and Sample were same identical with same retention time.

Precision

Repeatability:

System precision: Standard solution prepared as per test method and injected five times.

Method precision:

Prepared six sample preparations individually using single as per test method and injected each solution.

Acceptance Criteria:

The % relative standard deviation of individual Metformin and Vildagliptin, from the six units should be not more than 2.0%. The individual assays of Metformin and Vildagliptin should be not less than 98% and not more than 102.0%.

Observation:

Test results are showing that the test method is precise. Refer tables 7 and 8 for system precision and for method precision refer table 9 & 10.

Intermediate precision (analyst to analyst variability):

A study was conducted by two analysts as per test method

Acceptence Criteria:

The individual assays of Metformin and Vildagliptin should be not less than 98% and not more than 102% and %RSD of assays should be NMT2.0% by both analysts.

Observation: Individual %assays and %RSD of Assay are within limit and passes the intermediate precision, Refer table: 11 & 12.

Accuracy (Recovery):

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Metformin and Vildagliptin into each volumetric flask for each spike level to get the concentration of Metformin and Vildagliptin equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Metformin and Vildagliptin were calculated.

Acceptance Criteria: The mean % recovery of the Metformin and Vildagliptin at each spike level should be

not less than 98.0% and not more than 102.0% for both the drugs separately.

Observation:

$$\% \text{ Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

The recovery results indicating that the test method has an acceptable level of accuracy. Refer table: 13 & 14.

Linearity of Test Method:

A Series of solutions are prepared using Metformin and Vildagliptin working standards at concentration levels from 20ppm to 80ppm of target concentration .Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times.

Acceptance Criteria: Correlation Coefficient should be not less than 0.9990. % of y- Intercept should be ±2.0. % of RSD for level 1 and Level 6 should be not more than 2.0%.

Observation: The linear fit of the system was illustrated graphically. The results are presented in table 15 & 16.

Ruggedness of Test Method:

System to system variability:

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability.

Acceptance Criteria:

The % relative standard deviation of Metformin and Vildagliptin from the six sample preparations should be not more than 2.0%. The % assay of Metformin and Vildagliptin should be between 98.0%-102.0%.

Observation:

The % RSD was found within the limit.

b) Column to column variability: Column to column variability study was conducted by using different columns. Six samples were prepared and each was analyzed as per test method.

Acceptance Criteria:

The %RSD of Metformin and Vildagliptin tablets should be NMT2.0%. The %assay of Metformin and Vildagliptin should be between 98.0% and 102.0% for individual drugs.

Robustness:

a) Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow. Metformin and Vildagliptin and was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

Acceptance Criteria: The Tailing Factor of Metformin and Vildagliptin standards should be NMT 2.0 for Variation in Flow.

Observation:

The tailing factor for Metformin and Vildagliptin was found to be within the limits.

b) Effect of variation of temperature:

A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 20ºC temperature. The system suitability parameters were evaluated and found to be within the limits for a temperature change of 20ºc. Similarly sample solution was chromatographed at 25ºC temperature. Metformin and Vildagliptin were resolved from all other peaks and the retention times were comparable with those

Acceptance Criteria:

The Tailing Factor of Metformin and Vildagliptin standard and sample solutions should be NMT 2.0 for Variation in temperature.

Limit of Detection and Quantitation (LOD and LOQ):

From the linearity data calculate the limit of detection and quantitation, using the following formula.

$$LOD = \frac{3.3\,\sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

$$LOQ = \frac{10\,\sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

3. Results and Discussion

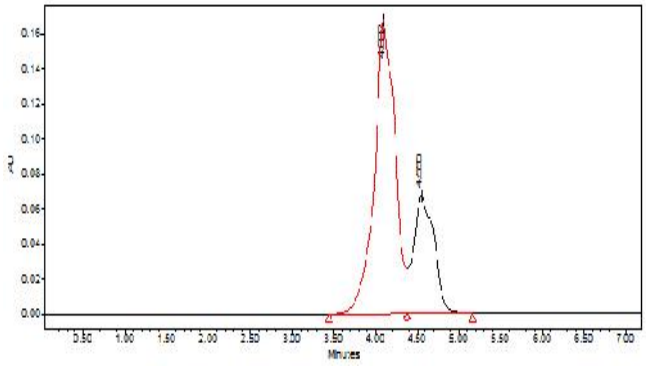

Figure 3: Chromatogram of Trial I

Table 2: Chromatogram of Trial I

S.NO	Name of the peak	Retention time (min)
1	Metformin	4.092
2	Vildagliptin	4.550

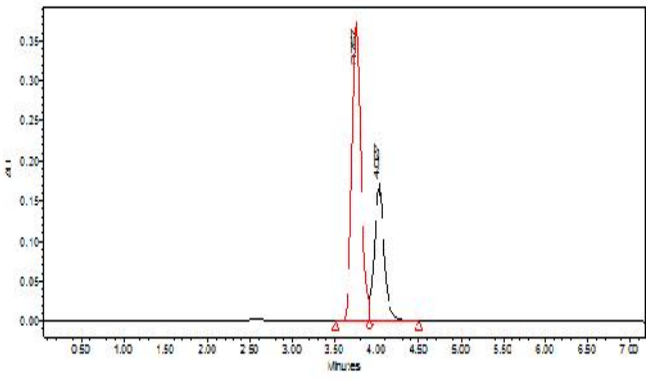

Figure 4: Chromatogram of Trial II

Table 3: Chromatogram of Trial II

S.NO	Name of the peak	Retention time (min)
1	Metformin	3.757
2	Vildagliptin	4.027

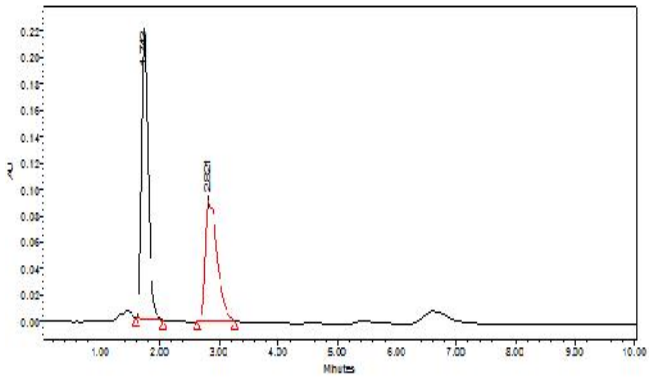

Figure 5: Chromatogram of Trial III

Table 4: Chromatogram of Trial III

S.NO	Name of the peak	Retention time (min)
1	Metformin	1.749
2	Vildagliptin	2.821

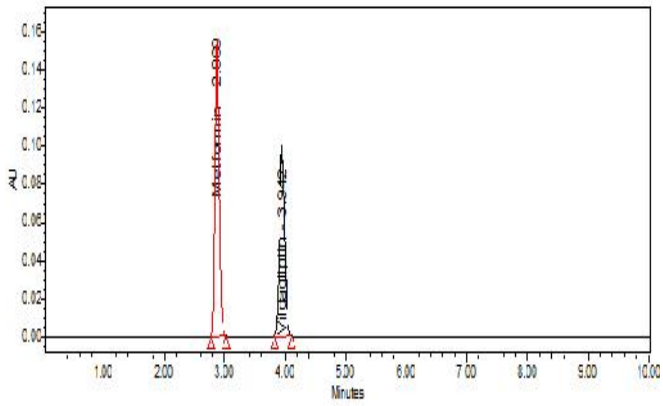

Figure 6: Chromatogram of standard

Table 5: Chromatogram of standard

S.NO	Name of the peak	Retention time (min)
1	Metformin	2.869
2	Vildagliptin	3.942

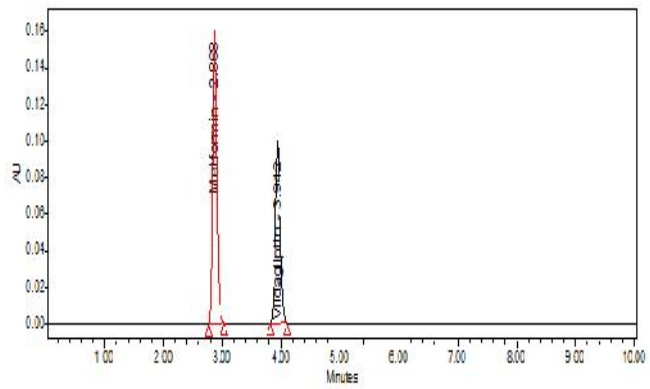

Figure 7: Chromatogram of sample

Table 6: Chromatogram of sample

S.NO	Name of the peak	Retention time (min)
1	Metformin	2.869
2	Vildagliptin	3.942

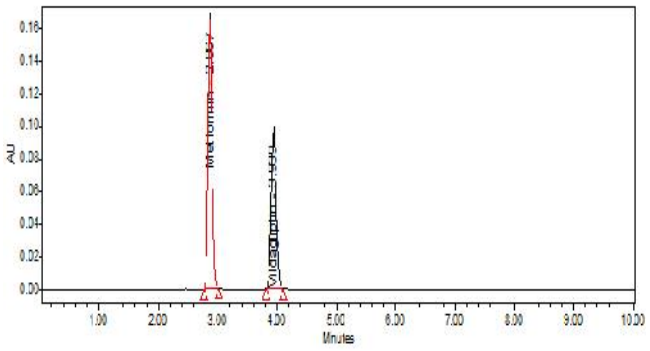

Figure 8: Chromatograms of system precision inject -1

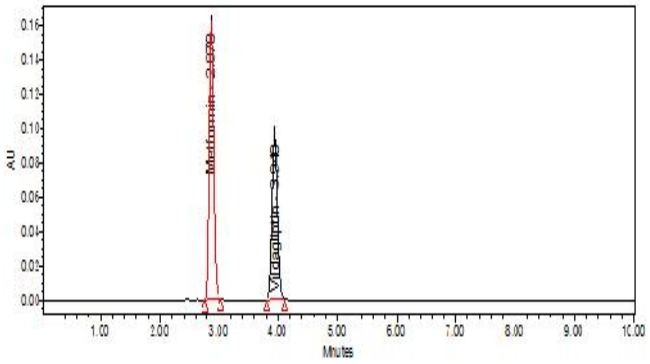

Figure 9: Chromatograms of system precision inject -2

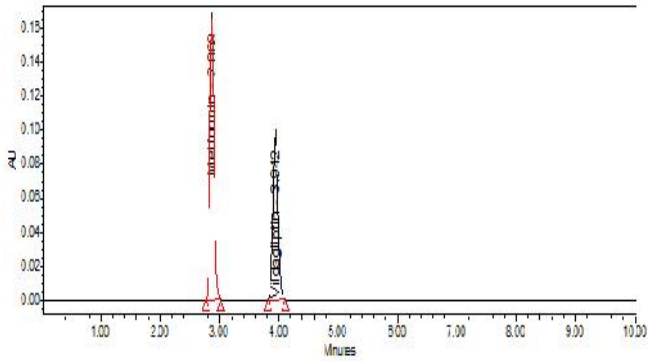

Figure 10: Chromatograms of Repeatability inject- 1

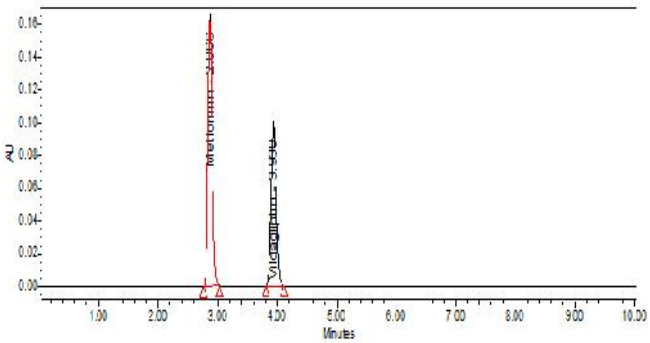

Figure 11: Chromatograms of Intermediate precision inj-3

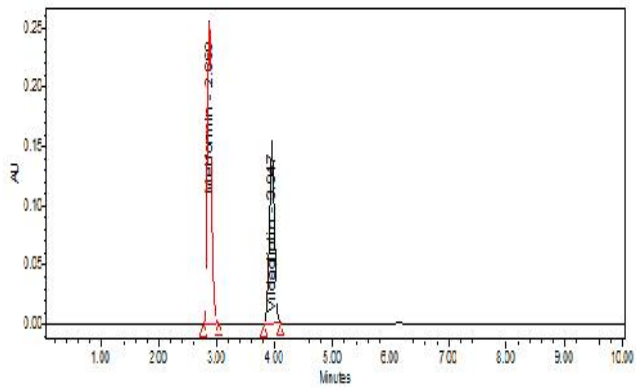

Figure 12: Chromatograms for Accuracy (150%)

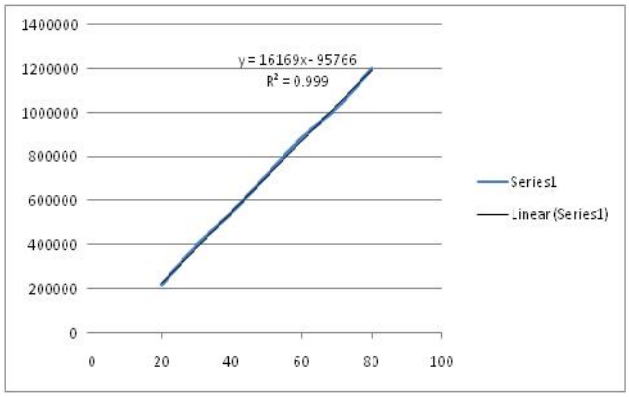

Figure 13: Linearity Plot (Concentration Vs Response) of Vildagliptin

4. Conclusion

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 233nm for Metformin and 296nm for Vildagliptin. Common wavelength will be 263nm and the peaks purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertsil C_{18}, ODS chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase were studied, mobile phase with ratio of 60:40 Methanol: Water was fixed due to good symmetrical peaks and for good resolution. So this mobile phase was used for the proposed study.

The system and method precision was found to be accurate and well within range. Detection limit was found to be 0.33 Metformin and 0.32 for Vildagliptin. Linearity study was, correlation coefficient and curve fitting was found to be 0.999. The analytical method was found linearity over the range of 20-80ppm of the target concentration for both the drugs. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory. The recovery results indicating that the test method has an acceptable level of accuracy was found to be 99.91% for metformin.99.93% for vildagliptin.

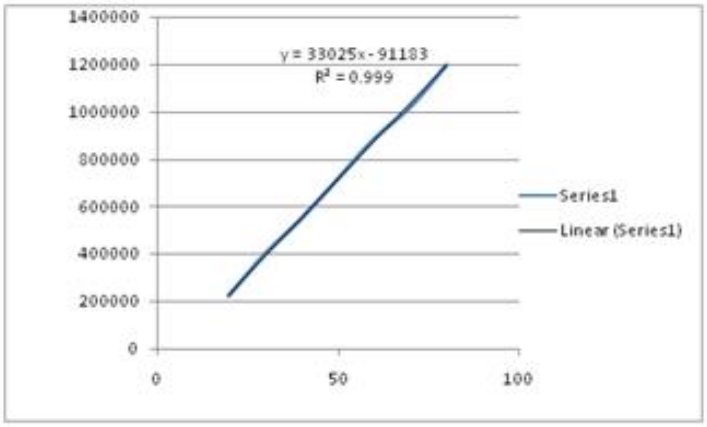

Figure 14: Linearity Plot (Concentration Vs Response) of Metformin

Table 7: Data of Repeatability (System precision) for Metformin

	Injection	Peak Areas of Metformin	% Assay
Concentration 40ppm	1	1239678	99.35
	2	1243389	99.63
	3	1264984	99.54
	4	1248352	99.25
	5	1256493	99.48
Statistical Analysis	**Mean**	1250579	99.4
	SD	10222.12	0.3546
	% RSD	0.817391	0.143

Table 8: Data of Repeatability (System precision) for Vildagliptin

	Injection	Peak Areas of Vildagliptin	% Assay
Concentration 40ppm	1	549407	99.98
	2	547265	99.30
	3	553482	99.60
	4	551981	99.84
	5	551495	99.72
Statistical Analysis	**Mean**	550726	99.71
	SD	6031.135	0.425
	% RSD	0.439932	0.240

Table 9: Data of Repeatability (Method precision) for Metformin

	Injection	Peak Areas of Metformin	% Assay
Concentration 40ppm	1	1243389	98.6
	2	1264984	99.02
	3	1248352	98.12
	4	1256493	98.31
	5	1239664	98.81
Statistical Analysis	**Mean**	1243411	98.36
	SD	1250579	98.48
	% RSD	10222.12	0.352647

Table 10: Data of Repeatability (Method precision) for Vildagliptin

	Injection	Peak Areas of Vildagliptin	% Assay
Concentration 40ppm	1	547265	98.55
	2	553782	98.88
	3	551981	99.40
	4	551495	99.30
	5	547437	100.53
Statistical Analysis	**Mean**	549117	98.28
	SD	550726	99.278
	% RSD	2422.819	0.827236

Table 11: (i) Data of Intermediate precision (Analyst 2) for Metformin

	Injection	Peak Areas of Metformin	% Assay
Concentration 40ppm	1	1243389	98.6
	2	1264984	99.02
	3	1248352	98.12
	4	1256493	98.31
	5	1239664	98.81
Statistical Analysis	**Mean**	1243411	98.36
	SD	1250579	98.48
	% RSD	10222.12	0.352647

Table 12: (ii) Data of Intermediate precision (Analyst 2) for Vildagliptin

	Injection	Peak Areas of Vildagliptin	% Assay
Concentration 40ppm	1	547265	98.55
	2	553782	98.88
	3	551981	99.40
	4	551495	99.30
	5	547437	100.53
Statistical Analysis	**Mean**	549117	98.28
	SD	550726	99.278
	% RSD	2422.819	0.827236

Table 13: (i) Data of Accuracy for Metformin

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% - Injection 1	20	19.95	99.75	**MEAN**	99.81
50% - Injection 3	20	20.08	100.4	**%RSD**	0.55
100 % - Injection 1	40	40.14	100.35	**MEAN**	99.91
100 % - Injection 2	40	39.96	99.9		
100% - Injection 3	40	39.80	99.5	**%RSD**	0.42
150% - Injection 1	60	59.89	99.81	**MEAN**	100.067
150% - Injection 2	60	60.04	100.06		
150% - Injection 3	60	60.09	100.15	**%RSD**	0.17

Table 14: (ii) Data of Accuracy for Vildagliptin

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% - Injection 1	20	19.98	99.9	**MEAN**	99.9
50% - Injection 2	20	19.94	99.7		
50% - Injection 3	20	20.02	100.1	**%RSD**	0.20
100 % - Injection 1	40	39.86	99.65	**MEAN**	99.9
100 % - Injection 2	40	40.05	100.125		
100% - Injection 3	40	39.98	99.95	**%RSD**	0.23
150% - Injection 1	60	59.90	99.83	**MEAN**	99.93
150% - Injection 2	60	59.97	99.95		
150% - Injection 3	60	60.02	100.03	**%RSD**	0.10

5. References

1. Ahrén, B; Landin-Olsson, M; Jansson, PA; Svensson, M; Holmes, D; Schweizer, A (2004). "Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes". The Journal of Clinical Endocrinology and Metabolism. **2004**, 89 (5): 2078–84.
2. Mentlein, R; Gallwitz, B; Schmidt, WE. "Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36) amide, peptide histidine methionine and is responsible for their degradation in human serum". European journal of biochemistry / FEBS. **1993**, 214 (3): 829–35.
3. EU approves Novartis's Eucreas diabetes drug. Reuters, February 25, **2008**.
4. www.ema.europa.eu.
5. Matveyenko AV, Dry S, Cox HI et al. "Beneficial endocrine but adverse exocrine effects of sitagliptin in the human islet amyloid polypeptide transgenic rat model of type 2 diabetes: interactions with metformin". Diabetes. **2009**, 58 (7): 1604–15.
6. Butler AE, Campbell-Thompson M, Gurlo T, Dawson DW, Atkinson M, Butler PC. "Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors". Diabetes. **2013**, 62 (7): 2595–604.
7. Egan, Amy G.; Blind, Eberhard; Dunder, Kristina; De Graeff, Pieter A.; Hummer, B. Timothy; Bourcier, Todd; Rosebraugh, Curtis. "Pancreatic Safety of Incretin-Based Drugs-FDA and EMA Assessment- NEJM". New England Journal of Medicine. **2014**, 370 (9): 794–7.
8. NPS - better medicines, better health (August **2010**). "Vildagliptin for type 2 diabetes". Retrieved 27 August 2010.
9. Pro B, Dang NH. "CD26/dipeptidyl peptidase IV and its role in cancer". Histol. Histopathol. **2004**, 19 (4): 1345–51.
10. Wesley UV, McGroarty M, Homoyouni A "Dipeptidyl peptidase inhibits malignant phenotype of prostate cancer cells by blocking basic fibroblast growth factor signaling pathway". Cancer Res. **2005**, 65 (4): 1325–34.
11. Wesley, U; et al (**2004**). "Role for dipeptidyl peptidase IV in tumor suppression of human non small cell lung carcinoma cells." Int J Cancer. **2004**, 109 (6): 855–866.
12. Clinical Guidelines Task Force, International Diabetes Federation. "Glucose control: oral therapy". In: Global Guideline for Type 2 Diabetes. Brussels: International Diabetes Federation, **2005**, 35–8.